UNITED STATES
CONSUMER PRODUCT SAFETY COMMISSION
Bethesda, MD 20814

Memorandum

Date: April 1, 2010

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THROUGH: Mary Ann Danello, Ph.D., Associate Executive Director, Directorate for Health Sciences
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SUBJECT : Toxicity Review of **Di(2-ethylhexyl) Phthalate (DEHP)**

The following memo provides the U.S. Consumer Product Safety Commission's (CPSC's) Health Sciences staff assessment of the potential toxicity associated with **DEHP**.

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 illness or injury during or as a result of reasonably foreseeable handling or use." Therefore, exposure and risk must be considered in addition to toxicity when assessing potential hazards under the FHSA (CPSC, 1992; summarized at 16 CFR 1500.135)

The FHSA addresses both acute and chronic hazards. While the FHSA does not require manufacturers to perform any specific battery of toxicological tests to assess the potential risk of chronic health hazards, the manufacturer is required to label a product appropriately according to the requirements of the FHSA. The first step in the risk assessment process is hazard identification, that is, a review of the available toxicity data for the chemical under consideration and a determination of whether the chemical is considered "toxic". Chronic toxicity data (including carcinogenicity, neurotoxicity, and reproductive and developmental toxicity) are

* These comments are those of the CPSC staff, have not been reviewed or approved by, and may not necessarily represent the views of, the Commission.

(including carcinogenicity, neurotoxicity, and reproductive and developmental toxicity) are assessed by the CPSC staff using guidelines issued by the Commission (CPSC, 1992). If it is concluded that a substance is "toxic" due to chronic toxicity, then a quantitative assessment of exposure and risk is performed to evaluate whether the chemical may be considered a "hazardous substance". This memo represents the first step in the risk assessment process; that is, the hazard identification step.

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Abbreviations

α_{2u}-globulin	alpha-2-urinary globulin
ADI	Acceptable daily intake
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BMD	Bench Mark Dose
BMDL	Bench Mark Dose Lower confidence limit
BUN	Blood urea nitrogen
CDC	Centers for Disease Control and Prevention (U.S.)
CERHR	Center for the Evaluation of Risks to Human Reproduction, National Toxicology Program (U.S.)
CHAP	Chronic Hazard Advisory Panel
CI	Confidence interval
CMA	Chemical Manufacturers Association
CPN	Chronic progressive nephropathy
CPSC	U.S. Consumer Product Safety Commission
CSTEE	Scientific Committee on Toxicity, Ecotoxicity, and the Environment, European Commission
DEHP	Di(2-ethylhexyl) phthalate
DINP	Diisononyl phthalate
DnOP	Di-*n*-octyl phthalate
DOP	Di-octyl phthalate (DEHP)
ECB	European Chemicals Bureau
F344	Fischer 344
FHSA	Federal Hazardous Substances Act
Gd	Gestational day
GJIC	Gap junction intercellular communication
GL	Guideline study
GLP	Good Laboratory Practices
IUR	Inventory and Update Reporting database
JRC	Joint Research Centre, European Commission, Ispra, Italy
Ld	Lactation day
LOAEC	Lowest observed adverse effect concentration
LOAEL	Lowest observed adverse effect level
LOEL	Lowest observed effect level
MAFF	Ministry of Agriculture, Fisheries, and Food, United Kingdom
MEHP	Mono(2-ethylhexyl) phthalate
MINP	Mono(isononyl) phthalate
MNCL	Mononuclear cell leukemia
N/A	Not available or specified
NERI	Danish National Environmental Institute
NOAEC	No observed adverse effect concentration
NOAEL	No observed adverse effect level
NOEL	No observed effect level
PCNA	Proliferating cell nuclear antigen
PNd	Postnatal day
PPARα	Peroxisome proliferator-activated receptor, alpha isoform
PPd	Postpartum day
PPRE	Peroxisome proliferator response element

PVC	Polyvinyl chloride
PWG	Pathology working group
RIVM	Rijksinstituut voor Volksgesondheid en Milieu (National Institute of Public Health and Environment), the Netherlands
SD	Sprague-Dawley
SER	Smooth endoplasmic reticulum
T½	Half-life
T3	Triiodothyronine
T4	Thyroxine
TBG	Thyroxine-binding globulin
TH	Thyroid hormones
TNO	Nederlandse Organisatie voor toegepast-natuurwetenschappelijk onderzoek (Netherlands Organisation for Applied Scientific Research)
TRα-1	Thyroid homone receptor alpha-1
WY	WY-14,643

Executive Summary

DEHP is a commonly used plasticizer found in a variety of consumer products.

When considering regulations in the Federal Hazardous Substances Act (FHSA), animal data were sufficient to support the conclusion that DEHP was not acutely toxic following single oral exposures. Sufficient animal data and limited human data also supported the conclusion that DEHP was not corrosive or a primary ocular or dermal irritant. There was inadequate evidence to designate DEHP as an acute exposure dermal or inhalation toxicant. Similarly, there was inadequate evidence to designate DEHP as a sensitizer. Sufficient animal data existed to support the conclusion that DEHP had acute, subchronic, and chronic toxicity in a variety of organs. DEHP-induced adverse effects were reported in animal test subject's reproductive organs, liver, kidney, and thyroid in numerous published studies. Sufficient animal data also existed to support the conclusion that DEHP was a carcinogen and a reproductive and developmental toxicant. DEHP-induced carcinogenic effects were reported noted in animal liver, testes, and blood. DEHP-induced reproductive effects were reported in both male and female animal reproductive tissues. DEHP-induced developmental effects in animals occurred following doses that were not maternally toxic. There was inadequate evidence to support the conclusion that DEHP was a neurotoxicant, a respiratory irritant, or a direct acting genotoxicant.

In summary, data supports the conclusion that DEHP can be considered "toxic" under the FHSA due to its toxicity following short-term, intermediate-term, and long-term exposures. This conclusion was based on the sufficient evidence in animals of DEHP-induced toxicity to the liver, kidney, testes, uterus, ovary, fetus, and thyroid.

When considering FHSA criteria, products that contain DEHP may be considered "hazardous" if short-term, intermediate-term, or long-term exposures to the general population during "reasonably foreseeable handling and use" exceed the short-duration, intermediate-duration, or long-term ADI's for the general population (0.1, 0.024, and 0.058 mg DEHP/kg bw-day, respectively).

In addition, products that contain DEHP may be considered "hazardous" if intermediate-term or long-term exposures to male populations during "reasonably foreseeable handling and use" exceed the intermediate-duration or long-term ADI's for reproduction (0.037 and 0.0058 mg DEHP/kg bw-day, respectively).

In addition, products that contain DEHP may be considered "hazardous" if exposures to reproductively viable female populations (13 to 49 years of age) during "reasonably foreseeable handling and use" exceed the ADI for development (0.011 mg DEHP/kg bw-day).

Insufficient evidence (hazard data) precluded the generation of ADI's for inhalation or dermal exposures or for cancer endpoints.

Introduction

This document is a review of current hazard information for di(2-ethylhexyl) phthalate (DEHP). It is intended to be utilized as part of an individual and cumulative phthalate risk assessment. This assessment was prepared from a variety of review articles (NICNAS, 2008; ATSDR, 2002; CPSC, 1985; IARC, 2000; ECB, 2008) as well as supplemental independent studies retrieved from literature searching.

Nomenclature-related confounding issues exist for DEHP. DEHP is commonly termed di-octyl phthalate (DOP) in the published literature and marketing/supplier information reports (i.e., IPL, 2008). DEHP, however, is distinct from DnOP (di-*n*-octyl phthalate; a straight carbon chain analogue), in both hazard and exposure potential. For this reason, DnOP hazards have been detailed in a separate report.

2. Physico-chemical Characteristics

DEHP is the branched chain analog to DnOP and is comprised of a pair of eight-carbon esters linked to a benzene-dicarboxylic acid ring. The branched ester side chains are in an *ortho* configuration, in contrast to those found in isophthalates (*meta*) or terephthalates (*para*). DEHP is one of a variety of plasticizers (Appendix 4) used in the production of polyvinyl chloride plastics, polyvinyl acetate, rubbers, cellulose plastics, and polyurethane resins.

Structural descriptors, names and synonyms, registry numbers, and physico-chemical characteristics of DEHP can be seen in Tables 2.1, 2.2, 2.3, and 2.4, respectively.

Table 2.1 Structural Descriptors and Molecular Formulas of DEHP (ChemIDplus Lite, 2009)

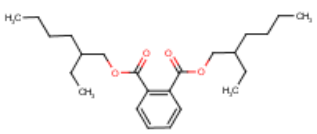
lnChl notation	InChI=1/C24H38O4/c1-5-9-13-19(7-3)17-27-23(25)21-15-11-12-16-22(21)24(26)28-18-20(8-4)14-10-6-2/h11-12,15-16,19-20H,5-10,13-14,17-18H2,1-4H3
Smiles notation	c1(c(C(OC[C@@H](CCCC)CC)=O)cccc1)C(OC[C@@H](CCCC)CC)=O
Molecular	 $C_{24}H_{38}O_4$; $C_6H_4[COOCH_2CH(C_2H_5)(CH_2)3CH_3]_2$; MW = 390.56

Table 2.2 Names and Synonyms of DEHP (ChemIDplus Lite, 2009)	
Synonyms	4-09-00-03181 (Beilstein Handbook Reference), AI3-04273, BRN 1890696, Bis(2-ethylhexyl) 1,2-benzenedicarboxylate, Bis(2-ethylhexyl) phthalate, Bis-(2-ethylhexyl)ester kyseliny ftalove, Bis-(2-ethylhexyl)ester kyseliny ftalove [Czech], Bisoflex 81, Bisoflex DOP, CCRIS 237, Caswell No. 392K, Celluflex DOP, Compound 889, DEHP, DOF, DOF [Russian plasticizer], Di(2-ethylhexyl) orthophthalate, Di(2-ethylhexyl) phthalate, Di(2-ethylhexyl)orthophthalate, Di(2-ethylhexyl)phthalate, Diacizer DOP, Diethylhexyl phthalate, Dioctyl phthalate, EINECS 204-211-0, EPA Pesticide Chemical Code 295200, Ergoplast FDO, Ergoplast FDO-S, Etalon, Etalon (plasticizer), Ethylhexyl phthalate, Eviplast 80, Eviplast 81, Fleximel, Flexol DOP, Flexol Plasticizer DOP, Good-rite GP 264, HSDB 339, Hatcol DOP, Hercoflex 260, Jayflex DOP, Kodaflex DEHP, Kodaflex DOP, Mollan O, Monocizer DOP, NCI-C52733, NSC 17069, Nuoplaz DOP, Octoil, PX-138, Palatinol AH, Pittsburgh PX-138, Plasthall DOP, Platinol AH, Platinol DOP, RC Plasticizer DOP, RCRA waste number U028, Reomol D 79P, Reomol DOP, Sansocizer DOP, Sansocizer R 8000, Sconamoll DOP, Sicol 150, Staflex DOP, Truflex DOP, Vestinol AH, Vinicizer 80, Witcizer 312
Systematic Name	1,2-Benzenedicarboxylic acid, 1,2-bis(2-ethylhexyl) ester, 1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester, Bis(2-ethylhexyl) phthalate, Di(2-ethylhexyl) phthalate, Phthalic acid, bis(2-ethylhexyl) ester
Superlist Name	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester, Bis(2-ethylhexyl) phthalate, DEHP, Di(2-ethylhexyl) phthalate, Di-(2-ethylhexyl) phthalate, Di-2-ethylhexyl phthalate, Di-2-ethylhexylphthalate, Di-sec-octyl phthalate, Diethylhexyl phthalate, Ethyl hexyl phthalate, Octyl phthalate, Phthalic acid, bis(2-ethylhexyl) ester, RCRA waste no. U028

Table 2.3 Registry Numbers for DEHP (ChemIDplus Lite, 2009, ATSDR, 2002)	
CAS Registry Number	117-81-7
Other CAS Registry Numbers	109630-52-6, 126639-29-0, 137718-37-7, 205180-59-2, 275818-89-8, 40120-69-2, 50885-87-5, 607374-50-5, 8033-53-2
System Generated Number	000117817

Table 2.4 Physico-chemical Properties of DEHP	
Purity	**99.6% min** (IPL, 2009)
Color	**Light Colored** (ChemIDplus Lite, 2009); Clear, 30 max on Pt/Co scale (IPL, 2009)
Odor	**Odorless** (ChemIDplus Lite, 2009); Slight odor (ATSDR, 2002)
Physical State	**Oily liquid** (NICNAS, 2008)
Water Solubility	**0.003 mg/L** (Staples *et al.,* 1997); 0.27 mg/L @ 25C (ChemIDplus Lite, 2009); 0.041mg/L @ 25 C (NICNAS, 2008; ATSDR, 2002); 0.0006 to 1.2 mg/L (Staples *et al.,* 1997)
Vapor Pressure	**$1x10^{-7}$ mmHg @ 25C** (Staples *et al.,* 1997; ATSDR, 2002); 1.42E-07 mm Hg @ 25C (ChemIDplus Lite, 2009); $1.33x10^{-8}$ kPa @ 25 C (NICNAS, 2008); $4.8*10^{-8}$ to $1.4*10^{-4}$ (Staples *et al.,* 1997)
Melting Point	**-47 C** (Staples *et al.,* 1997; NICNAS, 2008; ATSDR, 2002); -55C (ChemIDplus Lite, 2009)
Boiling Point	**384C** (ChemIDplus Lite, 2009; NICNAS, 2008; ATSDR, 2002), 387 C (CPSC, 1985)
Flash Point	**196 C** (NICNAS, 2008); 384.8 F (196 C; ATSDR, 2002)
Specific Gravity (g/mL)	**0.986 @ 20 C** (Staples *et al.,* 1997)
Log P (octanol-water; K_{ow})	**7.5** (Staples *et al.,* 1997; NICNAS, 2008; ATSDR, 2002); 7.6 (ChemIDplus Lite, 2009); 9.64 (Leyder and Boulanger, 1983 cited in CPSC, 1985); 4.2 to 8.39 (Staples *et al.,* 1997)
K_{oc} (L/kg; soil/sediment)	**87,420** to **510,000** (Staples *et al.,* 1997)
K_{oc} (L/kg; suspended solids)	**22,000** to **$1*10^{6}$** (Staples *et al.,* 1997)
Henry's Law Constant	**$1.71x10^{-5}$ atm-m^3/mole @ 25 C** (Staples *et al.,* 1997; NICNAS, 2008; ATSDR, 2002); 2.70E-07 atm-m^3/mole @ 25C (ChemIDplus Lite, 2009)
Atmospheric OH Rate Constant	**2.20E-11 cm^3/molecule-sec @ 25C** (ChemIDplus Lite, 2009)
Density	**984 kg/m^3 (g/ml) @ 20C** (NICNAS, 2008;ATSDR, 2002); 0.986 g/cm^3 (IPL, 2009)
Refractive Index	**1.485 ± 0.003 at 20 C** (IPL, 2009)
Storage Stability	"No observable changes in dietary concentrations [in feeds prepared monthly] were observed on storage [at room temperature]" (Poon *et al.,* 1997); "2.7% loss after 21 days of storage at room temperature" (Reel *et al.,* 1984 cited in Poon *et al.,* 1997); No significant difference in the concentration of DEHP in DEHP/rodent chow samples stored for 2 weeks at -20, 5, 25, and 45°C
Packaging	**200 kg in drums**, bulk (IPL, 2009)
Autoignition temperature	**735 F** (390 C; HSDB, 1990 cited in ATSDR, 2002)

3. Manufacture, Supply, and Use

In general, DEHP is manufactured commercially in a closed system by catalytically esterifying phthalic anhydride with 2-ethylhexanol. Unreacted alcohols are recovered and reused, and the DEHP mixture is purified by vacuum distillation or activated charcoal. The purity of DEHP using this method has been reported historically at over 99% (CPSC, 1985). The remaining fraction of the DEHP commercial mixture is comprised of the impurities isophthalic acid (CAS No. 121-91-5), terephthalic acid (CAS No. 100-21-0), and maleic acid (CAS No. 110-16-7; Thomas *et al.*, 1978 cited in CPSC, 1985). DEHP can also contain bisphenol A (CAS No. 80-05-7) at concentrations ranging from 0.025 to 0.5% (ECB, 2008).

DEHP is also naturally produced in red (*Bangia atropurpurea*; Chen, 2004), but not green (*Ulva* sp.) or brown algae (*Undaria pinnatifida*, *Laminaria japonica*; Namikoshi *et al.*, 2006). DEHP produced in red algae is not, however, used commercially.

The 2006 EPA non-confidential Inventory Update Reporting (IUR) database listed three producers (Eastman Chemical Company; Sterling Chemicals, Inc; and Sunoco, Inc) and eight importers (BASF Corporation; Chemcentral Corporation; GNC Corporation, Incorporated; Kyowa Hakko USA, Inc; LG Chem America, Inc; Polyone Corporation; Teknor Apex; and Tremco Incorporated) of DEHP into the United States. Market analysis reporting suggested that 2 additional producers (BASF Corporation and Exxon Mobil) currently compete with Eastman Chemical Company as the major U.S. producers of DEHP (Tecnon Orbichem, 2007).

Annual production estimates for DEHP in 2002 were approximately 240 million pounds (TURI, 2008). The 2006 IUR estimated that the aggregated U.S. national production volume of DEHP was between 100 and < 500 million pounds. These production estimates far exceeded the 2006 estimated annual imports (~ 69 million pounds) and exports (~ 13 million pounds) from the United States, suggesting that local incorporation into products was very important in the overall distribution of DEHP (Table 3.1). Other factors, such as the limited availability of other phthalates and chemicals (i.e., DINP and 2-ethylhexanol) and price fluctuations (i.e., 80/85 to 87/92 ¢/pound from March to June 2007) also affected the demand for and incorporation of DEHP into products (Tecnon Orbichem, 2007, 2009).

DEHP and di-iso-nonyl phthalate (DINP) were the two primary phthalates in the world chemical markets (Tecnon Orbichem, 2007, 2009). South Korea and Taiwan were the two major exporters of DEHP, and China was the predominate importer (Table 3.1). Annual importation of DEHP into China in 2006 to 2007 was approximately 672 to 746 million pounds compared to approximately 69 million pounds in the U.S. (Tecnon Orbichem, 2007, 2009).

Table 3.1 Worldwide Import and Export of DEHP

(average thousands of pounds/month; Tecnon OrbiChem, 2007, 2009)

Contributing Country	U.S. Imports[1] 2006/2007/2008	Chinese Imports[2] 2006/2007	Japanese Imports[1] 2006/2007	U.S. Exports[1] 2006/2007/2008	Chinese Exports[2] 2006/2007	Japanese Exports[1] 2006/2007	Taiwan Exports[1] 2006/2007	South Korean Exports[1] 2006/2007
Mexico	456/561-747/339	- /283	-	-	-	-	-	-
Brazil	1400/14-17/349	-	-	-	-	-	-	-
South Korea	3748/ -/359	31921/31421	2728/371	- /403-677/-	-	-	-	-
Canada	-	-	-	1045/761-822/628	-	-	-	-
Iran	-	- /1045	-	-	-	-	-	-
Malaysia	-	2320/3020	2699/ -	-	-	-	-	- /85
Singapore	-	3147/26	-	-	-	-	-	-
Taiwan	-	15838/25322	-	-	-	-	-	-
Hong Kong	-	-	-	-	235/711-802/530	-	-	-
United States	-	-	-	-	-	-	2585/ -	-
India	-	-	-	-	-	-	32/1333	1730/851
Phillippines	-	-	-	-	-	-	842/746	640/330
Vietnam	-	-	-	-	-	-	892/1197	85/138
China	-	-	-	-	-	-	9348/23555	28492/30371
Thailand	-	-	-	-	-	1236/935	-	-
Peru	-	-	-	-	-	-	-	678/554
Algeria	-	-	-	-	-	-	-	464/357
Egypt	-	-	-	-	-	-	-	995/808
Ethiopia	-	-	-	-	-	-	-	224/352
Kenya	-	-	-	-	-	-	-	896/806
Nigeria	-	-	-	-	-	-	-	896/3264
Syria	-	-	-	-	-	-	-	426/1397
UAE	-	-	-	-	-	-	-	181/245
Japan	-	-	-	-	-	-	-	2365/993
Others	119/44-45/65	419/281	5/ -	14/5-11/156	184/274-317/118	370/297	1449/684	3840/2560

[1] = averaged from data on January to March or January to October of the respective year

[2] = averaged from data on January to April of the respective year

Production or release of DEHP was reported in the Toxics Release Inventory (TRI; EPA, 2009). TRI figures suggested that the number of facilities associated with DEHP, total release into air and land, and the total offsite waster transfer of DHEP has been declining in the U.S. since the late 1980's (Figure 3.1, 3.2).

Figure 3.1 2006 Toxics Release Inventory Estimates for DEHP

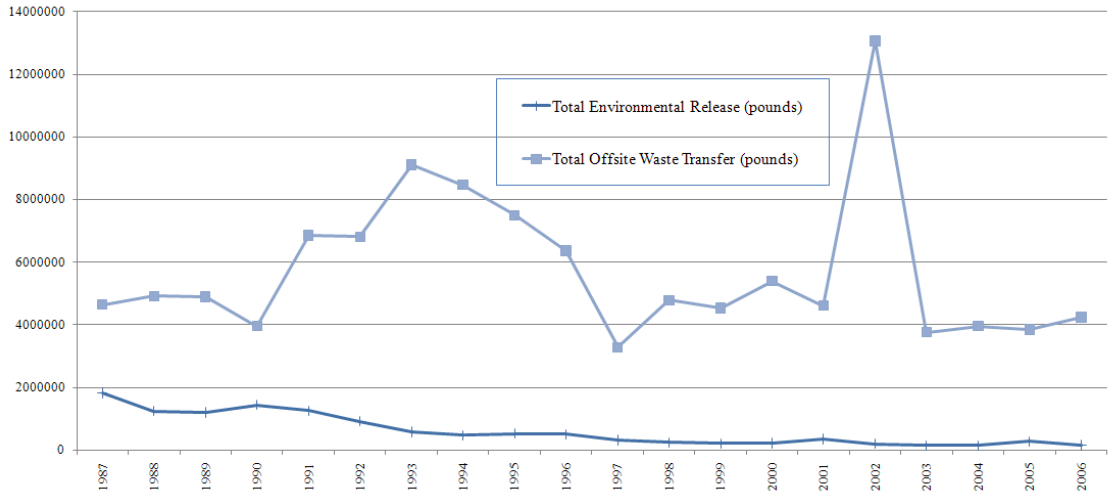
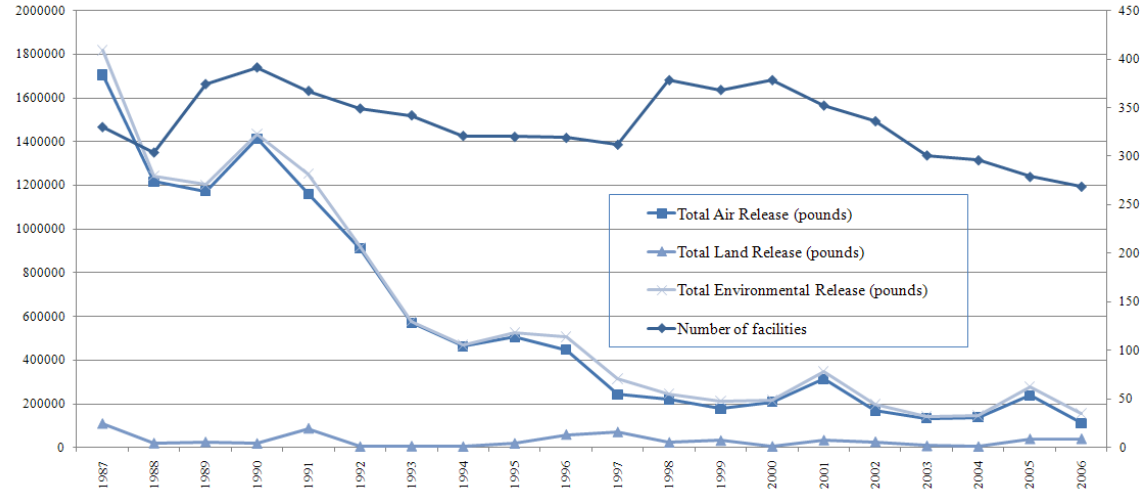


Figure 3.2 2006 Toxics Release Inventory Estimates for DEHP



DEHP uses can be divided into two categories: 1) use as a polymer, and 2) use as a non-polymer.

As a polymer, DEHP has been primarily used as a general purpose plasticizer in plastics production (97% of all DEHP). It has had additional uses in the creation of PVC, polyvinyl acetate, rubbers, cellulose plastics and polyurethane. As a polymer, DEHP imparts flexibility and other mechanical properties to various types of plastics found in consumer products, medical devices, and industrial/commercial products. Its use in medical devices (i.e., medical tubing and IV bags) and industrial/commercial products accounted for 25% and 45% of the overall consumption, respectively.

As a non-polymer, DEHP has been used in the formulation and industrial use of sealants, adhesives, paints, lacquers, printing inks, dielectric fluids, and ceramics. These uses constituted less than 3 to 5% of the national use of DEHP.

ATSDR (2002) reported the EPA Toxics Release Inventory data on the types of industries that used products containing DEHP. These included: "abrasive products, adhesives and sealants, agricultural chemicals, asbestos products, boat building and repair, cement, chemical preparations, chemicals and allied products, coated fabrics (not rubberized), crowns and closures, current carrying wire devices, custom compound purchased resins, electrical industrial apparatus, electromedical equipment, electronic capacitors, electronic components, fabric dress and work gloves, fabricated metal products, fabricated rubber products, gaskets, gray and ductile iron foundries, hand and edge tools, hard surface floor coverings, household laundry equipment, hydraulic, industrial inorganic chemicals, industrial organic chemicals, manufacturing industries, mattresses and bedsprings, meat packing plants, mechanical rubber goods, medicinals and botanicals, minerals (ground or treated), motor vehicles and car bodies, noncurrent carrying wiring devices, nonferrous wire drawing and insulating, nonmetallic mineral products, packing and sealing devices, paints and allied products, paper (coated and laminated), pharmaceutical preparations, photographic equipment and supplies, plastics foam products, plastics materials and resins, plastics pipe, plastics products, plating and polishing, refuse systems, rubber and plastic footwear, rubber and plastic hose and belting, sporting and athletic goods, surface active agents, surgical and medical instruments, tires and inner tubes, unsupported plastics film and sheet, unsupported plastics profile shapes, wood household furniture, wood products".

A variety of these uses were also cited in other publications. DEHP has also been used industrially and commercially to some extent as a dielectric fluid in small electrical capacitors (NOAA, 1985), and in other electronic component parts, extrudable molds and profiles, wire and cable coatings and jacketing, paper coatings, and aluminum foil coating/laminating (TURI, 2008), and hydraulic fluid, industrial and lubricating oils, defoaming agents during paper and paperboard manufacturing, and vacuum pump oil (IARC, 2000; Houlihan *et al.,* 2002; DiGangi *et al.,* 2002). DEHP has also been found in PVC medical devices (blood transfusion tubing, nasogastric tubing, endotracheal tubing, hemodialysis tubing, cardiopulmonary bypass and extracorporeal membrane oxygenation tubing, parenteral feeding tubes, blood, dialysis, and storage bags, catheters, PVC gloves, PVC dentures; PVC oxygen tents, syringes) (Houlihan *et al.,* 2002; DiGangi *et al.,* 2002; FDA, 2001; SCENIHR, 2007), in food packaging materials (IARC, 2000), food wrap (SCENIHR, 2007), cellophane, resinous and polymeric coatings used in food packaging, as a "flow promoter" in food contact surfaces, as a surface lubricant used in the manufacture of metallic articles that contact food, as a plasticizer for packaging for foods with high water content, in PVC straws, in PVC squeeze bottles, and historically in tubing used for milking cows (IARC, 2000; Houlihan *et al.,* 2002; DiGangi *et al.,* 2002). DEHP has also been found in products used as linings for landfill waste disposal sites, as a solvent in paints and

printing ink for textiles (ECB, 2008; SCENIHR, 2007), in munitions (IARC, 2000), as a leak detector in respirator and air filtration testing (IARC, 2000), and as a plasticizer in polyvinyl butyral, natural and synthetic rubber, chlorinated rubber, ethyl cellulose, and nitrocellulose production (ATSDR, 2002). DEHP is/was also used in a wide variety of consumer products. These can be seen in Table 3.2.

Table 3.2 Products Reported to Contain DEHP*

Consumer Products* Historically or Currently Created with DEHP**									Other
Arts/Crafts	**Auto**	**Home Maintenance**	**Home Office**	**Inside Home**	**Landscape/Yard**	**Personal Care**	**Pesticides**	**Pet care**	
Adhesives (SCENIHR, 2007)	Vehicle seats (CPSC, 1985)	Sealing and adhesive systems (polyurethane and polysulphide) (IPL, 2009; Jensen and Knudsen, 2006)	PVC notebook covers (Houlihan *et al.*,2002; DiGangi *et al.*,2002)	PVC flooring (Houlihan *et al.*,2002; DiGangi *et al.*,2002; DiGangi *et al.*,2002; IPL, 2009)	PVC weight covers (Houlihan *et al.*,2002; DiGangi *et al.*,2002)	Cosmetics (CERHR, 2006; SCENIHR, 2007)	Solvent, carrier, plasticizer (CPSC, 1985; ATSDR, 2002)	Cat and dog toys (Müller *et al.*,2006; Nielsen *et al.*,2005)	Footwear (CPSC, 1985; IARC, 2000; TURI, 2008)
Paints (SCENIHR, 2007)	Auto upholstery and tops (ATSDR, 2002; CERHR, 2006)	PVaC-based adhesives and paint binders(IPL, 2009)	Paper coatings (TURI, 2006)	PVC wall coverings (IPL, 2009)	PVC umbrellas (Houlihan *et al.*,2002; DiGangi *et al.*,2002)	Rubbing alcohol (IARC, 2000)	Acaricide active ingredient (IARC, 2000; ATSDR, 2002)		Childrens toys and infant items (CPSC, 1985; IARC, 2000; CERHR, 2006; TURI, 2008; Stringer *et al.*,1997, 2000; "slimy toys", Svendsen *et al.*,2005)
Decorative inks (IARC, 2000)	Floor mats (Houlihan *et al.*,2002; DiGangi *et al.*,2002)	Roofing (TURI, 2006)	Electronic component parts (TURI, 2006)	Shower curtains (IARC, 2000; TURI, 2008)	Garden hoses (ATSDR, 2002)	Perfumes (Houlihan *et al.*,2002; DiGangi *et al.*,2002; CERHR, 2006)	Inert ingredient (IARC, 2000)		Medical devices (TURI, 2008; Karbaek, 2003; FDA, 2001)
	Auto undercoating (TURI, 2006)	Wire and cable coatings and jackets (IARC, 2000; Houlihan *et al.*,2002; DiGangi *et al.*,2002; TURI, 2008)	Lighting ballasts and electric capacitors (TURI, 2006)	Upholstery (CPSC, 1985; IARC, 2000)	Swimming pool liners (ATSDR, 2002)	Hairspray (CERHR, 2006)			Expanded or imitation leather (IARC, 2000; IPL, 2009)
	Fenders, car door arm rests (ECB, 2008)	Construction materials (CERHR, 2006)	Erasable ink (ATSDR, 2002)	Tablecloths (IARC, 2000)	Water wings, swimming rings, paddling pools (Houlihan *et al.*,2002; DiGangi *et al.*,2002; DME, 2004)	PVC gloves (CERHR, 2006)			PVC foam products (IPL, 2009; plastic sword, mask, floor puzzle, surf board, activity carpet, book, ball, Borling *et al.*,2006)
		PVC roofing film (Houlihan *et al.*,2002; DiGangi *et al.*,2002)	Cardboard (ATSDR, 2002)	Liquid detergents (IARC, 2000)		Nail polishes (CERHR, 2006)			Films (IPL, 2009)
		Wood finishes (CERHR, 2006)	Pencil case, erasers (Svendsen *et al.*,2007)	Carpet coverings (Houlihan *et al.*,2002; DiGangi *et al.*,2002)		Body shampoo/ bath gel containers (Poulson and Schmidt, 2007)			Infant care items (changing pads, bibs, vinyl/rubber pants, diaper pants, playpens; CPSC, 1985; Houlihan *et al.*,2002; DiGangi *et al.*,2002; Tønning *et al.*,2008)

Table 3.2 Products Reported to Contain DEHP*									
Consumer Products* Created with DEHP** continued									Other
Arts/Crafts	**Auto**	**Home Maintenance**	**Home Office**	**Inside Home**	**Landscape/Yard**	**Personal Care**	**Pesticides**	**Pet care**	
		PVC gaskets (Houlihan *et al.*,2002; DiGangi *et al.*,2002)		PVC drawer liner (Houlihan *et al.*,2002; DiGangi *et al.*,2002)					Rainwear (IARC, 2000)
		PVC insulation (Houlihan *et al.*,2002; DiGangi *et al.*,2002)		PVC furniture covers (Houlihan *et al.*,2002; DiGangi *et al.*,2002)					Orthodontic retainers and dental composites (CERHR, 2006)
				PVC inflatable furniture/mattresses (Houlihan *et al.*,2002; DiGangi *et al.*,2002)					PVC Ball (CERHR, 2006)
				PVC mattress pads (Houlihan *et al.*,2002; DiGangi *et al.*,2002)					Disposable diapers (ATSDR, 2002)
				PVC shades (Houlihan *et al.*,2002; DiGangi *et al.*,2002)					PVC stroller covers (Houlihan *et al.*,2002; DiGangi *et al.*,2002)
				PVC tarps (Houlihan *et al.*,2002; DiGangi *et al.*,2002)					PVC purses (Houlihan *et al.*,2002; DiGangi *et al.*,2002)
				PVC waterbeds (Houlihan *et al.*,2002; DiGangi *et al.*,2002)					PVC luggage (Houlihan *et al.*,2002; DiGangi *et al.*,2002)
				PVC mattress and pillow case covers (Houlihan *et al.*,2002; DiGangi *et al.*,2002)					PVC clothing, backpacks, aprons (Houlihan *et al.*,2002; DiGangi *et al.*,2002)
				Ceramics (TURI, 2006)					Infant formula (TURI, 2006)

Table 3.2 Products Reported to Contain DEHP*									
Consumer Products* Created with DEHP** continued									Other
Arts/Crafts	**Auto**	**Home Maintenance**	**Home Office**	**Inside Home**	**Landscape/Yard**	**Personal Care**	**Pesticides**	**Pet care**	
									Aluminum foil coating/lamination (TURI, 2006)
									Adult entertainment toys (Nilsson *et al.*,2006)
									Headphones (Schmidt *et al.*,2008)
									Surface glazing on wooden toys (Hansen and Pedersen, 2005)
									Cotton, wool, flax, PET, and viscose textiles (Jensen and Knudsen, 2006)
									Soft drink and mineral water plastic bottles (Bosnir *et al.*,2007)

* CPSC shares regulatory jurisdiction with other Federal agencies for some of the products referenced in this table

** Amended categories from the Consumer Product Information Database created by the U.S. Health and Human Services Commission, 2009

4. Toxicokinetics

CPSC staff has reviewed both human and animal studies that investigate absorption, distribution, metabolism, and excretion of DEHP from oral, dermal, inhalation, or other routes of exposure. These studies illustrated that the toxicokinetics of DEHP was variable and strongly dependent on the age, gender, test species, and dose of DEHP. The toxicokinetic studies that address these factors are in table format in Appendix 1. A general diagrammatic representation of DEHP metabolism in rats and humans has been provided in Figures 4.1 and 4.2, respectively. A list of metabolite names can be seen in Table 4.1.

Table 4.1 Metabolite Designations and Their Corresponding Chemical Name

Metabolite	Chemical Name	Other Designation
I	Mono (2-ethyl-3-carboxypropyl) phthalate	MECPrP
II	Mono (2-carboxyhexyl) phthalate	-
III	Mono (2-ethyl-4-carboxybutyl) phthalate	MECBP
IV	Mono (2-carboxymethyl)hexyl phthalate	2cx-MMHP or MCMHP
V	Mono (2-ethyl-5-carboxypentyl) phthalate	5cx-MEPP or MECPP
VI	Mono (2-ethyl-5-oxyhexyl) phthalate	5oxo-MEHP or MEOHP
VII	Mono (2-(2-hydroxyethyl))hexyl phthalate	MHEHP
VIII	Mono (2-ethyl-4-hydroxyhexyl) phthalate	-
IX	Mono (2-ethyl-5-hydroxyhexyl) phthalate	5OH-MEHP or MEHHP
X	Mono (2-ethyl-6-hydroxyhexyl) phthalate	MEHHP
XI	Mono (2-ethylhexyl) phthalate Mono (2-ethylpentyl) phthalate	MEHP
XII	Mono (2-ethyl-4-oxyhexyl) phthalate	-
XIII	Phthalic acid	PA
XIV	Mono (2-carboxymethyl-4-oxyhexyl) phthalate	-
XV	Mono-(2-ethyl-4-oxo-5-carboxypentyl) phthalate Mono-(2-ethyl-4-oxo-6-carboxyhexyl) phthalate	MEOCPP
XVI	Mono-(2-ethyl-4-hydroxy-5-carboxypentyl) phthalate Mono-(2-ethyl-4-hydroxy-6-carboxyhexyl) phthalate	MEHCPP
XVII	Mono (2-(1-hydroxyethyl))hexyl phthalate	MHEHP
XVIII	Mono (2-carboxymethyl-4-hydroxyhexyl) phthalate	-
XIX	Mono (2-(1-hydroxyethyl)-5-hydroxyhexyl) phthalate	-
XX	Mono (2-ethyl-4,6-dihydroxyhexyl) phthalate	-
XXI	Mono (2-carboxymethyl-5-hydroxyhexyl) phthalate	-
XXII	-	-
XXIII	-	-
XXIV	-	-
XXV	Mono (2-carboxymethyl-5-oxyhexyl) phthalate	-
XXVI	Mono (2-(1-oxyethyl)hexyl) phthalate	MOEHP

Figure 4.1 Metabolic Relationships of DEHP in Rat Urine (Albro, 1986)

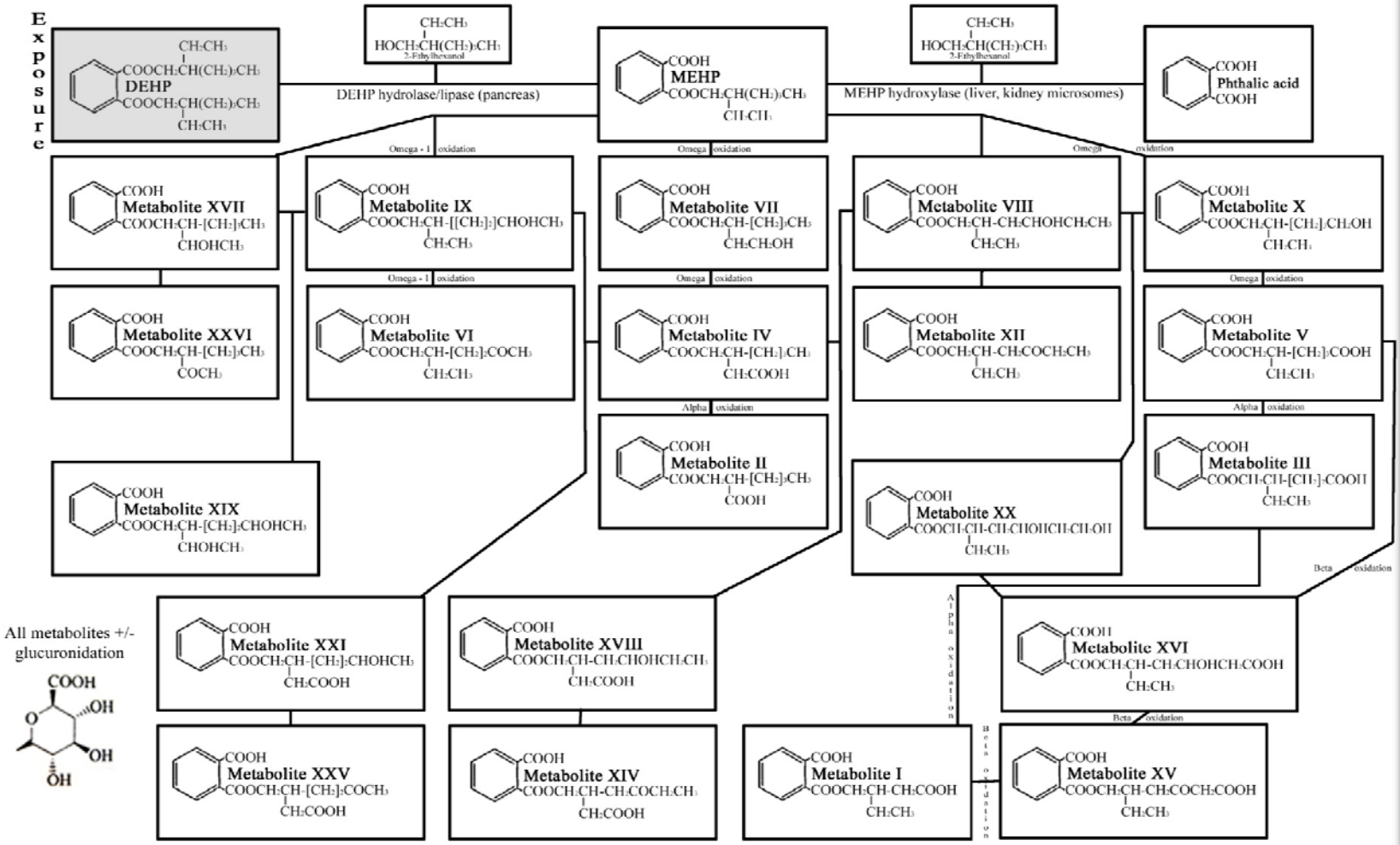
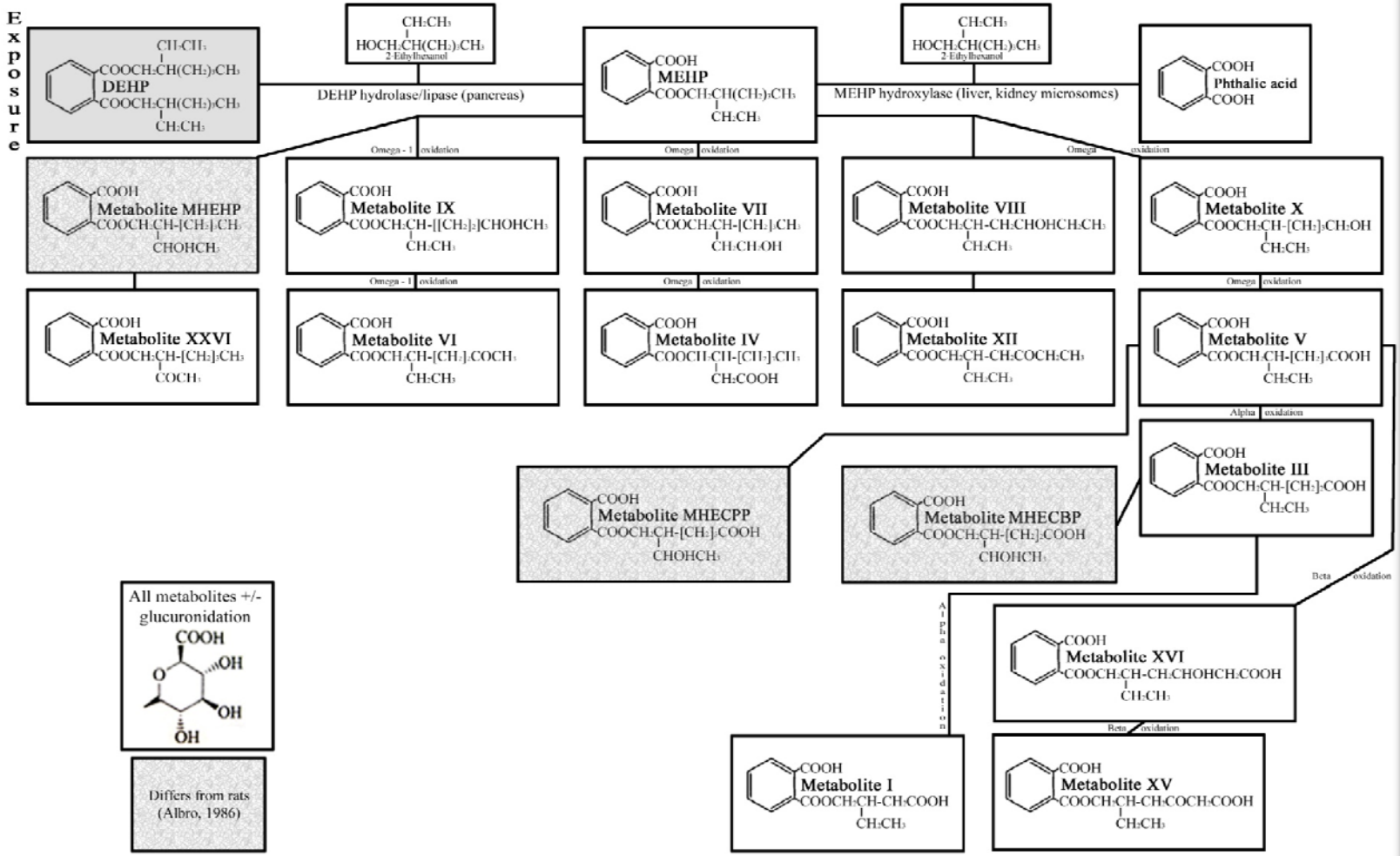


Figure 4.2 Metabolic Relationships of DEHP in Human Urine (Silva et al., 2006; Koch et al., 2006)



Absorption

Oral Exposure

Absorption following exposure has been investigated in a variety of toxicokinetic studies. Overall, these studies demonstrate that absorption is dependent on the test species, dose, dosing duration, and gut contents. Anecdotal observations from other ester compounds support the general conclusion that esterase-mediated metabolism (i.e., DEHP metabolism to MEHP) in the intestine can be highly species, site, compound, and presentation dependent (Inoue *et al.,* 1979; Van Gelder *et al.,* 2000).

Absorption from the gastrointestinal tract following oral ingestion is rapid for both DEHP (ECB, 2008; ICI, 1982a; Shell, 1982; Rhodes *et al.,* 1986; Pollack *et al.,* 1985a) and its primary metabolites mono-(2-ethylhexyl) phthalate (MEHP; Chu *et al.,* 1978) and 2-ethylhexanol (Albro, 1975).

Absorption of DEHP into the blood is a function of dose, however, and only larger oral doses result in parent DEHP partitioning into the systemic circulation. Numerous high dose studies report parent DEHP residues in blood, tissue, and excreta (Albro *et al.,* 1982a; Lake *et al.,* 1984b; Sjoberg *et al.,* 1985c; Pollack *et al.,* 1985a). *In vitro* experiments demonstrate that systemic transport of DEHP probably occurs following binding to serum proteins (Griffiths *et al.,* 1988).

With low dose exposures, DEHP is metabolized to MEHP and 2-ethylhexanol prior to absorption. Metabolism to MEHP is mediated by endogenous gut enzymes (esterases, hydrolases, pancreatic lipases) that are present in many tissues (Rowland, 1974; Rowland *et al.,* 1977; Kluwe, 1982). Because the majority of relevant metabolic enzymes originate in the pancreas, metabolism and hence, absorption occurs primarily in the small intestine.

Enzymes produced by gastrointestinal microflora or gut contents also metabolize DEHP to MEHP. Rat gut contents from the stomach, small intestine, and caecum have been shown to metabolize 1.0, 22.1, and 6.9%, respectively, of the DEHP dose following 16 hours of co-incubation (Rowland *et al.,* 1977). The proportion of metabolism occurring in human feces, in comparison, was lower (0.6%). In the rat, the ability of gut contents to metabolize DEHP was linked in-part to bacteria, since treatment with antibiotics during incubation reduced the overall DEHP metabolism (Roland, 1974).

Humans

In humans dosed with DEHP on food, peak absorption of DEHP or it metabolites occurred prior to a peak in serum concentration (2 hours following the oral administration of 0.64 mg DEHP/kg; Koch *et al.,* 2003). Only a small percentage (12 to 14%) of MEHP was absorbed when compared to other lower molecular weight phthalates (Anderson *et al.,* 2001).

Monkeys

In marmoset monkeys, absorption of DEHP or its metabolites also peaked rapidly and earlier than peak levels in the blood (1 and 1 to 3 hours following large single or multiple daily doses, respectively; 2000 mg/kg; ICI, 1982a; Shell, 1982; Rhodes *et al.,* 1986). In some circumstances, peak blood concentrations remained the same for at least 24 hours following the cessation of dosing, suggesting that there was either continued absorption from gastrointestinal compartments, or redistribution from tissues into systemic circulation.

Rats

In Sprague-Dawley rats, absorption of DEHP or its metabolites occurred prior to a peak in blood concentration (3 hours following dosing with 2000 mg/kg; Pollack *et al.,* 1985a; or 1 to 7 hours following dosing with 1000 mg/kg; Sjoberg *et al.,* 1985c). MEHP, the absorbed metabolite, had a peak plasma C_{Max} of 1 hour in most animals (range 3 to 7 hours; Sjoberg *et al.,* 1985c). Maximal absorption of MEHP occurred prior to peaks in blood levels (0.5 hours post-dose) and was sometimes followed by a secondary peak in blood levels a few hours later, possibly resulting from reabsorption of excreted metabolites (Chu *et al.,* 1978). MEHP in the blood was transported primarily as a complex bound to plasma proteins (Sjoberg *et al.,* 1985c).

In rats, the plasma concentration curves (MEHP AUCs) were higher for younger rats (25 day old; 1213 µg.hr/mL) than older ones (60 days old; 555 µg.hr/mL) when exposed to the same dose (1000 mg DEHP/kg) and dosing strategy, suggesting that differences in absorption or gut metabolism existed for different ages within a species (Sjoberg *et al.,* 1985c). Species or age differences may only apply to the effects following higher doses, however, since at low doses (i.e., 100 mg/kg), absorption for monkeys, rats, and mice was similar (CMA, 1982b; CMA, 1983; CMA, 1984a; Short *et al.,* 1987; Astill *et al.,* 1986).

General Comparison

A DEHP absorption threshold was reported to exist in some species such as the cynomolgous monkey, (Short *et al.,* 1987; Monsanto, 1988) and the marmoset monkey (Rhodes *et al.,* 1983; Rhodes *et al.,* 1986), but not in others such as CD-1 and $C3B6F_1$ mice (Albro *et al.,* 1982a). For species with a threshold, this meant that as oral doses increased, the percent of absorbed material decreased.

Metabolic adaptation may affect the absorption threshold. The rate of absorption has been shown to increase following chronic exposures, possibly because of an increased rate of conversion of DEHP to MEHP by intestinal contents and/or mucosa (Rowland, 1974; Rowland *et al.,* 1977).

Overall, the oral bioavailability of DEHP was summarized for 21 studies by the ECB (2008). Bioavailability in humans (37.5 to 46.5%) was similar to rats (30 to 50%), but higher on average than marmosets (2 to 45%) or Cynomolgous monkeys (6 to 50%).

Dermal Exposure

Dermal absorption rates have primarily been determined in animal studies. In the only human study reviewed by ECB (2008), dermal uptake was not able to be estimated because dermal contact durations were not reported. In animal studies, *in vivo* dermal absorption estimates of DEHP ranged from 6.5 to 26% in a variety of species (ECB, 2008; CPSC, 1985; Table 4.2).

Table 4.2 *In vivo* Dermal Absorption of DEHP
(ECB, 2008)

Test Animal (number)	Factors					
	Dose - Duration	Epidermis - whole	Epidermis - stratum corneum	Dermis	Hypodermis	Citation
Fischer 344 rat – male (3)	30 mg/kg (4.5 mg/cm^2) – single dose, non-occluded	At 5 days, 95% dose remained at application area; cumulative amount detected in excreta and tissues excluding dosed skin (**dermal absorption) = 9%**				Melnick *et al.,* 1987
Fischer 344 rat – male (?)	30-40 mg/kg (5-8 mg/cm^2 {9.3 mg/cm^2 -; CPSC, 1985}- single dose – occluded with a perforated cap	At 5 - 7 days, 86% dose remained at application area; cumulative amount detected in excreta and tissues excluding dosed skin (**dermal absorption) = 6.5 {6.9; CPSC, 1985} %**				Elsisi *et al.,* 1985, 1989
Hairless guinea pigs – female (5)	53 µg (13.2 µg/cm^2) – single dose – non-occluded	At 24 hours, cumulative amount detected in excreta and tissues excluding dosed skin (**dermal absorption) = 26%,** volatilization of dose over 7 days = 10%				Ng *et al.,* 1992
Hartley hairless guinea pigs – female (5)	119 µg/cm^2 for 24 hr 107 µg/cm^2 for 48 hr 442 µg/cm^2 for 7 days 529 µg/cm^2 for 14 days	At respective timepoints, cumulative amount detected in excreta and tissues including dosed skin (**dermal absorption) = 9.7 - 18%**				Chu *et al.,* 1996
Fischer 344 rat – male (8)	25.5 mg/cm^2 - single dose-occlusive	At 24 hours and 7 days, percutaneous **absorption rate** based on mass balance = **0.24 µg/cm^2/hr**				Deisinger *et al.,* 1998

In vitro permeability constants (K_p) and rates (J) ranged from 0.0105 to 94.6 *10^{-5} cm/hr and 0.02 to 22.37 µg/cm^2/hr, respectively, and were also reported for a variety of species (ECB, 2008; Table 4.3)

Table 4.3 *In vitro* Dermal Absorption of DEHP (ECB, 2008)						
Test Media (number)	Factors (Kp = *10^{-5} cm/hr, J = µg/cm^2/hr)					
	Dose - Duration	Epidermis - whole	Epidermis - stratum corneum	Dermis	Hypodermis	Citation
Human, nonviable skin; rat nonviable skin	70 mg/cm^2 @ 30C with glass diffusion cell; 50% aqueous ethanol as receptor fluid	Human K_p=0.57; Human J = 5.59, Rat K_p = 2.28, Rat J = 22.37; Human lag time = 3.1 hr, Rat lag time = 3.9 hr	-	-	-	Scott *et al.,* 1987
Human nonviable skin	288-576 mg/cm^2 @ 30C or 37C with Franz-type diffusion cell; isotonic saline as receptor fluid	-	Human K_p=0.0105; Human J = 0.1	-	-	Barber *et al.,* 1992
Fischer 344 rat full thickness skin	288-576 mg/cm^2 @ 30C or 37C with Franz-type diffusion cell; isotonic saline as receptor fluid	Rat K_p = 0.0431; Rat J = 0.42				Barber *et al.,* 1992
Guinea pig full thickness viable (FTV) and nonviable (NV) skin	53.2, 228, 468 µg/cm^2 FTV; 53.2 µg/cm^2 NV@ 37C with diffusion cell; HEPES-buffered HBSS, gentamicin and BSA as receptor fluid	Guinea pig J 53.2 µg/cm^2 (FTV) = 0.13, 53.2 µg/cm^2 (NV) = 0.11, 228 µg/cm^2 (FTV) = 0.23, 468 µg/cm^2 (FTV) = 0.49				Ng *et al.,* 1992
Sprague-Dawley rat – male epidermis and dermis	78.6 µg/cm^2 @ 31.5C with diffusion cell; PBS and 50% aqueous ethanol as receptor fluids	Rat K_p w/PBS = 1.3, Rat J w/PBS = 0.02; Rat K_p w/50% eth. = 94.6, Rat J w/50% eth. = 0.786	-	Rat K_p w/PBS = 4.76; Rat K_p w/50% eth. = 9.83	-	Pelling *et al.,* 1998
Porcine skin flaps - perfused	18.5 µg/cm^2 with a nonrecirculating chamber; undescribed perfusate	Porcine J = 0.34 µg/cm^2/hr				Wester *et al.,* 1998

Inhalation Exposure

The amount of DEHP absorbed through inhalation routes of exposure has received limited investigation in humans and rodents.

Humans

Human case studies involving preterm infants with tracheal intubations in a hospital setting (Roth *et al.,* 1988), workers at a DEHP manufacturing plant (Liss *et al.,* 1985), and workers in a boot and cable factory (Dirven *et al.,* 1993a) suggested that DEHP was absorbed via the inhalation route of exposure. In the studies reviewed, no details were given for the amount of DEHP absorbed or whether metabolic conversion ocurred prior to absorption.

Rats

Two rodent inhalation studies have been performed using DEHP as the test chemical (General Motors, 1982a, 1982b). Both studies demonstrated that inhaled DEHP was absorbed rapidly from Sprague-Dawley rat lungs (as demonstrated by concentrations of radioactivity in the blood) and that repeated inhalation dosings did not change the disposition kinetics of DEHP from that seen in the single dose experiment (General Motors, 1982a, 1982b cited in ECB, 2008). No futher study details were provided in the reviews consulted.

Other Exposure

Absorption following intravenous exposures is assumed to be complete since gastrointestinal and dermal barriers are bypassed and delivery to target tissues is rapid.

Intraperitoneal absorption of DEHP was slow and incomplete when compared to that from oral exposures. In a study comparing oral gavage, intravenous, and intraperitoneal exposure routes, Rhodes *et al.* (1983, 1986) demonstrated that the majority of a single DEHP dose (85% of 1000 mg/kg) remained in the peritoneal cavity of marmoset monkeys seven days post-dosing. In contrast, only minimal amounts of DEHP were found in the urine, feces, and tissues (10.0, 4.0, and 0.6%, respectively) at seven days post-dosing. As demonstrated in Sprague-Dawley rats, reduced intraperitoneal absorption can be exacerbated by multiple doses (Pollack *et al.,* 1985a).

Distribution

Oral Exposure

DEHP distribution following exposure and absorption was discussed in a substantial number of toxicokinetic studies. Overall, these studies demonstrated that DEHP and MEHP quickly partitioned into the blood and were systemically distributed to target tissues and organs. Systemic transport of both DEHP and MEHP occurred following binding to plasma proteins in *in vivo* and *in vitro* studies (Sjoberg *et al.,* 1985c; Griffiths *et al.,* 1988).

Humans

In humans, the absorption and distribution phase of DEHP toxicokinetics lasted approximately 4 to 8 hours when dosed with small amounts of DEHP on food (0.64 mg/kg; Koch *et al.,* 2003). No other information on the distribution of DEHP or its metabolites was found.

Monkeys

In monkeys, distribution of DEHP or DEHP metabolites to the blood compartment was fast and peaked from 1 to 3 hours following administration. Blood concentrations of DEHP or metabolites were dependent on the exposure dose, with plasma concentration curves (AUCs) averaging 208 and 466 µg-hr/ml for 100 and 500 mg/kg doses respectively. A doubling of plasma concentration (when a 5-fold increase was expected) suggested that there was a dose-dependent reduction in absorption from the gastrointestinal tract (Short *et al.,* 1987; Monsanto, 1988). Once in the blood, distribution of the DEHP or its metabolites in monkeys was primarily to the liver, kidneys, and gastrointestinal tract (Short *et al.,* 1987; Monsanto, 1988; CMA, 1982b; CMA, 1983; CMA, 1984a; Astill *et al.,* 1986; ICI, 1982a; Shell, 1982; Rhodes *et al.,* 1986).

Rats

In Sprague-Dawley rats, the majority of DEHP metabolites were located in the gastrointestinal tract immediately following dosing (50 mg/kg-day, Ikeda *et al.,* 1980). Some radiolabel activity was seen in other organs by 4 hours, with the liver having the highest concentration of metabolites (2% of the total dose). By 4 days following administration, the amount of metabolites in the intestinal tract was negligible. Less than 1% of the total dose was located in the bile.

DEHP peaked in the blood by 3 hours and was consistently lower in concentration than MEHP when measured at similar timepoints. Multiple daily doses did not change the concentration of DEHP in the blood (Pollack *et al.,* 1985a) or the maximum plasma concentrations and mean AUCs of MEHP, metabolites IX, VI, and V (Sjoberg *et al.,* 1986a) when compared to a single dose. In addition, the mean plasma elimination half-life of MEHP for multiple doses (1.8 hours) was not substantially different from that of single doses (3 hours; Sjoberg *et al.,* 1986a). Following a single dose, the liver and abdominal fat contained 6 and 4-

fold more DEHP metabolites, respectively, than the carcass and other tissues (Eastman Kodak Company, 1983). Ninety-six hours following dose administration, negligible concentrations of DEHP metabolites were found in the liver, kidney, or total gut contents (Lake *et al.,* 1984b).

Distribution of DEHP metabolites in Sprague-Dawley rats was age-dependent. Young rats (25 days old; 1213 µg.hr/mL) dosed with DEHP (1000 mg/kg) had higher mean AUCs than other older rats (40 and 60 days old, 611 and 555 µg.hr/mL) when exposed to similar doses and durations of exposure (Sjoberg *et al.,* 1985c). Mean plasma maximum concentrations (C_{max}; 1 to 7 hours) and mean plasma elimination half-lives (2.8 to 3.9 hours for clearance from blood to tissues) for MEHP were not different, however, among the age groups (Sjoberg *et al.,* 1985c).

In Fischer 344 rats, dosing concentration determined the overall distribution of intact DEHP in the body. Albro *et al.* (1982a) reported that intact DEHP reached the liver at 450 mg/kg doses and higher. By 4.5 days post-dose, DEHP metabolites were primarily in the intestinal contents, liver, fat, kidney, and adrenal gland. As with Sprague-Dawley rats, pretreatment with DEHP did not affect the distribution of DEHP metabolites in Fischer 344 rats (CMA, 1982a; Lington *et al.,* 1987; Short *et al.,* 1987; Astill *et al.,* 1986). DEHP metabolite accumulation was not observed in the livers or testis of Fischer 344 rats (Albro *et al.,* 1982a).

In Wistar rats, DEHP metabolites equilibrated quickly into the liver and abdominal fat following dosing (carbonyl ^{14}C-DEHP; 1000 or 5000 mg/kg for 35 and 49 days; $t_{½liver}$ = 1 to 2 days; $t_{½fat}$ = 3 to 5 days), but did not accumulate in the tissues (Daniel and Bratt, 1974). DEHP metabolites peaked in blood and testis (6 hours), liver and kidney (2 to 6 hours) and were not retained in the brain, heart, lungs, liver, spleen, kidney, stomach, intestine, testicle, blood, muscle, or adipose tissue (Tanaka *et al.,* 1975; Oishi, 1990). The half-life for MEHP in the blood (7.4 hours; AUC = 1497 µg.h per ml) was similar to that in the testes (8 hours; AUC = 436 µg.h per ml) even though AUCs were vastly different (Oishi, 1990). By 4 days post-administration of similar doses, < 0.1% of the DEHP metabolites remained in the tissues and organs (Lake *et al.,* 1975).

Administration of very high doses of DEHP (~9765 mg/kg) to JCL:Wistar rats resulted in DEHP and MEHP peak concentrations in blood and most tissues by 6 to 24 hours (Oishi and Hiraga, 1982). At 1 hour post-dosing, DEHP or metabolites were highest in the heart and lungs. By 6 hours post-dosing, the testes had the highest MEHP/DEHP ratio (2.1) when compared to blood (1.1) and other tissues (< 1.0). At 48 hours post-dosing, the concentration of DEHP peaked in the fat. During the first 24 hours, DEHP/metabolites were also detected at low levels in the brain and kidney. MEHP was also detected in the lungs and DEHP in the spleen. The half-life for DEHP in testicular tissue (8 hours) was less than both liver (24 hours) and epididymal fat (156 hours). The MEHP half-life in the blood (23 hours) was less than in the epididymal fat (68 hours; Oishi and Hiraga, 1982).

Distribution of ingested MEHP was similar to that of DEHP. Following oral gavage doses of 69 mg (7-^{14}C) MEHP/kg to Sprague-Dawley rats, MEHP concentrations peaked in the

blood at 0.5 hours and then peaked again at 5 hours post-dosing (Chu *et al.,* 1978). Subsequent dosing with 35 to 69 mg MEHP/kg resulted in an immediate accumulation in the blood, followed by a gradual decline at 10 and 20 minutes post-dose. At 20 minutes, MEHP or metabolites were distributed primarily to the liver, kidney, and bladder and secondarily to other tissues. By 24 hours, most of the DEHP metabolites were excreted, and negligible amounts were present in the liver, kidney, heart, lungs, intestine, and muscle.

Mice

In $B6C3F_1$ mice, oral gavage dosing of DEHP resulted in distribution throughout the whole body to target tissues. In mice, DEHP or metabolite residues were highest in the fat and marginal (< 1.0 mg/kg) in many other tissues (CMA, 1982b; CMA, 1983; CMA 1984a; Short *et al.,* 1987; Astill *et al.,* 1986). DEHP or its metabolites were not stored in any of these tissues for extended periods (Gaunt and Butterworth, 1982). In C57BL mice, DEHP or metabolites were primarily located in the stomach and small intestine 24 hours following dosing. Metabolites in these tissues declined to neglible amounts by 72 hours post-dose. Metabolites in the colon or feces reached a maximum by 2 and 4 hours, before declining substantially by day 3 post-dosing. DEHP metabolites were also found in cecal contents by 1 hour, reached a maximum by 2 hours, and declined significantly by 3 days post-dosing. In the kidney, DEHP metabolites were concentrated in the renal pelvis and renal papillae. Radiolabel activity in the kidney parenchyma [and testis] was comparable to general tissue levels. Bladder metabolite concentrations were highest from 1 to 24 hours post-dose and declined by day 3 (Gaunt and Butterworth, 1982). Pretreatment of C57BL mice with DEHP did not alter the distribution of DEHP or DEHP metabolites with subsequent dosing, except in the brown fat (Lindgren *et al.,* 1982). In young 3 to 20 day old NMRI mice, treatment with DEHP resulted in neglible radiolabel activity in the brain (Eriksson and Darnerud, 1985). Metabolite content in the liver, however, decreased from 27% to 2% as the mouse aged from 3 to 20 days old. This activity decreased even further following 7 days of recovery.

Hamsters

High doses of DEHP (1000 mg/kg) to Syrian golden hamsters followed by a 96 hour recovery period resulted in negligible concentrations of DEHP and DEHP metabolites in the liver, kidneys, or total gut (Lake *et al.,* 1984b).

Dogs and Miniature Pigs

In dogs and miniature pigs, large concentrations of DEHP and its metabolites were found in the gastrointestinal tract shortly after dosing. Metabolite concentrations decreased significantly in this tissue by day 4 post dosing (Ikeda *et al.,* 1980). DEHP was also found in piglet subcutaneous fat (0.42 mg/kg), renal fat (0.37 mg/kg), muscle (2.4 mg/kg), heart (< 0.2 mg/kg), lungs (0.25 mg/kg), and kidney (< 0.2 mg/kg), but not in the brain following low dose exposures (~ 125 mg/kg-day; Jarosova *et al.,* 1999). Radiolabel activity decreased 50% in

subcutaneous fat, renal fat, muscle, heart, and lungs by 14 days post-dosing, and to control levels in all tissues except renal fat and lungs by 28 days post-dosing. After administration of DEHP, MEHP concentrations increased in the liver, whole blood, and urine. MEHP tissue residues returned to control levels, however, by 14 days after exposure.

Hens

In broiler hens, both DEHP and MEHP were distributed throughout the body after low dose exposures (~135 mg/kg-day). DEHP partitioned into the mesenteric fat (0.33 mg/kg), skin (3.8 mg/kg), muscle (2.5 mg/kg), and liver (0.47 mg/kg; Jarosova *et al.,* 1999). As with piglets, the concentration of DEHP declined by 50% following a 14 day recovery period. MEHP, in contrast, was found primarily in the blood (7-fold higher than controls) and marginally in the liver (< 0.01 mg/kg) immediately after exposure. MEHP concentrations declined to control levels by 14 days after exposure.

Distribution into the Milk and Across the Placenta

Both MEHP and DEHP were also distributed/translocated across the placenta of pregnant rodents. Srivastava *et al.* (1989) reported that exposing pregnant rats to large doses of DEHP during gestation day (Gd) 6 to 15 resulted in detectable DEHP residues in fetal livers. Similar findings were reported for Fischer 344 rats. Uptake into C57BL mice fetuses also occurred following DEHP exposure to pregnant mice (Lindgren *et al.,* 1982). Exposure to dams at various times of gestation resulted in uptake into the yolk sac and embryo gut (4 hours post-dose on Gd 8), the embryo neuroepithelium and uterine fluid (24 hours post-dose on Gd 8), and the renal pelvis, urinary bladder, intestinal contents, skeleton, and liver (4 hours post-dose on Gd 16). On Gd 17 marginal radiolabel activity was seen in the fetuses (except for the renal pelvis, urinary bladder, and intestinal contents).

Both MEHP and DEHP can also be distributed into the milk of lactating rat dams. Sprague-Dawley rat dams exposed to large doses of DEHP (2000 mg/kg) during lactation days (Ld) 15 to 17 resulted in detectable milk concentrations of MEHP (25 µg/mL) and DEHP (216 µg/mL; Dostal *et al.,* 1987b; Table A3.28). This administration also resulted in MEHP, but "virtually no" DEHP in the plasma of pups 6 hours following multiple doses. Since pups typically also ingest feed and maternal feces by Ld14, the MEHP may not necessarily have been derived entirely from transfer from the milk (Tyl, personal communication, 2009). DEHP was also reported in the livers of 1 to 21 day old rat pups that had ingested milk produced by lactating dams exposed to DEHP (Parmar *et al.,* 1985).

Enterohepatic Recirculation

Enterohepatic recirculation or reabsorption of metabolites has not been well described for DEHP, but can increase the duration of exposures at target tissues. Chu *et al.* (1978) suggested that substantial resorption of DEHP metabolites occurred in the intestine of Sprague-Dawley

rats. Reabsorption of these metabolites may explain secondary peaks in metabolite blood concentrations observed hours after the primary peak (Chu *et al.,* 1978).

Dermal Exposure

Distribution in dermal exposures involves consideration of the local distribution of dose (percent remaining in/on skin) as well as the systemic distribution of the dose (organs, tissues). Elucidation of both types of information can greatly aid in the prediction of target sites.

Humans

The distribution of dermal DEHP doses in humans has been described in a limited sense by Wester *et al.* (1998) and ECB (2008). Overall, the percent of DEHP or DEHP metabolite recoveries were low following 24 hours of non-occlusive dosing when considering a skin surface wash (4.5%), cumulative urine excretion (1.1%), and tape recovery (0.15%). No additional details were provided in the reviewed study.

Rats

In rats, the majority of dermal DEHP doses remained at the site of application at 5 or 7 days after exposure (86 to 87%, Elsisi *et al.,* 1985, 1989; 95%, Melnick *et al.,* 1987). Of the dose absorbed, most was transported and retained in the muscles (1.17% and 1.2%, respectively). Retention in other tissues was low (≤ 0.3%) and overall, the amount of DEHP/metabolites remaining in the body was < 2% of the applied dose by 5 or 7 days post exposure. Distribution to the feces and urine in both studies was similar (2.1 and 3.0% for feces and urine {4.5% for both combined}, Elsisi *et al.,* 1985, 1989; 3% for feces and 5% for urine, Melnick *et al.,* 1987) after 5 or 7 days. In rat studies investigating the migration of DEHP from a plastic film, migration of DEHP occurred from the film at a rate of 0.725 and 1.4 μg/cm^2/hr by 24 hours after exposure (Deisinger *et al.,* 1998).

Guinea Pigs

In guinea pigs exposed dermally to DEHP, 31% of the dose was found in the 24 hour skin wash/rinsate and 13% in the protective skin pad. In addition, 21% of the dose was found in the urine and feces, 5% in the body tissues (liver, fat, muscle, skin), and 11.3% of the dose was recovered with tape stripping 7 days post-exposure. *In vitro* experiments with viable and nonviable skin confirmed that a lower amount of dose was captured in human skin washes when compared to rat studies (Ng *et al.,* 1992). For both skin types, the 24 hour percent recovery ranged from 38 to 50% (skin washes), 36 to 41% (skin disk) and 2.4 to 6.1% (receptor fluid). *In vitro* analysis also suggested that 10% of the dose may have volatilized after 7 days. In another study performed in guinea pigs (Chu *et al.,* 1996), the authors reported that “the amount of DEHP remaining in the skin after washing will eventually enter the systemic circulation and

should be considered as part of the total dose absorbed". The authors also commented that dermal penetration may be facilitated by hair follicles.

Pigs

In vitro experiments with pig skin demonstrated that the majority of a dermal dose remained in or on the skin (Wester *et al.,* 1998). Approximately 71% of the administered dose was recovered in the skin wash, 14.5% in skin strips, 3.8% in the skin, and 0.14% in the perfusate.

Inhalation Exposure

Humans

In humans, the ultimate distribution of DEHP following inhalation exposures was slightly different than that after oral exposures. Unmetabolized DEHP was found in the lungs and urine, but not liver, of preterm infants intubated with respiratory apparatus in a hospital setting. Furthermore, MEHP was not detected in the urine of these infants, or in the urine of workers exposed to aerosolized DEHP at a DEHP manufacturing plant (Roth *et al.,* 1988 and Liss *et al.,* 1985 cited in ECB, 2008). In other occupational settings involving potential inhalation exposures (a boot factory and a cable factory), the metabolites MEHP, V, VI, and IX were significantly increased in the urine of workers at the end of a work day when compared to concentrations at the beginning of the work day (Dirven *et al.,* 1993a, 1993b cited in ECB, 2008).

Rodents

In rodents, DEHP was absorbed relatively quickly following inhalation exposure and distributed to the carcass and skin (75%), the lung (10%), and all other tissues (2%; except the brain; General Motors, 1982a, 1982b). Distribution to the lung may have resulted from particles trapped in the mucocilliary escalator (ECB, 2008) or represented true partitioning to lung tissue, since this effect was also observed following intravenous dosing of marmosets (Rhodes *et al.,* 1983) and in preterm infants on medical ventilation (Roth *et al.,* 1988). Metabolite concentrations in the blood decreased in a log non-linear fashion following exposure, and the original burden of DEHP (and/or metabolites) redistributed as it cleared in the feces and urine. Only 6% of the original DEHP or metabolite burden remained in the tissues (lung and liver with small amounts, kidney with trace amounts), carcass, and skin after 72 hours. Forty percent of the metabolites were found in the feces and 52% in the urine after 72 hours.

Other Exposure

Intravenous exposure to low doses of DEHP in C57BL mice resulted in significant distribution of DEHP/metabolites to the gall bladder, intestinal contents, urinary bladder, liver, kidney, and brown fat by 4 hours post-administration. Moderate amounts of DEHP metabolites

also partitioned into the white fat, myocardium and muscle and marginal amounts distributed into the blood, bone, cartilage, testes, and nervous system (Lindgren *et al.,* 1982). Twenty-four hours post-dose, large amounts of DEHP or metabolites remained in the gall bladder, intestinal contents, urinary bladder, and brown fat. Activity at this time was lower, however, in the liver and kidney.

Similar activity was reported for Wistar rats. Following intravenous injection, DEHP or DEHP metabolites distributed from the blood to the liver and lungs (60 to 70% by 2 hours). At 4 days, 44% of the metabolites were in the urine, 29% in the feces, and 1% in the fat (Daniel and Bratt, 1974). In another study with Wistar rats, intravenous exposure to moderate doses of DEHP (500 mg/kg) resulted in a substantial quantity of metabolites (75%) in the liver by 1 hour post-dosing. This concentration declined to 50% by 2 hours and 0.17% by 189 hours post-dose. Intestinal concentrations were also high, and increased in proportion to declining liver levels. A moderate amount of DEHP or DEHP metabolites distributed to the heart, lungs, and spleen at 6 hours, and marginal concentrations were noted in the brain and testicles (Tanaka *et al.,* 1975).

Intra-arterial administration of DEHP to Sprague-Dawley rats resulted in similar effects. After exposure, 53% of the DEHP or DEHP metabolites distributed to the blood. Blood concentrations decreased to $1/3^{rd}$ and $1/5^{th}$ of the total concentration following 10 and 20 minutes, respectively. At 20 minutes, activity was highest in the liver, bladder, and kidney. Other tissues had lower concentrations of metabolites (Chu *et al.,* 1978). The large apparent distribution and high clearance rate of intra-arterially injected DEHP has been confirmed by others (Pollack *et al.,* 1985a).

Overall, the distribution of DEHP and its metabolites have been described in a variety of species. Studies suggested that following absorption into the blood, DEHP and MEHP were transported via plasma proteins to the liver, kidneys, fat, and other tissues. Distribution of residues to the tissues was short-lived and these did not accumulate. DEHP or DEHP metabolites also distributed across the placenta and into the milk of pregnant dams. This resulted in residues in fetal and neonatal tissues.

Metabolism

Oral Exposure

Metabolism of the diester phthalate, DEHP, to MEHP and 2-ethylhexanol occurred first by phase I biotransformation. A single ester link was cleaved hydrolytically in the small intestine by pancreatic non-specific lipase (DEHP hydrolase; CPSC, 1985; Albro *et al.,* 1973). The activity of DEHP hydrolase was both species and sex specific. Mice had higher activity than rats (M > F), guinea pigs, and hamsters, and male rats had higher activity than female rats (Albro *et al.,* 1973).

A minor amount of phthalic acid was also created by hydrolysis of the second ester linkage in MEHP (ATSDR, 1997; MOE, 2005). This was thought to occur primarily in the liver and involve the enzyme, alkaline liver lipase (ALL). The overall amount of phthalic acid created was low because ALL activity in metabolizing the second ester linkage was only 2% when compared to activity involved in metabolizing the first ester linkage (DEHP to MEHP and 2-ethylhexanol; Albro 1973). Further metabolism of ethylhexanol was reported in Albro (1975). Administration of labeled 1-ethylhexanol resulted in the creation of the oxidative metabolites 2-ethyl-5-hydroxyhexanoic acid, 2-ethyl-5-ketohexanoic acid, and 2-ethyl-1,6-hexandioic acid. A small proportion (3%) was also excreted unchanged. Further β-oxidation reduced the metabolites to acetate and carbon dioxide.

Enzymes produced by gastrointestinal microflora or gut contents also metabolized DEHP to MEHP. Rat gut contents from the stomach, small intestine, and caecum metabolized 1.0, 22.1, and 6.9%, respectively, of the DEHP dose following 16 hours of co-incubation (Rowland *et al.,* 1977). The proportion of metabolism occurring in human feces, in comparison, was 0.6%. In the rat, the ability of gut contents to metabolize DEHP has been linked in-part to bacteria, since treatment with antibiotics during incubation reduces the overall DEHP metabolism (Roland, 1974).

MEHP was further metabolized through oxidation to other products, which were in turn conjugated with glucuronic acid (glucuronidation; in some species) and excreted via the urine.

Humans

In humans, single or repeat dosing with DEHP resulted in the creation of between 12 and 21 urinary metabolites (Schmid and Schlatter, 1985; Bronsch, 1987). Primary metabolites included MEHP (6.4 to 12.7%), metabolite I (1.9 to 2.1%), IV (1.8 to 3.7 %), V (25.6 to 33.8%), VI (19.7 to 24.0%), VII (4.0 to 5.3%), IX (25.9 to 33.0%) and others (< 1%; Schmid and Schlatter, 1985). The serum half-life of MEHP and metabolites VI and IX was short (< 2 hours; Koch *et al.,* 2003). In addition, a significant portion of these urinary metabolites were conjugated to β–glucuronic acid, with estimates ranging from 65% (Schmid and Schlatter, 1985) to 99% (Bronsch, 1987).

Monkeys

Studies involving cynomolgous and marmoset monkeys have identified additional metabolites following oral DEHP exposures. Twenty-four hour monkey urine samples contained MEHP (1^o; 11%), phthalic acid (1^o), metabolites I, III, IV, V(1^o), VI, VII, IX(1^o), X(1^o), XII(1^o), XIII, XIV, and a variety of unidentified metabolites (Short *et al.,* 1987; Monsanto, 1988; CMA, 1982b; CMA, 1983; CMA, 1984a; Short *et al.,* 1987; Astill *et al.,* 1986). The ω-oxidation pathway was primarily responsible for metabolizing MEHP into I, V, and X (collective 34% of activity), and the ω-1 oxidation pathway was responsible for metabolizing MEHP into VI and IX (collective 19% of activity). The β-oxidation pathway was not very important in creating metabolites in monkeys. Urinary metabolites were conjugated prior to excretion. Glucuronide conjugates were estimated to range from 16 to 26% of the excreted activity (CMA, 1982b; CMA, 1983; CMA, 1984a; Short *et al.,* 1987; Astill *et al.,* 1986). Forty-eight hour fecal metabolites were similar to those in the urine (MEHP, phthalic acid, metabolites I to IV, VI, VII, IX, X, XII, XIV). Fecal metabolites also contained DEHP, however, as the primary metabolite (up to 98% of the total activity; ICI, 1982a; Shell, 1982; Rhodes *et al.,* 1986).

Rats

DEHP or MEHP dosed via oral gavage to Sprague-Dawley rats resulted in the systemic creation of twenty urinary metabolites (Albro *et al.,* 1983). These findings were more comprehensive than earler studies in Sprague-Dawley and Wistar rats that reported the creation of only 4 urinary metabolites (Tanaka *et al.,* 1975; Chu *et al.,* 1978). Some of the primary urinary metabolites in rats were identified as phthalic acid (< 3%), and metabolites I, V, VI, IX, but not MEHP (Albro *et al.,* 1973; Lhuguenot *et al.,* 1985). At high doses, metabolites I and V increased in relative concentration, and metabolites VI and IX decreased in relative concentration (Lhuguenot *et al.,* 1985). Unlike other mammalian species, rat metabolites were not conjugated (glucuronidated or glucosidated; Albro *et al.,* 1973, 1982b, 1983; Lhuguenot *et al.,* 1985). Fecal metabolites were approximately 50% DEHP at 24 hours post gavage (Lake *et al.,* 1984b).

Administration of DEHP in the feed to Fischer 344 rats resulted in the creation of 14 urinary and 15 fecal metabolites (CMA, 1982a; Lington *et al.,* 1987; Short *et al.,* 1987; Astill *et al.,* 1986). Urinary metabolites were identified as phthalic acid, and metabolites I, II, III, IV, V, VI, VII, IX, X, XII, XIII, and XIV. Metabolites I and V were highest in concentration, followed by phthalic acid and metabolite IX. DEHP or MEHP were not present as urinary metabolites. This finding contrasted with those of Ikeda *et al.* (1980) in which only five urinary metabolites, including DEHP, were identified in Sprague-Dawley rats, and Daniel and Bratt (1974) in which 14 urinary metabolites including MEHP, phthalic acid, and metabolites IV, V, VI, and IX were found in Wistar rats following the administration of large doses of DEHP.

DEHP, MEHP, phthalic acid, metabolites I to V (pooled), VI, VII, IX, X, XII, XIII, and XIV were present in the feces following dosing. Fecal metabolites with the highest concentration

were MEHP, metabolites I to V, VI, and IX. Urinary metabolite percent composition was dependent on the dose and prior exposure. Metabolite I increased with prior exposure but not dose, and metabolite V increased with dose, but not prior exposure (CMA, 1982a; Lington *et al.,* 1987; Short *et al.,* 1987; Astill *et al.,* 1986).

Mice

In mice, the major urinary DEHP metabolites created were dimethyl phthalate, MEHP (19% of total), and metabolites I, VI, and IX (CD-1 mice; Albro *et al.,* 1982b). In another study, 15 metabolites were identified in 0 to 24 hour urine samples and 10 metabolites were identified in 0 to 24 hour fecal samples (CMA, 1982b; CMA, 1983; CMA 1984a; Short *et al.,* 1987; Astill *et al.,* 1986). In the urine, the primary metabolites were MEHP, phthalic acid, and metabolites I, VI, IX, and XII, with lesser quantities of metabolite II, III, IV, V, VII, X, XII, and XIV. In the feces, the primary metabolites were DEHP and MEHP, with lesser quantities of phthalic acid and metabolites I to IV, VI, VII, IX, X, XII, and XIII.

Exposure to MEHP resulted in the creation of primarily MEHP and metabolite glucuronide conjugates in mice. Lower amounts of three less important β-glucose conjugates were also found glucosidated in the urine (3% of administered dose; Egestad and Sjoberg, 1992; Egestad *et al.,* 1996).

Guinea Pigs

In Dunkin-Hartley guinea pigs, labeled DEHP was primarily metabolized to MEHP (70% of all metabolites) and then glucuronidated (Albro *et al.,* 1982b). Glucuronidation, and not the creation of β-glucose conjugates, was also the major conjugation pathway for guinea pig exposures to MEHP (Egestad *et al.,* 1996).

Hamsters

Metabolism of DEHP was different in Syrian golden hamsters compared to guinea pigs. Administration of labeled DEHP resulted in the metabolic creation of MEHP (5% of total label), dimethyl phthalate, and metabolites I, V, VI, and IX in the urine (Albro *et al.,* 1982b). Twenty-four hour metabolites in the feces, however, were primarily DEHP (95%), with the remainder being MEHP and other oxidative metabolites (Lake *et al.,* 1984b).

Dogs and Miniature Pigs

Limited information was presented regarding metabolites in other species. Ikeda *et al.* (1980) identified three metabolites and the parent compound DEHP in dog urine, and five metabolites and DEHP in miniature pig urine. No other information on this was available in the referenced document (ECB, 2008).

Conjugation (Glucuronidation and Glucosidation)

As mentioned, MEHP and other oxidized metabolites react with glucuronic acid to form glucuronide conjugates (phase II biotransformation). Glucuronidation is important in making DEHP metabolites more polar and water soluble prior to excretion. Rats can glucuronidate DEHP, but do not excrete glucuronide conjugated metabolites (Albro 1986). The distribution of glucuronide-bound and free metabolites is important to understand, since the relative toxicity of DEHP is determined to a large extent by the free metabolites and enterohepatic recirculation of deconjugated metabolites (Silva *et al.,* 2003).

The ability to glucuronidate and deglucuronidate can differ significantly within a species, age class, or disease state. Dwivedi *et al.* (1987) demonstrated that rat strains can differ as much as 2-fold in serum and liver β–glucuronidase activity. Miyagi and Collier (2007) revealed that development of human neonatal hepatic "total" UDP-glucuronosyltransferase (UGT) enzyme activity did not mature until 20 months of age and also that β-glucuronidase activity (responsible for hydrolysis/enterohepatic recirculation) was highest in the neonatal liver and decreased to adult levels by 4 months. Surprisingly, it has been reported that no significant differences in UGT activities were seen between the different genders or ethnicities.

The shift from metabolic cleavage and recirculation to conjugation and clearance in the neonate is an important consideration and directly impacts the choice of animal models for further metabolic and toxicological research. The rat model may be a good choice for early human life stages, since rats do not excrete glucuronide-conjugates of higher MW phthalates (i.e., DEHP). Other species that do excrete glucuronide conjugates (i.e., mice) might better represent later human developmental stages.

With DEHP, the route of administration controlled the formation of MEHP and other metabolites. Pollack *et al.* (1985a) reported that 80% of DEHP was converted to MEHP following oral exposure, compared to only 1% following intra-arterial or intra-peritoneal exposures.

Dermal Exposure

In general, skin is known to possess cytochrome P-450, epoxide hydrolase, and glutathione-s-transferase activity which can metabolize benzo (a) pyrene and similar compounds (CPSC, 1985; Bickers *et al.,* 1981).

Homogenates of rat skin have been reported to hydrolyze DEHP at a level corresponding to 1% of that in the liver (CPSC, 1985; Albro *et al.,* 1984). Metabolism following dermal exposure has also been demonstrated to a limited extent in guinea pig skin *in vitro* experiments (Ng *et al.,* 1992). In this study, pretreatment of the perfusate and guinea pig skin with phenyl methyl sulfonyl fluoride (PMSF), an esterase inhibitor, decreased the amount of DEHP or metabolites that passed into the receptor fluid in 24 hours (3.36% to 2.67%). The relative amount

of MEHP decreased from 2.36 to 1.23% in the PMSF treatment group. Even though changes were marginal, this information suggested that dermal transport was modulated somewhat by the metabolites present.

Inhalation Exposure

A variety of different DEHP-associated metabolites have been reported following inhalation exposures in humans. Preterm infants exposed to DEHP from ventilation tubing were reported to have DEHP in the urine and lung tissue, but not the liver (Roth *et al.,* 1988 cited in ECB, 2008). MEHP was not found in the urine of these infants. In workers at a DEHP manufacturing plant, DEHP metabolites (excluding MEHP) were found in the urine (Liss *et al.,* 1985 cited in ECB, 2008). These results contrasted with those from a study by Dirven *et al.* (1993a, 1993b; cited in ECB, 2008) in which four DEHP metabolites (MEHP, V, VI, and IX) were found in urine samples from workers at a boot factory and cable factory. The relative amounts of these metabolites were higher post-shift when compared to pre-shift, suggesting that DEHP exposure and metabolism was occurring in the workplace. In another study by Dirven (1993b; cited in ECB, 2008), the relative amounts of metabolites were determined in humans following presumed inhalation exposures (MEHP, 26.2%; V, 21.8%; VI, 18.2%; and IX, 33.8%). These metabolites were conjugated to a varying extent (MEHP, 0 to 80%; V, 32 to 45%; VI, 80 to 95%; and IX, 88 to 100%) and had a pattern of absolute concentrations that suggested that ω-1 oxidation of metabolites (VI and IX) was more predominant than ω-oxidation (V).

Other Exposure

Metabolite production from other exposure routes such as intraperitoneal or intravenous is expected to be similar in quality, but different in quantity when compared to oral exposures. Quantitative differences in the various metabolites are expected because of different target organ effects and because they bypass gastrointestinal absorption.

Excretion

Oral Exposure

Excretion of DEHP and its metabolites occurred via urine, feces, respiration, and sweat.

Humans

In humans, urinary excretion of DEHP metabolites was multiphasic. Following low doses, the 8 to 16 hour half-lives for the primary metabolites (MEHP; metabolite IX; and metabolite VI) were estimated to be 2 hours. At 14 to 18 hours post-dose, the half-lives increased to 5 hours for MEHP and 10 hours for metabolite IX and VI (Koch *et al.,* 2003). As illustrated, the ratio of the metabolites changed over time.

Estimates for the urinary elimination of DEHP metabolites varied depending on the dose or species. In humans, approximately 16% of a small oral dose (3 mg/kg) was eliminated by 4 hours post-dose. Eleven to 28% of the dose was elimated in the urine by 24 hours post-dose. Twenty-seven to 31% of the administered dose was eliminated in urine by 47 hours post-dose (Bronsch, 1987; Schmid and Schlatter, 1985). At 47 hours post-dose, three metabolites were predominate; MEHP, 7.3%; metabolite IX, 24.7%, and metabolite VI, 14.9% (Koch *et al.,* 2003). Elimination half-lives from these data and others (Schmid and Schlatter, 1985) have been estimated to range from 12 to 24 hours.

Monkeys

In male cynomolgous monkeys, most DEHP metabolite excretion occurred in the first 24 to 48 hours following exposure. At 96 hours, excretion following low doses of DEHP was similar for the urine (20 to 55%) when compared to the feces (39 to 49%). With increased dose, this relationship changed, and fecal excretion (56 to 69%) was greater than that in the urine (4 to 13%; Short *et al.,* 1987; Monsanto, 1988). The latter relationship was supported by additional data demonstrating that fecal excretion was 49% and urinary excretion was 28.2% of the total DEHP activity by 48 and 24 hours, respectively (CMA, 1982b; CMA, 1983; CMA, 1984a; Short *et al.,* 1987; Astill *et al.,* 1986).

Dose-related increases in fecal excretion were also reported for marmoset monkeys (Rhodes *et al.,* 1983; Rhodes *et al.,* 1986). Urinary excretion following low dose exposures to DEHP was marginally higher than that observed in the feces (20 to 40% versus 25%, respectively). As in cynomolgous monkeys, this trend reversed following high dose exposures (4 and 84% of the activity in the urine and feces, respectively, with 0.6% remaining in the tissues). Increased fecal excretion was thought to result from less absorption of the DEHP following high dose administration. This relation was relevant for both male and female marmoset monkeys. High doses of DEHP resulted in minimal excretion of metabolites in the urine (1 and 2% for males and females, respectively) and higher excretion in the feces (64 and 75% for males and

females, respectively) by day six of dosing. A similar pattern was reported following extended dosing up to 13 days (ICI, 1982a; Shell, 1982; Rhodes *et al.,* 1986).

Rats

In Sprague-Dawley rats, excretion of DEHP metabolites was variable and dependent on the dose, animal strain, and age of the study animal. With low dose exposures, DEHP metabolites were primarily excreted in the urine (51%), followed by the feces (43%) in some studies (Lake *et al.,* 1984b). This relationship was reversed following administration of high DEHP doses to young rats and low DEHP doses to rats that had been fasted or rats that had been exposed to unlabeled DEHP for 21 to 28 days. In these cases, the majority of radiolabel activity was excreted in the feces (53 to 62%), followed by urine (27 to 37%) and air (4%). Less than 2% of the activity was recovered in the carcass and tissues (Lake *et al.,* 1984b, Eastman Kodak Co., 1983) and excretion was largely complete by 2 to 4 days (Chu *et al.,* 1978; Ikeda *et al.,* 1980). In addition, urinary radiolabel activity decreased (from 44 to 26%) when comparing 25 day old rats to 60 day old rats at 72 hours post-dose (Sjoberg *et al.,* 1985c).

Enterohepatic recirculation may account for a portion of the DEHP metabolites (activity) found in the urine and other compartments. Chu *et al.* (1978) reported that 40 to 52% of DEHP metabolites were excreted in the bile eight hours following administration. This information suggested that substantial resorption of DEHP or DEHP metabolites was occurring in the gastrointestinal tract.

In Fischer 344 rats, oral feeding of low, moderate, and high doses of DEHP resulted in the excretion of more urinary metabolites (53, 62 to 66, 66 to 69%, respectively) at 24 hours post dose than fecal metabolites (35 to 38, 26 to 30, 24 to 28%, respectively) at 48 hours post-dose. As with other rats, clearance of the DEHP metabolites was fast, and by 96 hours < 1% of the dose remained in the tissues (CMA, 1982a; Lington *et al.,* 1987; Short *et al.,* 1987; Astill *et al.,* 1986). In addition, no DEHP metabolites were detected in Wistar rat excreta 96 hours post-exposure to large doses of DEHP (Lake *et al.,* 1975). In contrast, DEHP was detected in the feces of Wistar rats following gavage dosing, but neither MEHP nor DEHP were detected in the urine of rats (Tanaka *et al.,* 1975).

Results derived from dosing Fischer 344 rats with DEHP in feed contrasted with that reported in oral gavage studies. In gavage studies, excretion mechanisms were not saturated by gavage doses up to 180 mg/kg, and 200 mg/kg was the estimated maximum concentration of DEHP that could have been gavage dosed without substantially increasing the amount of excreted unabsorbed DEHP (Albro *et al.,* 1982a). Although dose determined the proportion of metabolites excreted in the urine or feces, prior exposure to DEHP did not affect the rate or extent of excretion (CMA, 1982a; Lington *et al.,* 1987; Short *et al.,* 1987; Astill *et al.,* 1986), suggesting that DEHP metabolites did not accumulate in the blood or body tissues.

Prior exposure also did not affect the relative amount of urinary or fecal DEHP metabolite excretion in Wistar rats (Daniel and Bratt, 1974; Lake *et al.,* 1975). Repeated administration of MEHP instead of DEHP, however, increased the amount of metabolites excreted in the urine from 50 to 60% up to 70 to 80% (Lhuguenot *et al.,* 1985).

Administration of labeled 1-ethylhexanol also resulted in rapid clearance of metabolites (by 28 hours) and the excretion of metabolites in the urine (80 to 82%), feces (8 to 9%), and lungs as carbon dioxide (6 to 7%; Albro, 1975).

Excretion of DEHP metabolites from the bile has not been explored in great depth, but may impact total amounts perceived to be unabsorbed because of enterohepatic circulation. Daniel and Bratt (1974) and Tanaka *et al.* (1975) estimated that 5 to 10% of the DEHP metabolites (not DEHP or MEHP) were excreted in the bile following gavage exposure of Wistar rats to low doses of DEHP.

Mice

Mice (CD-1 and B6C3F_1) excreted glucuronide conjugates of DEHP metabolites (Albro *et al.,* 1982b) primarily in the first 12 to 24 hours, with feces being the primary route (52.0%), followed by the urine (37.3%; CMA, 1982b; CMA, 1983; CMA 1984a; Short *et al.,* 1987; Astill *et al.,* 1986).

Hamsters

In Syrian golden hamsters, primary excretion occurred by 24 hours, with low doses favoring excretion in the urine (53%) rather than feces (31%) and high doses favoring excretion in the feces (48%; Lake *et al.,* 1984b). Excretion of the labeled metabolites was primarily as a glucuronide conjugate (Albro *et al.,* 1982b).

Dogs and Pigs

In dogs and pigs, the primary route of excretion differed with time. At twenty-four hours, dogs and pigs excreted urinary (12, 37%) and fecal (56, 0.1%) metabolites, respectively, which were less than that at 96 hours in the urine (21, 75%) and feces (79, 26%; Ikeda *et al.,* 1980). By 96 hours, excretion was virtually completes.

Dermal Exposure

Specific studies outlining DEHP/metabolite excretion following dermal doses have not been found.

Inhalation Exposure

Humans

In some circumstances, excretion of DEHP/metabolites from inhalation exposures in humans was different than that from oral exposures. DEHP, not MEHP, was detected in the urine of preterm infants with ventilation tubing and in workers involved in the manufacture of DEHP (Roth *et al.,* 1988 and Liss *et al.,* 1985 cited in ECB, 2008). In other studies, MEHP and metabolites V, VI, and IX were detected in the urine of workers of a boot factory and cable factory (Dirven *et al.,* 1993a, 1993b). In the urine, the majority of these metabolites were present in their conjugated form.

Rats

In rat studies, inhaled DEHP was excreted primarily in the urine (52%) and feces (40%) at 72 hours post exposure (General Motors, 1982ab). Fecal excretion of DEHP/metabolites followed first order rate kinetics during this period, resulting in an elimination half-life of 22 hours and an elimination rate constant (Ke) of 0.032 hr^{-1}. Urinary excretion of DEHP was biphasic, with the rapid phase lasting 30 hours ($t_{½}$ = 10 hours, Ke = 0.069 hr^{-1}) followed by a slower phase ($t_{½}$ of 22 hours). In another study in which rats were dosed for 2 weeks with unlabeled DEHP and then exposed to a last dose of ^{14}C-labeled DEHP, 50% of the DEHP/metabolites were excreted in the urine and 40% in the feces. In this case, urinary excretion followed first order rate kinetics, had a half-life of 25 hours, and was initially slower than single inhalation exposures. After 24 hours, however, the excretion was similar to that from single dose inhalation studies.

Other Exposure

As with metabolism, excretion of DEHP metabolites created from other exposure routes such as intraperitoneal or intravenous was expected to be mostly similar in quality, but different in quantity when compared to oral exposures. A quantitative difference in the excretion for different exposure routes was expected because each exposure route will impact target organs in a different sequence and at different concentrations.

Rhodes *et al.* (1983, 1986) demonstrated this principle by administering DEHP to marmoset monkeys via three different exposure routes (oral, 100 and 2000 mg/kg; intravenous, 100 mg/kg; intraperitoneal, 1000 mg/kg). Exposure to DEHP via oral routes of administration resulted in the excretion of urinary (20-40%, 100 mg/kg; 4%, 2000 mg/kg) and fecal (25%, 100 mg/kg; 84%, 2000 mg/kg) metabolites. The reduced proportion of urinary metabolites and increased fecal metabolites suggested that an absorption threshold existed for the 2000 mg/kg dose. Exposure to DEHP via intravenous and intraperitoneal routes of administration resulted in dissimilar amounts of urinary (40 and 10%, respectively) and fecal (20 and 4%, respectively)

metabolites. Tewnty-eight percent of the intravenously administered dose remained in the lungs and 85% of the intraperitoneally administered dose remained in the peritoneal cavity. These two compartments were not significantly affected in oral dosing experiments.

In a Wistar rat study comparing oral and intravenous routes of exposure, 80% of the metabolites were excreted in the urine and feces by 5 to 7 days post-dosing, regardless of exposure route (with activity higher in the urine than feces). Only 5% of the dose was excreted in the bile 24 hours following oral dosing, when compared to 24% recovered following intravenous dosing (Tanaka *et al.,* 1975).

These studies suggested that the exposure-route also affected systemic distribution and excretion of DEHP metabolites.

Overall Toxicokinetic Conclusions

Oral Exposure

DEHP toxicokinetics have been determined in numerous animal and a few human studies. Low dose exposures to DEHP were rapidly metabolized to MEHP by intestinal contents, pancreatic lipases, and/or other esterases. Intact DEHP was systemically available following high dose exposures. Absorbed DEHP and metabolites were transported to target tissues such as the liver, kidneys, and fat and were oxidatively metabolized to approximately 15 to 20 metabolites. Metabolites were then conjugated and eliminated in a species-dependent manner in the urine and feces. Distribution to the tissues was short-lived and DEHP and or metabolites did not accumulate. DEHP and or metabolites also distributed across the placenta and into the milk of pregnant dams. This resulted in residues in fetal and neonatal tissues.

Potential DEHP metabolites have been discussed extensively in the presented papers. DEHP metabolite absorption, distribution (organ compartments), metabolic rates, excretion, conjugation states (bound or free), and enterohepatic recirculation have been described to some extent, for different species including humans.

Dermal Exposure

Relatively few dermal studies have been performed when compared to the oral route of exposure. *In vivo* dermal absorption has been estimated to range from 6.5 to 26%. Subsequent processes are thought to be similar to those following oral exposure.

Inhalation Exposure

As with dermal studies, relatively few inhalation studies have been performed when compared to the oral route of exposure. Minor differences from oral exposures included the deposition of DEHP-containing particles in the lung. Systemic process involved in the subsequent distribution, metabolism, and excretion of DEHP following inhalation exposures were expected to be the same as that from oral exposures.

Intraperitoneal or Intravenous Exposure (injection exposures)

Intravenous or intra-arterial administration of DEHP resulted in a higher level of intact DEHP reaching the target tissues. Subsequent metabolism and excretion was similar to oral exposure processes. Intraperitoneal exposures resulted in decreased absorption of DEHP. As with other routes of exposure, metabolism and excretion were expected to be similar to oral exposures.

5. Hazard Information

This section contains brief hazard summaries of the adverse effects of DEHP in a variety of animal and bacterial species. More detailed discussions of the studies can be viewed in the Appendices. When evaluating hazard study data, CPSC staff utilized the definitions for toxicity as presented in regulation (16 CFR §1500.3(c)(2)(ii)) and the chronic hazard guidelines (16 CFR §1500.135) in the Federal Hazardous Substances Act (FHSA; 15 U.S.C. 1261-1278). When considering the FHSA, substances that are "known" or "probable" toxicants are "toxic" and substances that are considered "possible" toxicants are "not toxic" (Table 5.1).

Table 5.1 Classification of Chronic Hazards (as per the FHSA)		
Evidence	Human Studies	Animal Studies
Sufficient evidence	**Known**	**Probable**
Limited evidence	**Probable**	Possible
Inadequate evidence	Possible	---

When considering FHSA criteria, evidence did not support DEHP as "acutely toxic". Median lethal doses (LD_{50}'s) for acute oral DEHP exposure were 9800 mg/kg or greater. These LD_{50}'s were far in excess of the oral LD_{50} range (50 to 5000 mg/kg) necessary to be termed "acutely toxic". DEHP was also not corrosive, a dermal or ocular irritant, or a sensitizer. Negative, conflicting, or insufficient data contributed to this decision. Evidence did, however, support the conclusion that DEHP was a chronic toxicant as defined by the FHSA. Studies provided data to support the conclusion that DEHP was: 1) a known animal and possible human carcinogen, 2) a known animal and possible human reproductive toxicant, and 3) a known animal and possible human developmental toxicant.

In the following discussions, hazard information was divided into sections thought to be of interest for regulatory matters (i.e., for labeling and other mitigation measures) as well as for biological and pathological consistency. More specifically, hazards were divided into whether the exposure was singular or repeated. Hazards associated with repeated exposures were further divided into groupings based on the affected organ system (i.e., hepatic, neurological, hematologic, etc) and discussed in terms of the exposure duration (***acute***, 14 days or less; ***intermediate-term*** or ***subchronic***, 15 to 364 days; ***long-term*** or ***chronic***, greater than 365 days; and ***multigenerational***; ATSDR, 2007). Discrete study information can be reviewed in the Appendices.

Acute Single-Dose Toxicity

Acute oral toxicity

Both human and animal studies have been reviewed for acute oral toxicity. In general, the acute oral toxicity of DEHP following single exposures was regarded as low.

In humans, ingestion of 10 grams of DEHP (Lowest Observed Adverse Effect Level (LOAEL) = 142.8 mg/kg, assuming 70kg b.w.) caused mild gastric disturbances and "moderate catharsis". Ingestion of 5 grams of DEHP did not, however, result in any clinical symptoms (No Observed Adverse Effect Level (NOAEL) = 71.4 mg/kg-day, assuming 70kg b.w.; Shaffer *et al.,* 1945).

The acute oral toxicities of DEHP in other animals were also low. The median lethal dose (LD_{50}) concentrations ranged from > 9800 to > 40,000 mg/kg in rats, (Shaffer *et al.,* 1945; Nuodex, 1981a; NTP, 1982; BASF, 1953, 1961; Shibko and Blumenthal, 1973; NICNAS, 2008), > 9860 to > 31,360 mg/kg in mice (BASF, 1941; Lawrence *et al.,* 1975; Nuodex, 1981b; NTP, 1982), 33,900 mg/kg in rabbits (Shaffer *et al.,* 1945), and 26,000 mg/kg in guinea pigs (Krauskop, 1973, NICNAS, 2008). The actual LD_{50} for neonatal and suckling animals may be lower than these presented, however. Dostal *et al.* (1987a) demonstrated that DEHP-induced mortality in younger rats (< 25 days old) was substantially higher than that in older animals (42 to 90 days old).

Acute oral toxicities were somewhat greater than intraperitoneal exposure lethal doses in rats (LD_{50}'s = 4900 to 147,000 mg/kg; ECB, 2008; Shaffer *et al.,* 1945; NICNAS, 2008) and mice (LD_{50}'s = 2800 to > 128,000 mg/kg; ECB, 2008; Lawrence *et al.,* 1975; Woodward *et al.,* 1986) Acute oral LD_{50}'s were also greater than intravenous exposure lethal doses in rats (LD_{50}'s = 200 to 2080 mg/kg; ECB, 2008; Schulz *et al.,* 1975; Rubin and Chang, 1978; Schmidt *et al.,* 1975; NICNAS, 2008) and mice (LD_{50}'s = 1060 to 1370 mg/kg; ECB, 2008; Health Canada, 2002; NICNAS 2008).

DEHP induced other clinical signs and pathologies in animals following single-dose exposures. In rats, a single oral gavage dose caused adverse clinical signs and other pathologies such as a rough hair coat, decreased activity and debilitation, a wet posterior, depression (LOAEL = 5000 mg/kg; Nuodex, 1981a), and general debilitation (LOAEL = 5000 mg/kg; NOAEL = 1500 mg/kg; Moser *et al.,* 1995). In mice, a single oral gavage dose primarily induced behavioral depression, rough fur, and a humped appearance (LOAEL = 9860 mg/kg; Nuodex, 1981b).

Methodological details involving the conduct of the human study and many of the acute oral toxicity animal studies were not provided in the review publications. Details omitted included the number and strain of animals, DEHP doses, timing of mortality, and clinical signs. Oral gavage was the preferred method for dose delivery in acute oral studies. In one

circumstance, the dose of DEHP (up to 40 mL/kg; Nuodex, 1981a) was 4 times higher than recommended maximum volumes for standard toxicity tests (10 mL/kg; Hayes, 2001). High dose volumes can induce diarrhea in animals and potentially limit absorption by enhancing excretion, both of which affect the lethal concentrations.

Methodological deficiencies were not thought to be significant enough to overshadow the observation that all of the acute rat and mouse oral LD_{50}'s cited for DEHP were consistently higher than the oral LD_{50} range (50 to 5000 mg/kg) that is considered toxic in FHSA criteria. The weight of evidence including **limited human and sufficient animal data supported the conclusion that DEHP did not fit the designation as an "acute oral toxicant" when considering FHSA criteria (16 CFR §1500.3(c)(2)(i)(A))**.

Acute dermal toxicity

The acute dermal toxicity of DEHP was reported in only one study. In this study, 24 hour dermal exposure to DEHP resulted in two mortalities out of six rabbits (LD_{50} of > 24,500 mg/kg; Shaffer *et al.,* 1945).

As with the acute oral studies, methodological details involving the conduct of the acute dermal toxicity study (i.e., incubation period and state) were not provided. This fact, combined with a lack of additional studies supported the conclusion **that there was "inadequate evidence" for the designation of DEHP as an "acute dermal toxicant" when considering FHSA criteria (16 CFR §1500.3(c)(2)(i)(C))**.

Acute inhalation toxicity

The acute inhalation toxicity of DEHP was reported in one GLP rat study. In rats, nose-only exposure to DEHP for 4 hours reduced body weight on day 2, induced a slightly unkempt appearance for 1 to 2 days, and stained perianal fur yellow (high-dose rats) without inducing any mortality. A median lethal concentration (LC_{50}) of > 10.62 mg/L was generated based on DEHP's technical limit for aerosol generation (Hüls, 1981). Pathological exam revealed that dark red foci and patches in the lungs were more common in treated animals (19/31 high-dose rats) when compared to controls following necropsy.

This study had sufficient methodological details to determine its validity. The dose level utilized (10.62 mg/L) was in excess of OECD's recommended limit for aerosols (5 mg/L) and EPA's "concern level" (2 mg/L), above which one might see increased particle size, particle aggregation, and airway dust loading. Dark red foci and patches on control lung tissues were also problematic, and suggested that non-treatment-associated pulmonary pathologies (infections?)

may have influenced acute inhalation results. These types of pathologies have not been typically observed following nose-only exposures in control rats that receive clean air only.

DEHP-induced respiratory effects via inhalation exposures were not correlated with excessive mortalities. Short-term exposure of rats to an ultrafine aerosol of DEHP (No Observable Adverse Effects Concentration (NOAEC) = 300 mg/m^3; Merkle *et al.,* 1988), intermediate length exposure of male or female rats to an aerosol of DEHP (NOAEC = 1000 mg/m^3; Klimisch *et al.,* 1991) and lifetime exposure of hamsters to a vapor of DEHP (NOAEC = 0.015 mg/m^3; Schmezer *et al.,* 1988) did not increase mortality rates in the studies reviewed.

Methodological issues and atypical control pathologies in the reviewed study supported the conclusion that **there was "inadequate evidence" for the designation of DEHP as an "acute inhalation toxicant" when considering FHSA criteria (16 CFR §1500.3(c)(2)(i)(B))**.

Primary skin irritation

In animals, DEHP was at worst a mild skin irritant when applied to patches of depilated guinea pig or rabbit skin for 4 to 24 hours (Hüls, 1981; Hüls, 1987a; BASF, 1986; Exxon, 1994). In the first of the OECD 404 guideline-compliant studies, no erythema or edema was found in three white Vienna rabbits dosed for 4 hours with undiluted DEHP on depilated skin (BASF, 1986). In another OECD-compliant study involving Little White Russian rabbits, dosing the depilated skin for 4 hours with undiluted DEHP resulted in a very slight erythema in all rabbits at 1 hour post-dosing (Hüls, 1987). Slight erythema progressed to a well defined erythema in one rabbit by 24 hours and persisted for 48 hours in the others. At 72 hours, treated skin was dry, and by 6 days scaly. All skin irritation resolved by 8 days after dosing. In an FDA GLP-compliant study, dosing the depilated skin of three New Zealand White rabbits (M&F) with undiluted DEHP induced a reversible, mild to moderate irritation by 24 hours post-dose (Hüls, 1981). The initial skin reaction was cleared by 72 hours, even in rabbits with abraded skin.

Qualification of DEHP as a minimal dermal irritant in animal studies was confirmed in a patch study involving 23 human volunteers. In this study, undiluted DEHP was not irritating when left in contact with skin for more than 7 days (Schaffer *et al.,* 1945).

Methodological details support conclusions generated by the studies. Two of the rabbit skin irritation studies were performed in accordance with OECD guideline 404 and had sufficient methodological details to determine their validity. One rabbit skin irritation study was performed in accordance with FDA and GLP recommended methods. Species, strain, patch coverage and duration and observations were all satisfactory for these studies. Animal skin irritation data was verified by patch testing in humans.

Study details demonstrated that DEHP is at worst, a mild skin irritant. The weight of evidence including human and animal data were sufficient to support the conclusion that **DEHP**

did not fit the definition of "corrosive" as outlined in the FHSA (16 CFR §1500.3(c)(3)) or designation as a "primary irritant" when considering FHSA criteria (16 CFR §1500.3(c)(4)). No significant dermal pathology scores (i.e., total combined edema and erythema severity scores of > 20 points) were observed in tests that most closely fulfill testing criteria as defined in 16 CFR §1500.41.

Primary eye irritation

No human studies indicating that DEHP was a primary eye irritant were found in the reviewed literature.

DEHP was a mild eye irritant in other animals. Administration of DEHP in a standard Draize-type testing strategy was mildly irritating to rabbit eyes in two OECD 405 guideline-compliant and one FDA GLP-compliant studies (Hüls, 1981; Hüls, 1987b; BASF, 1986). In the first of the OECD compliant studies, the average score for sample timepoints was 0.1 for conjunctival redness and 0.0 for corneal opacity, iritis, and conjunctival swelling in three white Vienna rabbits following conjunctival instillation of 0.1 ml of undiluted DEHP (BASF, 1986). In the other OECD-compliant study, mild conjuctival redness was evident in all rabbits, and mild discharge in one rabbit, at 1 hour post-dosing of the conjunctival sacs of three male Little White Russian rabbits with undiluted DEHP (Hüls, 1987). These mild effects resolved in later timepoints, and ultimately no corneal, iral, or conjunctival effects were noted. In the FDA GLP-compliant study, DEHP induced a mild conjunctival redness in 5 of 6 New Zealand White rabbits 1 hour following dosing with undiluted DEHP (Hüls, 1981). The mild conjunctival redness persisted in 3 of the rabbits for 24 hours and was completely resolved by 72 hours. No corneal or iral effects were noted at any point following dosing with DEHP.

Animal primary eye irritation studies were methodologically sufficient to determine their validity. Species, strain, eye instillation, duration of testing, and observations were all satisfactory for these studies.

Study details demonstrated that DEHP was at worst, a mild eye irritant. The weight of evidence including animal data were sufficient to support the conclusion that **DEHP did not fit the designation of an "eye irritant" when considering FHSA criteria (16 CFR §1500.3(c)(4))**. No "positive" results (i.e., ulceration or opacity of the cornea, inflammation of the iris, obvious swelling of the conjunctiva) were observed in tests that fulfill testing criteria as defined in 16 CFR §1500.42.

Respiratory irritation

Human or animal *in vivo* or *in vitro* studies directly assessing DEHP-induced lung irritation were not found in the reviewed literature. In Hüls (1981), nose-only inhalation exposures to DEHP and clean control air resulted in the development of dark red foci and patches in the lungs of test rats. These were more prevalent in DEHP-exposed rats. These pathologies were more than likely related to co-occurring non-treatment-related effects. In addition, the pathologies reported in Hüls study (1981) were not typical for acute irritant-induced lung effects, which include mucus hypersecretion, hypertrophy of the submucosal glands, and goblet cell hyperplasia or metaplasia (Cotran *et al.,* 1994).

The ability for DEHP to induce respiratory irritation can not be determined from the available studies.

Sensitization

A limited number of case reports of DEHP-induced sensitization were reported for humans. Contact urticaria resulting from headphone and PVC-gripped glove use was described by two authors (Walker *et al.,* 2000; Sugiura *et al.,* 2000, 2002). In reviews of these cases, the distinction between non-immunologic versus immunologic etiologies was not made.

In contrast to human data, sensitization responses were not evoked following DEHP exposure in animal studies. A sensitization response was not evoked in Dunkin-Hartley guinea pigs induced and then challenged with DEHP in a Magnusson-Kligman maximization-type test and a Buehler-type test performed according to Annex V and GLP Methods (Hüls, 1981; Exxon, 1994). In other animal tests, DEHP enhanced atopic dermatitis-like effects (dryness, eruption, wound, edema, ear thickening), enhanced the infiltration of eosinophils into dermatitis skin lesions, and increased the expression of MIP-1α and eotaxin (chemokines) in 8 week old NC/Nga mice in a dose-dependent fashion (0.8 to 20 µg per mouse; ~4.8 to 120 µg/kg-day; Takano *et al.,* 2006). Ear thickening, clinical scores, eosinophilic inflammation, and the expression of eotaxin were also increased in male NC/Nga mouse pups birthed from dams that were exposed to DEHP (600 µg/kg-day) via intraperitoneal injection during neonatal (lactation), but not fetal, periods (Yanagisawa *et al.,* 2008).

Guinea pig studies unequivocally demonstrated that DEHP was not a guinea pig skin sensitizer. Sensitization studies were sufficiently well described to determine their validity. Species, strain, dose administration via induction and challenge, and the duration of testing were all satisfactory for these studies (ECB, 2008).

Human case reports and other mouse skin testing suggested that modulation of sensitization-associated portions of the immune system may occur following DEHP exposure. Human case reports were not described, however, in sufficient detail to permit robust review.

Even though animal data illustrated that DEHP was not a sensitizer, human case-reports and mouse skin studies suggested that sensitization may occur following exposures. These contrasting findings supported the conclusion that **there was "limited or inadequate human and animal evidence" for the designation of DEHP as a "sensitizer" when considering FHSA criteria (16 CFR §1500.3(c)(5)(i))**.

Acute, Subchronic, and Chronic Single- and Repeat-Dose Toxicity

Mortality

Mortality occurring in repeat dose studies was reported for a variety of species. Overall, LOAELs for mortality ranged from 1000 to 5000 mg/kg-day for rats, rabbits, and mice exposed to DEHP for 5 to 14 days (Dostal *et al.,* 1987a; Cimini *et al.,* 1994; Parmar *et al.,* 1988, 1994; NTP, 1982). Mortality thresholds induced by 2 to 16 week subchronic exposures were similar to acute thresholds, and ranged from 2000 to 7900 mg/kg-day for rats, guinea pigs, rabbits, and mice (Parmar *et al.,* 1987, 1988, Eastman Kodak, 1992b; Ward *et al.,* 1998). Mortality thresholds following chronic exposures, in contrast, were substantially less than acute and subchronic mortality thresholds, and ranged from 50 to 1266 mg/kg-day for rats and mice exposed to DEHP for up to 2 years (Harris *et al.,* 1956; David *et al.,* 1999, 2000b; Moore, 1997).

General effects (i.e., food or water consumption, body weight, clinical signs)

Various general parameters such as body weight, clinical signs, and food consumption were adversely affected by exposure to DEHP.

Acute exposure

Multiple-dose gavage exposures to DEHP reduced body weights and body weight gains in adult rats (LOAEL = 1000 to 2000 mg/kg-day, NOAEL = 500 to 1500 mg/kg-day), guinea pigs (LOAEL = 2000 mg/kg-day), and marmoset monkeys (LOAEL = 2000 mg/kg-day), but not in mice (NOAEL = 1150 to 9860 mg/kg-day), cynomolgous monkeys (NOAEL = 500 mg/kg-day) or rabbits (NOAEL = 2000 mg/kg-day; James *et al.,* 1998; Adinehzadeh and Reo, 1998; Nuodex, 1981b; Dostal *et al.,* 1988; Parmar *et al.,* 1988; Oishi, 1989; Hazelton, 1983; Pugh *et al.,* 2000; Rhodes *et al.,* 1986; ICI, 1982b; Khaliq and Srivasta, 1993; Moser *et al.,* 1995). Acute body weight changes induced by DEHP gavage exposures in adult unmated rats were not substantially different than those from juvenile male (LOAEL = 2800 mg/kg-day; Gray and Butterworth, 1980) or lactating female (LOAEL = 2000 mg/kg-day; Dostal *et al.,* 1987b) rats.

Multiple DEHP exposures via feed resulted in higher acute effect levels than for gavage dosed rats (LOAEL = 1000 to 5000; NOAEL = 1905 mg/kg-day) but not mice (LOAEL = 630 to 5000 mg/kg-day; NOAEL = 1250 to 2500 mg/kg-day; Muhlenkamp and Gill, 1998; Mehrotra *et al.,* 1997; Van der Munckhof *et al.,* 1998; Shin *et al.,* 1999; Sjoberg *et al.,* 1986b; NTP, 1982). Exposure to DEHP via feed also acutely decreased food consumption in rats (LOAEL = 1200 to 2000 mg/kg-day; Adinehzadeh and Reo, 1998; Dostal *et al.,* 1987b) and induced clinical signs of toxicity in mice (LOAEL = 6000 mg/kg-day; Hazelton, 1983). As with gavage dosing, juvenile rats treated with DEHP via feed had similar effect levels (LOAEL = 1000 to 1700 mg/kg-day) to adult unmated rats (Sjoberg *et al.,* 1986a).

Repeated inhalation exposure to DEHP during gestation (NOAEL = 300 mg/m^3 = 138 mg/kg-day considering average rat weight of 0.3 kg and breathing rate of 5.74 L/hour) did not significantly alter the body weight of rats (Merkle *et al.,* 1988).

Subchronic exposure

Intermediate-term gavage exposures to DEHP resulted in decreased body weights and decreased body weight gain in rats (LOAEL = 2000 to 2500 mg/kg-day), guinea pigs (2000 mg/kg-day), and mice (LOAEL = 2000 mg/kg-day; NOAEL = 1171 mg/kg-day; Parmar *et al.,* 1988; Mangham *et al.,* 1981; Lee *et al.,* 1997). Longer-term exposures to DEHP via feed also resulted in decreased body weight and body weight gains, but at comparatively lower doses for rats (LOAEL = 200 to 2100 mg/kg-day; NOAEL = 50 to 1197 mg/kg-day;), mice (LOAEL = 100 to 7990 mg/kg-day; NOAEL = 44 to 2890 mg/kg-day), and hamsters (LOAEL = 1436 mg/kg-day; Tyl *et al.,* 1988; Mann *et al.,* 1985; Short *et al.,* 1987; CMA, 1984b; Barber *et al.,* 1987; Mochhiutti and Bernal, 1997; General Motors, 1982; Nuodex, 1981c; BIBRA, 1990; Eastman Kodak, 1992a, 1992b; Eagon *et al.,* 1994; Shaffer *et al.,* 1945; NTP, 1982; Poon *et al.,* 1997 (Table A3.70); Gray *et al.,* 1977 (Table A3.39); Lamb *et al.,* 1987 (Table A3.58); Ward *et al.,* 1998; Weghorst *et al.,* 1994; Maruyama *et al.,* 1994; CEFIC, 1982; Mitchell *et al.,* 1985a). In addition, dietary exposure to DEHP induced a rough hair coat and lethargy in mice (LOAEL of 91 mg/kg-day; NOAEL = 44 mg/kg-day; Tyl *et al.,* 1988).

Pregnant rat dams were more sensitive to DEHP-induced adverse effects on body weight than adult non-gestational rats. Maternal rat LOAELs (666 to 856 mg/kg-day) and NOAELs (357 mg/kg-day; Tyl *et al.,* 1988) were less than those for subchronically exposed rats not pregnant.

Chronic exposure

One-year chronic dietary exposure to rats from DEHP resulted in decreased absolute body weights and body weight gains at LOAELs ranging from 200 to 947 mg/kg-day. Rat NOAELs (60 mg/kg-day) from these studies paralleled observations reported in other chronic guinea pig and dog (capsule) studies (59 to 64 mg/kg-day; Carpenter *et al.,* 1953; Marsman *et al.,* 1988).

For longer period chronic exposures (1.5 to 2 years), decrements in body weight were, on average, more pronounced in rats (LOAEL = 70 to 2000 mg/kg-day; NOAEL = 7 to 322 mg/kg-day; Tamura *et al.,* 1990; Ganning *et al.,* 1987, 1991; Carpenter *et al.,* 1953; Rao *et al.,* 1990; Harris *et al.,* 1956) than in mice (LOAEL = 1266 to 1325 mg/kg-day; NOAEL = 354 to 672 mg/kg-day (NTP, 1982; David *et al.,* 1999, 2000b (Table A3.9)).

Multigeneration exposure

Multigenerational dietary exposure to DEHP in a continuous breeding study (NTP, 2004; CERHR, 2006) decreased Sprague-Dawley rat body weights in F_0 males (6%), F_1 male and female pups (9%; adjusted for litter size = 10%), F_1 male and female pups from PNd 1 to 21

(30% and 30%, respectively), F_1 females (19%), and F_1 breeder males (16%; LOAEL = 543 to 775 mg/kg-day; NOAEL = 392 to 592 mg/kg-day). DEHP also decreased body weights in F_1 non-breeder males (9%), F_2 live pups (10%; adjusted for litter size = 11%), F_2 male pups on PNd 1 and 4 (13% and 22%, respectively), F_2 female pups on PNd 1 and 21 (20% and 28%, respectively), F_2 breeder males (14%), and F_2 non-breeder males (14.5%; LOAEL = 392 to 592 mg/kg-day; NOAEL = 46 to 77 mg/kg-day; Bench Mark Dose $(BMD)_{10}$ = 5097 to 7496; BMD Lower confidence limit $(BMDL)_{10}$ = 2144 to 7119; $BMD_{1\ SD}$ = 1634 to 6788; $BMDL_{1\ SD}$ = 1229 to 3153 mg/kg). No decrements in body weight were observed in F_3 pups or adults.

Multigenerational exposures to DEHP also affected the body weights of Wistar rats (Schilling *et al.,* 2001; CERHR, 2006). Administration of DEHP decreased body weights or body weight gain in F_0 pregnant females (11%) and on Ld 21 (14%), F_1 male pups on PNd 1, 7, 14, 21 (6, 6, 26, 31%, respectively), F_1 female pups on PNd 7, 14, 21 (16, 27, 31%, respectively), F_1 pregnant females (15%) and on Ld 21 (21%), F_2 male pups on PNd 7, 14, 21 (11, 29, 35%, respectively), and F_2 female pups on PNd 7, 21 (11% and 33%, respectively; LOAEL = 1088 mg/kg-day; NOAEL = 340 mg/kg-day; BMD_{10} = 4085 to 9274; $BMDL_{10}$ = 2790 to 9034; $BMD_{1\ SD}$ = 3652 to 10,689; $BMDL_{1\ SD}$ = 2318 to 8389 mg/kg). Body weight decrements also occurred in F_2 female pups in this study (8%; LOAEL = 340 mg/kg-day; NOAEL = 113 mg/kg-day; BMD_{10} = 3691; $BMDL_{10}$ = 2724; $BMD_{1\ SD}$ = 3356; $BMDL_{1\ SD}$ = 2476 mg/kg).

Gastrointestinal toxicity

Gastrointestinal toxicity following exposures to DEHP was marginal.

In humans, ingestion of 10 grams of DEHP caused mild gastric disturbances and "moderate catharsis" (LOAEL = 142.8 mg/kg, assuming 70kg b.w.; NOAEL = 71.4 mg/kg-day; Shaffer *et al.,* 1945).

No gastric or intestinal effects were reported in marmoset monkeys, Sherman rats, Fischer 344 rats, and $B6C3F_1$ mice following subchronic and chronic gavage and dietary exposures to DEHP (NOAEL = 190 to 2500 mg/kg-day; Carpenter *et al.,* 1953; Kurata *et al.,* 1998; David *et al.,* 1999, 2000a, 2000b).

Pseudoductilar lesions (duct-like structures developed from acini; Scarpelli *et al.,* 1986) developed in the pancreas, however, following chronic exposures to DEHP in Fischer 344 rats (LOAEL = 2000 mg/kg-day; Rao *et al.,* 1990).

Hepatotoxicity

Orally administered DEHP and its associated metabolites were transported quickly to the liver following absorption from the gut, with peak liver concentrations found only a few hours

following dosing. These metabolites remained for a relatively extended duration in the liver (t½ of approximately 1 to 2 days). Rapid distribution and extended residence in the liver undoubtedly played a role in DEHP-induced hepatotoxicity.

In publications associated with DEHP-induced liver effects, enzymatic measurements were primarily associated with peroxisomal fatty acid β-oxidation and microsomal induction processes. These processes were chosen for investigation because metabolites of phthalates have been shown to induce the liver to inappropriately synthesize triglycerides and increase the synthesis of peroxisomal and microsomal fatty acid oxidases (Hinton *et al.,* 1986). Peroxisomal beta-oxidation is the mechanism by which long- and very-long chain fatty acids in the Acyl-CoA format are irreversibly metabolized in the peroxisome to generate Acetyl-CoA molecules. In peroxisomes, this activity is coupled to the generation of hydrogen peroxide (Reddy, 2001). Overall activity of the peroxisomal fatty acid β-oxidation cycle was determined by measuring CN-insensitive palmitoyl-CoA (fatty acid) oxidation (Lake, 1993). The function of β-oxidation was also assessed by determining the activity of carnitine acetyltransferase, an enzyme that transports activated acyl- groups into peroxisomes, and enoyl-CoA hydratase, a peroxisomal enzyme that facilitates the hydration of fatty acids. Microsomal induction and activity was determined by observing the hydrolysis of lauric acid, a substrate used for measuring the activity of CYP4A isoenzymes; 7-ethoxyresorufin O-deethylase, an enzyme for measuring CYP1A1; ethymorphine N-demethylase, an enzyme for measuring CYP3A; 7-ethoxycoumarin O-deethylase, a cytochrome P450-dependent microsomal enzyme (Tamasi *et al.,* 2004); and D-amino oxidase, a matrix-bound enzyme important in amino acid catabolism (Mannaerts and Van Veldhoven, 1993).

Acute exposure

Only one study investigated liver responses following a single gavage dose of DEHP. In this study, hepatic centrilobular necrosis or inflammation occurred following dosing of Fischer 344 rats (LOAEL = 1500 mg/kg-day; Berman *et al.,* 1995).

Numerous other repeat dose studies demonstrated a variety of hepatic pathologies in rats. Gavage exposures increased the absolute and relative liver weight and peroxisomal and microsomal enzyme activity in Sprague-Dawley rats (LOAEL = 25 to 1000 mg/kg-day; NOAEL = 10 to 25 mg/kg-day; Dostal *et al.,* 1987a; Lake *et al.,* 1984a; Lake *et al.,* 1984b; Lake *et al.,* 1986). In particular, palmitoyl-CoA oxidase activity and carnitine acetyl transferase activities were increased in these rats (LOAEL = 100 mg/kg-day; NOAEL = 10 mg/kg-day; Dostal *et al.,* 1987a). Increased relative liver weights and enzyme activities were also noted in Sprague-Dawley dams dosed during Ld's 2 to 6, 6 to 10, and 14 to 18 (LOAEL = 2000 mg/kg-day; Dostal *et al.,* 1987b (Table A3.24)). Gavage exposures in Fischer 344 and unspecified albino rats increased absolute and relative liver weights, changed hepatic enzyme activities, increased hepatic cellular mitosis and DNA synthesis 1300%, reduced hepatic apoptosis 20%, and altered liver profiles (LOAEL = 150 to 2000 mg/kg-day; James *et al.,* 1998; Adinehzadeh and Reo,

1998; Berman *et al.,* 1995; Parmar *et al.,* 1988). *In vitro* DEHP exposures involving hepatocytes isolated from Fischer 344 rats also resulted in increased peroxisomal palmitoyl-CoA oxidase activity (9-fold), catalase activity (2-fold), and decreased glutathione peroxidase activity (50%; LOAEL = 2000 mg/kg-day; Tomaszewski *et al.,* 1986). Gavage exposures also increased the absolute and relative liver weights of Wistar rats (LOAEL = 500 to 1000 mg/kg-day; NOAEL = 250 to 500 mg/kg-day; Oishi, 1989; Khaliq and Srivasta, 1993) and Alderley Park strains of Wistar rat (LOAEL = 2000 mg/kg-day; ICI, 1982; Rhodes *et al.,* 1986). Additional changes such as increased peroxisomal proliferation, increased proliferation of smooth endoplasmic reticulum, and altered mitochondria also occurred in the latter variety of rat (LOAEL = 2000 mg/kg-day; ICI, 1982b; Rhodes *et al.,* 1986). This concentration was not substantially different than the level at which increased palmitoyl-CoA oxidase activity, and decreased superoxide dismutase and decreased glutathione peroxidase activities were observed in specific, pathogen-free rat varieties (Alpk/AP; LOAEL = 1000 mg/kg-day; Elliot and Elcombe, 1987).

In $B6C3F_1$ mice treated with DEHP by gavage, the liver was enlarged in a dose-dependent fashion (Nuodex, 1981b; LOAEL = 1879 mg/kg-day), the absolute liver weight was increased by 9%, DNA synthesis by 248%, and hepatic apoptosis was decreased by 90% (James *et al.,* 1998; LOAEL = 1150 mg/kg-day). Similar exposures also induced a 21-fold increase in peroxisomal palmitoyl-CoA oxidase, a 3-fold increase in catalase, and a 35% decrease in glutathione peroxidase activity in *in vitro* experiments (LOAEL = 2000 mg/kg-day; Tomaszewski *et al.,* 1986).

Exposure to DEHP via gavage also increased liver weights and increased or decreased enzyme activity in guinea pigs (7 days exposure increased the activity of aniline hydroxylase, arylhydrocarbon hydroxylase, and ethylmorphine N-demethylase; 15 days exposure decreased the enzyme activities; LOAEL = 2000 mg/kg-day; Parmar *et al.,* 1988). Increases in liver weights (20%) and hepatic catalase (25%) were also induced by gavage dosing of DEHP for 14 days in marmoset monkeys (LOAEL = 2000 mg/kg-day; Rhodes *et al.,* 1986).

Dietary exposure to DEHP increased liver weights, peroxisomal proliferation, the induction of microsomal carboxylesterases, and NAD+ synthesis from tryptophan in Sprague-Dawley rats (LOAEL = 1905 to 2000 mg/kg-day; Hosokawa *et al.,* 1994; Shin *et al.,* 1999). Increased absolute and relative liver weights were also reported for Fischer 344 rats dosed via the diet, but at lower doses (LOAEL = 53 to 1600 mg/kg-day; NOAEL = 11 mg/kg-day; David *et al.,* 1999; Takagi *et al.,* 1992; Exxon, 1982a,b; Takagi *et al.,* 1990). In the study by Takagi *et al.* (1990), oxidized hepatic deoxyguanosine (in DNA) was also reported (LOAEL = 1200 mg/kg-day) following dietary exposures. DEHP dietary exposure also increased Wistar rat liver weights and peroxisomal proliferation at higher doses (LOAEL = 1894 mg/kg-day; Van den Munckhof *et al.,* 1998). Inhibition of gap junction intercellular communication also occurred following *in vitro* exposure of Wistar rat hepatocytes to MEHP (Lowest Observable Adverse Effects Concentration (LOAEC) = 57 µg/mL; Leibold *et al.,* 1994). The Alderley Park strain of Wistar rat may possess increased sensitivity to DEHP since increased liver weight,

morphological changes in bile ducts, peroxisome and smooth endoplasmic reticulum proliferation, increases in peroxisomal enzyme activity and the number of lipid filled lysosomes, induction of CYP 450s, mitochondrial changes, and glycogen depletion occurred at much lower doses than in other rats (LOAEL = 50 mg/kg-day; CEFIC, 1982; Mitchell *et al.,* 1985a).

Administration of DEHP via feed also increased the absolute and relative liver weights in $B6C3F_1$ mice (LOAEL = 188 to 1210 mg/kg-day; NOAEL = 188 to 250 mg/kg-day; David *et al.,* 1999; Eastman Kodak, 1992b) and increased hepatocellular hypertrophy (LOAEL = 6990 mg/kg-day; NOAEL = 2580 mg/kg-day; Eastman Kodak, 1992b). Increases in DNA synthesis may be tied to the strong induction of jun-B and jun-D, and small induction of c-fos and c-jun expression observed in BNL-CL.2 mouse liver epithelial cells shortly after dosing (LOAEC = 390 µg/mL; NOAEC = 39 µg/mL; Ledwith *et al.,* 1993).

Increases in absolute and/or relative liver weight and altered enzyme activities also occurred in other mouse strains (CD-1, C57BL/6, CH3/HeNCr) following DEHP exposure via gavage or feed (LOAELs = 191 to 4000 mg/kg-day; NOAEL = 91 mg/kg-day; Parmar *et al.,* 1988; Tyl *et al.,* 1988; Hosokawa *et al.,* 1994; Muhlenkamp and Gill, 1998; Lamb *et al.,* 1987 (Table A3.58); Weghorst *et al.,* 1994). Degenerative hepatic lesions occurred at higher doses overall in these mice (LOAEL = 2400 mg/kg-day; Ward *et al.,* 1998). DEHP exposure via gavage or diet also increased the liver weight of Chinese hamsters by 55% (LOAEL = 1000 mg/kg-day; Lake *et al.,* 1986) and Syrian golden hamsters by 36% (LOAEL = 2686 mg/kg-day; Hosokawa *et al.,* 1994).

Overall, the hepatic effects of DEHP in non-rodent species were less severe than that in rodents (Rhodes *et al.,* 1986). Gavage dosing cynomolgous monkeys with DEHP for 14 days (Pugh *et al.,*2000) and 25 days (Short *et al.,*1987) did not induce adverse hepatic effects (NOAEL = 500 mg/kg-day).

Hepatic responses to DEHP in rabbits were different than for other species. Repeat gavage dose, acute duration exposures decreased liver weights and decreased hepatic enzyme activities (LOAEL = 2000 mg/kg-day; Parmar *et al.,* 1988).

Subchronic exposure

Gavage dosing with DEHP increased absolute and relative liver weights, morphological and biochemical evidence of peroxisome proliferation and peroxisomal enzyme activation in Fischer 344 rats (LOAEL = 700 to 2000 mg/kg-day; Hodgson, 1987; Tenneco, 1981; Tomaszewski *et al.,* 1988). Increased relative liver weight, the number of peroxisomes and the proliferation of smooth endoplasmic reticulum were also noted in Wistar rats following gavage dosing (LOAEL = 2500 mg/kg-day; Mangham *et al.,* 1981 (Table A3.59)).

Dietary administration of DEHP to Sprague-Dawley rats induced hypertrophic responses in the liver, increased absolute and relative liver weights, the number of hepatic peroxisomes, the proliferation of smooth endoplasmic reticulum, and peroxisomal enzyme activity (LOAEL = 143

to 900 mg/kg-day; NOAEL = 37.6 mg/kg-day; Gray *et al.,* 1977 (Table A3.41; Table A3.42); General Motors, 1982; Poon *et al.,* 1997 (Table A3.70)). Hepatic effects in Gray *et al.* (1977) were not observed histopathologically in Sprague-Dawley rats (NOAEL = 1414 mg/kg-day). Fischer 344 rats dosed by diet also displayed increased absolute and relative liver weights, peroxisomal enzyme activity, hepatocellular hypertrophy, increased number of peroxisomes, hypolipidemia, biochemical evidence of cell proliferation, and biochemical and morphological evidence of peroxisome proliferation (LOAEL = 63 to 1200 mg/kg-day; NOAEL = 11 to 105 mg/kg-day; Tyl *et al.,* 1988; CMA, 1982c; Moody and Reddy, 1978; Barber *et al.,* 1987; CMA, 1984b; Short *et al.,* 1987; BIBRA, 1990; Nuodex, 1981c; Cattley *et al.,* 1988; Hodgson, 1987; David *et al.,* 1999; Eastman Kodak, 1992a; Eagon *et al.,* 1994). In Wistar rats, administration of DEHP in the feed also increased the absolute and relative liver weight, peroxisomal proliferation, peroxisomal enzyme activities, proliferation of smooth endoplasmic reticulum, and the number of altered mitochondria (LOAEL = 2%; LOAEL = 88 to 1730 mg/kg-day; NOAEL = 42 mg/kg-day; Fukuhara and Takabatake, 1977; Mann *et al.,* 1985; Mochhiutti and Bernal, 1997; Miyazawa *et al.,* 1980). Increases in both liver weight and peroxisomal enzyme activity were reversible following recovery periods (LOAEL = 2%; Miyazawa *et al.,* 1980). Dosing of Wistar rats by gavage also altered liver parameters. Significantly decreased aniline hydroxylase and ethylmorphine N-demethylase (LOAEL = 50 mg/kg-day) and cytochrome p450 (LOAEL = 100 mg/kg-day; NOAEL = 50 mg/kg-day) were observed following gavage dosing 25 day old Wistar rats (Parmar *et al.*, 1995; Table A3.69).

It is unknown why subchronic oral dosing to Wistar rats did not elicit any adverse hepatic effects in some studies (NOAEL = 1900 mg/kg-day; Shaffer *et al.,* 1945). With Sprague-Dawley rats, an increased number of peroxisomes and biochemical and morphological evidence of peroxisome proliferation were the most sensitive subchronic endpoints (LOAEL = 105 mg/kg-day; NOAEL = 11 mg/kg-day; CMA, 1984b; Barber *et al.,* 1987; Short *et al.,* 1987). Wistar rats also had increased numbers of peroxisomes and increased peroxisomal enzyme activation as the most sensitive adverse hepatic subchronic effects (LOAEL = 5 to 18 mg/kg-day; NOAEL = 5 mg/kg-day; RIVM, 1992).

Increased liver weights were not observed in marmoset monkeys when higher doses were used for 13 weeks (NOAEL = 2500 mg/kg-day; Kurata *et al.,* 1998). Dietary exposure also had no effect on hepatic parameters in Syrian golden hamsters (NOAEL = 1436 mg/kg-day; Maruyama *et al.,* 1994).

Chronic exposure

Chronic dietary dosing increased the relative liver weight, peroxisome proliferation, peroxisomal enzyme activity, and the number of mitochondria, lipofuschin deposits, and conjugated dienes in treated Sprague-Dawley rats (LOAEL = 7 to 1000 mg/kg-day; NOAEL = 7 mg/kg-day; Ganning *et al.,* 1987, 1991; Lake *et al.,* 1987). Liver necroses and fat infiltration was also observed in "a few rats" following chronic treatment (LOAEL = 200 mg/kg-day; BASF,

1960). Increases in hepatocellular carcinomas were observed in treated rats following long-term high-dose exposures to DEHP (LOAEL = 1377 mg/kg-day; Lake *et al.,* 1987). Hepatic tumors were not reported in other studies, however, following similar duration exposures at lower doses (NOAEL = 700 mg/kg-day; Ganning *et al.,* 1987, 1991). In Fischer 344 rats, dietary exposure to DEHP increased absolute and relative liver weight and DNA synthesis. It also increased peroxisomal enzyme activation, morphological and biochemical evidence of peroxisome proliferation, and fatty acid oxidase activity, but decreased catalase activity (LOAEL = 92 to 2444 mg/kg-day; Conway *et al.,* 1989; Marsman *et al.,* 1988; Rao *et al.,* 1987, 1990; Cattley *et al.,* 1987; David *et al.,* 1999, 2000a (Table A3.4)). Dietary exposure to DEHP also increased the incidence of spongiosis hepatis and clear cell foci (LOAEL = 147 to 322; NOAEL = 36 mg/kg-day; David *et al.,* 1999, 2000a (Table A3.4); Kluwe *et al.,* 1982). For long-term exposures, increased liver weight (M) and peroxisome proliferation were the most sensitive adverse endpoints (LOAEL = 28.9 mg/kg-day; NOAEL = 5.8 mg/kg-day; Moore, 1996).

Fischer 344 rats exposed to DEHP also developed hepatocarcinomas by week 78 (43%; LOAEL = 1579 mg/kg-day; Hayashi *et al.,* 1994), hepatocellular carcinomas (11/14 treated rats versus 1/10 control rats; LOAEL = 2%; Rao *et al.,* 1990; 1/4 rats and 2/4 rats by 52 and 78 weeks, repectively; LOAEL = 2%; Tamura *et al.,* 1990a,b), liver tumors (6/10 treated rats versus 0/10 control rats; LOAEL = 2%; Rao *et al.,* 1987), hepatocellular tumors (11/65 male treated rats and 22/80 female treated rats; $LOAEL_M$ = 147 mg/kg-day; $LOAEL_F$ = 939 mg/kg-day; David *et al.,* 1999, 2000a), and hepatic neoplastic lesions, hepatocellular carcinomas, adenomas, and neoplastic nodules (LOAEL = 147 to 550 mg/kg-day; NOAEL = 29 mg/kg-day; NTP, 1982 (Table A3.63); Moore, 1996 (Table A3.62); Cattley *et al.,* 1987; Kluwe *et al.,* 1982).

In Wistar rats, chronic exposure to dietary DEHP increased liver weights and changed peroxisomal enzyme activities (LOAEL = 300 to 867 mg/kg-day; NOAEL = 50 to 80 mg/kg-day; Tamura *et al.,* 1990; Harris *et al.,* 1956). Increased liver weights were present at 12 and 24 weeks during dosing, but not at 52 and 104 weeks (LOAEL = 300 to 400 mg/kg-day; NOAEL = 50 to 80 mg/kg-day; Harris *et al.,* 1956). In Sherman rats, DEHP increased liver weights at 52 weeks (LOAEL = 190 to 200 mg/kg-day; NOAEL = 60 mg/kg-day; Carpenter *et al.,* 1953). Increases in liver weights were reversed during DEHP-free recovery periods and no DEHP-induced hepatic neoplastic lesions were reported for this rat strain (NOAEL = 2%; Tamura *et al.,* 1990a, 1990b).

Chronic exposure to DEHP via feed also increased liver weights in $B6C3F_1$ mice (LOAEL = 98.5 to 292 mg/kg-day; NOAEL = 19.2 to 117 mg/kg-day; David *et al.,* 1999, 2000b (Table A3.10); Moore, 1997; NTP, 1982). Hepatic peroxisome proliferation was also noted in these mice (LOAEL = 98.5 mg/kg-day; NOAEL = 19.2 mg/kg-day; Moore, 1997). Chronic exposure also increased the incidence of hepatocellular neoplasms and carcinoma (LOAEL = 672 mg/kg-day; NTP, 1982 (Table A3.63); Kluwe *et al.,* 1982), the total number of adenomas and carcinomas in rats (M&F; partially reversible; LOAEL = 147 mg/kg-day; NOAEL = 29 mg/kg-day; Moore, 1997 (Table A3.62)), and hepatocellular tumors (27/65 male mice, and 19/65

female mice; $LOAEL_M$ = 292 mg/kg-day; $LOAEL_F$ = 354 mg/kg-day; David *et al.,* 1999, 2000b).

Chronic exposure to DEHP via the feed also increased guinea pig liver weights (LOAEL = 64 mg/kg-day; NOAEL = 19 mg/kg-day; Carpenter *et al.,* 1953).

Changes in hepatic parameters following long-term DEHP administration were not universal. Lifetime exposure of Syrian golden hamsters to DEHP via inhalation or interperitoneal injection did not increase the hepatic tumor incidence in treated hamster groups (NOAEC = 15 $\mu g/m^3$ and NOAEL = 3000 mg/kg-day, respectively; Schmezer *et al.,* 1988). Adverse hepatic effects were also not observed in dogs following chronic dosing with DEHP in capsules (NOAEL = 59 mg/kg-day; Carpenter *et al.,* 1953).

In vivo pathological evidence of DEHP-induced hepatic injury was lacking for humans. *In vitro* data suggested, however, that induction of palmitoyl-CoA oxidase and carnitine acetyltransferase does not occur following MEHP exposures to human hepatocytes (NOAEC = 56 µg/mL; Butterworth *et al.,* 1989).

Peroxisome proliferation

Peroxisomes are ubiquitous eukaryotic subcellular organelles. Larger peroxisomes (> 0.4 mm diameter) are found in hepatic parenchymal and kidney proximal tubule cells. Smaller peroxisomes (< 0.4 mm) are found in other tissues (Yokota *et al.*, 2008).

Two of the primary responsibilities of peroxisomes are the metabolism of fatty acids (β-oxidation) and oxidative reactions using hydrogen peroxide. In β-oxidation, peroxisomal membrane proteins transport fatty acids across the lipid bilayer membrane into the peroxisome. These fatty acids are then degraded two carbons at a time and converted into acetyl-CoA. Acetyl-CoA is then transported out of the peroxisome into the cytosol where it can be used in energy production and the biosynthesis of many other molecules (i.e., cholesterol, acetylcholine). In oxidative reactions, peroxisomes detoxify organic molecules (i.e., phenol, formic acid, formaldehyde, and alcohol) by using hydrogen peroxide to oxidize the substrate (Schrader and Fahimi, 2008).

Plasmalogen biosynthesis also occurs in the peroxisome and is facilitated by the enzymes dihydroxyacetonephosphate acyltransferase and alkyldihydroxyacetonephosphate synthetase. Plasmalogen "ether lipids" are of major importance as cell membrane components and antioxidants. Testicular tissue contains a large contingent of plasmalogens (Schrader and Fahimi, 2008).

Peroxisomes have additional roles in fatty acid α-oxidation, long/very long fatty acid activation, regulation of the acyl-CoA/CoA ratio, protein and amino acid metabolism, catabolism of purines, glyoxylate and dicarboxylate metabolism, hexose monophosphate pathway, glycerol

synthesis, nicotinate and nicotinamide metabolism, and retinoid metabolism (Schrader and Fahimi, 2008).

When stimulated by exogenous chemical or endogenous ligands, peroxisomes can "proliferate" (increase in size and number). Peroxisome proliferation can take place by budding from already existing peroxisomes or from de-novo construction with cytosolic cellular materials (Schrader and Fahimi, 2008). PEX 11 proteins play a critical role in the elongation of peroxisomes and formation of "beads on a string" appearance prior to division.

Endogenous ligands for PPARα include fatty acids (18 to 20 carbon polyunsaturated fatty acids and eicosanoids). Exogenous ligands such as phthalates, fibrates, thiazolidinediones, tetrachloroethylene, and perfluorooctanoic acid are also able to stimulate proliferation (Ito and Nakajima, 2008).

Hepatic peroxisome proliferation has been reported in many test species following exposure to DEHP. DEHP-induced peroxisome proliferation occurs through the stimulation of nuclear receptor proteins called peroxisome proliferator activated receptors (PPARs). There are 3 isoforms of PPAR; PPARα, PPARβ (δ), and PPARγ (Ito and Nakajima, 2008). All three PPARs are expressed in reproductive (gonads, uterus, prostate, mammary glands, pituitary) and central nervous tissue (Latini *et al.*, 2008). PPARα is also expressed in the liver, kidney, and heart, organs important in fatty acid catabolism (Ito and Nakajima, 2008). DEHP can also stimulate other peroxisomal membrane proteins (i.e., PEX) that may aid in proliferation. Schrader *et al.*, (1998) determined that PEX11α mRNA was induced greater than 10-fold in rat models in response to both clofibrate and DEHP. In contrast, PEX11β mRNA expression was not induced by exposure to these compounds, but was responsible for constituitive expression of peroxisomal abundance.

Metabolic processing of DEHP into MEHP and/or 2-ethylhexanoic acid or other metabolites is thought to be necessary prior to binding and activation of PPARs. 2-ethylhexanoic acid has been shown to be capable of activating mouse PPARα, PPARβ, and human PPARα, but not human PPARγ (Lampen *et al.*, 2003; Lapinskas *et al.*, 2004; Maloney and Waxman, 1999; cited in Corton and Lapinskas, 2005). Equivocal activation has also been reported for mouse PPARγ by 2-ethylhexanoic acid. MEHP, another primary metabolite of DHEP, has also been shown to activate mouse and human PPARα, PPARβ, and PPARγ (Hurst and Waxman, 2003; Lampen *et al.*, 2003; Lapinskas *et al.*, 2004; Maloney and Waxman, 1999; Gray *et al.*, 2000; Wine *et al.*, 1997; Gray *et al.*, 1999; Mylchreest *et al.*, 1999; Higuchi *et al.*, 2003; Ohtani *et al.*, 2000; Imajima *et al.*, 2001; Moore *et al.*, 2001; Parks *et al.*, 2000; Nagao *et al.*, 2000; Piersma *et al.*, 2000; Bility *et al.*, 2004; cited in Corton and Lapinskas, 2005). Scintillation proximity assays used to determine receptor binding have also indicated that MEHP is able to bind human PPARα and PPARγ receptors (Ki's = 15 and 12 μM, respectively; Lapinskas *et al.*, 2004; cited in Corton and Lapinskas, 2005). DEHP itself, only marginally activated mouse PPARα and PPARγ (Lampen *et al.*, 2003; Lapinskas *et al.*, 2004; Maloney and Waxman, 1999; cited in Corton and

Lapinskas, 2005) and did not bind to or activate human PPARα and PPARγ (Maloney and Waxman, 1999; cited in Corton and Lapinskas, 2005) or mouse PPARβ (Lampen *et al.*, 2003; Lapinskas *et al.*, 2004; cited in Corton and Lapinskas, 2005). The ability to bind PPARα and PPARγ for MEHP, phthalic acid, and to a lesser extent, DEHP, has been verified in docking model simulations, with only MEHP and phthalic acid being able to occupy the PPAR active sites (Kambia *et al.*, 2008). Overall differences in activation data suggest that mouse PPARs are more sensitive to activation than human PPARs (Bility *et al.*, 2004).

In brief, when DEHP binds to the PPAR, the combination induces it to heterodimerize with the liver X receptor and then the retinoid-x-receptor-α (RXR). The RXR heterodimer then binds to peroxisome proliferator hormone response element (PPRE) DNA target regions and either induces or decreases transcription of target genes. The RXR heterodimer can also form a heterodimer with other ligands such as thyroid hormone.

Exposure to DEHP leads to adaptation in the liver such as hepatocellular hypertrophy and hyperplasia. Chronic exposure can lead to liver tumors in male and female rats and mice (Corton and Lapinskas, 2005; see Carcinogenicity section). The adaptive consequences are mediated by PPARs.

Human Relevance

Currently, scientific consensus is that PPARα-mediated peroxisome proliferation and hepatocellular tumors (seen in DEHP-exposed rodents) have little or no relevance to humans (CERHR, 2006). This conclusion is supported by a variety of federal and international agencies (ATSDR, Health Canada, European Commission, IARC, CPSC, NICNAS).

Species differences that might contribute to differences in susceptibility have been reviewed extensively (Klaunig *et al.*, 2003; Rusyn *et al.*, 2006; Ito and Nakajima, 2008). Many authors have noted that there is: less PPARα mRNA in human livers when compared to rodents (Palmer *et al.*, 1998; Tugwod *et al.*, 1998), generally lower PPARα protein levels in humans when compared to the mouse (Walgren *et al.*, 2000), a reduced or absent PPARα-mediated peroxisome proliferation response in human livers when compared to rodents (David *et al.*, 1999; Doull *et al.*, 1999), a reduced ability for hepatocyte cell proliferation or suppression of apoptosis in humans (Perrone *et al.*, 1998; Doull *et al.*, 1999), a reduced ability for PPARα-humanized transgenic mice to induce hepatomegaly and hepatocyte proliferation following exposure to fenofibrate when compared to wild-type mice (Yang *et al.*, 2007), more inactive or pleiomorphic forms of PPARα in the human liver (Palmer *et al.*, 1998), a difference in the binding to or recognition of PPREs when comparing rodents and humans (Ito and Nakajima, 2008), a difference in the tissue localization of PPARα, with rodents expressing primarily in the liver and kidney, and humans in the kidney and skeletal muscle (Guyton *et al.*, 2009), and a 10- to 20-fold reduction in PPARα response in humans to agonists such as Wy-14,643 (Maloney and Waxman, 1999).

Species-specific metabolic differences have also been used to support negligible human relevance. Intestinal lipase activity has been reported to be 150- to 360-fold lower in marmosets (a human analogue) when compared to mice. Lipase mRNA was also significantly lower in marmosets when compared to rats and mice ($P < 0.05$). In addition, the affinity of DEHP for lipase was lower than in rats or mice (Ito et al., 2007; Ito and Nakajima, 2008). This evidence suggests that lowered metabolic concentration and activity will result in a lower MEHP concentration in the blood of marmosets, when compared to rodents.

Epidemiological evidence also supports the conclusion that humans may be refractory to DEHP-induced (peroxisome proliferator-induced) hepatic cancers. A clinical trial investigating the ability of gemofibrozil to lower serum lipids in men with elevated serum cholesterol did not report any differences in total death rate or the incidence of liver cancer between placebo or treated groups (2030 and 2051 men, respectively; reviewed in Klaunig *et al.*, 2003). In another clinical trial conducted by WHO, men with high cholesterol were treated with clofibrate or a placebo (high and low cholesterol groups) for an average of 5.3 years (5745, 5000, and 5000 men, respectively). Follow-up at 4.3 years post-treatment reported a statistically increased treatment-related age-adjusted total mortality when compared to the high cholesterol control group. In this case, excess mortalities were due to diseases of the liver, gallbladder, pancreas, and intestines, and included malignant neoplasms. In the final follow-up at 7.9 years, differences in the number or rate of cancer deaths between treatment and control groups were not significant (reviewed in Klaunig *et al.*, 2003). In the final epidemiological study, no associated increase in cancer risk was reported in groups exposed to fibrate therapy. This study was of limited size and poorly described, however, limiting its usefulness (reviewed in Klaunig *et al.*, 2003).

A few publications (Melnick *et al.*, 2001, 2002; NAS, 2008; Guyton *et al.*, 2009; Caldwell *et al.*, 2008) have questioned whether PPARα-induced peroxisome proliferation is the sole and obligatory pathway for phthalate-induced carcinogenicity. They have also postulated that a non-PPARα-dependent pathway might exist for phthalate-induced carcinogencity. For support, the authors have noted that: DEHP has been reported to induce liver tumors in PPARα-null mice after 2 years exposure to 100 and 500 mg/kg doses (Ito *et al.*, 2007), DEHP induced a significant trend increase in total liver tumors in PPARα-null mice with the Sv129 genetic background (Lee *et al.*, 1995, reported in Ito *et al.*, 2007), a statistical reanalysis of Ito *et al.*, 2007 and David *et al.*, 1999 data suggested that PPARα-null mice, but not wild-types, had significantly increased adenomas and adenomas and carcinomas at 500 mg/kg doses, and that null and wild-type mice had significant dose-response trends for adenomas and adenomas and carcinomas (Guyton *et al.*, 2009), a few DEHP-induced transcriptional changes (6%; induced genes *Cyp2b10*, *Cyp3a11*, *metallothionine-1*, *Cyp8b1*, *Gstm4*, and *Gstm7*) in wild-type, PPARα-null, and constituitive activated receptor(CAR)-null mice were PPARα-indpendent (Ren *et al.*, 2010), many PPARα activators are pleotrophic and can induce genotoxicity (Melnick, 2001), epigenetic alterations (Pogribny *et al.*, 2007), oxidative stress (Obrien *et al.*, 2007), and activate other cellular receptors (Guo *et al.*, 2007) or organelles (Lundgren *et al.*, 1987), and additional DEHP-induced effects occur in PPARα-free tissues.

These conclusions have also recently been reiterated by Ito and Nakajima (2008). These authors remarked that evidence suggested the existence of multiple non-PPARα pathways in DEHP-induced carcinogenesis. They further noted that species differences might exist because PPAR function, PPAR constituitive expression, and lipase activity differed among rodents and humans, making extrapolation from rodents to humans difficult. Ito and Nakajima concluded by supporting the further use of PPARα-null mice or mice with human PPARα to tease out these relationships.

The studies that suggest that DEHP may induce rodent liver tumors by a PPARα-independent pathway are limited by low doses, low tumor incidence, a lack of tumors in wild-type mice, and few animals per dose group. More studies would be needed in order to confirm these findings and change the scientific consensus that rodent liver tumors are not relevant to humans.

Thyroid effects in/on the liver

The thyroid may play a role in DEHP-induced liver hepatomegaly and other associated effects (Badr, 1992). DEHP has been reported to induce the activity of malic acid and carnitine acetyltransferase in the absence of thyroid hormones (TH). This effect has been termed "thyromimetic", since the pattern of malic enzyme gene expression in the liver of neonatal rats is typically affected by thyroid hormone status (Sood *et al.,* 1996). Malic enzyme is involved in fatty acid biosynthesis, is absent in fetal rat livers, is typically detectable several days after birth, but does not reach significant levels until after weaning (Madvig and Abraham, 1980).

Further evidence suggested that the thyroid may participate in DEHP-induced liver changes. A WY-induced increase in relative liver weight ("hepatomegaly") was mitigated in rats that were thyroidectomized. Administration of thyroid hormones T_3 or T_4 to thyroidectomized rats did not, however, re-establish WY-induced increases in relative liver weight (Miller *et al.,* 2001). This suggested that a non-TH-associated function also participated in this type of liver pathology.

Thyroid-modulated changes in liver DNA replication have also been reported for WY. In intact rats, treatment with WY induced a time-dependent increase in the percent of hepatocytes expressing nuclear proliferating cell nuclear antigen (PCNA). This increase was blunted significantly for both control and WY-treated rats that were thyroidectomized (Miller *et al.,* 2001).

Expression of the thyroid hormone receptor alpha-1 (TRα-1) in the liver also increased following 13 weeks of exposure to WY, GEM (another drug that induces hypolipidemia), and DBP (Miller *et al.,* 2001). This increase was not associated with changes in mRNA levels.

Badr (1992) also found that the induction of liver catalase activity by DEHP was dependent on the presence of thyroid hormones and that the induction of other peroxisomal β-oxidizing enzymes was not dependent on thyroidal status of the rats. Exposure to WY for up to 72 hours induced a significant time-dependent increase in acyl-CoA oxidase activity that was similar in both thyroidectomized and intact rats (Miller *et al.,* 2001). Acyl-CoA oxidase is an important enzyme in the peroxisomal β-oxidation pathway (Bronfman *et al.,* 1984).

Overall

Overall, data suggested that DEHP induces liver pathologies in animals through multiple mechanisms including changes in fatty acid metabolism, peroxisome proliferation, mitochondrial dysfunction, receptor activation, and cell proliferation. The weight of evidence from the above studies supported the conclusion that **there was "sufficient animal evidence" for the designation of DEHP as a "probable hepatotoxicant"**.

Renal toxicity

Exposure to DEHP induced adverse kidney effects in a variety of animal species.

Acute exposure

Exposure to DEHP via gavage (LOAEL = 1000 to 2000 mg/kg-day) or feed (LOAEL = 1200 to 1600 mg/kg-day) increased kidney weights in Sprague-Dawley, Fischer 344, and Alderly Park rats (ICI, 1982b; Rhodes *et al.,* 1986; Dostal *et al.,* 1987a; Exxon 1982a,b; Takagi *et al.,* 1990). Kidney weight increases in Dostal *et al.*'s study (1987a) were observed in 21, 42, and 86 day-old rats, but not those that were younger (6 to 16 day old rats). Younger rats (< 21 days) were probably being exposed to DEHP or its metabolites in milk as well. No histopathologies were noted in kidneys with increased weights in Fischer 344 rats dosed with DEHP via feed (NOAEL = 1600 mg/kg-day; Exxon 1982a, 1982b). Biochemically, however, a 2-3 fold increase in kidney microsomal lauric acid omega-hydroxylation activity was observed in rats acutely exposed to a similar dose (Sharma *et al.,* 1989).

Adverse effect data in monkeys contrasted with results reported for rodents. High-dose gavage exposures with DEHP did not affect the relative kidney weight in female Marmoset monkeys (NOAEL = 2000 mg/kg-day; Rhodes *et al.,* 1986). Male cynomolgous monkeys gavage dosed with DEHP at a slightly lower dose also did not develop changes in kidney weight (NOAEL = 500 mg/kg-day; Pugh *et al.,* 2000). No adverse effects were noted in kidneys of rats following lower doses of DEHP (NOAEL = 100 mg/kg-day; Dostal *et al.,* 1987a).

Subchronic exposure

Dietary exposure to DEHP increased the absolute and/or relative kidney weights in both Sprague-Dawley and Fischer 344 rats (LOAEL = 261 to 1892 mg/kg-day; NOAEL = 37.6 to

302.0 mg/kg-day; General Motors, 1982; Poon *et al.,* 1997 (Table A3.70); CMA, 1984b; Barber *et al.,* 1987; Eastman Kodak, 1992a) and Syrian golden hamsters (LOAEL = 1436 mg/kg-day; Maruyama *et al.,* 1994). As with short-term dosing of Fischer 344 rats, no histopathological alterations were noted in hamster kidneys that had increased weights (Maruyama *et al.,* 1994). In contrast, decreases in absolute kidney weight, increased inflammation, and degenerative kidney lesions were reported for $B6C3F_1$ and Sv/129 mice (LOAEL = 1210 to 2400 mg/kg-day; NOAEL = 250 mg/kg-day; Eastman Kodak, 1992b; Ward *et al.,* 1998). Exposure to large doses of DEHP via gavage, however, did not induce any kidney changes in Marmoset monkeys (NOAEL = 2500 mg/kg-day; Kurata *et al.,* 1998) or Wistar rats (NOAEL = 1900 mg/kg-day; Shaffer *et al.,* 1945).

Chronic exposure

Gavage dosing with DEHP for 12 months increased the incidence of focal cystic kidneys (0% in control, 37% in DEHP; $P < 0.04$), possibly type A tubular ectasia (enlarged collecting ducts; ~20% in control, ~45 to 55% in DEHP), and decreased creatinine clearance in male rats (from ~ 1mL/min to 0.5 mL/min; $P < 0.01$; LOAEL = 2.1 mg/kg-day; Crocker *et al.,* 1988). Increased absolute and/or relative kidney weights were also observed following dietary administration of DEHP to Sherman rats, Fischer 344 rats, and Wistar rats (LOAEL = 146.6 to 400 mg/kg-day; NOAEL = 28.9 to 80 mg/kg-day; Harris *et al.,* 1956; Carpenter *et al.,* 1953; David *et al.,* 1999, 2000a (Table A3.5); Moore, 1996). In contrast to Crocker *et al.*'s study (1988), creatinine clearance, urine volume, urine creatinine concentration, or other urinalysis parameters were not altered when Fischer 344 rats were exposed to DEHP (NOAEL = 789 to 938.5 mg/kg-day; David *et al.,* 2000a).

Dietary exposure to DEHP increased the incidence of kidney nephroses (LOAEL = 200 mg/kg-day), mineralization of the renal papilla, tubule cell pigmentation, irreversible chronic progressive nephropathy (CPN; LOAEL = 789 mg/kg-day; NOAEL = 146.6 mg/kg-day) and lipofuschin pigments in the tubular epithelium (LOAEL = 2000 mg/kg-day) in Fischer 344 and Sprague-Dawley rats in other studies (BASF, 1960; Rao *et al.,* 1990; Moore, 1996). Increased blood urea nitrogen (BUN; 78 weeks), marginally increased incidence of renal papilla mineralization (M&F; 78 weeks), and marginally increased severity of CPN (78 and 105 weeks; M) and renal tubule pigmentation (78 and 105 weeks; M&F) were also reported in David *et al.*'s (2000a) study (LOAEL = 789 to 938.5 mg/kg-day; NOAEL = 146.6 to 181.7 mg/kg-day (Table A3.5)). This was in addition to a dose-dependent increase in the incidence of mineralization of renal papilla at much lower doses (LOAEL = 5.8 mg/kg-day; M). Complete reversal of alterations in BUN, partial reversal of male and female absolute and relative body weights, and minimal or no reversal in the incidence and severity of mineralization of the renal papilla, CPN, or renal tubule pigmentation was noted in rats allowed to recover for 26 weeks following week 78 of dosing (David *et al.,* 2001; Table A3.17).

Long-term exposure effects in mice paralleled that reported for intermediate duration exposures. Dietary exposure to DEHP for 2 years decreased absolute and relative kidney weights and increased BUN in a dose-related fashion (LOAEL = 98.5 to 1458.2 mg/kg-day; NOAEL = 19.2 to 98.5 mg/kg-day; David *et al.,* 2000b (Table A3.11)), increased the incidence and severity of CPN (LOAEL = 292.2 to 1458.2 mg/kg-day; NOAEL = 98.5 to 354.2 mg/kg-day; David *et al.,* 2000b (Table A3.11); Moore, 1997), and increased inflammation (LOAEL = 1325 mg/kg-day; NOAEL = 672 mg/kg-day; NTP, 1982; Kluwe *et al.,* 1982) in $B6C3F_1$ mice. Partial reversal of decreased absolute and relative body weights and the severity of CPN, and minimal or no reversal in female absolute and relative body weights and incidence of CPN were noted in mice allowed to recover for 26 weeks following week 79 of exposure (David *et al.,* 2001; Table A3.16).

In contrast to mice, long-term administration of DEHP in capsules did not induce any adverse effects to the kidneys of dogs (NOAEL = 59 mg/kg-day; Carpenter *et al.,* 1953). In addition, kidney effects were not observed following dosing of low doses of DEHP to Sherman rats and guinea pigs (NOAEL = 190 and 64 mg/kg-day, respectively; Carpenter *et al.,* 1953).

Data suggested that adverse effects may occur in the kidneys following DEHP exposure and that these effects were species and gender specific. Increased absolute and relative kidney weights were observed following short-, intermediate-, and long-term dosing in most rat species, but not monkeys. Increases in kidney weight in rats may be related to peroxisomal proliferation (David *et al.,* 2000a), since this has been reported in the kidneys following exposure to DEHP in some studies (Ohno *et al.,* 1992; Cimini *et al.,* 1994; Price *et al.,* 1987). The reversible nature of some of the kidney pathologies and that of peroxisomal proliferation also suggested that some aspects of kidney effects may be mediated via this mechanism (David *et al.,* 2001). Some of the kidney effects may also have been mediated in part through PPARα independent mechanisms, since PPARα-null mice have been reported to have less frequent and severe renal lesions following exposure to DEHP (Ward *et al.*, 1998).

Increased incidence of renal papilla mineralization in male rats may be related to the intra-renal precipitation and accumulation of alpha-2-urinary-globulin (α_{2u}-globulin; David *et al.,* 2000a). Mineralization in linear profiles (within the loops of Henle) is one of many histological alterations induced by α_{2u}-globulin (Doi *et al.,* 2007). Further, accumulation of α_{2u}-globulin in male rat kidneys has been reported following DINP exposures (Caldwell *et al.,* 1999). Increased mineralization of the renal tubules did not occur in DEHP-exposed mice (David *et al.,* 2000b), indirectly supporting α_{2u}-like mechanisms. Pathologies involving α_{2u} are specific for male rats and no other gender or species, including humans (Swenberg, 1993). Empirical data demonstrating DEHP-induced hyaline droplet formation, α_{2u}-globulin precipitation, renal tubule regeneration, or lysosomal dysfunction were lacking for rats, however, complicating definitive conclusions regarding this pathology (David *et al.,* 2000a; Doi *et al.,* 2007).

An exacerbation of CPN was identified as an additional histological alteration in α_{2u}-globulin mediated pathologies (Doi *et al.,* 2007). DEHP-induced increases in the severity of CPN occurred in high dose male rats (David *et al.,* 2000a). Increased incidence and severity of CPN also occurred in male and female mice (David *et al.,* 2000b), however, suggesting that α_{2u} may not directly influence this type of pathology. David *et al.* (2001) attributed the development of CPN to factors associated with peroxisome proliferation. Increases in cell death may be mediated via MEHP, since it is reported that this metabolite can decrease cultured kidney epithelium cell viability, increase cell swelling at low doses, increase cell shrinkage at high doses, and alter the cytoskeletal network of F-actin *in vitro* (Rothenbacher *et al.,* 1998).

Decreased absolute and relative kidney weights were reported for mice following subchronic (Eastman Kodak, 1992b; Ward *et al.,* 1998) and chronic (David *et al.,* 2000b) dosing. The pathological mechanisms involved in this eliciting this adverse effect were unknown.

Decreased creatinine clearance and an increase in the incidence of Type A tubular ectasia and focal cystic kidneys were reported in Crocker *et al.* (1988). These pathologies were not observed in other subchronic or chronic studies. This fact, and the observation that very few animals (4 to 8) were used for each timepoint, suggests that this study may be less reliable for hazard endpoint selection.

Increased BUN was also consistently observed in rats dosed with large concentrations of DEHP (David *et al.,* 2000a, David *et al.,* 2001). Repeated evidence of this pathology suggests that it was not random and may correlate to other pathologies (such as chronic kidney disease) observed in these studies. In this case, increased BUN was suggestive of impaired kidney function, since other modulating factors for BUN such as excessive protein break down, increased protein consumption, gastrointestinal bleeding, and increased dehydration were not noted in chronic tests. Although BUN is not typically elevated until substantial kidney damage has occurred, increased concentrations may be related to the ability of DEHP to alter the kidneys ability to concentrate and dilute urine (reported in female rats exposed to 2% DEHP in diet for 17 weeks – Gray *et al.,* 1977).

Overall, data suggested that DEHP induced kidney pathologies through both non- α_{2u} and α_{2u}-like mechanisms. Since non- α_{2u}-like mechanisms can induce toxicity in humans, the weight of evidence from the above studies supported the conclusion that **there was "sufficient animal evidence" for the designation of DEHP as a "probable renal toxicant"**.

Neurotoxicity

A variety of toxicology studies identified DEHP-induced adverse neurological effects.

DEHP-induced neurotoxic effects were observed in some adult rodents. Dhanya *et al.* (2003) reported that neurodegenerative areas were present in the rat brain following exposure to

DEHP. Hetereogeneous changes in brain weights also occurred following long-term oral dosing with DEHP in Fischer 344 rats and $B6C3F_1$ mice (David *et al.,* 2000a, 2000b). Significant increases in relative brain weight were observed at high doses in male and female rats (LOAEL = 789.0 to 938.5 mg/kg-day; NOAEL = 146.6 to 181.7 mg/kg-day) and male mice (LOAEL = 1266.1 mg/kg-day; NOAEL = 292.2 mg/kg-day). A significant decrease in relative brain weight of male mice was also noted (LOAEL = 19.2 mg/kg-day). No significant patterns for relative brain weight were observed in female mice. Absolute brain weight was significantly decreased in $B6C3F_1$ male mice (LOAEL = 292.2 mg/kg-day; NOAEL = 98.5 mg/kg-day), and increased in female rats (LOAEL = 7.3 mg/kg-day) in a non-dose-related fashion. No significant patterns for absolute brain weights were reported for female mice and male rats. In these studies, increased weights were not correlated to histological alterations in the brain, peripheral nerves, spinal nerves, or spinal cord in rats or mice (NOAEL = 939 to 1458 mg/kg-day).

Fetal and neonatal rodent brains may be particularly sensitive to neurotoxicity mediated through DEHP. Administration of DEHP to pregnant mouse dams produced adverse neurobehavior in mice offspring (Tanaka, 2002; oral feeding; LOAEL = 60 to 473 mg/kg-day) and inhibited sexually dimorphic CNS development following *in utero* and lactational exposure (Moore *et al.,* 2001; oral gavage). Gavage dosing female Wistar dams during Gd 6 to Ld 21 also altered aromatase activity in the hypothalamic/preoptic area primarily of female offspring brains in a dose-dependent fashion (Andrade *et al.,* 2006). In this study, activity was inhibited at low doses and increased at high doses ($LOAEL_{inhibition}$ = 0.135 mg/kg-day; $NOAEL_{inhibition}$ = 0.045 mg/kg-day; $LOAEL_{increase}$ = 15 mg/kg-day; $NOAEL_{increase}$ = 5 mg/kg-day). Aromatase activity is important in the conversion of androstenedione to estrone and testosterone to estradiol. Both are important in male behavior patterning and excess activity can lead to gynecomastia (males) or precocious puberty and/or gigantomastia (females). Oral gavage dosing of Sprague-Dawley rat dams during gestation days 0 to 19 also altered the lipid metabolomic profile of fetal rat brains (LOAEL = 1500 mg/kg-day; Yan *et al.,* 2007). The total lipid concentration (i.e., free cholesterol and sphingomyelin) and fatty acid composition in fetal rat brains decreased following DEHP exposure in this study. Specifically, docosahexaenoic acid decreased significantly ($P < 0.05$) in cholesterol esters, and diacylglycerol, phosphatidylserine, lysophosphatidylcholine, and sphingomyelin while arachidonic acid decreased in cholesterol esters and lysophosphatidylcholine.

DEHP-induced neurological effects may be mediated through peroxisomal-induced alterations in the placental and fetal lipid/fatty acid supply (Xu *et al.,* 2005, 2006). In the developing fetus, polyunsaturated fatty acids are derived primarily from the dam after being transported across the placenta via carrier proteins. A rapid increase in lipid-requiring neurogenesis (and brain weight) occurs during approximately Gd 15 to birth in a fetal rat and myelination of the CNS occurs in mid-gestation.

In vitro studies also suggested that DEHP-induced intracellular ionic changes may also affect neurons. In one study, DEHP increased the intracellular levels of Ca^{2+} in rat neurohypophysial nerve terminals and rat pheochromocytoma cells (Tully *et al.,* 2000).

Intravenous exposures to DEHP were also implicated in neurological effects. IV bag perfusates (containing DEHP) inhibited human α1 rat β2 γ-aminobutyric acid type A ($GABA_A$) receptors and potentiated human α1-glycine receptors expressed on *Xaenopus laevis* oocytes (Yang *et al.,* 2007).

Neurotoxic effects have not been reported for some species following exposure to DEHP. Moser *et al.* (1995) reported the ocurrence of general clinical debilitation following a single oral gavage dose of DEHP (LOAEL = 5000 mg/kg) to Fischer 344 rats. This dose and multiple gavage doses did not result in alterations in autonomic function, sensorimotor function, neuromuscular function, and excitability and activity in a functional observational battery assessment (NOAEL = 1500 mg/kg-day). In contrast to changes in rat and mouse brain weight reported by David *et al.* (2000a, 2000b), there were no reported changes in the brain weights of rats or marmoset monkeys when acutely dosed via gavage (Rhodes *et al.*, 1986; NOAEL = 2000 mg/kg-day). A lack of neurotoxic activity may be related to the inability of DEHP to partition into neural tissue in some circumstances. General Motors (1982a,b) reported that carboxyl-^{14}C-DEHP (particle size ~ 0.4 to 0.5 μm) did not distribute to the Sprague-Dawley rat brain following acute inhalation exposures (NOAEC = 129 mg/m^3).

The lack of comprehensive neurotoxicity studies and contrary information presented for the reviewed studies supported the conclusion that **there was "limited animal evidence" for the designation of DEHP as a "neurotoxicant"**.

Respiratory toxicity

Inhalation, oral, and intravenous routes of exposure of DEHP induced adverse effects in respiratory tissue of animal models.

Acute exposure

Nose-only inhalation exposures to DEHP increased the incidence of dark red foci and patches in the lung (19/30) when compared to controls (2/10; LOAEC = 3.39 mg/L), but did not change relative lung weights in the same rats (NOAEC = 10.62 mg/L; Huls, 1981).

Pregnant female rats dosed with DEHP in their diet during their last week of pregnancy and for 2 days following birth had pups with a substantial decrease in the number of lung parenchymal airspaces, a significant increase in the airspace mean size, and an increase in the number of type II pneumocytes (Magliozzi *et al.,* 2003). Similar "alveolar simplification" (increased alveolar volume and decreased number/septation of alveoli) and increased epithelial

and mesenchymal cell proliferation was also reported in the distal lung parenchyma of rat pups treated under similar conditions (Rosicarelli and Stefanini, 2009).

Intravenous exposures to DEHP also induced respiratory pathologies. Intravenous administration of DEHP to rats resulted in edema of alveolar walls, hemorrhage, and leukocytic infiltration into lung tissue (Schulz *et al.,* 1975; Rubin and Chang, 1978).

Subchronic exposure

Inhalation exposure to an aerosol of DEHP reversibly increased lung weights, thickened alveolar septa, and induced a proliferation of foam cells in male Wistar rats (LOAEC = 1000 mg/m^3; NOAEC = 50 mg/m^3; Klimisch *et al.,* 1991). These effects were not reported for female rats at any dose in this study, or in Marmoset monkeys dosed via gavage with DEHP (Kurata *et al.,* 1998).

Chronic exposure

Dietary exposure to DEHP induced a significant dose-dependent increase in mean relative lung weight in both male mice and rats (LOAEL = 146.6 to 1266.1 mg/kg-day; NOAEL = 28.9 to 292.2 mg/kg-day; David *et al.,* 2000a, 2000b (Table A3.6; Table A3.12)). Non-significant increases in relative lung weight were also reported in female rats and mice of the high dose group. These effects were not observed, however, in Sherman rats following 52 or 104 week dietary exposures (NOAEL = 190 to 200 mg/kg-day; Carpenter *et al.,* 1953), or in Fischer 344 rats following 108 weeks of exposure via the diet (NOAEL = 2000 mg/kg-day; Rao *et al.,* 1990) to DEHP.

Data suggest that DEHP induced lung pathologies through oral, inhalation, and intravenous routes of exposure. The pathologic mechanisms for both routes are unknown and may include the alveolarization process in exposed newborn rats. In view of this, the weight of evidence from the above studies supported the conclusion that **there was "sufficient animal evidence" for the designation of DEHP as a "probable respiratory toxicant"**.

Endocrine activity

A variety of endocrine-related effects were described in animals following exposure to DEHP. In female Fischer 344 rats, estradiol metabolism and the function of estrogen receptors were altered following subchronic dietary exposure to DEHP (LOAEL = 1054 mg/kg-day; Eagon *et al.,* 1994). Chronic exposure of Fischer 344 rats to DEHP in feed also resulted in anterior pituitary hypertrophy in male rats (LOAEL = 674 mg/kg-day; NOAEL = 322 mg/kg-day; NTP, 1982; Kluwe *et al.,* 1982) and increased numbers of pituitary castration cells in rats (30/60; LOAEL = 789 mg/kg-day; NOAEL = 147 mg/kg-day; Moore, 1996). Gavage dosing pregnant Sprague-Dawley rats on Gd 14 to 18 also resulted in the significant reduction of

insulin-like peptide 3 (Insl3) mRNA levels in fetal testes and the concentration of testosterone (but not progesterone) in media incubated with exposed testes (LOAEL = 750 to 1000 mg/kg-day; Wilson *et al.*, 2004). Decreased testosterone production has also been reported in Leydig cell primary cultures following exposure to MEHP (Jones *et al.*, 1993).

DEHP-induced endocrine-related changes were not universal in all species, however. Large single doses (5000 mg/kg-day) and repeated gavage dose exposures (1500 mg/kg-day daily for 14 days) of DEHP did not induce any endocrine-related deficits in Fischer 344 rats (NOAEL = 1500 to 5000 mg/kg-day; Berman *et al.,* 1995). Similarly, subchronic duration gavage dosing of DEHP to marmoset monkeys did not result in any adverse endocrine effects (NOAEL = 2500 mg/kg-day; Kurata *et al.,* 1998). It is unknown why endocrine-related effects were not observed in these studies. Wilson *et al.* (2008) commented that the severity of changes in reproductive tissue and endocrines were dependent on the DEHP dose, rat strain, and target tissue. Differences in metabolism and receptor binding and activation have also been postulated to affect DEHP-induced reproductive outcomes.

The mechanistic pathway for DEHP-mediated endocrine related effects has been reviewed by Corton and Lapinskas (2005), Foster (2005), Latini *et al.* (2008), and Wilson *et al.* (2008). *In utero* exposure to DEHP alters Sertoli cells in the fetal testis biochemically and functionally. Alterations occur through the inhibition of FSH-stimulated proliferation and destruction of intermediate filament cellular structure. Perturbations in Sertoli cells adversely affect germ cells, resulting in increased germ cell apopotosis, abnormal gonocyte differentiation and the creation of multinucleated cells. Both Stem-cell factor (SCF) and its receptor c-kit contribute to this effect.

In utero exposure to DEHP also delays Leydig cell maturation, resulting in Leydig cell hyperplasia. Hyperplastic cells are not developmentally mature enough to produce sufficient concentrations of androgens (testosterone) for normal testicular development. Decreased testosterone contributes to "incomplete masculinization", typified by malformations of the epididymides and seminal vesicles and cryptorchidism. Decreased testosterone output also leads to reductions in dihydrotestosterone, which in-turn lead to prostate malformations, malformations of the external genitalia (hypospadias), areola and nipple retention, and decreased anogenital distance. Molecular contributors to this pathway may include the high-density lipoprotein receptor SRB-1, the steroidogenic acute regulatory protein (StAR), cyp17 [17α-hydroxylase C17,20-lyase], cyp11a, and steroidogenic factor (SF-1).

DEHP also interferes with Insl3 production. Decreased Insl3 causes agenesis of the gubernacular ligaments, resulting in testicular cryptorchidism in test animals. Polymorphic varieties of this receptor (LGR8) have been associated with human cryptorchidism.

Thyroid toxicity

Structural and functional changes in the thyroid were observed following exposure to DEHP. Reported changes were, however, dependent on the test species and the route of administration.

Acute exposure

Only one study has been reviewed that described DEHP-induced changes in thyroid organ weight. In this study, a slight non-significant decrease in the relative thyroid/parathyroid weight (and a significant decrease in clofibrate treatment organ weight) was reported following short-term gavage dosing of cynomolgous monkeys (Pugh *et al.,* 2000; Table A3.71).

Histopathologic changes in DEHP-exposed thyroids were also described. A "reactional hyperplasia" was reported for female Wistar rats administered DEHP via intraperitoneal injection once every other day for 14 days (Gayathri *et al.*, 2004 (Table A3.37).

Subchronic exposure

Longer-term (13 week) dietary treatments with DEHP reduced the thyroid follicle size and colloid density in male and female Sprague-Dawley rats (Poon *et al.,* 1997; Table A3.70). Decreased follicular size contrasted follicular cell hypertrophy reported in male rats exposed to peroxisome proliferators for 22 weeks (Miller *et al.*, 2001).

DEHP (and clofibrate or fenofibrate) -induced shrunken colloid was sometimes accompanied by calcium rich inclusions (Price *et al.,* 1988). Decreased colloid density was consistent with the "thyroid changes associated with an increased rate of thyroglobulin turnover" observed by Howarth *et al.* (2001) following dietary administration of DEHP to Wistar rats and also unpublished results from Miller *et al.* (2001) which reported diminished colloid in male rats exposed to peroxisome proliferators for 22 weeks.

Investigations of ultrastructure also revealed DEHP-induced effects in the thyroid. Studies utilizing electron microscopy reported that short-term (3 week) DEHP exposures increased the number and size of lysosomes, enlarged Golgi apparatus, and damaged the mitochondria of thyroids in rats (Hinton *et al.,* 1986). Price *et al.* (1988) reported that 12 week exposures caused similar ultrastructural alterations (increased number and size of lysosomes, hypertrophy of the Golgi apparatus) and also an increase in the dilation of the rough endoplasmic reticulum. These changes were thought to be representative of "persistent hyperactivity".

DEHP-induced structural changes were accompanied by biochemical alterations in the thyroid. Dietary administration of DEHP to male Wistar rats for 3 to 21 days resulted in a non-significant time- and dose-dependent increase in serum triiodothyronine (T_3) and a non-significant time-dependent decrease in thyroxine (T_4; Hinton *et al.,* 1986 (Table A3.54)). A decrease in serum T_4 also occurred following subcutaneous injection of DEHP into immature

intact and hypophysectomized Fischer 344 rats (Sekiguchi *et al.,* 2006; Table A3.72). Biochemical changes paralleled those reported following the administration of 0.4% clofibrate, a drug that induces hypolipidemia and peroxisome proliferation (Hinton *et al.,* 1986). Thyroid hormone changes were also similar in-part to data reported following short-term gavage dosing intact or thyroidectomized Sprague-Dawley rats with WY-14643 (WY), an experimental drug that induces hypolipidemia (Miller *et al.,* 2001 (Table A3.61)).

Reductions in T_4 have not been universally observed following exposure to DEHP. Gayathri *et al.* (2004) reported significant increases in T_3 and T_4 and a marginal decrease in TSH following intraperitoneal exposures to female Wistar rats (Table A3.37). Decrements in T_3 concentration (and T_4 to some extent) were reversed following a 7 day recovery period.

DEHP-induced alterations in thyroid structure and function have historically been termed a "persistent hyperactive response" by certain authors (Hinton *et al.,* 1986; Price *et al.,* 1988; Boas *et al.,* 2006). This term is misleading in the context of thyroid function, however, since many different thyroid functions or disease states can be termed overactive or "hyperactive". Biochemically, a "hyperactive" thyroid is typified by a persistent elevation of T_3 and T_4 hormones, such as occurs in Graves' disease (Cotran *et al.,* 1994). Clearly, hormonal results following oral exposures illustrating decreased T_4 and mildly elevated T_3 did not fit this definition, regardless of structural pathology and in fact, were more suggestive of systemic hypothyroidism. Intraperitoneal exposures to DEHP were different, however, and result in elevated T_3 and T_4 (Gayathri *et al.,* 2004). This effect occurred with a marginal decrease in TSH, suggesting that the negative control feedback loop for TH production was still functioning following intraperitoneal exposures.

Pathological and ultrastructural observations were somewhat representative of increased thyroid activity. DEHP-induced decreases in follicle size and colloid density as described in Price *et al.* (1988), Howarth *et al.* (2001), and Poon *et al.* (1997) are commonly seen in hyperactive thyroid follicles actively synthesizing or secreting thyroglobulin and thyroid hormones (Krstic, 1991). Diminished colloid has also been observed following exposure to other peroxisome proliferators such as WY (Miller *et al.,* 2001). Thyroid colloid serves as a reservoir of materials for thyroid hormone production and as a storage site for thyroid hormones. DEHP-induced ultrastructural alterations including increased the number and size of lysosomes, hypertrophy of the Golgi apparatus, increased number of microvilli, and dilation of the rough endoplasmic reticulum as described in Price *et al.* (1988) and Hinton *et al.* (1986) have also been commonly observed in active thyroids (Krstic, 1991; Krupp and Lee, 1986). The absence of increased apical vesicles (typical for hyperactive follicular cells that are synthesizing hormones; Krstic, 1991; Tsujio *et al.,* 2007) or colloid vacuoles (Nilsson, *et al.,* 1988), and the presence of all other ultrastructural and pathological changes did not enable, however, the distinction of whether biosynthetic or secretory mechanisms were primarily targeted/affected by DEHP exposures.

Additional diagnostic thyroidal effects have not been demonstrated in the above studies and cast doubt on the designation that DEHP induces hyperthyroidism. Clinical effects that are typical of hyperthyroid patients or animals (weight loss, increased food consumption, polyuria-polydipsia) were not observed or reported in the reviewed studies. Mitochondrial damage (Hinton *et al.,* 1986), calcium rich cellular inclusions (Price *et al.,* 1988), and follicular cell hypertrophy reported in male rats exposed to WY (Miller *et al.,* 2001) were also not typically observed in studies investigating hyperthyroid conditions.

Another uncertainty regarding DEHP-induced thyroidal effects is that data derived from rodent studies may not be directly translatable to humans. Rodents do not possess thyroxine-binding globulin (TBG), a binding protein with high affinity for T_4 and lower affinity for T_3 hormones. The plasma half-life of T_4 is also much shorter in rats (0.5 to 1 day) than in humans (5 to 9 days). Reproductive hormone induced modulation of the levels of TBG produced by the liver has also been reported, with estrogen increasing the hepatic production of TBG (thus increasing the total serum T_3 and T_4) and androgens decreasing the hepatic production of TBG (thus decreasing the total serum T_3 and T_4). TBG production levels probably are not a large factor when considering rat effects, since they do not possess TBG, but may contribute to thyroidal effects in exposed humans.

Alterations in thyroid function may be related to DEHP interactions with thyroid receptors. Wenzel *et al.* (2005) demonstrated *in vitro* that non-cytotoxic exposures to DEHP (100µM to 1mM) enhanced the uptake of iodide in a rat thyroid FRTL-5 cell line. This enhancement was specifically due to modulation of the sodium-iodide symporter (NIS; Wenzel *et al.,* 2005). In contrast, others have demonstrated that DEHP did not increase the expression of rat endogenous sodium/iodide symporter mRNA or increase the activity of human NIS promoter constructs (Breous *et al.,* 2005). Conflicting data prevented any conclusions based on receptor data.

The weight of evidence from the above studies supported the conclusion that **there was "sufficient animal evidence" for the designation of DEHP as a "probable thyroid toxicant"**.

Reproductive toxicity

Repeat dose administration of DEHP adversely affected test animal reproductive tissue structure and function. Both males and females of many test species were affected, with *in utero* and early post-natal reproductive system development of males being the most sensitive endpoint. Non-human primates did not respond in a reproductively adverse manner to DEHP exposure. Issues with the number of test primates, their health and growth, and untested sensitive stages of development may account for this observation.

Acute exposure

In male Wistar rats, daily gavage administration of DEHP for 7 days resulted in a 38% decrease in testes weight, shrunken seminiferous tubules with necrotic debris, and aspermatogenesis (LOAEL = 2000 mg/kg-day; Oishi, 1994). Decreased testes weight and increased atrophy was also reported in Alderley Park rats at the same dose (ICI, 1982b; Rhodes *et al.,* 1986). Short-duration repeat gavage dosing to female Sprague-Dawley rats suppressed ovulation, decreased preovulatory follicle granulosa cells 25%, and decreased serum estradiol (LOAEL = 2000 mg/kg-day; Davis *et al.,* 1994a). Dietary administration of DEHP for 10 days also resulted in a 20 to 25% change in the xenobiotic enzyme activity in the testes of male Sprague-Dawley rats (LOAEL = 1740 mg/kg-day; Mehrotra *et al.,* 1997). Testicular atrophy was also reported in Fischer 344 rats dosed with DEHP via the diet for 14 days (LOAEL = 1250 mg/kg-day; NOAEL = 630 mg/kg-day; NTP, 1982).

DEHP-induced adverse effects in the reproductive system were not observed in all species or at all dose levels. Daily gavage administration of DEHP for 4 to 14 days did not induce any significant reproductive deficits in Sprague-Dawley rats, marmoset or cynomolgous monkeys (NOAEL = 500 to 2000 mg/kg-day; Rhodes *et al.,* 1986; Zacharewski *et al.,* 1998; Pugh *et al.,* 2000; Sjoberg *et al.*, 1986a). Similarly, daily gavage administration to Sprague-Dawley rats during PPd 6 to 10, 14 to 18, 21 to 25, 42 to 46, and 86 to 90 did not result in toxicologically significant changes in fertility (NOAEL = 1000 mg/kg; Dostal *et al.*, 1988). Dosing with DEHP via the feed also had no histopathologic effects on the testes of Fischer 344 rats (NOAEL =1600 mg/kg-day; Exxon, 1982a, 1982b). Detailed information on these studies were not available in the reviews used in this hazard assessment. Dosing levels were sufficient, however, to induce changes in reproductive organs in other studies.

Subchronic exposure

Gavage administration of DEHP daily for 15 days to male Wistar rats decreased testicular weight (33%; LOAEL = 50 mg/kg-day; Parmar *et al.,* 1987 (Table A3.64)), increased testicular germ cell damage (57%; LOAEL = 250 mg/kg-day; Parmar *et al.,* 1987 (Table A3.65)), and decreased testicular weight, changed tubule morphology, damaged spermatogenic cells, and reduced sperm counts (LOAEL = 2000 mg/kg-day; Parmar *et al.,* 1987 (Table A3.66)). Dose-dependent increases in testicular lactate dehydrogenase, gamma glutamyl transpeptidase, and glucuronidase activity, and decreased acid phosphatase and SDH were reported for male 25-day old Wistar rats following exposure to DEHP for 30 days (Parmar *et al.,* 1995 (Table A3.68); ECB, 2008). Dietary administration of DEHP to Sprague-Dawley rats decreased absolute and relative testes weights, induced testicular atrophy, and increased seminiferous tubule atrophy (LOAEL = 375.2 mg/kg-day; NOAEL = 37.6 mg/kg-day; Poon *et al.,* 1997 (Table A3.70)), and also increased the amount of mild to moderate vacoulation in Sertoli cells (LOAEL = 37.6 mg/kg-day; NOAEL = 3.7 mg/kg-day; Poon *et al.,* 1997 (Table A3.70)).

Decreased absolute or relative testis weight and testicular atrophy also occurred in other studies in which Fischer 344 rats were dosed with DEHP in the feed for 1 to 13 weeks (LOAEL = 630 to 2496 mg/kg-day; NOAEL = 261 to 1224 mg/kg-day; CMA, 1984b; Barber *et al.,* 1987; BIBRA, 1990; Eastman Kodak, 1992a; NTP, 1982). Dosing female Fischer 344 rats with similar levels of DEHP did not result in the development of reproductive pathologies (NOAEL = 1250 mg/kg-day; NTP, 1982). In Wistar rats, adverse effects to male reproductive organs included reduced seminal vesicle and ventral prostate weight, tubule atrophy and degeneration, and testicular lesions (LOAEL = 900 to 1200 mg/kg-day; NOAEL = 339 to 400 mg/kg-day; Shaffer *et al.,* 1945; Gray and Butterworth, 1980 (Table A3.38); Schilling *et al.,* 1999). Decreased absolute testis weight, testis atrophy, and ***increased*** relative testis weight were reported in earlier studies using Sprague-Dawley rats (LOAEL = 737 mg/kg-day; NOAEL = 143 mg/kg-day; Gray *et al.,* 1977).

In some studies, intermediate-term repeat dosing of $B6C3F_1$ mice with DEHP had similar effects to those seen in rats. Decreased absolute and relative testis weight and testicular atrophy were observed following dosing in one study (LOAEL = 2580 to 6990 mg/kg-day; NOAEL = 1210 to 2580 mg/kg-day; Eastman Kodak, 1992b). An absence of corpora lutea was also noted in female mice at similar doses (LOAEL = 7900 mg/kg-day; NOAEL = 2890 mg/kg-day; Eastman Kodak, 1992b). In other mouse strains, decreased male fertility (Cr1:CD-1; LOAEL = 140 mg/kg-day; NOAEL = 14 mg/kg-day; Lamb *et al.,*1987 (Table A3.56)), and degenerative testicular lesions (Sv/129; LOAEL = 2400 mg/kg-day; Ward *et al.,*1998) were induced by dosing with DEHP. The need for relatively higher doses to elicit reproductive pathologies (when compared to rats) may explain incidences in which no effects on reproduction were reported in male or female mice (NTP, 1982).

Chronic exposure

Dietary administration of DEHP to male Sprague-Dawley rats for 102 weeks increased the incidence of testicular atrophy and inhibited spermatogenesis (LOAEL = 7 mg/kg-day; Ganning *et al.,* 1987, 1991). Testicular atrophy following 78 weeks exposure to DEHP in feed has also been reported for Wistar rats, but at a much higher dose (LOAEL = 2000 mg/kg-day; Price *et al.,* 1987). In Fischer 344 rats, testicular atrophy and severe seminiferous tubule degeneration were also observed following DEHP exposures (LOAEL = 322 to 674 mg/kg-day; NOAEL = 322 mg/kg-day; Kluwe *et al.,* 1982; NTP, 1982). At similar doses (LOAEL = 789 mg/kg-day; NOAEL = 147 mg/kg-day), DEHP also reduced testes weights, increased the incidence and severity of bilateral aspermatogenesis, increased the immature or abnormal forms of sperm in the epididymis, induced hypospermia in the epididymis, and decreased the incidence of testicular interstitial cell neoplasms (Moore, 1996). Bilateral testicular aspermatogenesis was also induced by lower concentrations of DEHP in the feed (LOAEL = 5.8 mg/kg-day; David *et al.,* 2000a (Table A3.7)). In male rats, removal of DEHP from the feed resulted in partial recovery of absolute and relative testes weights. The incidence of aspermatogenesis, interstitial

cell tumors of the testes, and pituitary castration cells did not change substantially, however, following a 26 week recovery period (David *et al.,* 2001 (Table A3.18)).

Exposure to DEHP in the feed for 104 weeks did not induce reproductive effects in all species. In Sherman rats, reproductive effects were not reported following long-term dosing (NOAEL = 190 to 328 mg/kg-day; Carpenter *et al.,* 1953). DEHP administered to Wistar rats via inhalation daily for 6 hours, 5 days a week, for 4 weeks also had no effect on any reproductive parameters (NOAEC = 1000 mg/m^3; Klimisch *et al.,* 1991).

Dietary administration of DEHP to $B6C3F_1$ mice for 104 weeks resulted in testicular atrophy and seminiferous tubule degeneration (LOAEL = 1325 mg/kg-day; NOAEL = 672 mg/kg-day; NTP, 1982; Kluwe *et al.,* 1982) and reduced testes weight and increased the incidence and severity of bilateral hypospermia and immature/abnormal epididymal sperm (LOAEL = 98.5 to 1266 mg/kg-day; NOAEL = 19.2 to 292.2 mg/kg-day; Moore, 1997; David *et al.,* 2000b (Table A3.13)). Reductions in female uterus weights were also seen following long-term DEHP dosing (LOAEL = 1458.2 mg/kg-day; NOAEL = 354.2 mg/kg-day; David *et al.,* 2000b (Table A3.13)). In mice, removal of DEHP from the feed resulted in partial recovery of absolute and relative testes and uterine weights, bilateral hypospermia, and hypospermia of the epididymis. The incidence of immature/abnormal epididymal sperm did not change following a 26 week recovery period (David *et al.,* 2001; Table A3.19).

Daily administration of DEHP via gavage to marmoset monkeys for 65 weeks resulted in an increase in relative and absolute ovary and uterine weight and elevations in serum 17β-estradiol (LOAEL = 500 mg/kg-day; NOAEL = 100 mg/kg-day; Mitsubishi, 2003; CERHR, 2006 (Table A3.3)). These dose-related increases in ovary and uterine weights were used in a subsequent reanalysis to create Benchmark Dose levels (CERHR, 2006; Table A3.3).

Studies assessing DEHP-induced reproductive effects in marmosets were confounded by numerous factors (CERHR, 2006). Confounders included the assessment of a life stage (90 to 115 days old) that was not the most sensitive in other species, uncertain decision criteria for excluding certain monkeys, health-related issues which culminated in the replacement of 1 to 3 animals per group, failure to collect testicular weights in some animals, a marmoset-specific short transit time for DEHP in the gut and diarrhea (both of which would limit intestinal absorption), the necessity for high vitamin C concentrations in the diet (which has protective effects against DEHP-induced testicular effects in rats and mice), a lack of lutenizing hormone (LH), and high circulating levels of free steroids (which would impair the ability to distinguish reduced steroid levels and resultant effects of reduced steroid levels). All of these factors may have contributed to the reduced response to DEHP observed in marmoset monkeys.

Multigeneration exposure

In a three-generation Wistar rat reproduction study (Schilling, 2001; CERHR, 2006), dietary administration of DEHP at decreased the number of F_0 and F_1 males with confirmed

fertility (12% and 24%, respectively), increased the number of F_0 females with stillborn pups (4-fold), decreased the F_1 male and female absolute brain, kidney, testis, ovary, and uterus weights on postnatal day (PNd) 21 (7, 33, 37, 32, 22%, respectively), increased in the F_1 male and female relative brain weights on PNd 21 (38%), decreased the F_1 male and female relative thymus weights on PNd 21 (12%), decreased the growth of follicles and corpora lutea, decreased grip strength and increased hind limb splay (M&F), increased the percent of abnormal sperm in F_1 males (27%), decreased the F_1 litter size (F_2 pups per litter; 19%), decreased the F_2 male and female absolute brain, thymus, spleen, kidney, testis, and ovary weights on PNd 21 (7, 39, 53, 30, 38, 26%, respectively), increased the F_2 male and female relative brain and uterus weights on PND 21 (39% and 28%, respectively), and decreased the F_2 male and female relative thymus, spleen, and testis weights (12, 32, 10%, respectively; LOAEL = 1088 mg/kg-day; NOAEL = 340 mg/kg-day; BMD_{10} = 2467 to 11,077; $BMDL_{10}$ = 250 to 9201; $BMD_{1\ SD}$ = 4185 to 25, 588; $BMDL_{1\ SD}$ = 2963 to 9379 mg/kg). Administration of DEHP also decreased the number of F_1 pups surviving during PNd 0 to 4 (4%), increased the number of F_1 females with stillborn pups (3-fold), decreased the F_1 male and female absolute thymus weights on PNd 21 (12%), increased the F_1 male and female absolute liver weights on PNd 21 (17%), and decreased the number of F_2 pups surviving (15%; LOAEL = 340 mg/kg-day; NOAEL = 113 mg/kg-day; BMD_{10} = 1271 to 11,399; $BMDL_{10}$ = 805 to 8541; $BMD_{1\ SD}$ = 2713 to 3443; $BMDL_{1\ SD}$ = 1817 to 2728 mg/kg). Exposure to DEHP also decreased the F_1 male and female absolute spleen weights on PNd 21 (15%), increased the F_1 male and female relative liver weights on PNd 21 (8%), decreased the F_1 male and female relative spleen weights on PNd 21 (11%), increased the F_2 male and female absolute liver weights on PNd 21 (14%), increased the F_2 male and female relative liver and kidney weights on PNd 21 (12% and 6%, respectively), and increased the incidence of focal tubular atrophy in the F_0 and F_1 generations (0, 1, 3, 6 males and 2, 7, 4, 13 males, respectively; LOAEL = 113 mg/kg-day; BMD_{10} = 939 to 3450; $BMDL_{10}$ = 636 to 2224; $BMD_{1\ SD}$ = 1184 to 4300; $BMDL_{1\ SD}$ = 956 to 2801 mg/kg). In this study, adverse effects were not noted in the following parameters; the number of F_0 and F_1 males with confirmed mating, F_0 sperm parameters, F_0 litter size (F_1 pups per litter), F_1 and F_2 pups surviving during PNd 4 to 21 (on a per pup basis), live F_1 and F_2 pups per litter on PNd 4, 7, 14, and 21, F_1 and F_2 pup sex ratio, F_1 and F_2 female anogenital distance, F_1 relative testis, ovary and uterus weights on PNd 21, F_1 sperm count (testis, epidiymis), F_1 percent motile sperm, F_2 postmiplantation loss per F_1 female, F_2 absolute uterus weight on PNd 21, and relative ovary weight in F_2 females on PNd 21 (NOAEL = 1088 mg/kg-day).

The CERHR (2006) felt that the lowest effective dose for reproduction from this study was 113 mg/kg-day. For F_2 pup survival, the lowest reproductive BMD_{10} was 2325 mg/kg (263 mg/kg-day) and the lowest reproductive $BMDL_{10}$ was 2045 mg/kg (231 mg/kg-day).

In a pilot reproduction study conducted by the NTP (2004), dietary exposure of Sprague-Dawley rats to DEHP from 7 days premating through PNd 21 decreased vaginal, uterine, and cervical weights in PNd 21 pups (LOAEL = 321.4 mg/kg-day). The ratio of anogenital distance

to pup weight was also increased in female pups (LOAEL = 644.0 mg/kg-day; NOAEL = 321.4 mg/kg-day).

In a continuous breeding study conducted by the NTP (2004), DEHP exposures increased the cumulative days to deliver in F_0 females (litter 1), decreased the number of spermatids per testis and sperm veolocity in F_0 males (31% and 11%, respectively), decreased the absolute cauda epididymis, epididymis, and testis weights in F_0 males (19, 16, and 23%, respectively), increased the number of F_0 males with a small right testis (2/10; atrophy of seminiferous tubules with a loss of germ cells), decreased the proportion of liveborn pups in F_0 matings (4%), inhibited the production of any litters with mated F_1 males and females, increased estrous cycle effects (0.4 days) and relative uterine and ovarian weight in F_1 females (36% and 35%, respectively), decreased the absolute seminal vesicle and dorsolateral prostate weight in F_1 breeder males (29% and 29%, respectively), decreased the relative epididymis and cauda epididymis weights in F_1 non-breeder males (42% and 33.8%, respectively), increased the number of F_1 non-breeder males with small epididymides (21/21) and cauda epididymides (21/21), and decreased the epididymal sperm density in F_1 non-breeder males (99.6%; LOAEL = 543 to 775 mg/kg-day; NOAEL = 392 to 592 mg/kg-day; BMD_{10} = 6389; $BMDL_{10}$ = 2819; $BMD_{1\ SD}$ = 6181; $BMDL_{1\ SD}$ = 2310 mg/kg).

Multigenerational administration of DEHP also decreased the live pups per litter (F_{1a}) and live males per litter in F_0 matings (F_{1a}; 18% and 20%, respectively), decreased the pregnancy index for the first two F_1 male and female litters (71% and 59%), decreased the absolute cauda epididymis, epididymis, testis, and ventral prostate weights in F_1 breeder males (37, 35, 51, 28%, respectively), increased the number of F_1 breeder males with small testes and epididymides (8/10 and 2/10, respectively) and seminiferous tubule atrophy and sperm release failure, decreased the absolute epididymis, cauda epididymis, testis, and relative testis weights in F_1 non-breeder males (20, 20, 34, 28%, respectively), increased the number of F_1 non-breeder males with small testes (9/30), decreased the number of spermatids per testis and sperm per cauda in F_1 non-breeder males (69 and 61%, respectively), decreased the male pup and combined survival for PNd 1 to 21 in F_1 matings (20% and 19%, respectively), decreased the pregnancy index for the first two F_2 male and female litters (53% and 47%), decreased the number of litters per pair of F_2 males and females, decreased the absolute epididymis, seminal vesicle, testis, cauda epididymis, and relative testes weights in F_2 breeder males (36, 24, 60, 63, 53%, respectively), increased the number of F_2 breeder males with small testes (8/10), epididymides (8/10), and cauda epididymides (8/10), decreased the absolute epididymis, testis, cauda epididymis, and relative testis, and cauda epididymis weights in F_2 non-breeder males (27, 49, 32, 40, 20%, respectively), increased the number of F_2 non-breeder males with small testes, epididymides, and cauda epididymides (11/20, 7/20, and 6/21, respectively), decreased the F_2 male non-breeder number of spermatids per testis, sperm per cauda, epididymal sperm density, and percent motile sperm (74, 72, 64, and 25%, respectively), decreased the F_3 male absolute dorsolateral prostate, testis and relative testis and epididymis weight (41, 45, 48, 35%, respectively), and decreased the number of spermatids per testis, sperm per cauda, and epididymal sperm density in F_3 males (79, 95,

94%, respectively; LOAEL = 392 to 592 mg/kg-day; NOAEL = 46 to 77 mg/kg-day; BMD_{10} = 787 to 7293; $BMDL_{10}$ = 728 to 6408; $BMD_{1\ SD}$ = 554 [9 to 15 mg/kg-day] to 7802; $BMDL_{1\ SD}$ = 412 to 3504 mg/kg;).

Crossover breeding studies clarified reproductive effects (lack of litters in the F_1 543 to 775 mg/kg-day treatment group and the non-significant decrease in F_3 pup body weights in the 392 to 592 mg/kg-day treatment group) in the continuous breeding study. Results from the crossover study revealed that the pregnancy, fertility, and mating indices were wholly impaired in the male 543 to775 mg/kg-day group (0 fertile, 0 mated, 0 pregnant). Matings of F_1 female rats treated with the same dose and control males resulted in decreased live male pup weight and male pup anogenital distance (13.6% and 16.9%, respectively) and increased female anogenital distance/bw (17.6%). As with the F_1 group, matings with F_2 females dosed with 392 to 592 mg/kg-day and control males resulted in decreased adjusted live male pup weights (8.2 to 12.3%) and male pup anogenital distances (11.5%). Matings with a group of F_2 males treated with 392 to 592 mg/kg-day and control females resulted in decreased fertility and pregnancy indices, implantation sites (54.5%), and live female pups per litter (31%).

From these results, the CERHR concluded that the lowest BMD_{10} (787 mg/kg) was based on a 95% decrease in sperm of the cauda of F_3 males. This equated to 36 to 61 mg/kg-day. The lowest $BMD_{1\ SD}$ (554 mg/kg) was based on a 25% decrease in motile sperm in the F_2 non-breeder males. This equated to 9 to 15 mg/kg-day. The CERHR panel also noted that increases in Sertoli cell vacoulation observed in the orginal CERHR evaluation of DEHP were not observed in this study. Sertoli cell vacoulation was the primary endpoint LOAEL in the previous evaluation.

The CERHR panel also stated that 300 and 1000 mg/kg (14 to 23 and 46 to 77 mg/kg-day) were part of the DEHP dose-response for testicular abnormalities, resulting in a NOAEL of 100 mg/kg (3 to 5 mg/kg-day).

The NTP study was one of only two studies "that provide a comprehensive assessment of phthalate syndrome in a large enough number of male offspring to detect adverse reproductive effects at low dose levels" (Gray *et al.,* 2009). Increases in the incidence of small testes, epididymides, seminal vesicles, ventral prostate, and cauda epididymides from F_1 non-breeder males and increases in the incidence of small testes, epididymides, and cauda epididymides from F_2 non-breeder males were not, however, demonstrated in the 300 and 1000 mg/kg dose groups, nor in higher doses (except the incidence of small testes in the F_1 male treatment group). The actual incidence of adverse effects in these groups (F_1 = 2 to 7%; F_2 = 4 to 5%) was also lower than that typically considered to be biologically relevant (10%). Both observations increase the uncertainty associated with CERHR endpoint conclusions and suggest that alternative endpoints should also be considered for risk assessment purposes.

Overall, animal studies have demonstrated that DEHP induces male reproductive deficits in many species including rats and mice. Adverse reproductive effects such as morphological changes in Sertoli cells (single exposure LOAEL = 2800 mg/kg-day), testicular atrophy (short-

term exposure LOAEL = 1250 mg/kg-day), vacoulation of Sertoli cells (intermediate-duration exposure LOAEL = 37.6 mg/kg-day), testicular atrophy and inhibition of spermatogenesis (long-term exposure LOAEL = 5.8 to 7.0 mg/kg-day), and changes in sperm parameters, decreases in testis, seminal vesicle, dorsolateral prostate, and epididymides weight, and tubular atrophy (multigenerational exposure LOAEL = 113 to 1088 mg/kg-day) illustrated DEHP's potential to induce adverse effects in a variety of male animal reproductive organs.

Female reproductive deficits have also been observed in animal models following DEHP exposures. Adverse effects such as suppressed ovulation, decreased preovulatory follicle granulose cells, and decreased serum estradiol (short-term exposure in rats; LOAEL = 2000 mg/kg-day), an absence of corpora lutea (intermediate duration exposure LOAEL = 7900 mg/kg-day; mice), increased ovary and uterine weights and serum estradiol (long-term exposure LOAEL = 500 mg/kg-day; marmosets), and decreased ovary, vaginal, cervical, and uterine weights, follicle growth, and the number of corpora lutea, and increased ano-genital distance (F_1 or F_2 rat pups; (multigenerational exposure LOAEL = 321.4 to 1088 mg/kg-day) have been reported.

DEHP-induced reproductive effects are less well described in humans than in animal models. Studies associating DEHP exposure to human male fertility have received the most attention. In an epidemiology study, Rozati *et al.* (2002) concluded that a significant inverse correlation existed with mean seminal plasma phthalate concentration and normal sperm morphology ($P < 0.001$). In the same study, a positive correlation was observed between mean phthalate concentration and percent acid-denaturable sperm chromatin ($P < 0.001$). Denaturation is typically enhanced in sperm nuclei that have abnormal chromatin structure (Ernepreiss *et al.,* 2001). Sperm DNA damage has also been associated with urinary MEHP concentration (after adjusting for the oxidative metabolites MEHHP and MEHP (Hauser *et al.*, 2007) and a slight increase in odds-ratios was reported for MEHP and sperm motility (OR = 1.4; CI = 0.7-2.9, adjusted for age, abstinence, and smoking; Duty *et al.*, 2003).

Alterations in human sperm parameters may have been caused by decreased testosterone. Duty *et al.* (2005) reported a statistically significant negative Spearman's correlation coefficient (-0.17) for MEHP concentration and serum testosterone ($P \leq 0.05$). An MEHP-induced decrease in testosterone was consistent with other DEHP-induced decrements in testosterone observed in rodent toxicology studies. Changes in normal sperm morphology, nuclear chromatin, and DNA damage also suggested that effects may also be observed in fertility. Rozati *et al.* (2002) demonstrated that a significant inverse correlation was observed between mean seminal plasma phthalate concentration and infertility in 21 men (infertile group – 2.03 ± 0.214 µg/mL; fertile group – 0.06 ± 0.02 µg/mL; $P < 0.05$).

Human studies are not uniformly positive when relating DEHP exposures to reproductive deficiencies. Rozati *et al.* (2002) concluded that there were no associations between total phthalates and seminal phthalate concentration and ejaculate volume, sperm concentration,

progressive motility, sperm vitality or osmoregulation, or sperm nuclear chromatin decondensation. Studies have also provided evidence that there is no significant correlation between MEHP urine concentration and semen parameters such as sperm concentration, percent motility, and morphology (Duty *et al.,* 2003) or sperm DNA damage (Duty *et al.,* 2003). A lack of correlation was also reported for MEHP urine concentration and serum sex hormone-binding globulin, inhibin B, FSH, and LH (Duty *et al.,* 2005). This finding was verified in part by Jönsson *et al.* (2005), who concluded that urinary MEHP concentration was not correlated with seminal plasma neutral α-glucosidase, zinc, prostate-specific antigen and fructose, serum FSH, LH, sex hormone-binding globulin, testosterone, inhibin, or 17β estradiol, or seminal sperm concentration, motility, and chromatin structure. In addition, Cobellis *et al.* (2003) reported that endometriosis was not significantly correlated to DEHP or MEHP concentrations in the plasma or peritoneal cavity and Modigh *et al.* (2002) reported no association between DEHP exposure and a prolongation in time-to-pregnancy.

In general, human studies assessing potential DEHP-induced reproductive effects were limited by small samples sizes, confounders (such as BMI, age, fish consumption, low response rates), and sampling methodologies (including limiting analysis to MEHP, but not other DEHP metabolites). Overall, however, human studies have weakly correlated changes in a variety of sperm parameters (morphology, chromatin structure, and mobility) to DEHP or MEHP exposures.

Peroxisome proliferation

Peroxisome proliferation and the PPARs expression have also been implicated in DEHP-induced adverse effects noted in the male and female reproductive tracts. The specific contribution that peroxisome proliferation has to reproductive or development toxicity is still, however, uncertain.

PPAR mRNA expression has been described in both rat and human reproductive cells. Human PPARα mRNA expression has been found in Leydig cells, spermatocytes, and the whole testis, but not Sertoli cells (Schultz *et al.*, 1999; Elbrecht *et al.*, 1996; cited in Corton and Lapinskas, 2005). Elbrecht *et al.* (1996) also revealed that testis expressed mRNA for human PPARβ (cited in Corton and Lapinskas, 2005). Conflicting data has been reported for the expression of human PPARγ mRNA (Elbrecht *et al.*, 1996; Hase *et al.*, 2002; cited in Corton and Lapinskas, 2005). Positive mRNA expression data was reported in rats for PPARα and PPARβ (Leydig cells, Sertoli cells, whole testis; Gazouli *et al.*, 2002; Braissant *et al.*, 1996; Schultz *et al.*, 1999; Xing *et al.*, 1995; cited in Corton and Lapinskas, 2005). Equivocal or negative data was reported for rat PPAR mRNA expression in spermatocytes and PPARγ mRNA expression in Leydig and Sertoli cells. In seminiferous tubules of early postnatal rats, PPARα expression was high on PNd 1, declined until PNd 30, and then increased at PDd 60.

PPARα, β, and γ have been discovered in the ovaries and gestational tissues of rodents. Expression of PPARs is dependent on the stage reproductive cycle. PPARγ is predominately

expressed in granulose cells and preovulatory follicles, with expression declining following the LH surge. It is also expressed to a lesser extent in granulosa-thecal cells and in the corpus luteum. Expression levels increase in these tissues following ovulation, but decrease following regression of the corpus luteum. PPARγ is also expressed in the uterus and blastocyst. The distribution of expression of PPARs suggests that PPARγ is involved in follicular development, ovulation, corpus luteum progression, and placental maturation, PPARα is involved in sperm fertility, and PPARβ is involved in embryo implantation (Latini *et al.*, 2008)

Even though PPARs are expressed in testicular tissue, they might not be necessary for fertility and testicular development. Mice that were PPARα-null (Lee *et al.*, 1995; cited in Corton and Lapinskas, 2005) and PPARβ-null (Peters *et al.*, 2000; cited in Corton and Lapinskas, 2005) were viable and fertile. Testicular effects may also have been mediated in part through a PPARα independent mechanism, since PPARα-null mice have been reported to have less frequent and severe testicular lesions following exposure to DEHP (Ward *et al.*, 1998). In other cases, PPARβ-null animals had impaired fertility and PPARγ-null mutations were lethal in the embryonic stage.

The expression of PPAR genes is not constant, and cells in different stages exhibit different expression levels. In seminiferous tubules, PPARα was expressed most strongly during stages II to VI in the spermatocyte differentiation cycle and stages XIII to I in Sertoli cell nuclei (Schultz *et al.*, 1999; cited in Corton and Lapinskas, 2005). This expression could be modulated by FSH during all stages, suggesting that FSH partly controlled the expression of PPARα.

The weight of evidence from the above studies supported the conclusion that **there was "limited human evidence and sufficient animal evidence" for the designation of DEHP as a "probable reproductive toxicant"**.

Pre- and Post-natal toxicity

DEHP exposure during the gestational period of animals resulted in significant adverse effects to fetuses of the exposed dams.

Single large exposures of DEHP (4882 mg/kg-day) during gestation day (Gd) 12 slightly increased the dead, resorbed, and malformed fetuses in Wistar rats (Ritter *et al.,* 1987). Lower dose exposures during Gd 3 also increased the number of abnormal gonocytes and reduced Sertoli cell proliferation (LOAEL = 100 mg/kg-day; NOAEL = 20 mg/kg-day; Li *et al.,* 2000).

Multiple-dose administration of DEHP via gavage to Wistar rat dams during Gd 6 to 15 increased fetal death and increased the incidence of external, soft tissue, and skeletal malformations (LOAEL = 1000 mg/kg-day; NOAEL = 200 mg/kg-day; Hellwig *et al.,* 1997 (Table A3.48; Table A3.49). Gavage doses to Sprague-Dawley rats during Gd 14 to 21 and

postpartum days 1 to 3 also significantly delayed male reproductive system maturation, increased testicular degeneration, reduced the weight of sex organs in adult males, decreased male fetal testosterone synthesis during male sexual differentiation, and altered sexual differentiation (LOAEL = 750 mg/kg-day; Gray *et al.,* 1999, 2000; Parks *et al.,* 2000).

Administration of DEHP to male Sprague-Dawley rats during Gd 3 to 21 and PPd 1 to 21 decreased testes and anterior prostate weights and altered sexual differentiation in offspring (LOAEL = 375 mg/kg-day; Moore *et al.,* 2001). In Wistar rats, administration during preGd 90 to Gd 1 decreased fetus weight (10%) and decreased placenta weight (8%; LOAEL = 1700 mg/kg-day; NOAEL = 340 mg/kg-day; Nikonorow *et al.,* 1973). Dosing of Fischer 344 rats during Gd 0 to 20 increased prenatal and perinatal mortalities (LOAEL = 313 mg/kg-day; NOAEL = 164 mg/kg-day; Price *et al.,* 1986), increased fetal resorptions (LOAEL = 1055 mg/kg-day; Tyl *et al.,* 1988), and decreased fetal body weights (LOAEL = 666 mg/kg-day; NOAEL = 357 mg/kg-day; Tyl *et al.,* 1988).

DEHP-induced developmental changes in rats were not universal. Gavage administration of DEHP to Sprague-Dawley rats during PPd 40 to 53, 60 to 73 (and feed), and 105 to 114 did not result in any reproductive or developmental alterations (NOAEL = 1000 to 2800 mg/kg-day; Sjoberg *et al.,* 1986a).

Exposure to DEHP also induced developmental alterations in mice. Multiple exposures via gavage administration to C57BL/6NxS, Sic-ICR, and ddY-Sic mice during Gd 6 to 10 decreased fetal viability, increased resorptions and external malformations (LOAEL = 1000 mg/kg-day; NOAEL = 250 mg/kg-day; Peters *et al.,* 1997; Shiota and Mima, 1985) and increased fetal lethality (11.2% at a NOAEL = 50 mg/kg-day; 60% at a LOAEL = 1000 mg/kg-day; Yagi *et al.,* 1980; Tomita *et al.,* 1982a). Dosing during Gd 0 to 17 or Gd 1 to 18 increased the number of external, visceral, and skeletal abnormalities (CD-1; LOAEL = 91 mg/kg-day; NOAEL = 44 mg/kg-day; Tyl *et al.,* 1988), increased prenatal and perinatal mortality (CD-1; LOAEL = 95 mg/kg-day; NOAEL = 48 mg/kg-day; Price *et al.,*1988c) and increased the percent resorptions and dead fetuses (ICR; LOAEL = 170 mg/kg-day; NOAEL = 83 mg/kg-day; Shiota *et al.,* 1980).

Administration of DEHP to lactating dams also induced adverse effects in pups. Multiple gavage doses of DEHP changed the milk composition in Sprague-Dawley rats at high doses (2000 mg/kg-day) when administered during lactation days (Ld) 15 to 17 (Dostal *et al.,* 1987b (Table A3.26)). Dosing dams during Ld days 2 to 6, 6 to 10, and 14 to 18 also decreased Sprague-Dawley pup body weight (26, 20, and 14%, respectively; LOAEL = 2000 mg/kg-day; Dostal *et al.,* 1987b (Table A3.23)). Oral gavage doses during PPd 1 to 21 also increased the peroxisome proliferation in Fischer 344 rat pup liver and kidneys (LOAEL = 1000 mg/kg-day; Stefanini *et al.,* 1995). Absolute and relative testes weight and the number of Sertoli cells were also decreased in Sprague-Dawley rats following the administraton of DEHP on PPd 6 to 10 (LOAEL = 500 to 1000 mg/kg-day; NOAEL = 100 to 200 mg/kg-day; Dostal *et al.,* 1988 (Table

A3.32)). Spermatid maturation was also delayed at 4 weeks after dosing (LOAEL = 200 mg/kg-day; NOAEL = 100 mg/kg-day (Table A3.31)).

Gavage administration of DEHP to Sprague-Dawley rats during PPd 25 to 38, 28 to 37, and 70 to 79 resulted in testicular damage, decreased testes weight and increased testicular atrophy, and testicular damage and decreased seminal prostate weight, respectively (LOAEL = 1000 to 2800 mg/kg-day; Gray and Butterworth, 1980; Sjoberg *et al.,* 1986a). Similar effects were reported in male Wistar rats. Administration during PPd 28 to 37, 30 to 39, and 70 to 79 resulted in a decrease in relative testis weight (33%), a reduction in seminal vesicle and ventral prostate weight, a loss of germinal cells (Gray and Butterworth, 1980), aspermatogenesis with a reduction in testes, seminal vesicle, and ventral prostate weights, decreased testes zinc (Oishi, 1990), and reduced seminal vesicle and ventral prostate weights and tubule damage (Gray and Butterworth, 1980), respectively (LOAEL = 2000 to 2800 mg/kg-day). Gavage administration during PPd 86 to 90 also resulted in a loss of spermatids and spermatocytes and decreased testicular zinc concentration (LOAEL = 1000 mg/kg-day; NOAEL = 100 mg/kg-day; Dostal *et al.,* 1988 (Table A3.30)).

Administration of DEHP in the feed to Sprague-Dawley rats during PPd 25 to 38 decreased testicular weight (21% at 1000 and 79% at 1700 mg/kg-day), increased tubule damage, and increased severe testes damage. Dietary exposure to DEHP during PPd 40 to 53 decreased testes weight (43%) and increased seminiferous tubule damage (LOAEL = 1700 mg/kg-day; NOAEL = 1000 mg/kg-day; Sjoberg *et al.,* 1986a). Gavage dosing of Fischer 344 rats with DEHP decreased pup body weights (24%; LOAEL = 500 mg/kg-day; Cimini *et al.,* 1994).

In a two generation Fischer 344 rat reproduction study (Schilling, 2001; CERHR, 2006), administration of DEHP in the feed resulted in developmental effects including increased postimplantation loss per F_0 female (2.1-fold), decreased F_1 male anogenital distance (14%), increased number of F_1 males with nipples/areola per litter (38-fold), increased F_1 days to vaginal opening (12%), and increased F_1 days to preputial separation (19%; LOAEL = 1088 mg/kg-day; NOAEL = 340 mg/kg-day; BMD_{10} = 5780 to 7921; $BMDL_{10}$ = 813 to 6534; $BMD_{1\ SD}$ = 3986 to 9070; $BMDL_{1\ SD}$ = 2592 to 7659 mg/kg). Decreased male F_2 anogenital distance on PND 1 (9%) and an increased number of F_2 males with nipples/areolas per litter (45-fold) were also reported (LOAEL = 340 mg/kg-day; NOAEL = 113 mg/kg-day; BMD_{10} = 1610 to 8810; $BMDL_{10}$ = 6204; $BMD_{1\ SD}$ = 6597; $BMDL_{1\ SD}$ = 3981 mg/kg).

Similar effects were reported in a Sprague-Dawley rat continuous breeding study performed by NTP (2004; CERHR, 2006). Multigenerational exposure to DEHP in feed increased the female F_1 pup anogenital distance relative to body weight (10 to 17%), increased the age of vaginal opening in F_1 females (8 days), increased the age of preputial separation in F_1 males and females (11 days), and increased the age of testes descent in F_1 males (6 days; LOAEL = 543 to 775 mg/kg-day; NOAEL = 392-592 mg/kg-day). Exposure to lower concentrations of

DEHP also decreased the male F_{1a} and F_{1b} pups' anogenital distance (7% and 8%, respectively), increased the age of vaginal opening in F_1 females (3 days), increased the age of preputial separation in F_1 males and females (4 days), increased the age of testes descent in F_1 males (3 days), decreased the anogenital distance in male F_{2a} and F_{2c} pups (13% and 18%, respectively), increased the age of vaginal opening in F_2 females (6 days), decreased the anogenital distance of male F_{3a} pups (13%), increased the age of vaginal opening in F_3 females (6 days), increased the incidence of retained nipples in F_3 males (11%), increased the age of preputial separation in F_3 males (5.1 days) and increased the age of testes descent in F_3 males (2.5 days; LOAEL = 392 to 592 mg/kg-day; NOAEL = 46 to 77 mg/kg-day; BMD_{10} = 5375 to 10566; $BMDL_{10}$ = 3284 to 7631; $BMD_{1\ SD}$ = 1093 to 6846; $BMDL_{1\ SD}$ = 963 to 2661 mg/kg). Lower doses of DEHP increased the age of preputial separation in F_2 males and females (6.5 days), and increased the age of testes descent in F_2 males (3.4 days; LOAEL = 46 to 77 mg/kg-day; NOAEL = 14 to 23 mg/kg-day).

In subsequent crossover studies, F_1 female rats treated with DEHP (543 to 775 mg/kg-day) were mated with control males. Mating resulted in decreased male pup anogenital distance (16.9%) and increased female pup anogenital distance relative to body weight (17.6%). F_2 female rats dosed with DEHP (392 to 592 mg/kg-day) were also mated to control males. This mating resulted in decreased male pup anogenital distance (11.5%). Matings involving treated F_1 or F_2 males and control females either produced no litters or litters with no changes in anogenital distance.

Swan *et al.* (2005, 2009) have attempted to epidemiologically correlate *in utero* phthalate exposure (urinary phthalate concentration) to reduced anogenital distance in humans.

In Swan *et al.*'s first paper (2005), the urinary concentration of some phthalate metabolites (MEP, MBP, MBzP, and MiBP) were negatively associated with an anogenital index (a calculated function containing anogenital distance). Significant negative associations were not, however, made for DEHP metabolites. Two of these metabolites, MEOHP and MEHHP, did have anogenital index regression coefficients of comparable size to MEP and MBzP, but the significance of this finding is still uncertain (P = 0.114, P = 0.145). Methodological controversies such as the small study size (85 mothers with pre-natal urine samples), the estimation of exposure using only a single urinary sample obtained during pregnancy (instead of many samples covering the important stages of pregnancy), and uncommon normalization for anogenital distance (the anogenital index), increased the uncertainties associated with this studies' conclusions. A lack of statistically significant correlations between reduced anogenital index and DEHP metabolites also detracted from the studies' conclusions, since DEHP is one of the most potent reproductive toxicants among the phthalates.

In Swan *et al.*'s later study (2009), concentrations of MEOHP, MEHHP, and the sum of MEOHP, MEHHP, and MEHP (DEHP metabolites) were associated with a decreased "masculinity" score (P=0.02, 0.04, and 0.04, respectively). MEHP concentration was not

negatively associated with testosterone concentration. As with the 2005 study, a reduced sample group size (n=71 to 74), the estimation of exposure using only a single urinary sample obtained during pregnancy, subjective measures of preschool activity (masculine versus feminine) and parental activities, and the novel estimation of a "composite" score increased uncertainties associated with the study. FSH and inhibin production also did not fit patterns estimated for other phthalate metabolites (MBP, MBzP), further detracting from study conclusions.

The weight of evidence from the above studies supported the conclusion that **there was "sufficient animal and limited human evidence" for the designation of DEHP as a "probable developmental toxicant"**.

Genotoxicity

DEHP has been tested for genotoxicity in a variety of systems. In bacterial, eukaryotic, and mammalian *in vitro* systems, DEHP and its metabolites were largely negative for mutagenic or other direct acting genotoxic effects with or without metabolic activation (Table 5.2; IARC, 2000; ATSDR, 2002; ECB, 2008). Positive results were primarily associated with various mammalian cell systems in which cell transformation and gap junction intercellular communication were measured in the absence of metabolic activation. A Summary of this information can be seen in Table 5.2 and specific genotoxicity test results can be seen in Appendix 5.

Table 5.2 Summary *In vitro* Genotoxic Effects of DEHP and Select Metabolites (ECB, 2008; ATSDR, 2002; IARC, 2000)				
Species/Test System (Strain)	End Point	Compound	Conclusion WITH activation Neg/Weak-Equiv/Pos	Conclusion WITHOUT activation Neg/Weak-Equiv/Pos
Bacterial Systems				
Salmonella typhimurium	Gene mutation	DEHP	73/0/1	72/1/0
		MEHP	10/0/0	10/0/0
		5OH-MEHP	4/0/0	4/0/0
		5oxo-MEHP	4/0/0	4/0/0
		5cx-MEPP	4/0/0	4/0/0
		2-ethylhexanol	5/0/0	5/0/0
Escherichia coli	Gene mutation	DEHP	3/0/0	3/0/0
S. typhimurium	Azaguanine resistance	DEHP	1/0/0	1/0/0
Bacillus subtilis (rec assay)	DNA damage – differential toxicity	DEHP	N/A	1/0/0
		MEHP	N/A	0/0/1
		2-ethylhexanol	N/A	1/0/0
		phthalic acid	N/A	1/0/0
DEHP and Select Metabolite Total Assays			**104/0/1**	**106/1/1**
Eukaryotic Systems				
S. cerevisae	Gene conversion	DEHP	5/2/1	5/1/1
S. cerevisae	Mitotic aneuploidy	DEHP	0/0/1	1/0/2
S. cerevisae	Homozygosis	DEHP	2/0/0	2/0/0
S. cerevisae	Mitotic segregation	DEHP	N/A	2/0/0
S. cerevisae	Gene mutation	DEHP	13/1/0	14/1/0
Schizosaccharomyces pombe (P1)	Gene mutation	DEHP	1/1/0	1/1/0
S. cerevisae	DEL assay, ICR recombination	DEHP	1/0/0	1/0/0
Aspergillus niger	Mitotic segregation	DEHP	1/0/0	N/A
Aspergillus nidulans	Haploid, mutation	DEHP	N/A	1/0/0
Aspergillus nidulans	Non-disjunction	DEHP	N/A	1/0/0
Aspergillus nidulans	Mitotic crossing-over	DEHP	N/A	1/0/0
Drosphila melanogaster	Crossing-over/recombination	DEHP	N/A	1/0/0
D. melanogaster	Somatic mutation	DEHP	N/A	0/3/1
DEHP and Select Metabolite Total Assays			**23/4/2**	**30/6/4**
Mammalian Systems				
Mouse lymphoma L5178Y cells	Gene mutation	DEHP	8/0/1	6/2/1
Mouse lymphoma L5178Y cells	Gene mutation	**MEHP**	1/0/0	1/0/0
Mouse lymphoma L5178Y cells	Gene mutation	**2-ethylhexanol**	1/0/0	1/0/0
CHO-Ki-BH4 – Chinese hamster ovary cells	Gene mutation	DEHP	1/0/0	1/0/0
BALB/c-3T3 mouse cells	Gene mutation	DEHP	1/0/0	N/A
Human lymphocytes	Gene mutation	DEHP	2/0/0	2/0/0

Table 5.2 Summary *In vitro* Genotoxic Effects of DEHP and Select Metabolites (ECB, 2008; ATSDR, 2002; IARC, 2000)				
Species/Test System (Strain)	End Point	Compound	Conclusion WITH activation Neg/Weak-Equiv/Pos	Conclusion WITHOUT activation Neg/Weak-Equiv/Pos
Mammalian Systems continued				
Rat hepatocytes	DNA single strand breaks	DEHP	N/A	1/0/0
Syrian hamster hepatocytes	DNA single strand breaks	DEHP	N/A	1/0/0
Human primary hepatocytes	DNA repair - UDS	DEHP	N/A	1/0/0
Human primary hepatocytes	DNA repair - UDS	**MEHP**	N/A	1/0/0
Rat primary hepatocytes	DNA repair - UDS	DEHP	N/A	6/0/0
Rat hepatocytes	DNA single strand breaks	DEHP	N/A	1/0/0
CHO cells	DNA single strand breaks	DEHP	N/A	1/0/0
SHE cells	DNA single strand breaks	DEHP	N/A	0/1/0
$B6C3F_1$ Mouse primary hepatocytes	DNA repair - UIA, UDS	DEHP	N/A	1/0/0
$B6C3F_1$ Mouse primary hepatocytes	DNA repair - UIA, UDS	**MEHP**	N/A	1/0/0
V79 cells	DNA repair	DEHP	1/0/0	N/A
CH Don cells	Sister chromatid exchange	DEHP	N/A	1/0/0
CHO cells	Sister chromatid exchange	DEHP	4/0/1	1/1/0
Rat liver (RL-4)	Sister chromatid exchange	DEHP	N/A	1/0/0
Human lymphocytes	Sister chromatid exchange	DEHP	1/0/0	1/0/0
Human lymphocytes (co-culture with rat liver cells)	Sister chromatid exchange	DEHP	0/1/0	1/0/0
Chinese hamster V79 cells	Sister chromatid exchange	**MEHP**	N/A	0/0/1
Human hepatocytes	Chromosomal aberrations	DEHP	N/A	1/0/0
Human leucocytes	Chromosomal aberrations	DEHP	N/A	1/0/0
Human fetal lung cells	Chromosomal aberrations	DEHP	N/A	1/0/0
CHO cells	Chromosomal aberrations	DEHP	N/A	1/0/0
Rat liver (RL4)	Chromosomal aberrations	DEHP	N/A	1/0/0
CH Don cells	Chromosomal aberrations	DEHP	N/A	1/0/0
CH lung cells	Chromosomal aberrations	DEHP	N/A	1/0/0
CH liver cells	Chromosomal aberrations	DEHP	N/A	1/0/0
CHO cells	Chromosomal aberrations	DEHP	1/0/0	1/0/0
Chinese hamster lung fibroblasts	Chromosomal aberrations	DEHP	1/0/0	1/0/0
SHE cells	Chromosomal aberrations	DEHP	0/0/1	1/0/0
SHE cells	Chromosomal aberrations	**MEHP**	0/0/1	1/0/0
Human lymphocytes	Chromosomal aberrations	DEHP	N/A	1/0/0
CHO cells	Micronucleus formation	DEHP	1/0/0	1/0/0
Rat hepatocytes	Micronucleus formation	DEHP	N/A	1/0/0
SHE cells	Micronucleus formation	DEHP	N/A	0/0/1
CH SV40-transformed liver cells	Selective DNA amplification	DEHP	1/0/0	N/A
CHO cells	Cell transformation	DEHP	0/0/1	NS
Mouse JB6 epidermal cells	Cell transformation	DEHP	0/0/1	N/A
SHE cells	Cell transformation	DEHP	1/0/1	0/1/5
SHE cells	Cell transformation	**MEHP**	0/1/0	1/0/1
Mouse C3H/10T½ fibroblasts	Cell transformation	DEHP	0/1/0	1/1/0
Mouse C3H/10T½ fibroblasts	Cell transformation	**MEHP**	N/A	1/0/0

Table 5.2 Summary *In vitro* Genotoxic Effects of DEHP and Select Metabolites (ECB, 2008; ATSDR, 2002; IARC, 2000)				
Species/Test System (Strain)	End Point	Compound	Conclusion WITH activation Neg/Weak-Equiv/Pos	Conclusion WITHOUT activation Neg/Weak-Equiv/Pos
Mammalian Systems continued				
Primary rat tracheal epithelial cells	Cell transformation	DEHP	N/A	0/0/1
BALB/3T3 mouse cells	Cell transformation	DEHP	2/0/0	2/0/0
RLV/Fischer rat	Cell transformation	DEHP	N/A	0/0/1
SA7/Syrian hamster embryo cells	Cell transformation	DEHP	N/A	0/0/1
SHE Cells	Cell transformation	DEHP	N/A	0/0/3
Mouse Balb/c-3T3 clone I13 C14 cells	Cell transformation	DEHP	N/A	2/0/0
Mouse Balb/c-3T3 clone A31 cells	Cell transformation	DEHP	1/0/0	0/0/1
Chinese hamster V79 fibroblasts	Gap junction intercellular communication	DEHP	N/A	2/0/4
Syrian hamster embryo cells	Gap junction intercellular communication	DEHP	N/A	0/0/1
Chinese hamster V79 fibroblasts and Syrian hamster embryo cells	Gap junction intercellular communication	DEHP	N/A	0/0/1
Chinese hamster V79 fibroblasts and Syrian hamster embryo cells	Gap junction intercellular communication	**MEHP**	N/A	0/0/1
Rat hepatocytes	DNA binding	DEHP	1/0/0	N/A
Human fetal lung cells	Aneupoidy	DEHP	N/A	1/0/0
CH liver cells	Aneupoidy	DEHP	N/A	0/1/0
Rat liver (RL4)	Polyploidy, aneuploidy	DEHP	N/A	1/0/0
CH1-L primary liver cells	Mitotic aberrations	DEHP	N/A	0/0/2
SHE cells	Ornithine decarboxylase superinduction	DEHP	N/A	1/0/0
Human blood - leucocytes	Comet assay	DEHP	1/0/0	0/0/1
Body fluids	Sprague-Dawley rat urine, microbial mutagenicity	DEHP	1/0/0	1/0/0
DEHP and Select Metabolite Total Assays			**31/3/7**	**58/7/26**

DEHP and its select metabolites were also largely negative for mutagenic or other direct-acting genotoxic effects in mammalian and insect *in vivo* systems (Table 5.3; IARC, 2000; ATSDR, 2002; ECB, 2008). Positive results were primarily associated with replicative DNA synthesis and the Dominant Lethal test in mammalian test systems. As with *in vitro* tests, a summary of this information can be seen in Table 5.3 and specific *in vivo* genotoxicity test results can be seen in Appendix 5.

Table 5.3 Summary *In vivo* Genotoxic Effects of DEHP and Select Metabolites (ECB, 2008; ATSDR, 2002; IARC, 2000)			
Species/Test System (Strain)	End Point	Compound	Conclusion WITHOUT activation (Neg/Weak-Equiv/Pos)
Mammalian Systems			
Human leucocytes	Chromosomal aberrations	DEHP	1/0/0
SH embryos	Chromosomal aberrations	DEHP	0/0/1
Fischer 344 rat bone marrow	Chromosomal aberrations	DEHP	1/0/0
SH embryos	Cell transformation	DEHP	0/0/1
Hamster embryo cells	8AG/6TG-resistant mutation	DEHP	0/0/1
Rat bone marrow	Micronucleus formation	DEHP	1/0/0
Rat bone marrow	Mitotic Index	DEHP	1/0/0
Mouse	Dominant lethal test	DEHP	1/0/1
ICR Swiss mouse	Dominant lethal test	DEHP	0/0/2
CD-1 mice	Dominant lethal test	DEHP	1/0/0
ICR SIM mice	Dominant lethal test	DEHP	1/0/0
Mouse bone marrow	Micronucleus formation	DEHP	1/0/0
$B6C3F_1$ Mouse erythrocytes	Micronucleus formation	DEHP	1/0/0
Mouse bone marrow	Micronucleus formation	DEHP	1/0/0
Fischer 344 rats	DNA binding	DEHP	0/1/0
Fischer 344 rat liver DNA	DNA binding - covalent	DEHP	3/1/0
Fischer 344 rat hepatocyte DNA	DNA binding - covalent	DEHP	1/0/0
Fischer 344 rat hepatocytes	DNA repair- UDS	DEHP	2/0/0
Sprague-Dawley rat hepatocytes	DNA repair - UDS	DEHP	1/0/0
Rat liver	DNA repair	DEHP	0/0/1
$B6C3F_1$ mouse hepatocytes	DNA repair - UDS	DEHP	1/0/0
Primary rat hepatocytes	Replicative DNA synthesis	DEHP	0/0/1
Fischer 344 rats	Replicative DNA synthesis	DEHP	1/0/3
Alderley Park rats	Replicative DNA synthesis	DEHP	0/0/1
Marmosets	Replicative DNA synthesis	DEHP	1/0/0
$B6C3F_1$ mice	Replicative DNA synthesis	DEHP	0/0/1
Rat liver	DNA strand breaks	DEHP	1/0/0
Wistar rat liver	DNA strand breaks	DEHP	1/0/0
Wistar rat liver	DNA strand breaks	**MEHP**	1/0/0
Fischer 344 rat liver	DNA single-strand breaks	DEHP	0/1/0
Fischer 344 rat liver	DNA base modification – DNA oxidative damage	DEHP	1/0/1
Rat liver	Tetraploid nuclei	DEHP	0/0/1
Fischer rat hepatocytes	Aneupoidy	DEHP	1/0/0
Rat kidney	Tumor promotion	DEHP	0/0/1
S. typhimurium (TA100): Rat host-mediated assay	Gene mutation	DEHP	1/0/0
C57BL/6f lacl transgenic mouse liver	Gene mutation	DEHP	1/0/0
Cynomolgous monkey liver cells	Gap junction intercellular communication	DEHP	1/0/0
$B6C3F_1$ mice	Sperm morphology	DEHP	1/0/0
Sprague-Dawley rats	Sperm morphology	DEHP	1/0/0
Insect Systems			
D. melanogaster	Sex-linked recessive lethal mutation	DEHP	2/0/0
D. melanogaster	DNA double strand breakage	DEHP	1/0/0
D. melanogaster	DNA repair test	DEHP	1/0/0
D. melanogaster	Wing spot test, mutation	DEHP	1/0/0
DEHP and Select Metabolite Total Assays			**35/3/16**

Data from both *in vitro* and *in vivo* bacterial, eukaryotic, and mammalian genetic toxicity studies were found for DEHP. Although certain studies may have inconsistencies in methodology or data, the overwhelming majority were useful for generating conclusions regarding DEHP mutagenicity and genotoxicity. The breadth and number of genotoxicity studies also covered what is considered a normal genotoxicity testing battery of studies (FDA, 2000).

A substantial amount of bacterial, eukaryotic, insect, and mammalian genetic toxicity data supported the conclusion that **DEHP is not a "known or probable direct-acting genotoxicant"**.

Carcinogenicity

Genotoxicity

Genotoxicity can initiate, modulate, or perpetuate the development of cancer, so should be considered in all evaluations of carcinogenicity. Previously discussed genotoxicity information is re-summarized below.

Overall, DEHP and its metabolites were largely negative for mutagenic or other genotoxic effects when tested in bacterial, eukaryotic, and mammalian *in vitro* systems with or without metabolic activation (Table 5.2; IARC, 2000; ATSDR, 2002; ECB, 2008). DEHP and its metabolites were also largely negative for mutagenic or other genotoxic effects in mammalian and insect *in vivo* systems (Table 5.3; IARC, 2000; ATSDR, 2002; ECB, 2008).

Positive results in *in vitro* studies were primarily associated with various mammalian cell systems in which cell transformation and gap junction intercellular communication were measured in the absence of metabolic activation. In *in vivo* studies, positive results were primarily associated with replicative DNA synthesis and the Dominant Lethal test in mammalian test systems.

Initiation and promotion

Initiation and promotion studies revealed that DEHP itself has limited ability to initiate carcinogenesis. In Fischer 344 rats, single gavage and 12 weeks of dietary administration of DEHP (10,000 mg/kg and 600 mg/kg-day, respectively) did not initiate hepatic tumor activity when followed by the promoters 2-acetyl-aminofluorene or phenobarbital (Garvey *et al.,* 1987). A similar result was seen in $B6C3F_1$ mice exposed to a single gavage dose of DEHP (25,000 or 50,000 mg/kg) and promoted with phenobarbital (Ward *et al.,* 1983). Further, administration of dietary DEHP (12,000 mg/kg, ~ 550 mg/kg-day) for 24, 26, or 78 weeks also did not initiate hepatic tumor activity when followed by phenobarbital (Ward *et al.,* 1986; Williams *et al.,* 1987).

DEHP did have the ability, however, to promote the development of hepatic and kidney cancer, once initiated by other chemical agents. In Sprague-Dawley rats, initiation with diethylnitrosamine followed by promotion with DEHP resulted in a 2-fold increase in the number and area of ATPase-deficient liver foci in one study (200 and 500 mg/kg; Oesterle and Deml, 1988), and an increase in the number and area of ATPase-deficient foci and GGTase-positive liver foci in another study (50 and 200 mg/kg; Gerbracht *et al.,* 1990). Further, in Fischer 344 rats, initiation with N-ethyl-N-hydroxyethylnitrosamine followed by promotion with 12,000 mg/kg DEHP (~600 mg/kg-day) resulted in an increase in the incidence of renal adenomas and adenocarcinomas, and an increase in the number of tumors per kidney (Kurokawa *et al.,* 1988).

Stronger promoting effects were seen in mouse studies. In $B6C3F_1$ mice, initiation with diethylnitrosamine followed by promotion with 3000 to 12,000 mg/kg DEHP (600 – 2400 mg/kg-day) resulted in an increase in hepatic foci and neoplasms (Ward *et al.,* 1983), significant time-dependent increases in liver focal proliferative lesions after exposure for 28 days, and significant increase in incidence of liver tumors at 168 days (Ward *et al.,* 1984), an increase in liver tumors (Schuller and Ward, 1984), and increased incidence and area of liver focal proliferative lesions (Hagiwara *et al.,* 1986). Increased numbers of liver focal proliferative lesions including hyperplastic foci, hepatocellular adenomas, and carcinomas were also seen following transplacental initation with N-nitrosoethylurea and promotion with 6000 mg/kg DEHP (~1200 mg/kg-day; Ward *et al.,* 1990). Initiation with N-nitrosodiethylamine and promotion with 12,000 mg/kg DEHP (~2400 mg/kg) also resulted in an increase in hepatic liver tumors in C3H/HeNCr mice (Weghorst *et al.,* 1993, 1994).

The use of the rat liver foci assay has been criticized by some authors as being inappropriate for estimating carcinogenic activity in peroxisome-inducing compounds (Milman and Weisburger, 1994). This is because some peroxisome proliferators (i.e., Wy-14, 643) that induce altered hepatic foci and hyperplastic nodules do not have any GGT+ activity in the liver (Milman and Weisburger, 1994). Use of the GGT+ foci/cm^2 (instead of foci/cm^3) has also been criticized because of biases inherent in cutting and interpreting specimens for pathological assessment. For this reason, promotion data using GGT+ activity should be considered preliminary.

The promotion of liver cancer was not seen in all cases. Initiation with diethylnitrosamine, N-nitrosodiethylamine, and 2-fluorenylacetamide followed by promotion with 3000 to 12,000 mg/kg DEHP (~150 to 600 mg/kg-day) did not result in significantly increased numbers of hepatic cancers in Fischer 344 rats (Ito *et al.,* 1988; Ward *et al.,* 1986; Popp *et al.,* 1985; Maruyama *et al.,* 1990; Williams *et al.,* 1987). Similarly, initiation with N-butyl-N-(4-hydroxybutyl) nitrosamine followed by promotion with 3000 to 12,000 mg/kg DEHP (~250 to 600 mg/kg-day) did not result in significantly increased cancers in the kidneys or urinary bladder of Fischer 344 rats (Hagiwara *et al.,* 1990).

Carcinogenicity studies

A number of studies have assessed the carcinogenicity of DEHP exposures in animals. Increases in hepatocellular carcinomas were observed in treated Sprague-Dawley rats following long-term high dose exposures to DEHP (LOAEL = 1377 mg/kg-day; Lake *et al.,* 1987). Hepatic tumors were not reported in other Sprague-Dawley rat studies, however, following similar duration exposures at lower doses (NOAEL = 700 mg/kg-day; Ganning *et al.,* 1987, 1991).

In Fischer 344 rats, exposure to DEHP increased the incidence of hepatocarcinomas (43% by week 78; LOAEL = 1579 mg/kg-day; Hayashi *et al.,* 1994), hepatocellular carcinomas (11/14 treated rats versus 1/10 control rats; LOAEL = 2%; Rao *et al.,* 1990), liver tumors (6/10 treated rats versus 0/10 control rats; LOAEL = 2%; Rao *et al.,* 1987), hepatocellular tumors (11/65 male rats and 22/80 female rats; $LOAEL_M$ = 147 mg/kg-day; $LOAEL_F$ = 939 mg/kg-day; David *et al.,* 1999, 2000a), hepatocellular carcinomas (1/4 rats and 2/4 rats by 52 and 78 weeks, respectively; LOAEL = 2%; Tamura *et al.,* 1990a, 1990b), hepatic neoplastic lesions, hepatocellular carcinomas, adenomas, and neoplastic nodules (LOAEL = 147 to 550 mg/kg-day; NOAEL = 29 mg/kg-day; NTP, 1982 (Table A3.63); Moore, 1996 (Table A3.62); Cattley *et al.,* 1987; Kluwe *et al.,* 1982).

Long-term exposure to DEHP also increased the incidence of hepatocellular neoplasms and carcinomas (LOAEL = 672 mg/kg-day; NTP, 1982 (Table A3.63); Kluwe *et al.,* 1982), the total number of adenomas and carcinomas in rats (M&F; partially reversible; LOAEL = 147 mg/kg-day; NOAEL = 29 mg/kg-day; Moore, 1997 (Table A3.62)), and hepatocellular tumors (27/65 male mice, and 19/65 female mice; $LOAEL_M$ = 292 mg/kg-day; $LOAEL_F$ = 354 mg/kg-day; David *et al.,* 1999, 2000b).

A sequence of key events for the development of liver carcinogenesis in rodents was described by Rusyn *et al.* (2006). This included: i) metabolism of DEHP and systemic distribution of MEHP and other secondary metabolites, ii) activation of hepatic macrophages which increases the production of oxidants (receptor-independent), iii) PPARα activation in hepatocytes and increased expression of peroxisomal and non-peroxisomal genes related to metabolism (see "Liver Toxicity" section), iv) peroxisomal and mitochondrial enlargement, v) a transient increase in cell proliferation and decrease in apoptosis, vi) sustained liver enlargement, vii) oxidative stress and DNA damage over the long-term, viii) clonal expansion of initiated cells, ix) pre-neoplastic nodule development, and x) development of adenomas and carcinomas. These events have been described in more detail in Babich *et al.* (2010).

Overall, DEHP-induced (PPARα-mediated) hepatocarcinogenic effects are thought to have little or no relevance to humans. Species differences in PPARα expression, binding, localization, and activation pathways support the conclusion of limited human relevance. Differences in metabolic capacities (enzyme composition and quantity) and intestinal processing of phthalates (when comparing humans and rodents) have also been reported, supporting this conclusion. A limited number of studies have provided evidence that additional non-PPARα

mechanisms may also contribute to DEHP-induced hepatic cancer. These hypotheses need further investigation.

The weight of evidence from the above studies supported the conclusion that **there was "sufficient animal evidence" for the designation of DEHP as a "probable carcinogen"**. The carcinogenic relevance to humans in this case, however, is thought to be neglible.

Lowest Hazard Endpoints by Organ System and Exposure Duration

Sufficient data has been presented to select hazard endpoints on the basis of organ system and exposure duration. These endpoints were used in ranking the relative effects on the organ systems as well as in the selection of exposure duration-related hazard endpoints.

Overall, short-term exposure effects occurred in the liver at doses lower than other organ systems. In intermediate duration exposures, the liver, biochemical attributes of the liver, and testicular morphologies were affected more than other organ systems. In long-term exposures, the kidney, the liver, biochemical functions of the liver, and testicular structure and function were affected at lower concentrations than other organ systems.

Overall Uncertainty

The hazard database for DEHP consisted of hundreds of robust studies and numerous less well described studies. Exposure durations in these studies ranged from acute to chronic and multigeneration. In the majority of studies reviewed, the routes of exposures were oral. Additional information featuring the inhalation route of exposure has been reviewed for a few species.

A primary uncertainty associated with the DEHP hazard evaluation concerns the extrapolation of data from non-human primates and other animals to humans.

Currently, there is a scientific consensus regarding DEHP-induced cancers and its lack of human relevance. Recent publications demonstrate, however, that alternate “human-relevant” carcinogenic pathways may also contribute to DEHP-induced cancers. The relevance of many of the other DEHP-induced pathologies (i.e. reproductive, kidney, thyroid, etc) are also uncertain.

The adverse effect of parenteral exposures (through non-consumer products such as medical devices) is an additional data gap. There is a small amount of data demonstrating that metabolism of DEHP following parenteral exposures is substantially different from oral routes.

Another uncertainty or data gap concerns the effects of DEHP on populations with genetic inborn errors of metabolism. At least 25 disorders related to peroxisomes have been reviewed by Ito and Nakajima (2008). These disorders affect populations ranging from prenatal to adult in age, with some polymorphisms being very prevalent. Deficiencies in the ability to produce “normal” human peroxisomes, such as in Zellweger’s spectrum diseases (Zellweger’s syndrome, infantile Refsum disease, neonatal adrenoleukodystrophy [NALD]), may affect the clinical and pathological sequela of DEHP. This area of concern has not been investigated.

Overall Acceptable Daily Intakes

Acceptable daily intakes values (ADI's) are calculated when a given chemical is considered "toxic" and sufficient toxicity information is available. The ADI is the amount of a chemical that one may be exposed to on a daily basis without posing a significant risk of health effects to consumers. ADI's were estimated for relevant exposure durations for the general population (non-reproductive endpoint) and for males (reproductive endpoint). An additional ADI was estimated for developmental effects (maternal exposures resulting in developmental effects).

General population ADI's

Short-term oral exposures – general population

For short-duration oral exposures, the NOAEL of 10 mg/kg-day (Dostal *et al.,* 1987a, 1987b; ATSDR, 2002; ECB, 2008) was chosen as the representative overall hazard endpoint. This endpoint is derived from two studies in which male Sprague-Dawley rats were gavage dosed with DEHP once daily for 5 days. In these guideline studies, DEHP doses of 100 mg/kg-day (LOAEL) increased the absolute and relative liver weight in 2, 3, 6, and 12 week old rats, increased biochemical functions associated with the liver (i.e., palmitoyl-CoA oxidase activity, carnitine acetytransferase activity, peroxisomal proliferation, peroxisomal enzyme activity), and decreased serum triglycerides and cholesterol.

Choice of hepatic study data for use as a hazard endpoint is supported by additional studies with slightly higher hazard effect levels. In the first study, the relative liver weight was increased in male Fischer 344 rats following dietary exposure to DEHP for 7 days (David *et al.,* 1999; ATSDR, 2002). The LOAEL based on this adverse effect was slightly below (53 mg/kg-day), and the NOAEL was slightly higher (11 mg/kg-day) than that in the Dostal studies. Increased hepatic peroxisomal enzyme activity was also reported in male Sprague-Dawley rats dosed daily by gavage for 14 days (Lake *et al.,* 1984b; ECB, 2008). Increased activity occurred at a lower LOAEL (25 mg/kg-day) than increases in relative liver weight (LOAEL = 100 mg/kg-day; NOAEL = 25 mg/kg-day) in these rats. Increased peroxisomal enzyme activity alone was not chosen as the representative hazard endpoint, however, because of published information supporting the concept that peroxisomal activity may not be relevant to the development of human pathologies and also the lack of supporting hepatic structural or biochemical changes at the same dose level.

All other DEHP-induced changes occurred at much higher doses. Changes in the thyroid (LOAEL = 2000 mg/kg-day), fetal lethality (NOAEL = 50 mg/kg-day), milk composition (LOAEL = 2000 mg/kg-day), ovulation and serum estradiol (LOAEL = 2000 mg/kg-day), testicular structure and function, kidney weight, body weight and mortality (NOAEL = 100

mg/kg-day), the immune system (LOAEL = 1500 mg/kg-day), food consumption (LOAEL = 1200 mg/kg-day), and clinical toxicity (6000 mg/kg-day) have been reported.

The NOAEL of 10 mg/kg-day was used to generate an ADI for all populations by dividing by an uncertainty factor of 100 (10X for interspecies variation, 10X for intraspecies variation and sensitive populations). This "safety factor" is typically applied by CPSC to the lowest NO[A]EL for animal data in which developmental, reproductive, or neurotoxicological effects have been determined (16 CFR§1500.135(d)(4)(B)). **The short-term exposure oral ADI for the general population was calculated to be 0.1 mg/kg-day.**

Intermediate-term oral exposures – general population

For intermediate-duration oral exposures, the dose LOAEL of 24.0 mg/kg-day (BIBRA, 1990; ECB, 2008) was chosen as the representative overall hazard endpoint for general toxicity induced by intermediate-term exposures. This endpoint was derived from a GLP study in which male Fischer 344 rats were dosed with DEHP in the feed for 28 days. DEHP doses of 24.0 mg/kg-day (LOAEL) increased the relative liver weight in these rats.

Increased hepatic peroxisomal enzyme activity (RIVM, 1992), increased triglycerides (CMA, 1984b; Barber *et al.,* 1987; ECB, 2008) and increased number of peroxisomes (RIVM, 1992) were also reported in male Wistar and Fischer 344 rats exposed to DEHP in the diet for 14, 21, or 28 days. These effects occurred at lower LOAELs (5, 11, and 18 mg/kg-day, respectively) than increases the relative liver weight in BIBRA's study. Peroxisomal enzyme activity, and increased peroxisome number were not chosen as representative hazard endpoints, however, because of published information supporting the concept that peroxisomal activity may not be relevant to the development of human pathologies. All three effects also lacked supporting hepatic structural or biochemical changes at the same dose level.

Choice of liver study data for use as a hazard endpoint was supported by additional studies with slightly higher hazard effect levels for the liver. In the first study, liver weight and peroxisomal enzyme activity increased in male and female Fischer 344 rats following dietary exposure to DEHP for 7 or 21 days (CMA, 1982c; ECB, 2008). The LOAEL based on this adverse effect was 3.5-fold above (80 mg/kg-day) that in the BIBRA (1990) study. Increased liver weight, changes in bile ducts, peroxisome proliferation, SER proliferation, increased peroxisome enzyme activity, lipid filling of lysosomes, glycogen depletion induction of cytochrome P-450s, and mitochondrial changes were also observed in male and female albino Alderley Park rats exposed to DEHP in the diet for 3, 7, 14, 28 days, and 36 weeks (CEFIC, 1982; Mitchell *et al.,* 1985a; ECB, 2008; ATSDR, 2002). Both of these sets of effects occurred at a higher LOAEL (50 mg/kg-day) than that in the BIBRA's study. Finally, increased absolute and relative liver weight was reported following dietary exposure of Fischer 344 rats to DEHP

for 13 weeks (Eastman Kodak, 1992a; ECB, 2008). As with the other studies, the LOAEL (63 mg/kg-day) was slightly above that reported for BIBRA (1990).

All other DEHP-induced changes occurred at much higher doses. Changes in the thyroid (NOAEL = 37.6 mg/kg-day), mortality (LOAEL = 2000 mg/kg-day), clinical behavior (NOAEL = 44 mg/kg-day), body weight (NOAEL = 50 mg/kg-day), food consumption (LOAEL = 357 mg/kg-day), hematology (NOAEL = 37.6 mg/kg-day), the immune system (NOAEL = 1900 mg/kg-day), the thymus (NOAEL = 2580 mg/kg-day), musculskeletal systems (NOAEL = 2500 mg/kg-day), the kidney (NOAEL = 37.6 mg/kg-day), development (NOAEL = 40 mg/kg-day), the ovary (NOAEL = 797 mg/kg-day), the gastrointestinal system (NOAEL = 2500 mg/kg-day), the cardiovascular system (NOAEL = 1900 mg/kg-day), the lung (NOAEL = 2500 mg/kg-day), and neurobehavior (LOAEL = 60 mg/kg-day) were reported.

The LOAEL of 24 mg/kg-day was used to generate an Acceptable Daily Intake (ADI) by dividing by an uncertainty factor of 1000 (10X for interspecies variation, 10X for intraspecies variation and sensitive populations, 10X for conversion of a LOAEL to a NOAEL). The 1000-fold "safety factor" is typically applied by CPSC to the lowest LO[A]EL for animal data in which developmental, reproductive, or neurotoxicological effects have been determined (16 CFR§1500.135(d)(4)(B)). Other federal agencies such as ATSDR have also historically used 10X factors each for intraspecies, interspecies, and LOAEL to NOAEL extrapolation (Pohl and Abadin, 1995). This magnitude of factor for LOAEL to NOAEL extrapolation has also been shown to be protective for 99% of the responses to mild adverse effects from other routes of exposure (inhalation; Alexeeff *et al.,* 2002). The relative mildness of the adverse effect in this case (liver), however, encourages the future consideration of lower uncertainty factors for LOAEL to NOAEL extrapolation (Pohl and Abadin, 1995; Dourson *et al.,* 1996). **The intermediate-term exposure oral ADI for the general population was calculated to be 0.024 mg/kg-day.**

Long-term oral exposures – general population

For long-duration oral exposures, the dose NOAEL of 5.8 mg/kg-day (David *et al.,* 2000a; Moore, 1996; ECB, 2008) was chosen as the representative overall hazard endpoint for general toxicity. This endpoint was derived from a well conducted study in which male and female Fischer 344 rats were dosed with DEHP in the feed for 104 weeks. DEHP doses of 28.9 mg/kg-day (LOAEL) increased the absolute and relative liver weight and peroxisome proliferation in male rats.

Increased hepatic peroxisomal enzyme activity (Ganning *et al.,* 1987, 1991) was reported in male Sprague-Dawley rats exposed to DEHP in the diet for 102 weeks. This effect occurred at a lower LOAEL (7 mg/kg-day) than increases in the liver weight in Moore's study. Peroxisomal enzyme activity was not chosen as a representative hazard endpoint, however, because of

published information supporting the concept that peroxisomal activity may not be relevant to the development of human pathologies and a lack of supporting hepatic structural or biochemical changes at the same dose level.

Two additional studies previously discussed in "*Long-term Oral Exposures – Reproduction"* also reported renal effect levels below that in Moore's study (1996). As discussed, these studies were not used as hazard endpoints because adverse effect findings were not verified in other studies, and the renal changes may be related to α_{2u} globulin. These, therefore may not be relevant to the development of human pathologies.

Choice of liver study data for use as a hazard endpoint was supported by additional studies with slightly higher hazard effect levels. In the first study, an increase in liver weight was reported in guinea pigs following dietary exposure to DEHP for 52 weeks (Carpenter *et al.,* 1953; ATSDR, 2002). The NOAEL based on this effect was 4-fold higher (19 mg/kg-day) than that in Moore's study. Increased hepatic peroxisome proliferation and the number of mitochondria have also been observed in male Sprague-Dawley rats dosed with DEHP in the diet for 102 weeks (Ganning *et al.,* 1987, 1991; ECB, 2008; ATSDR, 2002). The NOAEL based on this effect was slightly higher (7 mg/kg-day) than in Moore's study. The final study demonstrated that DEHP administered in the feed for 104 weeks increased the liver weight in male $B6C3F_1$ mice and also increased peroxisome proliferation (Moore, 1997; ECB, 2008). These effects occurred at a NOAEL approximately 3-fold higher (19.2 mg/kg-day) than in his rat study.

All other DEHP-induced changes occurred at much higher doses. Changes in mortality (LOAEL = 50 to 80 mg/kg-day), body weight (NOAEL = 7 mg/kg-day), food consumption (LOAEL = 322 mg/kg-day), the immune system (NOAEL = 64 mg/kg-day), musculskeletal systems (NOAEL = 939 mg/kg-day), fertility (NOAEL = 46 mg/kg-day), the ovary and uterus (NOAEL = 100 mg/kg-day), the pituitary (NOAEL = 146.6 mg/kg-day), the gastrointestinal system (NOAEL = 190 mg/kg-day), the cardiovascular system (NOAEL = 190 mg/kg-day), and the lung (NOAEL = 28.9 mg/kg-day) were reported.

The NOAEL of 5.8 mg/kg-day was used to generate an Acceptable Daily Intake (ADI) by dividing by an uncertainty factor of 100 (10X for interpescies variation, 10X for intraspecies variation and sensitive populations). This "safety factor" is typically applied by CPSC to the lowest NO[A]EL for animal data in which developmental, reproductive, or neurotoxicological effects have been determined (16 CFR§1500.135(d)(4)(B)). **The long-term exposure oral ADI for the general population was calculated to be 0.058 mg/kg-day.**

Reproductive ADI's

Intermediate-term oral exposures – reproduction

For intermediate-duration oral exposures to adults, the dose NOAEL of 3.7 mg/kg-day (Poon *et al.,* 1997 (Table A3.70); ECB, 2008) was chosen as the representative overall hazard endpoint for male reproduction. This endpoint was derived from a GLP study in which young adult Sprague-Dawley rats were dosed with DEHP in the feed (0, 0.4, 3.7, 37.6, 375.2 mg/kg-day; M) for 13 weeks. DEHP doses of 37.6 mg/kg-day (LOAEL) induced mild vacoulation of Sertoli cells in the testes of 7 out of 10 male rats in this study.

Choice of reproduction study data for use as a hazard endpoint was supported by additional studies with slightly higher hazard effect levels. In the first study, testicular weight was decreased 33% in male Wistar rats following daily gavage exposure for 30 days (Parmar *et al.,* 1995 (Table A3.67); ATSDR, 2002).The LOAEL based on this adverse effect was slightly above (50 mg/kg-day) that in the Poon *et al.* (1997) study. Decreased testicular zinc concentration, epididymal sperm density and motility, and increased number of abnormal sperm were also discovered in Fischer 344 rats exposed to DEHP in the diet for 8 weeks (Agarwal *et al.,* 1986a, 1986b; ECB, 2008). These effects occurred at a slightly higher LOAEL (69 mg/kg-day) and NOAEL (18 mg/kg-day) than increases in Sertoli cell vacoulation. Finally, dose-dependent decreases in the number of litters and the proportion of Crl:CD-1 mice pups born alive were reported following exposure to dietary DEHP for 18 weeks (Lamb *et al.,* 1987 (Table A3.56); ECB, 2008; ATSDR, 2002). Litter and survival effects occurred at a higher LOAEL (140 mg/kg-day) and NOAEL (14 mg/kg-day) than in Poon *et al.*'s study (1997). The original NTP review (2001) of DEHP commented that "it is reasonable to conclude that these values are indistinguishable [from the Lamb *et al.* NOAEL of 14 mg/kg-day] given the wide dose spacing and inherent variability in the endpoints. It is the panel's view that the existing data support a NOAEL within the range of 3.7 to 14 mg/kg-day for oral exposure in rats". Sertoli cell vacoulation determined in Poon *et al.*'s study was not observed, however, in a CERHR re-evaluation of DEHP (NTP, 2006).

All other DEHP-induced changes occurred at much higher doses. Affected organ systems and lowest effector dose can be seen in "***Intermediate-term Oral Exposures – General Population***".

The NOAEL of 3.7 mg/kg-day was used to generate an Acceptable Daily Intake (ADI) by dividing by an uncertainty factor of 100 (10X for interpescies variation, 10X for intraspecies variation and sensitive populations). This "safety factor" is typically applied by CPSC to the lowest NO[A]EL for animal data in which developmental, reproductive, or neurotoxicological

effects have been determined (16 CFR§1500.135(d)(4)(B)). **The intermediate-term exposure oral ADI for male reproduction was calculated to be 0.037 mg/kg-day.**

Long-term oral exposures – reproduction

For long-term oral exposures to adults, the chronic LOAEL of 5.8 mg/kg-day (David *et al.*, 2000a) was chosen as the representative overall hazard endpoint for male reproduction. This endpoint was derived from a guideline study in which adult Fischer 344 rats were dosed with DEHP (0, 100, 500, 2500, or 12,500 mg DEHP/kg feed; 5.8, 28.9, 146.6, 789.0 mg/kg-day for M; 7.3, 36.1, 181.7, and 938.5 mg/kg-day for F) for 104 weeks. DEHP doses of 5.8 mg/kg-day (LOAEL) induced a substantial dose-dependent increase in the incidence of aspermatogenesis by 104 weeks (M; +6%). This increase was statistically significant in the 28.9 mg/kg-day treatment group.

Choice of reproduction study data for use as a hazard endpoint was supported by additional studies with slightly higher hazard effect levels. In the first study, increased incidence of testicular tubule atrophy and inhibited spermatogenesis was observed in male Sprague-Dawley rats following dietary exposure to DEHP for 102 weeks (Ganning *et al.,* 1991; ECB, 2008). The LOAEL based on this adverse effect was 1.2-fold above that in the David *et al.* (2000a) study (7 mg/kg-day; NOAEL = 0.7 mg/kg-day). Dose-dependent changes in testicular parameters and development were also reported for Sprague-Dawley rats exposed to dietary DEHP for 3 generations (Wolfe *et al.,* 2003; ECB, 2008). These parameters occurred at a LOAEL 2.4-fold higher (LOAEL = 14 mg/kg-day; NOAEL = 4.8 mg/kg-day) than that in David *et al.*'s report (2000a).

Choice of this study was also supported indirectly by mechanistic information. Physiologically, Sertoli cells support spermatogenesis. It is expected, therefore, that adverse effects to Sertoli cells (Poon *et al.*, 1997) will be reflected in the number, density or quality of sperm (David *et al.*, 2000a; Ganning *et al.*, 1991), especially when durations of exposure are increased.

Two studies reported toxic effect levels that were at or below that in David *et al.*'s paper (2000a). In the first study, exposure to DEHP by gavage for 3, 6, 9, or 12 months increased the incidence of focal cystic kidneys and decreased creatinine clearance in rats (Crocker *et al.,* 1988; ECB, 2008). These effects occurred at a LOAEL of 0.9 mg/kg-day. A dose-dependent increase in the mineralization of the renal papilla was also reported in male Fischer 344 rats exposed to Dietary DEHP for 78 or 105 weeks (David *et al.,* 2000a). This change occurred at 105 weeks at a LOAEL of 5.8 mg/kg-day. These studies were not used as hazard endpoints, however, because adverse effect findings were not repeated in other studies and also because the pathologies may be related to α_{2u} globulin, and are therefore not be relevant to the development of human pathologies.

All other DEHP-induced changes from long-term exposure occurred at much higher doses. Affected organ systems and lowest effector dose can be seen in "***Long-term Oral Exposures – General Population***".

The LOAEL of 5.8 mg/kg-day was used to generate an Acceptable Daily Intake (ADI) by dividing by an uncertainty factor of 1000 (10X for interspecies variation, 10X for intraspecies variation and sensitive populations, 10X for conversion of a LOAEL to a NOAEL). The 1000-fold "safety factor" is typically applied by CPSC to the lowest LO[A]EL for animal data in which developmental, reproductive, or neurotoxicological effects have been determined (16 CFR§1500.135(d)(4)(B)). Other federal agencies such as ATSDR have also historically used 10X factors each for intraspecies, interspecies, and LOAEL to NOAEL extrapolation (Pohl and Abadin, 1995). This magnitude of factor for LOAEL to NOAEL extrapolation has also been shown to be protective for 99% of the responses to mild adverse effects from other routes of exposure (inhalation; Alexeeff *et al.,* 2002). The relative severity of the adverse effect in this case (testicular atrophy) does not support the use of lower uncertainty factors for LOAEL to NOAEL extrapolation (Pohl and Abadin, 1995; Dourson *et al.,* 1996). **The long-term exposure oral ADI for male reproduction was calculated to be 0.0058 mg/kg-day.**

Developmental ADI

Maternal exposures – developmental effects

For developmental effects, the maternal dose LOAEL of 11 mg/kg-day was chosen as the representative overall hazard endpoint (Gray *et al.*, 2009; Table A3.46; Table A3.47). This endpoint was derived from a well conducted study in which pregnant Sprague-Dawley rat dams were dosed with DEHP via gavage (0, 11, 33, 100, 300 mg/kg-day; M) during Gd 8 to Ld17. Maternal DEHP doses of 11 mg/kg-day (LOAEL) significantly increased the incidence of pups with phthalate syndrome (PS; morphological and functional changes in reproductive organs similar to human TDS; 11.3% of pups affected). These doses did not induce any maternal toxicity.

Choice of the developmental study data for use as a hazard endpoint was supported by additional studies with slightly higher hazard effect levels. In the first study (Foster *et al.*, 2006b) DEHP-induced combined reproductive tract malformations (PS effects) were revealed in a RACB study that included additional animals from the non-bred cohort. The LOAEL based on these effects was slightly higher (15 mg/kg-day; NOAEL = 5 mg/kg-day (Table A3.34)) than that in the Gray *et al.* (2009) study. In the second study (NTP, 2006), the CERHR Panel determined that DEHP induced the formation of testicular abnormalities. The LOAEL from this experiment (14 to 23 mg/kg-day; NOAEL = 4.8 mg/kg-day) was higher than that in the Gray *et al.* (2009) study.

All other DEHP-induced developmental changes occurred at much higher doses (see Appendix 2).

The LOAEL of 11 mg/kg-day was used to generate an Acceptable Daily Intake (ADI) by dividing by an uncertainty factor of 1000 (10X for interspecies variation, 10X for intraspecies variation and sensitive populations, 10X for conversion of a LOAEL to a NOAEL). The 1000-fold "safety factor" is typically applied by CPSC to the lowest LO[A]EL for animal data in which developmental, reproductive, or neurotoxicological effects have been determined (16 CFR§1500.135(d)(4)(B)). Other federal agencies such as ATSDR have also historically used 10X factors each for intraspecies, interspecies, and LOAEL to NOAEL extrapolation (Pohl and Abadin, 1995). This magnitude of factor for LOAEL to NOAEL extrapolation has also been shown to be protective for 99% of the responses to mild adverse effects from other routes of exposure (inhalation; Alexeeff *et al.,* 2002). The relative severity of the adverse effect in this case (increased incidence of PS) did not support the use of lower uncertainty factors for LOAEL to NOAEL extrapolation (Pohl and Abadin, 1995; Dourson *et al.,* 1996). **The maternal exposure oral ADI for development was calculated to be 0.011 mg/kg-day.**

Insufficient evidence (hazard data) precluded the generation of ADI's for inhalation or dermal exposures or for cancer endpoints.

Other regulatory levels

ADI's proposed by CPSC were appended to information on other regulatory levels as summarized by GFEA (2007). This information revealed that CPSC ADI's were within the range of regulatory levels proposed by other federal and international organizations (Table 5.4). Toxicological endpoints used to generate CPSC's ADI's were also similar to those selected by other regulatory agencies.

Table 5.4 Summarized and Amended Regulatory Levels as Presented by GFEA (2007)

Term, Institution	Value (µg/kg-day)	Underlying NOAEL (mg/kg-day)	Uncertainty Factors	Reference; Source of Toxicology Endpoint
TDI[1] (Maximum Permissable Risk Level); RIVM	4	3.7	1000	RIVM, 2002; Poon *et al.*, 1997
ADI[2] for long-term oral exposures and reproductive deficits; CPSC	**5.8**	**5.8 (LOAEL)**	**1000**	**Carlson, 2010; David *et al.*, 2000a**
ADI[2] for maternal exposures during gestation and developmental deficits; CPSC	**11**	**11 (LOAEL)**	**1000**	**Carlson, 2010; Gray *et al.*, 2009**
RfD[3]; EPA	20	20 (LOAEL)	1000	EPA, 1991; Carpenter *et al.*, 1953
TDI[1]; ECB/EU (RAR-DEHP)	20 (0-3 mon. infants, women of childbearing age)	4.8	240	TDI; ECB/EU (RAR-DEHP), 2004; Wolfe *et al.*, 2003
ADI[2] for intermediate-term oral exposures of the general population; CPSC	**24**	**24 (LOAEL)**	**1000**	**Carlson, 2010; Bibra, 1990; ECB, 2008**
TDI[1]; ECB/EU (RAR-DEHP)	25 (3-12 mon. infants)	4.8	192	TDI; ECB/EU (RAR-DEHP), 2004; Wolfe *et al.*, 2003
TDI[1]; WHO	25	2.5	100	WHO, 2003
ADI[2] for intermediate-term oral exposures and reproductive deficits; CPSC, 2010	**37**	**3.7**	**100**	**Carlson, 2010; Poon *et al.*, 1997**
TDI[1]; EU CSTEE	37	3.7	100	EU CSTEE, 1998; Poon *et al.*, 1997; Arcadi *et al.*, 1998
TDI[1]; Health Canada	44	44	1000	Health Canada, 1994; Wolkowski-Tyl, 1984
TDI[1]; ECB/EU (RAR-DEHP)	48 (all other populations)	4.8	100	TDI; ECB/EU (RAR-DEHP), 2004; Wolfe *et al.*, 2003
TRD[4]; German UBA	50	2.9 (intake; 4.8)	58, 100	German UBA, 2003; Wolfe and

				Layton, 2003
TDI[1]; BfR and EFSA	50	4.8	100	BfR, 2005 and EFSA, 2005; Wolfe and Layton, 2003
ADI[2] for long-term oral exposures of the general population; CPSC	**58**	**5.8**	**100**	**Carlson, 2010; David *et al.*, 2000a; Moore, 1996**
MRL(chronic exposure)[5]; ATSDR	60	5.8	100	ATSDR, 2002; David *et al.*, 2000a;
ADI[2] for short-term oral exposures of the general population; CPSC	**100**	**10**	**100**	**Carlson, 2010; Dostal *et al.*, 1987a,b**
MRL(intermediate-term exposure)[5]; ATSDR	100	14	100	ATSDR, 2002; Lamb *et al.*, 1987

[1]Tolerable Daily Intake; [2]Acceptable Daily Intake; [3]Reference Dose (chronic exposure); [4]Tolerable Resorbed Dose; [5]Minimal Risk Level

Summary

The FHSA defines a "hazardous substance" as a substance that satisfies both parts of a two-part test. To be a hazardous substance, a product must first present one or more of the hazards enumerated in the statute (i.e., it must be "toxic", corrosive, flammable, an irritant, or a strong sensitizer, or generate pressure through decomposition, heat, or other means). Secondly, the product must have the potential to cause substantial personal injury or substantial illness during or as a result of any customary or reasonably foreseeable handling or use, including reasonably foreseeable ingestion by children. This hazard summary reviews evidence to support conclusions for the toxicity, corrosivity, irritancy, and sensitizing potential of DEHP.

When considering FHSA criteria, animal data were sufficient to support the conclusion that DEHP was not acutely toxic following single oral exposures. Acute oral toxicities (LD_{50}'s) for DEHP in rats (> 9800 to > 40,000 mg/kg), mice (> 9860 to > 31,360 mg/kg), rabbits (33,900 mg/kg), and guinea pigs (26,000 mg/kg) were in excess of the oral LD_{50} range (50 to 5000 mg/kg) necessary to be termed acutely toxic.

Sufficient animal data and limited human data also supported the conclusion that DEHP was not corrosive or a primary ocular or dermal irritant.

There was inadequate evidence to designate DEHP as an acute exposure dermal or inhalation toxicant. Each route of exposure had only one poorly described or performed study to evaluate when considering potential effects.

Similarly, there was inadequate evidence to designate DEHP as a sensitizer. Contrasting sensitization data were reported in animal (negative) and human (negative and positive) studies.

Sufficient animal data existed to support the conclusion that DEHP had acute, subchronic, and chronic toxicity in a variety of organs. DEHP-induced adverse effects were reported in animal test subject's reproductive organs, liver, kidney, and thyroid in numerous published studies.

Sufficient animal data existed to support the conclusion that DEHP was a rodent carcinogen and a reproductive and developmental toxicant. DEHP-induced carcinogenic effects were reported noted in animal liver, testes, and blood. DEHP-induced reproductive effects were reported in both male and female animal reproductive tissue. DEHP-induced developmental effects in animals occurred following doses that were not maternally toxic.

There was inadequate evidence to support the conclusion that DEHP was a neurotoxicant, a respiratory irritant, or a direct acting genotoxicant. Adverse effects were not observed or key evidence was missing in the majority of studies presented.

In evaluating the potential hazards presented by DEHP, the Commission has appropriately followed the definitions for toxicity (both acute and chronic), irritancy, and sensitization when considering the FHSA and its implementing regulations, 16 CFR §1500. At this time, there is insufficient data for the CPSC staff to conduct the second part of the analysis to determine what potential exposures to DEHP would be present if used in children's toys, child care articles, or other consumer products.

In summary, data supported the conclusion that DEHP can be considered "toxic" when considering FHSA criteria due to its toxicity following short-term, intermediate-term, and long-term exposures. This conclusion was based on the sufficient evidence in animals of DEHP-induced toxicity to the liver, kidney, testes, uterus, ovary, fetus, and thyroid.

When considering the FHSA criteria, products that contain DEHP may be considered "hazardous" if short-term, intermediate-term, or long-term exposures to the general population during "reasonably foreseeable handling and use" exceed the short-duration, intermediate-duration, or long-term ADI's for the general population (0.1, 0.024, and 0.058 mg DEHP/kg bw-day, respectively).

In addition, products that contain DEHP may be considered "hazardous" if intermediate-term, or long-term exposures to male populations during "reasonably foreseeable handling and use" exceed the intermediate-duration, or long-term ADI's for male reproduction (0.037 and 0.0058 mg DEHP/kg bw-day, respectively).

In addition, products that contain DEHP may be considered "hazardous" if exposures to reproductively viable female populations (13 to 49 years of age) during "reasonably foreseeable handling and use" exceed the ADI for development (0.011 mg DEHP/kg bw-day).

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Appendix 1. DEHP-induced Toxicokinetics

(retrieved from ECB, 2008; ATSDR, 2002; and IARC, 2000)

Toxicokinetic Studies with Discussion on Absorption					
Species (Gender Age Weight)	***Exposure Route***	***Dose (# test subjects)***	***Dose duration***	***Toxicokinetic Endpoint***	***Citation***
Cynomolgous monkey (M)	Oral gavage	Carbonyl^{14}C-DEHP; 100 or 500 mg/kg-day; (2 animals per group)	unlabeled once daily for 21 days, carbonyl^{14}C-labeled on day 22, unlabeled once daily for days 23-25	Absorption similar for two dose groups (as viewed by activity in urine), but AUC of higher dose higher than low dose - suggests dose-dependent reduction in absorption from tract or different or more efficient excretion pathway, or higher retention in body tissues at higher dose.	Short *et al.*,1987; Monsanto, 1988
Cynomolgous monkeys (M)	Oral gavage	Carbonyl-^{14}C-DEHP - 100 mg/kg (3 monkeys)	Once	Absorption is similar in monkeys, rats, and mice at dose of 100 mg/kg	CMA, 1982b; CMA, 1983; CMA, 1984a; Short *et al.*,1987; Astill *et al.*,1986
Marmoset monkeys (M/F; 12-18 months old)	Oral gavage	^{14}C-ring labeled-DEHP; 2000 mg/kg (3 M and 3 F per group)	14 days	At 24 hours - Uptake of activity into blood rapid, with peak at 1 hour (5 – M – 0.02% of total activity, 8 µg/g – F – 0.03% of total activity) At day 14 - Uptake of activity into blood rapid, with peak at 1 and 3hours for M and F (13 µg/g –M/F; 0.03% - M, 0.06% - F) – during recovery period 24 hours later these blood levels did not decline significantly Tissue activity in M/F were 29/47 µg/g (liver) and 15/35 µg/g (kidney)	ICI, 1982a; Shell, 1982; Rhodes *et al.*,1986
Marmoset monkeys (M)	Oral gavage Intravenous Intraperitoneal	^{14}C-ring labeled-DEHP; 100 and 2000 mg/kg – oral; 100 mg/kg – iv; 1000 mg/kg – ip (3 monkeys per group)	Once for each route	dose-dependent reduction in absorption from intestinal tract	Rhodes *et al.*,1983; Rhodes *et al.*,1986
Sprague-Dawley rats (25, 40, or 60 days old)	Oral gavage	1000 mg/kg (9-10 rats per group)	Once	Plasma DEHP (> 2mg/L) was found in some of the animals 1-7 hours post-dose. MEHP was detected in most plasma samples with the Cmax at 1 hour post-dose in most animals, but 3-7 hours in some (but no difference in Cmax between different age groups, mean Cmax=93µg/mL) – mean AUC of MEHP was 1213 (25 days old), 611 (40 days old), 555 (60 days old) µg.hr/mL – mean plasma elimination half-life of MEHP was same for all age groups (2.8-3.9 hours) – 98% of MEHP bound to plasma proteins at all doses	Sjoberg *et al.*,1985c
Sprague-Dawley rats (M)	Oral gavage; Intra-arterial; Intraperitoneal	2000 mg/kg; 100 mg/kg; 4000 mg/kg	Once via gavage, followed by a 30 hour recovery then once daily for 7 days	DEHP absorbed quickly from oral exposures; the rate and extent of absorption from the peritoneal cavity was decreased following multiple injections	Pollack *et al.*,1985a
Fischer 344 rats (M)	Oral gavage	1-^{14}C-ethylhexanol (2 rats)	?	2-EH absorbed rapidly	Albro, 1975
CD-1 mice	Oral gavage	1.8 to 1000 mg/kg	Once	6 hours post dose – absorption threshold could not be determined (b/c higher level of DEHP-hydrolase in intestines?)	Albro *et al.*,1982a
C3B6F$_1$ mice	Oral gavage	1.8 to 1000 mg/kg	Once	6 hours post dose – absorption threshold could not be determined (b/c higher level of DEHP-hydrolase in intestines?)	Albro *et al.*,1982a

Toxicokinetic Studies with Discussion on Distribution					
Species (Gender Age Weight)	***Exposure Route***	***Dose (# test subjects)***	***Dose duration***	***Toxicokinetic Endpoint***	***Citation***
Human (M, 61 yr, 75kg)	Oral feeding	Deuterium-labelled-DEHP; 48.1 mg (0.64 mg/kg bw; 1 person)	Once	Peak concentrations in serum at 2 hours – Adsorption and distribution phase of 4-8 hours	Koch *et al.*,2003
Cynomolgous monkey (M)	Oral gavage	Carbonyl^{14}C-DEHP; 100 or 500 mg/kg-day; (2 animals per group)	unlabeled once daily for 21 days, carbonyl^{14}C-labeled on day 22, unlabeled once daily for days 23-25	At 500 mg/kg, < 0.2% of total dose detected in liver and intestines - AUC (plasma concentration curve) for up to 48 hours was 133-283 µg-hr/ml (low dose), and 387-545 µg-hr/ml (high dose)	Short *et al.*,1987; Monsanto, 1988
Cynomolgous monkey (M)	Oral gavage	Carbonyl^{14}C-DEHP; 100 or 500 mg/kg-day; (2 animals per group)	unlabeled once daily for 21 days, carbonyl^{14}C-labeled on day 22, unlabeled once daily for days 23-25	Absorption similar for two dose groups (as viewed by activity in urine), but AUC of higher dose higher than low dose - suggests dose-dependent reduction in absorption from tract or different or more efficient excretion pathway, or higher retention in body tissues at higher dose.	Short *et al.*,1987; Monsanto, 1988
Cynomolgous monkeys (M)	Oral gavage	Carbonyl-^{14}C-DEHP - 100 mg/kg (3 monkeys)	Once	Activity detected in some tissues at a mean of < 1 mg/kg, with liver having the highest activity	CMA, 1982b; CMA, 1983; CMA, 1984a; Short *et al.*,1987; Astill *et al.*,1986
Marmoset monkeys (M/F; 12-18 months old)	Oral gavage	^{14}C-ring labeled-DEHP; 2000 mg/kg (3 M and 3 F per group)	14 days	At 24 hours - Uptake of activity into blood rapid, with peak at 1 hour (5 – M – 0.02% of total activity, 8 µg/g – F – 0.03% of total activity) At day 14 - Uptake of activity into blood rapid with peak at 1 and 3 hours for M and F (13 µg/g –M/F; 0.03% - M, 0.06% - F) – during recovery period 24 hours later these blood levels did not decline significantly Tissue activity in M/F were 29/47 µg/g (liver) and 15/35 µg/g (kidney)	ICI, 1982a; Shell, 1982; Rhodes *et al.*,1986
Sprague-Dawley rats (25, 40, or 60 days old)	Oral gavage	1000 mg/kg (9-10 rats per group)	Once	Plasma DEHP (> 2mg/L) was found in some of the animals 1-7 hours post-dose. MEHP was detected in most plasma samples with the Cmax at 1 hour post-dose in most animals, but 3-7 hours in some (but no difference in Cmax between different age groups, mean Cmax=93µg/mL) – mean AUC of MEHP was 1213 (25 days old), 611 (40 days old), 555 (60 days old) µg.hr/mL – mean plasma elimination half-life of MEHP was same for all age groups (2.8-3.9 hours) – 98% of MEHP bound to plasma proteins at all doses	Sjoberg *et al.*,1985c
Sprague-Dawley rats (M, 35 days old)	Oral gavage	2.7 mmol/kg (5 rats per group)	Once, and once daily for 7 days	MEHP metabolite plasma concentrations and mean AUC's were less than MEHP following single or multiple doses – max plasma concentrations of MEHP (0.55, 0.56 µmol/mL) and metabolites IX (0.15, 0.09 µmol/mL), VI (0.06, 0.07 µmol/mL), and V (0.06, 0.09 µmol/mL) and mean AUCs of MEHP (5.15, 3.44 µmol/mL) and metabolites IX (0.84, 0.46 µmol/mL), VI (0.44, 0.41 µmol/mL), and V (0.39, 0.43 µmol/mL) were not significantly different between animals given single or multiple doses of DEHP - Mean plasma elimination t½ of MEHP for multiple dose = 1.8 hours versus single dose=3 hours	Sjoberg *et al.*,1986a
Sprague-Dawley rats (M)	Oral gavage; Intra-arterial; Intraperitoneal	2000 mg/kg; 100 mg/kg; 4000 mg/kg	Once via gavage, followed by a 30 hour recovery then once daily for 7 days	DEHP peak in blood at 3 hours; bioavailability of DEHP = 13%; MEHP in blood at higher conc than DEHP; single dose blood concentrations of DEHP same as multiple dose blood concentrations; intra-arterial injections resulted in large apparent volume of distribution and high clearance rate	Pollack *et al.*,1985a

Sprague-Dawley rats (lactating)	Oral gavage	2000 mg/kg	Once daily for 3 days during lactation days 15-17	MEHP (76µg/mL), but "virtually no" DEHP, in plasma 6 hours post-3rd dose; MEHP (25µg/mL) and DEHP (216µg/mL) found in milk 6 hours following 3rd dose	Dostal *et al.*,1987a
Sprague-Dawley rats (M; fasted)	Oral gavage	2-hexyl-^{14}C-DEHP; 100 mg/kg	Once	Liver and abdominal fat had 6 and 4 times as much activity (respectively) as carcass and other tissues	Eastman Kodak Co., 1983
Sprague-Dawley rats (M; 5 weeks old)	Oral gavage	Carbonyl-^{14}C-DEHP; 100 mg/kg (5 rats), 1000 mg/kg (5 rats)	Once	Marginal activity in liver, kidney, or total gut contents at 96 hours post-dose	Lake *et al.*,1984b
Sprague-Dawley rats (M; adult; 200-300g)	Oral feeding	50 mg/kg-day unlabeled DEHP prior to 50 mg/kg carbonyl-^{14}C-DEHP; (6 rats per group)	Unlabeled dose for 21-28 days prior to single dose of labeled DEHP, then unlabeled dose until sacrificed	At day 1, substantial activity in gastrointestinal tract – At day 4, minimal activity in GI tract – Other organs have minimal activity – liver has highest activity (2% of total dose) after 4 hours – Bile accounted for < 1% of total dose	Ikeda *et al.*,1980
Sprague-Dawley rats (M)	Oral gavage and carotid artery cannula	7-^{14}C-MEHP/kg (69mg, 20µCi; 4 rats per group) (35 mg; 4 rats per group) (69mg, 20µCi; 6.9mg, 2µCi; 4 rats per group)	Once via gavage; Once via cannula; Once via gavage Once via gavage	With gavage, MEHP peaked in blood at 0.5 hours – Blood concentration also increased to a small extent 5 hours after dosing and then slowly decreased With IA exposures, 53% of the activity was in blood in first sample. After 10 and 20 minutes, activity was 1/3 and 1/5 in blood – 20 minutes after IA, activity high in liver, bladder, and kidney and other tissues had lower activity For high 69mg dose, activity almost gone from body and only marginal amounts left in kidney, liver, heart, lungs, intestine, and muscle For low 6.9 mg dose, no activity in tissues 24 hours post-dose	Chu *et al.*,1978
JCL:Wistar rats (M, 200 g)	Oral gavage	25 mmol DEHP/kg (~9765 mg/kg)	Once	DEHP and MEHP in blood and tissue increased to peak in 6-24 hours post dose; Highest conc of DEHP and MEHP in heart and lungs with 1 hour after dosing; DEHP and MEHP detected in brain and kidney at low conc; Low conc of MEHP in lungs and DEHP in spleen; DEHP in fat peaked at 48 hours post-dose; Liver DEHP t½ = 1 day; epididymal fat DEHP t½ = 6.5 day; at 6 hours post-dose, testes had highest MEHP/DEHP ratio (2.1), with blood 1.1, and other tissues < 1; DEHP t½ in testicular tissue (8 hours), in epididymal fat (156 hours); MEHP t½ in blood (23 hours) and epididymal fat (68 hours)	Oishi and Hiraga, 1982
Wistar rats (M, 35 days old)	Oral gavage	2000 mg/kg	Once	MEHP in blood and testes peaked at 6 hours; MEHP t½ in blood (7.4 hours; AUC = 1497 µg.h per ml) and testes (8.0 hours; AUC = 436 µg.h per ml)	Oishi, 1990
Wistar rats (M; young; 100-200g)	Oral gavage	Unlabelled and Carbonyl-^{14}C-DEHP - 2000 mg/kg	Once daily for 0, 6, or 13 days; followed by once with Radiolabeled DEHP	At 4 days post-dose, < 0.1% of activity in tissues and organs	Lake *et al.*,1975
Wistar rats (M; 150-250 g)	Oral gavage or intravenous	Carbonyl-^{14}C-DEHP; 500 mg/kg (gavage), 50 mg/kg (iv) (2 or 3 rats per group)	Once	At 6 hours post-dose peak activity in blood; At 2-6 hours, peak in liver and kidney; no retention in brain, heart, lungs, liver, spleen, kidney, stomach, intestine, testicle, blood, muscle, or adipose tissue	Tanaka *et al.*,1975
Wistar rats (M; 150-250 g)	Oral gavage or intravenous	Carbonyl-^{14}C-DEHP; 500 mg/kg (gavage), 50 mg/kg (iv) (2 or 3 rats per group)	Once	Post IV exposure, 75% of activity recovered in liver by 1 hour, 50% by 2 hours, and 0.17% by 7th day - Intestine also had highest activity which increased as liver decreased – activity in liver and kidneys peak at 2-6 hours following iv exposure –moderate activity also in heart lungs and spleen, with blood activity peaking at 6 hours – testicle and brain had lowest concentrations	Tanaka *et al.*,1975
Wistar rats (F)	Oral feeding	Carbonyl-^{14}C-DEHP; 1000, 5000 mg/kg	35 and 49 days respectively	Rapid equilibrium of activity in liver and abdominal fat without accumulation	Daniel and Bratt, 1974
Wistar rats (F)	Oral feeding	Carbonyl-^{14}C-DEHP; 1000, 5000 mg/kg	35 and 49 days respectively	Liver t½ = 1-2 days; fat t½ = 3-5 days	Daniel and Bratt, 1974
Wistar rats	Intravenous injection	^{14}C-DEHP/kg	Once	Activity moved quickly from blood; by 2 hours, 60-70% in liver and lung; by 4 days, 44% in urine, 29% in feces, 1% in fat	Daniel and Bratt, 1974

Fischer 344 rats (M)	Oral gavage	^{14}C; 1.8, 18, 180 mg/kg (1.8 mg/kg ^{14}C; 12 rats per group)	Ten daily for 1 day	DEHP did not accumulate in liver or testes (but testes had lower activity)	Albro *et al.*,1982a
Fischer 344 rats	Oral gavage	1.8 to 1000 mg/kg	Once	6 hours post dose – above threshold of 450 mg/kg dose, increasing amount of DEHP in liver ("intact DEHP will reach liver when concentration in diet exceeds 0.43%")	Albro *et al.*,1982a
Fischer 344 rats (M; 100-150 g)	Oral feeding	1000, 6000, 12000 mg/kg (~85, 550, 1000 mg/kg-day; 12 rats per group – 4 rats per time) followed by 24 hours of carbonyl-^{14}C	0, 6, or 20 days followed by 24 hours of Radiolabeled DEHP	At 112-116 hours after ^{14}C, primary activity in intestinal contents – other sources in liver, fat, kidney, and adrenal glands – pretreatment with unlabeled DEHP did not affect distribution of activity	CMA, 1982a; Lington *et al.*,1987; Short *et al.*,1987; Astill *et al.*,1986
Fischer 344 rats (pregnant)	Oral gavage	1000 mg/kg	Once daily during Gd 6-15	DEHP can cross placenta and was detected in fetal livers	Srivastava *et al.*,1989
Fischer 344 rats	Oral gavage	2000 mg/kg (5 mothers each group/7 pups each mother)	From day 1 to day 21 of birth	DEHP in livers of pups of treated dams (DEHP transfer through milk)	Parmar *et al.*,1985
C57BL mice (M; 10-12 g)	Oral gavage	Carbonyl^{14}C-DEHP; 6.72 mg (3 groups of 1 control animal, 8 treated)	Once	No evidence of storage in tissues, and whole body distribution – radioactivity largely in stomach and small intestine up to 24 hours post dose – only slight activity at 72 hours – ^{14}C in cecal contents by 1 hour, maximum at 2 hours, lasted for 1 day, and was only found in one animal by day 3. – No activity in colon or feces by 1 hour, maximum at 2 and 4 hours, respectively and minimal by 72 hours – In bladder, activity at 1-24 hours, and less activity by day 3 – In kidney, activity concentrated in the renal pelvis and papillae – In testes and kidney parenchyma, activity similar to general tissue levels	Gaunt and Butterworth, 1982
C57BL mice (M)	Intravenous	10 µCi (9.6 mg/kg) 2-ethylhexyl-1-^{14}C (2 mice); 10 µCi (3.6 mg/kg) carbonyl^{14}C (2 mice)	Once	4 hours post admin - Gall bladder, intestinal contents, urinary bladder, liver, kidney, and brown fat – large amount of activity - Medium activity in white fat, myocardium, and muscles. Low activity in the blood, bone, cartilage, testes, and nervous system 24 hours post admin. – gall bladder, intestinal contents, and urinary bladder still high, brown fat high, but liver and kidney was lower	Lindgren *et al.*,1982
C57BL mice (M)	Oral gavage	10 mg/kg ^{14}C-DEHP (4 mice) followed by 10 µCi DEHP at 24 hours after admin	Once daily for 5 days	24 hour distribution same in pretreated animals as in those without pretreatment – brown fat conc of DEHP higher in pretreatment group	Lindgren *et al.*,1982
C57BL mice (pregnant; gestation day 8, 16)	Oral gavage	10µCi ^{14}C-DEHP (six mice per group); 7.7 mg/kg (2-ethylhexyl-1-^{14}C and 2.9 mg/kg carbonyl^{14}C-DEHP (mice at Gd 8); 4.8 mg/kg (2-ethylhexyl-1-^{14}C and 1.8 mg/kg carbonyl^{14}C-DEHP (mice at Gd 8)	Once	At early gestation – uptake into yolk sac; at 4 hours – high activity in embryo gut on Gd 8; at 24 hours – high activity in embryo neuroepithelium and uterine fluid (other organs low); in late gestation, large activity in yolk sac; at 4 hours on Gd 16, large activity in renal pelvis, urinary bladder, and intestinal contents – moderate activity in skeleton and liver; on Gd 17, marginal activity in fetuses except for a high activity in renal pelvis, urinary bladder, and intestinal contents	Lindgren *et al.*,1982
NMRI mice (3-20 days old)	Oral gavage	7-^{14}C-DEHP; 0.7 mg/kg	Once	Activity and retention in brain low in 10 and 20 day old mice; At 24 hours activity in liver ranged from 27 to 2% in 3, 10, and 20 day mice, respectively; 7 days recovery induced significant decreases in liver activity	Eriksson and Darnerud, 1985

$B6C3F_1$ mice (M)				DEHP in many tissues at < 1 mg/kg; Highest conc in fat; total recovery of activity 90% (63-102%)	CMA, 1982b; CMA, 1983; CMA 1984a; Short *et al.*,1987; Astill *et al.*,1986
DSN Syrian golden hamsters (M; 5 week old)	Oral gavage	Carbonyl-^{14}C-DEHP; 100, 1000 mg/kg (3 or 5 hamsters per group)	Once (followed by 96 hours of recovery)	Negligible amounts of radioactivity in liver, kidneys, or total gut	Lake *et al.*,1984b
Dogs				A large amount of DEHP/metabolites in gastrointestinal tract at 1 day, only a small amount remains at day 4	Ikeda *et al.*,1980
Miniature pigs				A large amount of DEHP/metabolites in gastrointestinal tract at 1 day, only a small amount remains at day 4	Ikeda *et al.*,1980
Piglets (33-50 kg)	Oral feeding	5 g/day (~125 mg/kg-day; 4 piglets DEHP, 2 piglets control)	14 days with 0, 14, and 28 day recovery	Body/tissue/organ weights not affected – DEHP in subcutaneous fat (0.42 mg/kg), renal fat (0.37 mg/kg), muscle (2.4 mg/kg), heart (< 0.2 mg/kg), lungs (0.25 mg/kg), and kidney (< 0.2 mg/kg), but not in brain – in 14 day recovery, DEHP decreased 50% in subcutaneous fat, renal fat, muscle, heart, and lungs – in 28 days recovery, DEHP decreased to controls in all tissues except renal fat and lungs - MEHP increased in liver, whole blood, and urine (with great variation), returned to control levels by 14 days recovery (DEHP in organs possibly due partially to feed, which contained 0.4mg/kg DEHP – muscle biotransfer factor of 0.125d/kg)	Jarosova *et al.*,1999
Broiler hens (750g)	Oral feeding	100 mg/day (~135 mg/kg-day; 18 hens per group)	14 days with 0, 14, and 28 day recovery	DEHP in mesenteric fat (0.33 mg/kg), skin (3.8 mg/kg), muscle (2.5 mg/kg), liver (0.47 mg/kg). MEHP in liver (< 0.01 mg/kg whole tissue) whole blood (7X control level). Day 14 of recovery levels of DEHP > 50% less than after dosing for muscle, skin, fat, liver. MEHP in liver and blood to control levels by recovery day 14 (DEHP in organs possibly due to feed, which contained 1mg/kg DEHP)	Jarosova *et al.*,1999

Toxicokinetic Studies with Discussion on Metabolism

Species (Gender Age Weight)	***Exposure Route***	***Dose (# test subjects)***	***Dose duration***	***Toxicokinetic Endpoint***	***Citation***
Human (M; 34, 47 yr old)	Oral feeding	30 mg single dose; 10 mg/day repeat dose (2 people)	Once or once daily for 4 days	Single dose - 12 urinary metabolites - MEHP (6.4, 12.7%), Metabolite I (1.9, 2.1%), IV (3.7,1.8%), V (25.6, 33.8%), VI (24.0, 19.7%), VII (5.3, 4.0%), IX (33.0, 25.9%), other metab < 1% - 65% of metab conjugated Calculated conversion factors for MEHP (2.4%), Metabolite VI (5.5%), and IX (7.4%)	Schmid and Schlatter, 1985
Human	Oral feeding	213 mg single dose; 70 mg/day repeat dose (1 person)	Once or once daily for 3 days	Single dose – 55% of recovered metabolites = MEHP – 21 other metabolites identified (99% of these conj. to β–glucuronic acid)	Bronsch, 1987
Human (M, 61 yr, 75kg)	Oral feeding	Deuterium-labelled-DEHP; 48.1 mg (0.64 mg/kg bw; 1 person)	Once	MEHP primary metabolite – serum t½ of MEHP and metabolites VI and IX was < 2 hours – 5OH and 5oxo are major metabolites of DEHP	Koch *et al.*,2003
Cynomolgous monkey (M)	Oral gavage	Carbonyl^{14}C-DEHP; 100 or 500 mg/kg-day; (2 animals per group)	unlabeled once daily for 21 days, carbonyl^{14}C-labeled on day 22, unlabeled once daily for days 23-25	0-24 hour urine samples MEHP (1^o), phthalic acid (1^o), metabolites I, III, IV, V(1^o), VI, IX(1^o), X(1^o), XII(1^o), XIII, XIV, and unidentified Polar components (inc glucuronides) was a small percent of activity Metabolite VI believed to be the stimulant to induce peroxisome proliferation in rodents)	Short *et al.*,1987; Monsanto, 1988

Cynomolgous monkeys (M)	Oral gavage	Carbonyl-^{14}C-DEHP - 100 mg/kg (3 monkeys)	Once	0-24 hour urine samples – 14 metabolites – MEHP (1^o; 11%), phthalic acid, metabolites I, III, IV, V (1^o), VI, VII, IX (1^o), X (1^o), XII, XIII, XIV, and unidentified – glucuronide conjugates may be 15-26% of excreted activity MEHP metabolized into X, V, and I (collective 34% of activity) via the ω-oxidation pathway and IX and VI (collective 19%) via the ω-1 oxidation pathway - β-oxidation pathway not very important in monkeys 0-48 hour fecal samples – 10 metabolites – DEHP (1^o), MEHP, phthalic acid, metabolites I-IV, VI, VII, IX, X, XII, XIV	CMA, 1982b; CMA, 1983; CMA, 1984a; Short *et al.*,1987; Astill *et al.*,1986
Marmoset monkeys (M/F; 12-18 months old)	Oral gavage	^{14}C-ring labeled-DEHP; 2000 mg/kg (3 M and 3 F per group)	14 days	DEHP identified as 98% of activity in feces	ICI, 1982a; Shell, 1982; Rhodes *et al.*,1986
Sprague-Dawley rats (M)	Oral gavage; Intra-arterial; Intraperitoneal	2000 mg/kg; 100 mg/kg; 4000 mg/kg	Once via gavage, followed by a 30 hour recovery then once via gavage daily for 7 days	MEHP formation strongly dependent on route of administration; Following oral exposure, 80% of DEHP undergoes conversion to MEHP – Following intra-arterial or intraperitoneal exposures, 1% of DEHP undergoes conversion to MEHP	Pollack *et al.*,1985a
Sprague-Dawley rats (M; 250-350g)	Oral gavage	7-^{14}C-DEHP or 7-^{14}C MEHP; 100 mg	Once daily for 2 days	Twenty metabolites in urine with metabolites from DEHP and MEHP treatment being identical – Conjugates were not detected	Albro *et al.*,1983
Sprague-Dawley rats (M; 300g)	Oral gavage	7-^{14}C-DEHP; 100 mg (1 rat)	Once and then again after 7 days	Activity profiles qualitatively similar suggesting that metabolites were produced physiologically and not via bacteria in the urine	Albro *et al.*,1983
Sprague-Dawley rats (M; 300-400g; adult)	Oral gavage	7-^{14}C-DEHP; 200μL (196 mg)	Once daily for 2 days	Urinary metabolites identified as phthalic acid (< 3%), and metabolites I, V, VI, IX (resulting from ω- and ω-1 oxidation of MEHP; MEHP is metabolized like a fatty acid); MEHP and conjugates not in urine	Albro *et al.*,1973
Sprague-Dawley rats (M; adult; 200-300g)	Oral feeding	50 mg/kg-day unlabeled DEHP prior to 50 mg/kg carbonyl-^{14}C-DEHP; (6 rats per group)	Unlabeled dose for 21-28 days prior to single dose of labeled DEHP, then unlabeled dose until sacrificed	Four urinary metabolites identified – with a trace of DEHP in urine	Ikeda *et al.*,1980
Sprague-Dawley rats (M)	Oral gavage and carotid artery cannula	7-^{14}C-MEHP/kg (69mg, 20μCi; 4 rats per group) (35 mg; 4 rats per group); (69mg, 20μCi; 6.9mg, 2μCi; 4 rats per group); (69mg, 20μCi; 4 rats per group)	Once via gavage; Once via cannula; Once via gavage Once via gavage	Four DEHP metabolites idenftified	Chu *et al.*,1978
Wistar rats (M; 150-250 g)	Oral gavage or intravenous	Carbonyl-^{14}C-DEHP; 500 mg/kg (gavage), 50 mg/kg (iv) (2 or 3 rats per group)	Once	Four urinary metabolites	Tanaka *et al.*,1975
Wistar rats (F)	Oral feeding	Carbonyl-^{14}C-DEHP; 1000, 5000 mg/kg	35 and 49 days respectively	14 urinary metabolites; MEHP, no DEHP, phthalic acid, metabolites IV, V, VI, IX	Daniel and Bratt, 1974?
Wistar rats (M, 180-220 g)	Oral gavage	7-^{14}C-DEHP/^{14}C-MEHP; 50 or 500 mg/kg (3 rats per group)	Once daily for 3 days	No conjugates detected after DEHP or MEHP; Single dose – no dose differences - major metabolites in urine were I, V, VI, IX Multiple dose – at high dose – increased metabolites I, V, and decreased VI, IX	Lhuguenot *et al.*,1985

Fischer 344 rats (M; 100-150 g)	Oral feeding	1000, 6000, 12000 mg/kg (~85, 550, 1000 mg/kg-day; 12 rats per group – 4 rats per time) followed by 24 hours of carbonyl-^{14}C-DEHP	0, 6, or 20 days followed by 24 hours of radiolabeled DEHP	14 urinary metabolites identified as phthalic acid, metabolites I, II, III, IV, V, VI, VII, IX, X, XII, XIII, XIV, and unidentified, with the major metabolites I and V, and to a lesser extent IX and phthalic acid (NO DEHP or MEHP in urine) 15 fecal metabolites identified as DEHP, MEHP, phthalic acid, metabolites I-V (pooled), VI, VII, IX, X, XII, XIII, and XIV with major components being MEHP, metabolites I-V, VI, and IX Proportion of metabolites changed with dose and prior exposure to DEHP (primarily dif with 85 and 550 mg/kg-day; and with prior exposure of 0-6 days) Urinary excretion of metabolite I constant at all doses, but increased with prior exposure, of metabolite V increased with dose, but remained constant or decreased with prior exposure (suggests that at high doses rats ability to convert V to I is saturated; also suggests capacity for β-oxidation increases with repeat exposure to DEHP)	CMA, 1982a; Lington *et al.*,1987; Short *et al.*,1987; Astill *et al.*,1986
Fischer 344 rats	Oral gavage	carbonyl-^{14}C-DEHP; 180 mg/kg	Once daily for 2 days	Metabolites with carboxyl groups (I-V) primarily excreted (after 3-6 oxidative steps)	Albro *et al.*,1982b
Fischer 344 rats	Oral gavage	carbonyl-^{14}C-DEHP; 180 mg/kg	Once daily for 2 days	No conjugation of DEHP metabolites	Albro *et al.*,1982b
Fischer 344 rats (M)	Oral gavage	1-^{14}C-ethylhexanol (2 rats)	?	Identified metabolites as 2-ethyl-5-hydroxyhexanoic acid, 2-ethyl-5-ketohexanoic acid, 2-ethyl-1,6-hexandioic acid and 3% excreted unchanged – 2-EH metabolized through oxidation pathways (ω- and ω-1) and then β-oxidation to acetate and carbon dioxide	Albro, 1975
CD-1 mice (M)	Oral gavage	Carbonyl^{14}C-DEHP; 360 mg/kg	Once daily for 2 days	Dimethyl phthalate, MEHP, metabolites I, VI, and IX were primary metabolites; MEHP 19% of total metabolites	Albro *et al.*,1982b
B6C3F$_1$ mice (M)				0-24 hour urine samples 15 metabolites – MEHP, phthalic acid, metabolites I, II, III, IV, V, VI, VII, IX, X, XII, XIII, XIV, and unidentified – primary metabolites = MEHP, phthalic acid, metabolites I, VI, IX, and XIII 0-24 hour feces samples 10 metabolites – DEHP, MEHP, phthalic acid, metabolites I-IV, VI, VII, IX, X, XII, XIII, with DEHP and MEHP being the major metabolites	CMA, 1982b; CMA, 1983; CMA 1984a; Short *et al.*,1987; Astill *et al.*,1986
Mice	Oral gavage	^{14}C-MEHP; 400 mg/kg	Once	Primary metabolites were glucuronides; Three less important metabolites were conjugates of β-glucose (glucosidation)	Egestad and Sjoberg, 1992
Mice (M)	Oral gavage	Carbonyl^{14}C-MEHP; 400 mg/kg (11 mice); activity of 6.1μCi/mmol for mice	Once	MEHP primarily glucuronidated – MEHP glucuronide and metabolite glucuronides evenly split as primary conjugates; conjugates of β-glucose found in urine (3% of administered dose)	Egestad *et al.*,1996
Dunkin Hartley guinea pigs (M)	Oral gavage	Carbonyl-^{14}C-DEHP;	Twice, once each day	MEHP was primary metabolite (70% of total)	Albro *et al.*,1982b
Dunkin Hartley guinea pigs (M)	Oral gavage	Carbonyl-^{14}C-DEHP;	Twice, once each day	Glucuronide conjugates were the only conjugate detected	Albro *et al.*,1982b
Dunkin Hartley guinea pigs (M)	Oral gavage	Carbonyl-^{14}C-MEHP;	Once	Glucuronidation is a major conjugation pathway for MEHP – MEHP glucuronide is the dominating metabolite – No β-glucose conjugates	Egestad *et al.*,1996
Syrian golden hamsters (M)	Oral gavage	Carbonyl-^{14}C-DEHP;	Twice, once each day	Main metabolites dimethyl phthalate, metabolites I, V, VI, and IX – MEHP 5% of total	Albro *et al.*,1982b
DSN Syrian golden hamsters (M; 5 week old)	Oral gavage	Carbonyl-^{14}C-DEHP; 100, 1000 mg/kg (3 or 5 hamsters per group)	Once (followed by 96 hours of recovery)	0-24 hour fecal samples from high and low dose 95% DEHP, remainder MEHP and other metabolites	Lake *et al.*,1984b
Dogs				Three metabolites	Ikeda *et al.*,1980
Miniature pigs				Five metabolites	Ikeda *et al.*,1980

Toxicokinetic Studies with Discussion on Excretion

Species (Gender Age Weight)	***Exposure Route***	***Dose (# test subjects)***	***Dose duration***	***Toxicokinetic Endpoint***	***Citation***
Human (M; 34, 47 yr old)	Oral feeding	30 mg single dose; 10 mg/day repeat dose (2 people)	Once or Once daily for 4 days	Single dose - Excretion of DEHP primarily in first 24 hours with a urinary elimination t½ of 12 hours – 11 and 15% of admin. dose eliminated in urine Repeat dose – 15 and 25% of admin. dose eliminated in urine. Excretion of metabolites fluctuated daily	Schmid and Schlatter, 1985
Human	Oral feeding	213 mg single dose; 70 mg/day repeat dose (1 person)	Once or once daily for 3 days	Single dose – 16, 28, 31% of admin. dose eliminated in urine by 4, 24, and 47hr Repeat dose – 27% of admin. dose eliminated in urine by 47 hr	Bronsch, 1987
Human	Oral feeding	^{13}C-labelled-DEHP; 168-255 µg, 336-510 µg (each group with 8 people)	Once	Mean excretion for coeluting MEHP and mono-isooctylphthalate (MIOP) is 13% Calculated conversion factor for MEHP = 0-26%	Anderson *et al.*,2001
Human (M, 61 yr, 75kg)	Oral feeding	Deuterium-labelled-DEHP; 48.1 mg (0.64 mg/kg bw; 1 person)	Once	Multiphase excretion in urine – At 8-16 hours post-dose, urine elimination t½ = 2 hours for MEHP, 5OH-MEHP, 5oxo-MEHP – At 14-18 hours post-dose, urine elimination t½ = 5 hours for MEHP, 10 hours for 5OH-MEHP and 5oxo-MEHP – - the ratio of MEHP to the metabolites varies over time (1 to 4.9 during phase I at 8 to 16 hours post-dose; 1 to 14.3 during phase II at 16 to 24 hours post-dose; 1 to 74 during phase III at 44 hours post-dose) - 47% of DEHP activity excreted in urine as 3 metabolites (MEHP, 7.3%; 5OH, 24.7%, 5oxo, 14.9%) after 44 hours (ratio =1 to 5.4 [contrasting 1 to 8.7 and 1 to 14.1 reported by other authors)	Koch *et al.*,2003
Cynomolgous monkey (M)	Oral gavage	Carbonyl^{14}C-DEHP; 100 or 500 mg/kg-day; (2 animals per group)	unlabeled once daily for 21 days, carbonyl^{14}C-labeled on day 22, unlabeled once daily for days 23-25	At low dose and 96 hours, excretion 20-55% (urine) and 49-39% (feces) At high dose and 96 hours, excretion 4-13% (urine) and 69-56% (feces) Majority of activity excreted in first 24 hours and most of the remainder by 48 hours	Short *et al.*,1987; Monsanto, 1988
Cynomolgous monkeys (M)	Oral gavage	Carbonyl-^{14}C-DEHP - 100 mg/kg (3 monkeys)	Once	Urinary excretion – 28.2% of total activity during first 24 hours Fecal excretion – 49.0% of total activity during first 48 hours Total recovery of activity was 68-91%	CMA, 1982b; CMA, 1983; CMA, 1984a; Short *et al.*,1987; Astill *et al.*,1986
Marmoset monkeys (M)	Oral gavage Intravenous Intraperitoneal	^{14}C-ring labeled-DEHP; 100 and 2000 mg/kg – oral; 100 mg/kg – iv; 1000 mg/kg – ip (3 monkeys per group)	Once for each route	Intravenous exposure – 40% activity excreted in urine, 20% excreted in feces, 28% remained in lungs, minimal % in other tissues Intraperitoneal exposure – 10% activity in urine, 4% excreted in feces, 85% remained in peritoneal cavity, 0.6% in tissues Oral low dose – 20-40% activity in urine, 25% excreted in feces, Oral high dose – 4% activity in urine, 84% excreted in feces, < 0.1% remained in tissues (dose-dependent reduction in absorption from intestinal tract)	Rhodes *et al.*,1983; Rhodes *et al.*,1986
Marmoset monkeys (M/F; 12-18 months old)	Oral gavage	^{14}C-ring labeled DEHP; 2000 mg/kg (3 M and 3 F per group)	14 days	After day 6 dosing – M excreted activity in urine (1%) and feces (64%) – F excreted activity in urine (2%) and feces (75%) After day 13 dosing - M excreted activity in urine (1%) and feces (59%) – F excreted activity in urine (1%) and feces (71%)	ICI, 1982a; Shell, 1982; Rhodes *et al.*,1986
Sprague-Dawley rats (25 or 60 days old)	Oral gavage	Carbonyl-^{14}C-DEHP; 1000 mg/kg (6 rats per group)	Once	Urinary activity was 44 (25 day old rats) and 26% (60 day old rats) by 72 hours – 85% of urinary radioactivity detected within 24 hours - no unmetabolized DEHP or MEHP detected in urine	Sjoberg *et al.*,1985c
Sprague-Dawley rats (M; fasted)	Oral gavage	2-hexyl-^{14}C-DEHP; 100 mg/kg	Once	62% of activity excreted in the feces; 34% DEHP, 4% MEHP 30% of activity excreted in the urine as metabolites 4% of activity excreted in the air < 2% recovered in carcass and tissues	Eastman Kodak Co., 1983
Sprague-Dawley rats (M; 5 weeks old)	Oral gavage	Carbonyl-^{14}C-DEHP; 100 mg/kg (5 rats), 1000 mg/kg (5 rats)	Once	Most of the activity excreted by 24 hours In the low dose, excretion in the urine (51%), and feces (43%) In the high dose, excretion in the feces (53%)	Lake *et al.*,1984b

Sprague-Dawley rats (M; 5 weeks old)	Oral gavage	Carbonyl-^{14}C-DEHP; 100 mg/kg (5 rats), 1000 mg/kg (5 rats)	Once	0-24 hour feces samples in low and high dose group – approximately 50% activity was DEHP, remainder were metabolites	Lake *et al.*,1984b
Sprague-Dawley rats (M; adult; 200-300g)	Oral feeding	50 mg/kg-day unlabeled-DEHP prior to 50 mg/kg carbonyl-^{14}C-DEHP; (6 rats per group)	Unlabeled dose for 21-28 days prior to single dose of labeled DEHP, then unlabeled dose until sacrificed	First 24 hours excretion in urine (27%) and feces (57%) Four days excretion in urine (37%) and feces (53%) – Excretion rapid and almost complete by 4 days -	Ikeda *et al.*,1980
Sprague-Dawley rats (M)	Oral gavage and carotid artery cannula	7-^{14}C-MEHP/kg (69mg, 20µCi; 4 rats per group) (35 mg; 4 rats per group); (69mg, 20µCi; 6.9mg, 2µCi; 4 rats per group); (69mg, 20µCi; 4 rats per group); (35mg; 3.5mg; 4 rats per group);	Once via gavage; Once via cannula; Once via gavage Once via gavage Once via iv	81% of dose accounted for in urine – limited excretion after 48 hours By 8 hours 40-52% of dose excreted in bile (following 35 and 3.5 mg/kg doses, respectively) Suggests substantial resorption is occurring in intestine	Chu *et al.*,1978
Wistar rats (M; young; 100-200g)	Oral gavage	Unlabelled and Carbonyl-^{14}C-DEHP - 2000 mg/kg	Once daily for 0, 6, or 13 days; followed by once with radiolabeled DEHP	At 4 days post-dose excreta had no radioactivity; prior to this, activity in urine (52%) or feces (48%); in rats pretreated for 6 or 13 days, activity in urine (60%) or feces (40%)	Lake *et al.*,1975
Wistar rats (M; 150-250 g)	Oral gavage or intravenous	Carbonyl-^{14}C-DEHP; 500 mg/kg (gavage), 50 mg/kg (iv) (2 or 3 rats per group)	Once	At 5-7 days post-dose, 80% excreted in urine and feces for both oral and iv exposure routes; activity in urine higher than feces; in bile duct, 5% and 24% recovered in 24 hours post-dose from oral and iv routes of exposure, respectively; DEHP excreted in feces DEHP or MEHP not detected in urine or bile	Tanaka *et al.*,1975
Wistar rats (M)	Oral gavage and oral feeding prior to oral gavage	Carbonyl-^{14}C-DEHP; 2.9 mg/kg Unlabeled-DEHP; 1000 mg/kg; (5 rats per group)	Once and 7 days prior to administration of labeled DEHP	Single dose - Urinary excretion 42%, Fecal excretion 57% by 7 days Pre-exposure dose followed by single dose - Urinary excretion 57%, Fecal excretion 38% by 4 days	Daniel and Bratt, 1974
Wistar rats	Oral gavage	2.6 mg/kg	?	10% excreted in bile	Daniel and Bratt, 1974?
Wistar rats (M, 180-220 g)	Oral gavage	7-^{14}C-DEHP/^{14}C-MEHP; 50 or 500 mg/kg (3 rats per group)	Once daily for 3 days	By 4 days, activity in urine was 50 and 60% (DEHP low and high dose) and 70 and 80% (MEHP low and high dose) of total daily dose	Lhuguenot *et al.*,1985
Fischer 344 rats (M)	Oral gavage	^{14}C; 1.8, 18, 180 mg/kg (1.8 mg/kg ^{14}C; 12 rats per group)	Ten daily for 1 day	Excretion independent of dose after 4 days – Excretion mechanisms not saturated by doses up to 180 mg/kg-day	Albro *et al.*,1982a
Fischer 344 rats	Oral gavage	1.8 to 1000 mg/kg	Once	6 hours post dose – "maximum amount of DEHP that could be given as single oral dose without significant excretion of unabsorbed DEHP in feces was 200 mg/kg"	Albro *et al.*,1982a

Fischer 344 rats (M; 100-150 g)	Oral feeding	1000, 6000, 12000 mg/kg (~85, 550, 1000 mg/kg-day; 12 rats per group – 4 rats per time) followed by 24 hours of carbonyl-^{14}C-DEHP	0, 6, or 20 days followed by 24 hours of radiolabeled DEHP	In urine, the percentage excreted was 53%, 62-66%, and 66-69% at 85, 550, and 1000 mg/kg-day respectively and primarily occurred during first 24 hours In feces, the percentage excreted was 35-38%, 26-30%, and 24-28% at 85, 550, and 1000 mg/kg-day respectively and primarily occurred during first 48 hours Prior exposure to unlabeled DEHP did not affect extent or rate of excretion 4 days after exposure, < 1% of the dose remained in tissues	CMA, 1982a; Lington *et al.*,1987; Short *et al.*,1987; Astill *et al.*,1986
Fischer 344 rats (M)	Oral gavage	1-^{14}C-ethylhexanol (2 rats)	?	Activity excreted in urine (80-82%), feces (8-9%), and as carbon dioxide (6-7%) with almost all cleared by 28 hours	Albro, 1975
CD-1 mice (M)	Oral gavage	Carbonyl^{14}C-DEHP; 360 mg/kg	Once daily for 2 days	Excreted glucuronide conjugates	Albro *et al.*,1982b
B6C3F$_1$ mice (M)				Excreted 37.3% activity in urine, primarily in first 12 hours – Excreted 52.0% activity in feces, primarily in first 24 hours	CMA, 1982b; CMA, 1983; CMA 1984a; Short *et al.*,1987; Astill *et al.*,1986
Syrian golden hamsters (M)	Oral gavage	Carbonyl-^{14}C-DEHP;	Twice, once each day	excreted glucuronide conjugate	Albro *et al.*,1982b
DSN Syrian golden hamsters (M; 5 week old)	Oral gavage	Carbonyl-^{14}C-DEHP; 100, 1000 mg/kg (3 or 5 hamsters per group)	Once (followed by 96 hours of recovery)	Most of the radioactivity excreted within 24 hours – at the low dose, excretion in urine (53%) or feces (31%) – at the high dose, excretion primarily in feces (48%)	Lake *et al.*,1984b
Dogs				24 hour urine and feces excretion 12 and 56%, respectively – 96 hour urine and feces excretion 21 and 75%, respectively – excretion virtually complete in 96 hours – only a trace of DEHP in dog urine	Ikeda *et al.*,1980
Miniature pigs				24 hour urine and feces excretion 37and 0.1%, respectively – 96 hour urine and feces excretion 79 and 26%, respectively – excretion virtually complete in 96 hours – only a trace of DEHP in pig urine	Ikeda *et al.*,1980

Appendix 2. DEHP-induced Adverse Effect Levels

(retrieved from ECB, 2008; ATSDR, 2002; and IARC, 2000 and selected publications)

Summary Table of DEHP-induced Effects [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13]							
Species (Gender)	***Exposure Route***	***Dose***	***Dose Duration***	***Toxicological Endpoint***	***Toxicological Basis***	***Citation***	***Organ System***
Mortality							
Humans	Oral capsule	5 or 10 g (~71 or 143 mg/kg; based on 70 kg weight)	Once	No mortality; NOAEL = 143 mg/kg	No mortality	Shaffer *et al.*,1945; ECB, 2008; ATSDR, 2002	Mortality
Wistar rats (M)	Oral gavage	N/A	Once	LD_{50} = 30,600 mg/kg	Mortality	Shaffer *et al.*,1945; ATSDR, 2002	Mortality
Fischer 344 rats (M&F)	Oral gavage	5000, 10,000, 20,000, 40,000 mg/kg (5 rats per sex per group) in volumes up to 40 ml/kg; GLP)	Once	LD_{50} > 40,000 mg/kg	Mortality	Nuodex, 1981a; ECB, 2008	Mortality
Fischer 344 rats (M&F)	Oral gavage	800 to 20,000 mg/kg (5 rats per sex per group)	Once	LD_{50} > 20,000 mg/kg	Mortality	NTP, 1982; ECB, 2008	Mortality
Rats	Oral gavage	N/A	Once	LD_{50} = 26,000 mg/kg	Mortality	NTP, 1982	Mortality
Wistar rats (M)	Oral gavage	N/A	Once	LD_{50} > 34,000	Mortality	Hodge, 1943; NTP, 1982	Mortality
Rats	N/A	N/A	N/A	LD_{50} > 20,000 mg/kg	Mortality	BASF, 1953; ECB, 2008	Mortality
Rats	N/A	N/A	N/A	LD_{50} > 9800 mg/kg	Mortality	BASF, 1961; ECB, 2008	Mortality
ICR/SIM mice (M)	Oral gavage	100, 250, 500, 1000, 2500, 5000, 7500, 9860 mg/kg (5 – 10 males per group; GLP)	Once	LD_{50} > 9860 mg/kg	Mortality	Nuodex, 1981b; ECB, 2008	Mortality
$B6C3F_1$ mice (M&F)	Oral gavage	800 {1250} - 20,000 mg/kg (5 rats per sex per group)	Once	LD_{50} > 20,000 mg/kg	Mortality	NTP, 1982; ECB, 2008	Mortality
Mice	Oral gavage	N/A	Once	LD_{50} = 49,000 mg/kg	Mortality	NTP, 1982	Mortality
Mice	Oral gavage	N/A	Once	LD_{50} = 2600 mg/kg	Mortality	NTP, 1987	Mortality
Mice	N/A	N/A	N/A	LD_{50} > 31,360 mg/kg	Mortality	Lawrence *et al.*,1974; ECB, 2008	Mortality
Mice	N/A	5000, 10,000 mg/kg	N/A	LD_{50} > 10,000 mg/kg	Mortality	BASF, 1941; ECB, 2008	Mortality
Rabbit (N/A)	Oral gavage	N/A	Once	LD_{50} = 33,900	Mortality	Shaffer *et al.*,1945; ATSDR, 2002	Mortality
Rats	Intraperitoneal	N/A	N/A	LD_{50}s = 4900-147,000; 49,000; 30,600 mg/kg	Mortality	Shaffer *et al.*,1945; Singh *et al.*,1972; ECB, 2008	Mortality
Mice	Intraperitoneal	N/A	N/A	LD_{50}s = 5000 – > 128,000; 4200; 38,000 mg/kg	Mortality	Calley *et al.*,1966; NTP, 1982; ECB, 2008	Mortality
Rats	Intravenous	N/A	N/A	LD_{50}s = 250-2080 mg/kg	Mortality	Peterson *et al.*,1974; NTP, 1982; ECB, 2008	Mortality

Mice	Intravenous	N/A	N/A	LD_{50}s = 1060-1370 mg/kg	Mortality	Peterson *et al.*,1974; NTP, 1982; ECB, 2008	Mortality
Rats	Intravenous	NS	Once	LD_{50} = 200 mg/kg	Mortality	Schulz *et al.*,1975; Rubin and Chang, 1978; ECB, 2008	Mortality
Rats	Inhalation	0, 3.39, 6.82, 10.62 mg/L (5 male and 5 female per group)	4 hours, nose only	4h-LC_{50} > 10.62 mg/L	Mortality	Huls, 1981	Mortality
Rabbits	Dermal	Doses up to 20 mL/kg	24 hours	LD_{50} = ~25 mL/kg (~ 24,500 mg/kg)	20 mL/kg killed 2 of 6 rabbits	Shaffer *et al.*,1945	Mortality
Sprague-Dawley rats (M)	Oral gavage	0, 10, 100, 1000, 2000 mg/kg-day (10 rats per group; GL)	Once daily for 5 days on day 6, 14-16, 21, 42, 86 of age	LOAEL = 1000 mg/kg-day; NOAEL = 100 mg/kg-day	Mortality in 14-18 day old rats after 5 doses (68%), older rats less suceptible)	Dostal *et al.*,1987a, 1987b; ECB, 2008; ATSDR, 2002	Mortality
Sprague-Dawley rats (M)	Oral gavage	0, 10, 100, 1000, 2000 mg/kg-day (10 rats per group; GL)	Once daily for 5 days on day 6, 14-16, 21, 42, 86 of age	LOAEL = 2000 mg/kg-day; NOAEL = 1000 mg/kg-day	Mortality in all pups in 3 youngest age groups	Dostal *et al.*,1987b; ECB, 2008	Mortality
Sprague-Dawley rats (M&F)	Oral gavage	0, 10, 100, 1000, 2000 mg DEHP/kg bw (7-10 rats per group); 0,100, 200, 500, 1000 mg/kg (16 rat pups per group); 0, 200, 500, 1000 mg/kg (50 rat pups per group)	Once daily during PPd 6-10, 14-18, 21-25, 42-46, 86-90; Once daily for 5 days during PPd 6-10 – recovery for 4 weeks; Once daily for 5 days during PPd 6-10	LOAEL = 2000 mg/kg; NOAEL = 1000 mg/kg	Mortality in all rats dosed during PPd 21-25	Dostal *et al.*, 1988	Mortality
Sprague-Dawley rats (M&F)	Oral gavage	0, 10, 100, 1000, 2000 mg DEHP/kg bw (7-10 rats per group); 0,100, 200, 500, 1000 mg/kg (16 rat pups per group); 0, 200, 500, 1000 mg/kg (50 rat pups per group)	Once daily during PPd 6-10, 14-18, 21-25, 42-46, 86-90; Once daily for 5 days during PPd 6-10 – recovery for 4 weeks; Once daily for 5 days during PPd 6-10	LOAEL = 2000 mg/kg	Mortality in all rats dosed during PPd 14-18	Dostal *et al.*, 1988	Mortality
Fischer 344 rat (F)	Oral gavage	N/A	Once daily for 7 days during PPd 1-7	LOAEL = 5000 mg/kg-day	Mortality (25% within 1 week)	Cimini *et al.*,1994; ATSDR, 2002	Mortality
Wistar rats	Oral gavage	N/A	Once daily for 7 days	LOAEL = 2000 mg/kg-day	Mortality at 7 days in 3 week old rats (10%); 0% in untreated and treated older rats	Parmar *et al.*,1994; ATSDR, 2002	Mortality
Rabbit (N/A)	Oral gavage	N/A	Once daily for 7 days	LOAEL = 2000 mg/kg-day	Mortality (50%)	Parmar *et al.*,1988; ATSDR, 2002	Mortality

B6C3F$_1$ mice (M&F)	Oral feeding	0, 6300, 12,500, 25,000, 50,000, 100,000 mg/kg (0, 630, 1250, 2500, 5000, 10,000 mg/kg-day; 5 mice per sex per group)	14 days	LOAEL = 5000 mg/kg-day; NOAEL = 2500 mg/kg-day	Mortality; 100% at 10,000 mg/kg-day (M&F); 1/5(M) and 4/5(F) at 5000 mg/kg-day	NTP, 1982; ECB, 2008	Mortality
Fischer 344 rats	Oral feeding	0, 6300, 12,500, 25,000, 50,000, 100,000 mg/kg (0, 630, 1250, 2500, 5000, 10,000 mg/kg-day; 5 rats per sex per group)	14 days	LOAEL = 10,000 mg/kg-day; NOAEL = 5000 mg/kg-day	Mortality; 2/5 M and 4/5 F	NTP, 1982; ECB, 2008	Mortality
Wistar rats	Oral gavage	N/A	Once daily for 15 days	LOAEL = 2000 mg/kg-day	Mortality after 3 weeks (50%), subsequent mortality (100%)	Parmar *et al.*,1987; ATSDR, 2002	Mortality
Guinea pig (NS)	Oral gavage	N/A	Once daily for 15 days	LOAEL = 2000 mg/kg-day	Mortality (40%)	Parmar *et al.*,1988; ATSDR, 2002	Mortality
Rabbit (NS)	Oral gavage	N/A	Once daily for 15 days	LOAEL = 2000 mg/kg-day	Mortality (100%)	Parmar *et al.*,1988; ATSDR, 2002	Mortality
B6C3F$_1$ mice (M&F)	Oral feeding	0, 1000, 5000, 10,000, 25,000 mg/kg (0, 250, 1210, 2580, 6990 mg/kg-day(M); 0, 270, 1430, 2890, 7900 mg/kg-day (F); 10 mice per sex per group; GLP)	28 days	LOAEL = 6990 – 7900 mg/kg-day; NOAEL = 2580-2890 mg/kg-day	Mortality: 4/10 (M) and 3/10 (F)	Eastman Kodak, 1992b; ECB, 2008	Mortality
Sv/129 mice (M)	Oral feeding	N/A	16 weeks	LOAEL = 2400 mg/kg-day	Mortality between weeks 12 and 16 (100%)	Ward *et al.*,1998; ATSDR, 2002	Mortality
Wistar rats (M&F)	Oral feeding	0, 0.1, 0.5% (0, 50-80, 300-400 mg/kg-day; 43 rats per sex per group)	3, 6, 12, 24 months	LOAEL = 50-80 mg/kg-day	Overall mortality 85-96%	Harris *et al.*,1956; ECB, 2008	Mortality
B6C3F$_1$ mice (M)	Oral feeding	N/A	104 weeks	LOAEL = 1266 mg/kg-day	Reduced survival due to hepatocellular neoplasia (M; 45%)	David *et al.*,1999, 2000b; ATSDR, 2002	Mortality
B6C3F$_1$ mice (M&F)	Oral feeding	0, 100, 500, 1500, 6000 mg/kg (0, 19.2, 98.5, 292.2, 1266.1 mg/kg-day (M); 0, 23.8, 116.8, 354.2, 1458.2 mg/kg-day (F), or 6000 mg/kg for 78 weeks and a 26 week recovery period; 70-85 rats per sex per group; 55 rats per sex in recovery group)	104 weeks	LOAEL = 292.2 {1266.1} mg/kg-day; NOAEL = 98.5 {292.2} mg/kg-day	Decreased survival (M)	Moore, 1997; ECB, 2008	Mortality
Wistar rats (M&F)	Oral feeding	0, 1000, 3000, 9000 mg/kg (0, 113, 340, 1088 mg/kg-day; 20-25 rats per sex per group; GLP)	3 generations	LOAEL = 1060 mg/kg-day; NOAEL = 339 mg/kg-day	Decreased mortality in F1 generation adults (M&F)	Schilling *et al.*,2001; CERHR, 2006; ECB, 2008	Mortality
General/Body weight/Food consumption							
Rats	Inhalation	0, 3.39, 6.82, 10.62 mg/L (5 male and 5 female per group)	4 hours, nose only	LOAEC = 3.39 mg/L	Unkempt appearance 1-2 days post dosing	Huls, 1981	General
Rats	Inhalation	0, 3.39, 6.82, 10.62 mg/L (5 male and 5 female per group)	4 hours, nose only	LOAEC = 10.62 mg/L	Yellow fur staining	Huls, 1981	General

Fischer 344 rats (M&F)	Oral gavage	5000, 10,000, 20,000, 40,000 mg/kg (5 males and 5 females per group) in volumes up to 40 ml/kg	Once	LOAEL = 5000 mg/kg	During first days after dosing, rough coat, decreased activity, wet posterior, dose-related days of clinical symptoms	Nuodex, 1981a	General
Fischer 344 rats (M&F)	Oral gavage	5000, 10,000, 20,000, 40,000 mg/kg (5 males and 5 females per group) in volumes up to 40 ml/kg	Once	NOAEL = 5000 mg/kg	No abnormalities upon gross necropsy	Nuodex, 1981a	General
ICR/SIM mice (M)	Oral gavage	9860 mg/kg (10 males per group)	Once	LOAEL = 9860 mg/kg	Depression and rough fur in treated group for 2-3 days, a humped appearance after treatment lasting for one day	Nuodex, 1981b	General
ICR/SIM mice (M)	Oral gavage	9860 mg/kg (10 males per group)	Once	NOAEL = 9860 mg/kg	No abnormalities upon gross necropsy	Nuodex, 1981b	General
Human (M)	Oral capsule	5000 and 10,000 mg (71.4 mg/kg-day, 142.8 mg/kg-day; 2 adults)	Once	LOAEL = 10,000 mg	Mild gastric disturbances and "moderate catharsis"	Shaffer *et al.*,1945	General
Fischer 344 rats	Oral gavage	N/A	Once	LOAEL = 5000 mg/kg-day; NOAEL = 1500 mg/kg-day	Signs of general debilitation	Moser *et al.*,1995; ATSDR, 2002	General
CD-1 mice (F)	Oral gavage	0, 6000, 7690, 9860 mg/kg-day (10 mice per group; GLP)	Once daily for 8 days	LOAEL = 6000 mg/kg-day	Clinical toxicity	Hazelton, 1983; ECB, 2008	General
Wistar rats (F)	Oral gavage	0, 40, 200, 1000 mg/kg-day (9-10 litters per group)	Once daily during Gd 6-15	LOAEL = 1000 mg/kg-day; NOAEL = 200 mg/kg-day	Substantial increase in the number of dams with vaginal hemorrhage on Gd 15	Hellwig *et al.*, 1997	General
Fischer 344 rats	Oral gavage	N/A	Once daily for 14 days	NOAEL = 1500 mg/kg-day	No toxicologically significant general effects	Moser *et al.*,1995; ATSDR, 2002	General
CD-1 mice	Oral feeding	0, 0.025, 0.05, 0.1, 0.15% (0, 44, 91, 190.6, 292.5 mg/kg-day; 30-31 rats per group)	17 days during Gd 0-17	LOAEL = 91 mg/kg-day; NOAEL = 44 mg/kg-day	Rough coat; lethargy	Tyl *et al.*,1988; ATSDR, 2002	General
Wistar rats (M&F)	Oral gavage	0, 2500 mg/kg-day (6 rats per sex per group)	Once daily for 7 or 21 days	NOAEL = 2500 mg/kg-day	No toxicologically significant clinical signs	Mangham *et al.*,1981	General
Sprague-Dawley rats (M)	Oral feeding	0, 0.2, 1.0, and 2.0% (0, 143, 737, 1440 mg/kg-day; M; 0, 154, 797, 1414 mg/kg-day; F) (15 rats per group): 0, 1.0, and 2.0% (0, 737, 1440 mg/kg-day; M; 0, 797, 1414 mg/kg-day; F): 0 and 2.0% (0, 1440 mg/kg-day; M; 0, 1414 mg/kg-day; F) (10 rats per group)	17 weeks: 2 or 6 weeks:	LOAEL = 1414 mg/kg-day; NOAEL = 797 mg/kg-day	Significant loss of fur in the head and ventral areas by 17 weeks (F)	Gray *et al.*, 1977	General
Rats	Inhalation	0, 3.39, 6.82, 10.62 mg/L (5 male and 5 female per group)	Once for 4 hours, nose only	LOAEC = 10.62 mg/L	Reduced body weight gain on day 2 post exposure	Huls, 1981	Body weight
Fischer 344 rats	Oral gavage	N/A	Once daily for 2 days	NOAEL = 950 mg/kg-day	No toxicologically significant effects on body weight	James *et al.*,1998; ATSDR, 2002	Body weight
$B6C3F_1$ mice	Oral gavage	N/A	Once daily for 2 days	NOAEL = 1150 mg/kg-day	No toxicologically significant effects on body weight	James *et al.*,1998; ATSDR, 2002	Body weight
$B6C3F_1$ mice (M)	Oral gavage	0, 1879, 2844, 4304, 6514, 9860 mg/kg-day (10 rats per group; GLP)	Once a day for 5 days	NOAEL = 9860 mg/kg-day	No toxicologically significant effects on body weight or body weight gain	Nuodex, 1981b; ECB, 2008	Body weight

Sprague-Dawley rats (M&F)	Oral gavage	0, 10, 100, 1000, 2000 mg DEHP/kg bw (7-10 rats per group); 0,100, 200, 500, 1000 mg/kg (16 rat pups per group); 0, 200, 500, 1000 mg/kg (50 rat pups per group)	Once daily during PPd 6-10, 14-18, 21-25, 42-46, 86-90; Once daily for 5 days during PPd 6-10 – recovery for 4 weeks; Once daily for 5 days during PPd 6-10	LOAEL = 1000 mg/kg; NOAEL = 500 mg/kg	Significant decrease in body weight in rats dosed during PPd 6-10	Dostal *et al.*, 1988	Body weight
Sprague-Dawley rats (M&F)	Oral gavage	0, 10, 100, 1000, 2000 mg DEHP/kg bw (7-10 rats per group); 0,100, 200, 500, 1000 mg/kg (16 rat pups per group); 0, 200, 500, 1000 mg/kg (50 rat pups per group)	Once daily during PPd 6-10, 14-18, 21-25, 42-46, 86-90; Once daily for 5 days during PPd 6-10 – recovery for 4 weeks; Once daily for 5 days during PPd 6-10	NOAEL = 1000 mg/kg	No toxicologically significant change in the body weight of rats dosed during PPd 6-10 and allowed to recover for 4, 11, 12, 13, 16, or 23 weeks	Dostal *et al.*, 1988	Body weight
Sprague-Dawley rats (M&F)	Oral gavage	0, 10, 100, 1000, 2000 mg DEHP/kg bw (7-10 rats per group); 0,100, 200, 500, 1000 mg/kg (16 rat pups per group); 0, 200, 500, 1000 mg/kg (50 rat pups per group)	Once daily during PPd 6-10, 14-18, 21-25, 42-46, 86-90; Once daily for 5 days during PPd 6-10 – recovery for 4 weeks; Once daily for 5 days during PPd 6-10	LOAEL = 1000 mg/kg; NOAEL = 500 mg/kg	Significant decrease in the body weight of rats dosed during PPd 6-10 ($P < 0.05$); Substantial (7%) dose-dependent decrease in body weight of rats dosed during PPd 6-10 and recovered for 19 weeks	Dostal *et al.*, 1988	Body weight
Sprague-Dawley rats (F)	Oral gavage	2000 mg DEHP/kg bw (7-8 dams dosed per group, 10 pups per dam)	Once daily during Ld 2-6, 6-10, 14-18, 15-17	LOAEL = 2000 mg/kg	Significant decrease in maternal and suckling pup body weight in rats dosed during Ld 2-6, 6-10, 14-18 ($P < 0.05$; Pair-fed controls for Ld 14-18 also had significantly decreased maternal and suckling pup body weights)	Dostal *et al.*, 1987	Body weight
Sprague-Dawley rats (F)	Oral gavage	2000 mg DEHP/kg bw (7-8 dams dosed per group, 10 pups per dam)	Once daily during Ld 2-6, 6-10, 14-18, 15-17	NOAEL = 2000 mg/kg	No toxicologically significant change in body weights of maternal or suckling pup rats dosed during Ld 15-17	Dostal *et al.*, 1987	Body weight
C57BL/6 mice	Oral feeding	N/A	7 days	LOAEL = 3850 mg/kg-day	Decrease in final body weight (17%)	Muhlenkamp and Gill, 1998; ATSDR, 2002	Body weight
Mice (NS)	Oral gavage	N/A	Once daily for 7 days	NOAEL = 2000 mg/kg-day	No toxicologically significant effects on body weight	Parmar *et al.*,1988; ATSDR, 2002	Body weight
Guinea pig (NS)	Oral gavage	N/A	Once daily for 7 days	LOAEL = 2000 mg/kg-day	Decrease in body weight gain (39%)	Parmar *et al.*,1988; ATSDR, 2002	Body weight
Rabbit (NS)	Oral gavage	N/A	Once daily for 7 days	NOAEL = 2000 mg/kg-day	No toxicologically significant effects on body weight	Parmar *et al.*,1988; ATSDR, 2002	Body weight
Wistar rats	Oral gavage	N/A	Once daily for 7 days	NOAEL = 1500 mg/kg-day	No toxicologically significant effects on body weight	Oishi, 1989; ATSDR, 2002	Body weight

Albino rat	Oral gavage	N/A	Once daily for 7 days	LOAEL = 2000 mg/kg-day	Decrease in body weight gain (10%)	Parmar *et al.*,1988; ATSDR, 2002	Body weight
CD-1 mice (F)	Oral gavage	0, 6000, 7690, 9860 mg/kg-day (10 mice per group; GLP)	Once daily for 8 days	NOAEL = 9860 mg/kg-day	No toxicologically significant effects on body weight and body weight gain	Hazelton, 1983; ECB, 2008	Body weight
Wistar rats (F)	Oral gavage	0, 40, 200, 1000 mg/kg-day (9-10 litters per group)	Once daily during Gd 6-15	NOAEL = 1000 mg/kg-day	No toxicologically significant change in the body weight on day 6, 10, 15, or 20	Hellwig *et al.*, 1997	Body weight
Wistar rats (M; 4, 10, 15 week old)	Oral gavage; Oral feeding	0, 2800 mg/kg-day ± testosterone proprionate (200 µg/kg/day or FSH (100 U); 0, 2% (0, ~ 1200 mg/kg-day	10 days; 10 or 42 days and then recovery to 4 week old rats	LOAEL = 2800 mg/kg-day	Age-dependent decrease in the body weight (4 > 10 > 15 weeks old) following 10 days of dosing	Gray and Butterworth, 1980	Body weight
Wistar rats (F)	Inhalation (head-nose)	0, 0.01, 0.05, 0.3 mg/L (0, 10, 50, 300 mg/m^3)	10 days for 6 hours/day during Gd 6-15	NOAEC = 300 mg/m^3	No toxicologically significant effects on body weight	Merkle *et al.*,1988; ECB, 2008	Body weight
Sprague-Dawley rats (M)	Oral feeding	N/A	10 days	LOAEL = 1740 mg/kg-day	Decreased final body weight (22%)	Mehrotra *et al.*,1997; ATSDR, 2002	Body weight
Wistar rat	Oral feeding	N/A	14 days	LOAEL = 1894 mg/kg-day	Decreased final body weight (17%)	Van den Munckhof *et al.*,1998; ATSDR, 2002	Body weight
Sprague-Dawley rat	Oral feeding	N/A	14 days	NOAEL = 1905 mg/kg-day	No toxicologically significant effects on body weight	Shin *et al.*,1999; ATSDR, 2002	Body weight
Sprague-Dawley rat	Oral feeding	N/A	14 days on PPd 25-38, 40-53, 60-73	LOAEL = 1000 – 1700 mg/kg-day	Decreased body weight gain (22 - 26%)	Sjoberg *et al.*,1986a; ATSDR, 2002	Body weight
Sprague-Dawley rat	Oral feeding	N/A	14 days	LOAEL = 1000 mg/kg-day	Decreased body weight gain (22%)	Sjoberg *et al.*,1986b; ATSDR, 2002	Body weight
Cynomolgous monkeys (M)	Oral gavage	N/A	Once daily for 14 days	NOAEL = 500 mg/kg-day	No toxicologically significant effects on body weight	Pugh *et al.*,2000; ATSDR, 2002	Body weight
Marmoset Monkey (M&F; 250-400 g)	Oral gavage	0, 2000 mg/kg (5 monkeys per sex per group)	Once daily for 14 days	LOAEL = 2000 mg/kg-day	Decreased body weight (70%)	Rhodes *et al.*,1986; ATSDR, 2002	Body weight
Wistar - Alderley Park rats (M&F)	Oral gavage	0, 2000 mg/kg-day (10 rats per sex per group; GLP)	Once daily for 14 days	LOAEL = 2000 mg/kg-day	Decreased body weight gain (M; 40%)	ICI, 1982b; Rhodes *et al.*,1986; ECB, 2008; ATSDR, 2002	Body weight
Wistar rats (M)	Oral gavage	0, 250, 500, 1000 or 2000 mg/kg-day (5 rats per group)	Once daily for 14 days	LOAEL = 1000 mg/kg-day; NOAEL = 500 mg/kg-day	Decreased body weight	Khaliq and Srivastava, 1993; ECB, 2008	Body weight
$B6C3F_1$ mice (M&F)	Oral feeding	0, 6300, 12,500, 25,000, 50,000, 100,000 mg/kg (0, 630, 1250, 2500, 5000, 10,000 mg/kg-day; 5 mice per sex per group)	14 days	LOAEL = 2500 mg/kg-day; NOAEL = 1250 mg/kg-day	Decreased body weight (M)	NTP, 1982; ECB, 2008	Body weight
$B6C3F_1$ mice (M&F)	Oral feeding	0, 6300, 12,500, 25,000, 50,000, 100,000 mg/kg (0, 630, 1250, 2500, 5000, 10,000 mg/kg-day; 5 mice per sex per group)	14 days	LOAEL = 5000 mg/kg-day; NOAEL = 2500 mg/kg-day	Decreased body weight (F)	NTP, 1982; ECB, 2008	Body weight

$B6C3F_1$ mice (M&F)	Oral feeding	0, 6300, 12,500, 25,000, 50,000, 100,000 mg/kg (0, 630, 1250, 2500, 5000, 10,000 mg/kg-day; 5 mice per sex per group)	14 days	LOAEL = 630 mg/kg-day	Dose-dependent decreased body weight gain (M)	NTP, 1982; ECB, 2008	Body weight
Fischer 344 rats	Oral feeding	0, 6300, 12,500, 25,000, 50,000, 100,000 mg/kg (0, 630, 1250, 2500, 5000, 10,000 mg/kg-day; 5 rats per sex per group)	14 days	LOAEL = 2500 mg/kg-day; NOAEL = 1250 mg/kg-day	Decreased body weight gain (M)	NTP, 1982; ECB, 2008	Body weight
Fischer 344 rats	Oral feeding	0, 6300, 12,500, 25,000, 50,000, 100,000 mg/kg (0, 630, 1250, 2500, 5000, 10,000 mg/kg-day; 5 rats per sex per group)	14 days	LOAEL = 5000 mg/kg-day; NOAEL = 2500 mg/kg-day	Decreased body weight and body weight gain (F)	NTP, 1982; ECB, 2008	Body weight
Guinea pig (NS)	Oral gavage	N/A	Once daily for 15 days	LOAEL = 2000 mg/kg-day	Decrease in body weight gain (19%)	Parmar *et al.*,1988; ATSDR, 2002	Body weight
Albino rats	Oral gavage	N/A	Once daily for 15 days	LOAEL = 2000 mg/kg-day	Decrease in body weight gain (24%)	Parmar *et al.*,1988; ATSDR, 2002	Body weight
Mice (NS)	Oral gavage	N/A	Once daily for 15 days	LOAEL = 2000 mg/kg-day	Change in body weight gain (11%)	Parmar *et al.*,1988; ATSDR, 2002	Body weight
CD-1 mice {1-CR}	Oral feeding	0, 0.025, 0.05, 0.10, 0.15% (0, 44, 91, 190.6, 292.5 mg/kg-day; 30-31 mice per group)	17 days during Gd 0-17	LOAEL = 190.6 mg/kg-day; NOAEL = 91 mg/kg-day	Decreased maternal body weight gain (30%; primarily due to decreased uterine weights)	Tyl *et al.*,1988; NTIS, 1984; ECB, 2008; ATSDR, 2002	Body weight
Fischer 344 rats (CrlBr; F)	Oral feeding	0, 0.5, 1.0, 1.5, 2.0% (0, 357.2, 666, 856, 1055 mg/kg-day; 34-25 rats per group)	20 days during Gd 0-20	$LOAEL_{maternal}$ = 856 mg/kg-day	Decreased maternal body weight gain (39%)	Tyl *et al.*,1988; NTIS, 1984; ECB, 2008; ATSDR, 2002	Body weight
Fischer 344 rats (CrlBr; F)	Oral feeding	0, 0.5, 1.0, 1.5, 2.0% (0, 357.2, 666, 856, 1055 mg/kg-day; 34-25 rats per group)	20 days during Gd 0-20	$LOAEL_{maternal}$ = 666 mg/kg-day; $NOAEL_{maternal}$ = 357.2 mg/kg-day	Decreased maternal body weight gain (19%)	Tyl *et al.*,1988; NTIS, 1984; ECB, 2008; ATSDR, 2002	Body weight
Wistar rats (M&F)	Oral gavage	0, 2500 mg/kg-day (6 rats per sex per group)	Once daily for 7 or 21 days	LOAEL = 2500 mg/kg-day	Significant decrease in body weight after 10 and 20 days of exposure	Mangham *et al.*,1981	Body weight
Wistar rats (M)	Oral feeding	0, 2% (1830, 1650, and 1810 after 3, 10, 21 days; 4 rats per treatment group, 6 rats in control groups)	3, 10, and 21 days	LOAEL = 1650 mg/kg-day	Decreased body weight after 10-21 days	Mann *et al.*,1985; ECB, 2008	Body weight
Fischer 344 rats (M)	Oral feeding	0, 100, 1000, 6000, 12,000, 25,000 mg/kg (0, 11, 105, 667, 1223, 2100 mg/kg-day; 5 rats per group)	21 days	LOAEL = 2100 mg/kg-day; NOAEL = 1223 mg/kg-day	Decreased body weight gain	Short *et al.*,1987; ATSDR, 2002	Body weight
Fischer 344 rats (M&F)	Oral feeding	0, 0.01, 0.1, 0.6, 1.2, 2.5% (0, 11, 105, 667, 1224, 2101 mg/kg-day (M); 0, 12, 109, 643, 1197, 1892 mg/kg-day (F); 5 rats per sex per group; GLP)	21 days	LOAEL = 1892 mg/kg-day; NOAEL = 1197 mg/kg-day	Decreased final body weight (41%)	CMA, 1984b; Barber *et al.*,1987; ECB, 2008; ATSDR, 2002	Body weight
Wistar rats	Oral feeding	N/A	21 days	LOAEL = 1730 mg/kg-day	Decreased final body weight (28%)	Mocchiutti and Bernal, 1997; ATSDR, 2002	Body weight

Sprague-Dawley rats (M)	Oral feeding	0, 2% (900 mg/kg-day; 4 rats per group)	21 days	LOAEL = 900 mg/kg-day	Decreased body weight and body weight gain	General Motors, 1982; ECB, 2008	Body weight
Wistar rats (M)	Oral feeding	0, 60, 200, 600, 2000, 6000 mg/kg (0, 5, 18, 52, 182, 549 mg/kg-day; 6 rats per group)	14 or 28 days	LOAEL = 182 mg/kg-day; NOAEL = 52 mg/kg-day	Dose-related increase in absolute body weight following 2 or 4 weeks	RIVM, 1992	Body weight
Wistar rats (F)	Oral gavage	0, 0.015, 0.045, 0.135, 0.405, 1.215, 5, 15, 45, 135, 405 mg/kg-day; ? rats per group)	Once daily for 27 days during Gd6 to Ld 21, recovery til 144 days old	NOAEL = 405 mg/kg-day	No toxicologically significant effects on body weight	Andrade *et al.*, 2006b	Body weight
Fischer 344 rats (M&F)	Oral feeding	0, 0.2, 0.67, 2.0% (0, 150, 504, 1563 mg/kg-day (M); 0, 147, 490, 1416 mg/kg-day (F); 5 rats per sex per group; GLP)	28 days	LOAEL = 490 mg/kg-day; NOAEL = 147 mg/kg-day	Decreased body weight	Nuodex, 1981c; ECB, 2008	Body weight
Fischer 344 rats (M)	Oral feeding	0, 0.02, 0.05, 0.1, 0.5, 1.0, 2.5% (0, 24, 52, 115, 559, 1093, 2496 mg/kg-day; 5 rats per group, 10 rats in control; GLP)	28 days	LOAEL = 2496 mg/kg-day; NOAEL = 1093 mg/kg-day	Decreased body weight	BIBRA, 1990; ECB, 2008	Body weight
Fischer 344 rats	Oral feeding	N/A	28 days	NOAEL = 705 mg/kg-day	No toxicologically significant effects on body weight	Hodgson, 1987; ATSDR, 2002	Body weight
B6C3F$_1$ mice (M&F)	Oral feeding	0, 1000, 5000, 10,000, 25,000 mg/kg (0, 250, 1210, 2580, 6990 mg/kg-day(M); 0, 270, 1430, 2890, 7900 mg/kg-day (F); 10 mice per sex per group; GLP)	28 days	LOAEL = 1210 mg/kg-day; NOAEL = 250 mg/kg-day	Decreased body weight and body weight gain (M)	Eastman Kodak, 1992b; ECB, 2008	Body weight
B6C3F$_1$ mice (M&F)	Oral feeding	0, 1000, 5000, 10,000, 25,000 mg/kg (0, 250, 1210, 2580, 6990 mg/kg-day(M); 0, 270, 1430, 2890, 7900 mg/kg-day (F); 10 mice per sex per group; GLP)	28 days	LOAEL = 7900 mg/kg-day; NOAEL = 2890 mg/kg-day	Decreased body weight and body weight gain (F)	Eastman Kodak, 1992b; ECB, 2008	Body weight
ICR mice	Oral gavage	N/A	Once daily for 2 days a week for 4 weeks	NOAEL = 1171 mg/kg-day	No toxicologically significant effects on body weight	Lee *et al.*,1997; ATSDR, 2002	Body weight
Fischer 344 rats (M)	Oral feeding	0, 320, 1250, 5000, 20,000 mg/kg (0, 18, 69, 284, 1156 mg/kg-day; 24 rats per group)	8 weeks (60 days)	LOAEL = 284 mg/kg-day; NOAEL = 69 mg/kg-day	Dose-dependent decrease in total body weight	Agarwal *et al.*,1986a/b; ECB, 2008	Body weight
CRL:CD Sprague-Dawley rats (F)	Oral gavage	0, 11, 33, 100, 300 mg/kg-day (12-14 litters per group)	Once daily during Gd 8-17; recovery til maturity; continued dosing from 18 til 63-65	NOAEL = 300 mg/kg-day	No toxicologically significant change in dam body weight or body weight gain; 18-day body weight and body weight gain of F_1 pups dosed from Gd 8 to Pnd 64 In pups dosed from Gd 8 to Ld 17 then recovery, no toxicologically significant change in body weight	Gray *et al.*, 2009	Body weight
CRL:CD Sprague-Dawley rats (F)	Oral gavage	0, 11, 33, 100, 300 mg/kg-day (12-14 litters per group)	Once daily during Gd 8-17; recovery til maturity; continued dosing from 18 til 63-65	LOAEL = 300 mg/kg-day; NOAEL = 100 mg/kg-day	In pups dosed from Gd 8 to PNd 64; marginal decrease in body weight	Gray *et al.*, 2009	Body weight

Wistar rats	Oral feeding	N/A	12 weeks (90 days)	LOAEL = 400 mg/kg-day, NOAEL = 200 mg/kg-day	Decreased body weight gain	Shaffer *et al.*,1945; ATSDR, 2002	Body weight
Fischer 344 rats (M&F)	Oral feeding	0, 1000, 4000, 12,500, 25,000 mg/kg (0, 63, 261, 859, 1724 mg/kg-day (M); 0, 73, 302, 918, 1858 mg/kg-day (F); 10 rats per sex per group; GLP)	13 weeks	LOAEL = 918 mg/kg-day; NOAEL = 302 mg/kg-day	Decreased body weight gain (F)	Eastman Kodak, 1992a; ECB, 2008	Body weight
Fischer 344 rats (M&F)	Oral feeding	0, 1000, 4000, 12,500, 25,000 mg/kg (0, 63, 261, 859, 1724 mg/kg-day (M); 0, 73, 302, 918, 1858 mg/kg-day (F); 10 rats per sex per group; GLP)	13 weeks	LOAEL = 1724 mg/kg-day; NOAEL = 859 mg/kg-day	Decreased body weight gain (M)	Eastman Kodak, 1992a; ECB, 2008	Body weight
Fischer 344 rats (M&F)	Oral feeding	0, 1600, 3100, 6300, 12,500, 25,000 mg/kg (0, 80, 160, 320, 630, 1250 mg/kg-day; 10 rats per sex per group)	13 weeks	LOAEL = 630 {1250} mg/kg-day; NOAEL = 320 {630} mg/kg-day	Dose-dependent decreased body weight gain (M)	NTP, 1982; ECB, 2008	Body weight
Fischer 344 rats (M&F)	Oral feeding	0, 1600, 3100, 6300, 12,500, 25,000 mg/kg (0, 80, 160, 320, 630, 1250 mg/kg-day; 10 rats per sex per group)	13 weeks	LOAEL = 1250 mg/kg-day; NOAEL = 630 mg/kg-day	Dose-dependent decreased body weight gain (F)	NTP, 1982; ECB, 2008	Body weight
B6C3F$_1$ mice (M&F)	Oral feeding	0, 800, 1600, 3100, 6300, 12,500 mg/kg (0, 100, 200, 400, 800, 1600 mg/kg-day; 10 mice per sex per group)	13 weeks	LOAEL = 400 mg/kg-day; NOAEL = 200 mg/kg-day	Dose-dependent decreased body weight gain (M)	NTP, 1982; ECB, 2008	Body weight
B6C3F$_1$ mice (M&F)	Oral feeding	0, 800, 1600, 3100, 6300, 12,500 mg/kg (0, 100, 200, 400, 800, 1600 mg/kg-day; 10 mice per sex per group)	13 weeks	LOAEL = 100 mg/kg-day	Decreased body weight gain (F)	NTP, 1982; ECB, 2008	Body weight
Sprague-Dawley rats (M&F)	Oral feeding	0, 5, 50, 500, 5000 mg/kg (0, 0.4, 3.7, 37.6, 375.2 mg/kg-day (M); 0, 0.4, 4.2, 42.2, 414.3 mg/kg-day (F); 10 rats per sex per group; GLP)	13 weeks	NOAEL = 375.2 mg/kg-day	No toxicologically significant effects on body weight (M)	Poon *et al.*,1997; ATSDR, 2002	Body weight
Fischer 344 rats	Oral feeding	N/A	4-16 weeks	LOAEL = 1054 mg/kg-day	Decreased final body weight at 4 weeks (19%)	Eagon *et al.*,1994; ATSDR, 2002	Body weight
Sprague-Dawley rats (M&F)	Oral feeding	0, 0.2, 1.0, and 2.0% (0, 143, 737, 1440 mg/kg-day; M; 0, 154, 797, 1414 mg/kg-day; F) (15 rats per group): 0, 1.0, and 2.0% (0, 737, 1440 mg/kg-day; M; 0, 797, 1414 mg/kg-day; F): 0 and 2.0% (0, 1440 mg/kg-day; M; 0, 1414 mg/kg-day; F) (10 rats per group)	17 weeks: 2 or 6 weeks:	LOAEL = 737-797 mg/kg-day; NOAEL = 143-154 mg/kg-day	Significant dose-dependent decrease in body weight by day 27 (M&F), 55 (M), 90 (M&F), 120 (M&F; $P < 0.001$-0.05)	Gray *et al.*, 1977	Body weight

Sprague-Dawley rats (M&F)	Oral feeding	0, 0.2, 1.0, and 2.0% (0, 143, 737, 1440 mg/kg-day; M; 0, 154, 797, 1414 mg/kg-day; F) (15 rats per group): 0, 1.0, and 2.0% (0, 737, 1440 mg/kg-day; M; 0, 797, 1414 mg/kg-day; F): 0 and 2.0% (0, 1440 mg/kg-day; M; 0, 1414 mg/kg-day; F) (10 rats per group)	17 weeks: 2 or 6 weeks:	LOAEL = 1414-1440 mg/kg-day; NOAEL = 737-797 mg/kg-day	Significant dose-dependent decrease in body weight by day 55 (F; $P < 0.001$)	Gray *et al.*, 1977	Body weight
Sprague-Dawley rats (M&F)	Oral feeding	0, 0.2, 1.0, and 2.0% (0, 143, 737, 1440 mg/kg-day; M; 0, 154, 797, 1414 mg/kg-day; F) (15 rats per group): 0, 1.0, and 2.0% (0, 737, 1440 mg/kg-day; M; 0, 797, 1414 mg/kg-day; F): 0 and 2.0% (0, 1440 mg/kg-day; M; 0, 1414 mg/kg-day; F) (10 rats per group)	17 weeks: 2 or 6 weeks:	LOAEL = 737 mg/kg-day; NOAEL = 143 mg/kg-day	Significant dose-dependent decrease in the body weight (M 6, 17 weeks; $P < 0.01$)	Gray *et al.*, 1977	Body weight
Sprague-Dawley rats (M&F)	Oral feeding	0, 0.2, 1.0, and 2.0% (0, 143, 737, 1440 mg/kg-day; M; 0, 154, 797, 1414 mg/kg-day; F) (15 rats per group): 0, 1.0, and 2.0% (0, 737, 1440 mg/kg-day; M; 0, 797, 1414 mg/kg-day; F): 0 and 2.0% (0, 1440 mg/kg-day; M; 0, 1414 mg/kg-day; F) (10 rats per group)	17 weeks: 2 or 6 weeks:	LOAEL = 1414-1440 mg/kg-day; NOAEL = 737-797 mg/kg-day	Significant dose-dependent decrease in the body weight (M&F, 2, 6, 17 weeks; $P < 0.001$-0.01)	Gray *et al.*, 1977	Body weight
Cr1:CD-1 mice	Oral feeding	N/A	18 weeks (126 days)	NOAEL = 420 mg/kg-day	No toxicologically significant effects on body weight	Lamb *et al.*,1987; ATSDR, 2002	Body weight
Sv/129 mice (M)	Oral feeding	N/A	24 weeks	LOAEL = 2400 mg/kg-day	Decreased final body weight (50%; M)	Ward *et al.*,1998; ATSDR, 2002	Body weight
CH3/HeNCr mice	Oral feeding	N/A	24 weeks	LOAEL = 1953 mg/kg-day	Decreased final body weight (> 50%)	Weghorst *et al.*,1994; ATSDR, 2002	Body weight
Syrian golden hamsters	Oral feeding	N/A	30 weeks	LOAEL = 1436 mg/kg-day	Decreased final body weight (16%)	Maruyama *et al.*,1994; ATSDR, 2002	Body weight
Alderley Park rats (M&F)	Oral feeding	0, 50, 200, 1000 mg/kg-day (20 rats per sex per group; 30 rats per sex in control; GLP)	3, 7, 14, 28 days or 36 weeks (9 months)	LOAEL = 200 {1000} mg/kg-day; NOAEL = 50 {200} mg/kg-day	Decreased body weight gain (10-15%), decreased body weight at 9 months	CEFIC, 1982; Mitchell *et al.*,1985a; ECB, 2008; ATSDR, 2002	Body weight
Fischer 344 rats	Oral feeding	N/A	52 weeks	LOAEL = 947 mg/kg-day	Decreased final body weight (17%)	Marsman *et al.*,1988; ATSDR, 2002	Body weight
Sherman rats	Oral feeding	N/A	52 weeks	LOAEL = 200 mg/kg-day; NOAEL = 60 mg/kg-day	Decreased body weight gain at 52 weeks	Carpenter *et al.*,1953; ATSDR, 2002	Body weight

Guinea pig (NS)	Oral feeding	N/A	52 weeks	NOAEL = 64 mg/kg-day	No toxicologically significant effects on body weight	Carpenter *et al.*,1953; ATSDR, 2002	Body weight
Dog (NS)	Capsule	N/A	Once daily for 5 days/week for 52 weeks	NOAEL = 59 mg/kg-day	No toxicologically significant effects on body weight	Carpenter *et al.*,1953; ATSDR, 2002	Body weight
Wistar rats	Oral feeding	N/A	79 weeks	LOAEL = 867 mg/kg-day	Decreased body weight gain (21%)	Tamura *et al.*,1990; ATSDR, 2002	Body weight
Sprague-Dawley rats (M)	Oral feeding	0, 0.02, 0.2, 2.0% (0, 7, 70, 700 mg/kg-day; 520 total rats; GL)	102 weeks	LOAEL = 70{140} mg/kg-day; NOAEL = 7{14} mg/kg-day	Decreased final body weight (~ 10%)	Ganning *et al.*,1987, 1991; ECB, 2008; ATSDR, 2002	Body weight
Sprague-Dawley rats (M)	Oral feeding	0, 0.02, 0.2, 2.0% (0, 7, 70, 700 mg/kg-day; 520 total rats; GL)	102 weeks	LOAEL = 700{1400} mg/kg-day; NOAEL = 70 mg/kg-day	Decreased final body weight (~ 27%)	Ganning *et al.*,1987, 1991; ECB, 2008; ATSDR, 2002	Body weight
Fischer 344 rats (M&F)	Oral feeding	0, 6000, 12,000 mg/kg (0, 322, 674 mg/kg-day (M); 0, 394, 774 mg/kg-day (F); 50 rats per sex per group)	103 weeks	LOAEL = 322 mg/kg-day	Dose-dependent decreased body weight gain (M)	NTP, 1982; ECB, 2008	Body weight
Fischer 344 rats (M&F)	Oral feeding	0, 6000, 12,000 mg/kg (0, 322, 674 mg/kg-day (M); 0, 394, 774 mg/kg-day (F); 50 rats per sex per group)	103 weeks	LOAEL = 744 mg/kg-day; NOAEL = 394 mg/kg-day	Decreased body weight (F)	NTP, 1982; ECB, 2008	Body weight
B6C3F$_1$ mice (M&F)	Oral feeding	0, 3000, 6000 mg/kg (0, 672, 1325 mg/kg-day (M); 0, 799, 1821 mg/kg-day (F); 50 mice per sex per group; GLP)	103 weeks	LOAEL = 799 {1325, 1821} mg/kg-day; NOAEL = {672, 799} mg/kg-day	Dose-dependent decreased body weight gain from week 25 on (F)	NTP, 1982; ECB, 2008	Body weight
Sherman rats (M)	Oral feeding	N/A	104 weeks	LOAEL = 190 mg/kg-day; NOAEL = 60 mg/kg-day	Decreased body weight gain (M)	Carpenter *et al.*,1953; ATSDR, 2002	Body weight
Fischer 344 rats (M)	Oral feeding	N/A	104 weeks	LOAEL = 789 mg/kg-day	Decreased body weight gain (15%; M)	David *et al.*,1999, 2000a; ATSDR, 2002	Body weight
B6C3F$_1$ mice (M&F)	Oral feeding	0, 100, 500, 1500, or 6000 mg DEHP/kg feed (19.2, 98.5, 292.2, 1266.1 mg/kg-day for M; 23.8, 116.8, 354.2, 1458.2 mg/kg-day for F; 10-15 mice per sex per group)	79 or 105 weeks	LOAEL = 1266.1 – 1458.2 mg/kg-day; NOAEL = 292.2 – 354.2 mg/kg-day	Significant decrease in the terminal body weights (10%, M; 6%,F) at 105 weeks ($P \leq 0.05$)	David *et al.*, 2000b	Body weight
Fischer 344 rats	Oral feeding	N/A	108 weeks	LOAEL = 2000 mg/kg-day	Decreased in body weight gain (27%)	Rao *et al.*,1990; ATSDR, 2002	Body weight
Wistar rats (M&F)	Oral feeding	0, 0.1, 0.5% (0, 50-80, 300-400 mg/kg-day; 43 rats per sex per group	3, 6, 12, 24 months	LOAEL = 300-400 mg/kg-day; NOAEL = 50-80 mg/kg-day	Decrease in body weight	Harris *et al.*,1956; ECB, 2008	Body weight
Wistar rats (M&F)	Oral feeding	0, 1000, 3000, 9000 mg/kg (0, 113, 340, 1088 mg/kg-day; 20-25 rats per sex per group; GLP)	3 generations	LOAEL = 1088 mg/kg-day; NOAEL = 340 mg/kg-day	Decreased body weight gain in F_0 during pregnancy (11%), decreased body weight in F_1 adults during pregnancy (15%), on Ld 21 (14-21%), decreased body weight in F_1 M pups on PNd 1 (6%), 7 (6%), 14 (26%), 21 (31%), in F_1 F pups on PNd 7 (16%), 14 (27%), 21 (31%), in F_2 M pups on PNd 7 (11%), 14 (29%), 21 (35%), in F_2 F pups on PNd 7 (11%), 14 (21%), 21 (33%),	Schilling *et al.*,2001; CERHR, 2006; ECB, 2008	Body weight

Wistar rats (M&F)	Oral feeding	0, 1000, 3000, 9000 mg/kg (0, 113, 340, 1088 mg/kg-day; 20-25 rats per sex per group; GLP)	3 generations	LOAEL = 340 mg/kg-day; NOAEL = 113 mg/kg-day	Decreased body weight in F2 pups (8%)	Schilling *et al.*,2001; CERHR, 2006; ECB, 2008	Body weight
Fischer 344 rats	Oral gavage	N/A	Once daily for 3 days	LOAEL = 1200 mg/kg-day	Decreased food consumption (44%)	Adinehzadeh and Reo, 1998; ATSDR, 2002	Food consumption
Sprague-Dawley rats (F)	Oral gavage	2000 mg DEHP/kg bw (7-8 dams dosed per group, 10 pups per dam)	Once daily during Ld 2-6, 6-10, 14-18, 15-17	LOAEL = 2000 mg/kg	Significant decrease in food consumption in rat dams dosed during Ld 14-18 ($P < 0.05$)	Dostal *et al.*, 1987	Food consumption
Fischer 344 rats (CrlBr; F)	Oral feeding	0, 0.5, 1.0, 1.5, 2% (0, 357…mg/kg-day; 34-25 rats per group)	Once daily for 20 days during Gd 0-20	LOAEL = 357 mg/kg-day	Decreased maternal food consumption	Tyl *et al.*,1988; NTIS, 1984; ECB, 2008	Food consumption
Wistar rats (M&F)	Oral gavage	0, 2500 mg/kg-day (6 rats per sex per group)	Once daily for 7 or 21 days	LOAEL = 2500 mg/kg-day	Significant decrease in food consumption after 10 and 20 days of exposure	Mangham *et al.*,1981	Food consumption
Sprague-Dawley rats (M&F)	Oral feeding	0, 0.2, 1.0, and 2.0% (0, 143, 737, 1440 mg/kg-day; M; 0, 154, 797, 1414 mg/kg-day; F) (15 rats per group): 0, 1.0, and 2.0% (0, 737, 1440 mg/kg-day; M; 0, 797, 1414 mg/kg-day; F): 0 and 2.0% (0, 1440 mg/kg-day; M; 0, 1414 mg/kg-day; F) (10 rats per group)	17 weeks: 2 or 6 weeks:	LOAEL = 737 mg/kg-day; NOAEL = 737 mg/kg-day	Significant dose-dependent decrease in food consumption by day 1 (M), 55 (M; $P < 0.01$-0.05)	Gray *et al.*, 1977	Food consumption
Sprague-Dawley rats (M&F)	Oral feeding	0, 0.2, 1.0, and 2.0% (0, 143, 737, 1440 mg/kg-day; M; 0, 154, 797, 1414 mg/kg-day; F) (15 rats per group): 0, 1.0, and 2.0% (0, 737, 1440 mg/kg-day; M; 0, 797, 1414 mg/kg-day; F): 0 and 2.0% (0, 1440 mg/kg-day; M; 0, 1414 mg/kg-day; F) (10 rats per group)	17 weeks: 2 or 6 weeks:	LOAEL = 1414-1440 mg/kg-day; NOAEL = 737-797 mg/kg-day	Significant dose-dependent decrease in food consumption by day 1 (F), 27 (M&F), 55 (F), 90 (M&F), 120 (F; $P < 0.01$-0.05)	Gray *et al.*, 1977	Food consumption
Fischer 344 rats	Oral feeding	0, 6000, 12,000 mg/kg (0, 322, 674 mg/kg-day (M); 0, 394, 774 mg/kg-day (F); 50 rats per sex per group)	103 weeks	LOAEL = 322 mg/kg-day	Decreased food consumption (M and F; 14-25%)	NTP, 1982	Food consumption
Wistar rats (M&F)	Oral feeding	0, 1000, 3000, 9000 mg/kg (0, 113, 340, 1088 mg/kg-day; 20-25 rats per sex per group; GLP)	3 generations	LOAEL = 1088 mg/kg-day; NOAEL = 340 mg/kg-day	Decreased food consumption in F_0 adults during Ld 1-4 (18%), Ld 4-7 (21%), Ld 7-14 (33%), and F_1 rats during Gd 0-7 (7%), Gd 7-14 (9%), Ld 1-4 (32%), Ld 4-7 (31%), and Ld 7-14 (44%)	Schilling *et al.*,2001; CERHR, 2006; ECB, 2008	Food consumption
Wistar rats (M&F)	Oral feeding	0, 1000, 3000, 9000 mg/kg (0, 113, 340, 1088 mg/kg-day; 20-25 rats per sex per group; GLP)	3 generations	LOAEL = 340 mg/kg-day; NOAEL = 113 mg/kg-day	Decreased food consumption in F_1 rats during Ld 7-14	Schilling *et al.*,2001; CERHR, 2006; ECB, 2008	Food consumption
Wistar rats (M&F)	Oral gavage	0, 2500 mg/kg-day (6 rats per sex per group)	Once daily for 7 or 21 days	LOAEL = 2500 mg/kg-day	No toxicologically significant changes in water intake	Mangham *et al.*,1981	Water intake

Sprague-Dawley rats (M&F)	Oral feeding	0, 0.2, 1.0, and 2.0% (0, 143, 737, 1440 mg/kg-day; M; 0, 154, 797, 1414 mg/kg-day; F) (15 rats per group): 0, 1.0, and 2.0% (0, 737, 1440 mg/kg-day; M; 0, 797, 1414 mg/kg-day; F): 0 and 2.0% (0, 1440 mg/kg-day; M; 0, 1414 mg/kg-day; F) (10 rats per group)	17 weeks: 2 or 6 weeks:	LOAEL = 1414-1440 mg/kg-day; NOAEL = 737-797 mg/kg-day	Significant dose-dependent decrease in water intake by day 27 (M), Significant increase in water consumption by day 90 (M; $P < 0.01$)	Gray *et al.*, 1977	Water intake
Skin/Eye							
White Vienna rabbits	Dermal	Undiluted DEHP – 2.5 cm^2 patch (3 animals; GL)	Once for 4 hours, occlusive, post-wash	Not irritating	No evidence of erythema or edema (zero scores on ranking system)	BASF, 1986; ECB, 2008	Skin
Little White Russian rabbits (M)	Dermal	Undiluted DEHP – 6 cm^2 patch (3 M animals; GL)	Once for 4 hours, occlusive, post-wash	Slightly irritating	Very slight erythema by 1 hour and edema in one rabbit, well defined erythema in one rabbit by 24 hours, slight erythema in all rabbits by 48 and 72 hours with skin dryness at 72 hours, scaly skin by day 6, clearance by day 8	Hüls, 1987a; ECB, 2008	Skin
New Zealand White rabbits (M&F)	Dermal	Undiluted DEHP – 1 in^2 patch (3 animals each gender; intact and abraded skin; GLP)	Once for 24 hours, occlusive, post-wash	Slightly irritating	Mild to moderate reactions by 24 hours, with clearance by 72 hours	Hüls, 1981; ECB, 2008	Skin
Albino Dunkin-Hartley guinea pigs – formalin sensitive (F)	Dermal	40, 60, 80, 100% DEHP (1 animal per group)	Once for 6 hours of patch	No irritation	No toxicologically significant dermal effects	Exxon, 1994	Skin
Humans	Dermal	Undiluted DEHP (23 adults)	Twice as a patch test for 7 days then reapplied 10 days later	Not irritating	No erythema or other reactions	Shaffer *et al.*,1945; ECB, 2008	Skin
Albino Dunkin-Hartley guinea pigs (F)	Intradermal and dermal (Magnusson-Kligman test)	Induction – 10% DEHP in paraffin oil and Freund's adjuvant, 50% DEHP in paraffin oil for patch Challenge – 50% DEHP patch	Induction – intradermal injection followed by 48 hour dermal patch application 7 days later Challenge – 14 days after induction 24 hour patch test	Not a sensitizer according to the Magnusson-Kligman guinea pig maximization test	Not irritating or sensitization following challenge	Hüls, 1981; ECB, 2008	Skin
Albino Dunkin-Hartley guinea pigs (F)	Dermal	Undiluted DEHP (20 animals in control group, 20 animals in treatment group; GLP)	Induction – three, 6-hour patch applications on days 1, 8, and 15 Challenge – three, 6-hour patch applications on days 1, 8, and 15 of challenge	Not a sensitizer according to the Buehler test	No sensitization	Exxon, 1994; ECB, 2008	Skin

Marmoset monkey	Oral gavage	N/A	Once daily for 13 weeks	NOAEL = 2500 mg/kg-day	No toxicologically significant dermal effects	Kurata *et al.*,1998; ATSDR, 2002	Skin
White Vienna rabbits	Ocular	Undiluted DEHP – 0.1 ml in conjunctival sac (3 animals; GL)	Once, no post-wash	Slightly irritating	0.1 score for conjunctiva redness, 0.0 for corneal opacity at 24, 48, and 72 hours	BASF, 1986; ECB, 2008	Eye
Little White Russian rabbits (M)	Ocular	Undiluted DEHP – 0.1 ml in conjunctival sac (3 animals; GL)	Once, no post-wash	Slightly irritating	Conjunctiva of all rabbits mild redness at 1 hour with one rabbit showing mild discharge, no cojunctival reactions, chemosis, corneal opacity, or iris lesions at subsequent times	Hüls, 1987b; ECB, 2008	Eye
New Zealand White rabbits (M&F)	Ocular	Undiluted DEHP – 0.1 ml in conjunctival sac (3 animals each gender; GLP)	Once, no post-wash	Slightly irritating	No reactions with cornea or iris at any time, conjunctiva of 5 rabbits mildly red at 1 hour, one rabbit mildly red iris, mild redness in conjunctiva of 3 rabbits at 24 hours, no redness by 72 hours or 7 days	Hüls, 1981; ECB, 2008	Eye
Marmoset monkey	Oral gavage	N/A	Once daily for 13 weeks	NOAEL = 2500 mg/kg-day	No toxicologically significant ocular effects	Kurata *et al.*,1998; ATSDR, 2002	Eye
Clinical chemistry/Hematology							
Sprague-Dawley rats (M)	Oral gavage	0, 10, 100, 1000, 2000 mg/kg-day (10 rats per group; GL)	Once daily for 5 days (from D6, 14-16, 21,42, and 86 of age)	LOAEL = 100 mg/kg-day; NOAEL = 10 mg/kg-day	Decreased triglycerides and decreased cholesterol	Dostal *et al.*,1987b; ECB, 2008	Clinical chemistry
Sprague-Dawley rats (M)	Oral gavage	0, 10, 100, 1000, 2000 mg/kg-day (10 rats per group; GL)	Once daily for 5 days (from D6, 14-16, 21,42, and 86 of age)	LOAEL = 1000 mg/kg-day; NOAEL = 100 mg/kg-day	Decreased plasma cholesterol in weanlings and adults	Dostal *et al.*,1987b; ECB, 2008	Clinical chemistry
Sprague-Dawley rats (F)	Oral gavage	2000 mg DEHP/kg bw (7-8 dams dosed per group, 10 pups per dam)	Once daily during Ld 2-6, 6-10, 14-18, 15-17	LOAEL = 2000 mg/kg	Significant decrease in plasma cholesterol in maternal rats dosed during Ld 2-6, 6-10, and 14-18 ($P < 0.05$)	Dostal *et al.*, 1987	Clinical chemistry
Sprague-Dawley rats (F)	Oral gavage	2000 mg DEHP/kg bw (7-8 dams dosed per group, 10 pups per dam)	Once daily during Ld 2-6, 6-10, 14-18, 15-17	LOAEL = 2000 mg/kg	Significant decrease in plasma triglycerides in maternal rats dosed during Ld 2-6 and 14-18 ($P < 0.05$)	Dostal *et al.*, 1987	Clinical chemistry
Sprague-Dawley rats (F)	Oral gavage	2000 mg DEHP/kg bw (7-8 dams dosed per group, 10 pups per dam)	Once daily during Ld 2-6, 6-10, 14-18, 15-17	LOAEL = 2000 mg/kg	Significant increase in solids, protein, lipids, and significant decrease in lactose ($P < 0.05$; Pair-fed control had significant increase in protein and decrease in lactose)	Dostal *et al.*, 1987	Clinical chemistry – milk composition
Sprague-Dawley rats (F)	Oral gavage	2000 mg DEHP/kg bw (7-8 dams dosed per group, 10 pups per dam)	Once daily during Ld 2-6, 6-10, 14-18, 15-17	LOAEL = 2000 mg/kg	Substantial increase in DEHP and MEHP in lactating rat dam plasma and milk; Marginal increase in suckling pup rat MEHP concentration	Dostal *et al.*, 1987	Clinincal chemistry
Sprague-Dawley rats (F)	Oral gavage	2000 mg DEHP/kg bw (7-8 dams dosed per group, 10 pups per dam)	Once daily during Ld 2-6, 6-10, 14-18, 15-17	NOAEL = 2000 mg/kg	No toxicologically significant change in suckling pup DEHP plasma level	Dostal *et al.*, 1987	Clinincal chemistry
Fischer 344 rats (M)	Oral feeding	0, 2% (1600 mg/kg-day; 8 rats per group; GL)	7 days	LOAEL = 1600 mg/kg-day	Decreased serum cholesterol and triglycerides	Exxon, 1982a,b; ECB, 2008	Clinical chemistry
Cynomolgous monkeys (M)	Oral gavage	N/A	Once daily for 14 days	NOAEL = 500 mg/kg-day	No toxicologicall significant effects on clinical chemistry	Pugh *et al.*,2000; ATSDR, 2002	Clinical chemistry
Wistar - Alderley Park rats (M&F)	Oral gavage	0 and 2000 mg/kg-day (10 rats per sex per group; GLP)	Once daily for 14 days	LOAEL = 2000 mg/kg-day	Decreased serum cholesterol (M) and decreased triglycerides (M)	ICI, 1982b; Rhodes *et al.*,1986; ECB, 2008	Clinical chemistry

Fischer 344 rats (M&F)	Oral feeding	0, 0.1, 0.6, 1.2% (0, 80, 480, 960 mg/kg-day; 4-5 rats per sex per group; GLP)	7 days or 21 days	LOAEL = 80 mg/kg-day	Decreased serum triglycerides	CMA, 1982c; ECB, 2008	Clinical chemistry
Fischer 344 rats (M&F)	Oral feeding	0, 0.1, 0.6, 1.2% (0, 80, 480, 960 mg/kg-day; 4-5 rats per sex per group; GLP)	7 days or 21 days	LOAEL = 480 mg/kg-day; NOAEL = 80 mg/kg-day	Decreased serum cholesterol	CMA, 1982c; ECB, 2008	Clinical chemistry
Fischer 344 rats (M)	Oral feeding	0, 2% (4-5 rats per group)	21 days	LOAEL = 2%	Hypolipidemia	Moody and Reddy, 1978; ECB, 2008	Clinical chemistry
Sprague-Dawley rats (M)	Oral feeding	0, 2% (900 mg/kg-day; 4 rats per group)	21 days	LOAEL = 900 mg/kg-day	Increasing trend for cholesterol and triglycerides	General Motors, 1982; ECB, 2008	Clinical chemistry
Fischer 344 rats (M&F)	Oral feeding	0, 0.01, 0.1, 0.6, 1.2, 2.5% (0, 11, 105, 667, 1224, 2101 mg/kg-day (M); 0, 12, 109, 643, 1197, 1892 mg/kg-day (F); 5 rats per sex per group; GLP)	21 days	LOAEL = 667 mg/kg-day; NOAEL = 105 mg/kg-day	Decreased trigycerides (M)	CMA, 1984b; Barber *et al.*,1987; ECB, 2008	Clinical chemistry
Fischer 344 rats (M&F)	Oral feeding	0, 0.01, 0.1, 0.6, 1.2, 2.5% (0, 11, 105, 667, 1224, 2101 mg/kg-day (M); 0, 12, 109, 643, 1197, 1892 mg/kg-day (F); 5 rats per sex per group; GLP)	21 days	LOAEL = 11 mg/kg-day	Increased triglycerides (M)	CMA, 1984b; Barber *et al.*,1987; ECB, 2008	Clinical chemistry
Fischer 344 rats (M&F)	Oral feeding	0, 0.01, 0.1, 0.6, 1.2, 2.5% (0, 11, 105, 667, 1224, 2101 mg/kg-day (M); 0, 12, 109, 643, 1197, 1892 mg/kg-day (F); 5 rats per sex per group; GLP)	21 days	LOAEL = 1197 mg/kg-day; NOAEL = 643 mg/kg-day	Increased trigycerides (F)	CMA, 1984b; Barber *et al.*,1987; ECB, 2008	Clinical chemistry
Fischer 344 rats (M&F)	Oral feeding	0, 0.2, 0.67, 2.0% (0, 150, 504, 1563 mg/kg-day (M); 0, 147, 490, 1416 mg/kg-day (F); 5 rats per sex per group; GLP)	28 days	LOAEL = 147 mg/kg-day	Decreased total lipids	Nuodex, 1981c; ECB, 2008	Clinical chemistry
Sprague-Dawley rats (M&F)	Oral feeding	0, 5, 50, 500, 5000 mg/kg (0, 0.4, 3.7, 37.6, 375.2 mg/kg-day (M); 0, 0.4, 4.2, 42.2, 414.3 mg/kg-day (F); 10 rats per sex per group; GLP)	13 weeks	LOAEL = 375.2 – 414.3 mg/kg-day; NOAEL = 37.6 – 42.2 mg/kg-day	Significant increase in albumin (M) and albumin/globulin (M&F; $P < 0.01$) and potassium ($P < 0.05$), platelet count	Poon *et al.*,1997; ATSDR, 2002	Clinincal chemistry
Sprague-Dawley rats (M&F)	Oral feeding	0, 5, 50, 500, 5000 mg/kg (0, 0.4, 3.7, 37.6, 375.2 mg/kg-day (M); 0, 0.4, 4.2, 42.2, 414.3 mg/kg-day (F); 10 rats per sex per group; GLP)	13 weeks	LOAEL = 345.0 – 411.0 mg/kg-day	Significant increase in calcium (M; $P < 0.05$), inorganic phosphate (M&F; $P < 0.05$), total protein (M&F; $P < 0.05$), albumin (F; $P < 0.05$) (mg/dL)	Poon *et al.*,1997; ATSDR, 2002	Clinical chemistry
Sprague-Dawley rats (M&F)	Oral feeding	0, 5, 50, 500, 5000 mg/kg (0, 0.4, 3.7, 37.6, 375.2 mg/kg-day (M); 0, 0.4, 4.2, 42.2, 414.3 mg/kg-day (F); 10 rats per sex per group; GLP)	13 weeks	LOAEL = 0.4 mg/kg-day	Significant decrease in aminotransferase (M; $P < 0.01$-0.05); Substantial decrease in aminotransferase (F)	Poon *et al.*,1997; ATSDR, 2002	Clinical chemistry

Sprague-Dawley rats (M&F)	Oral feeding	0, 5, 50, 500, 5000 mg/kg (0, 0.4, 3.7, 37.6, 375.2 mg/kg-day (M); 0, 0.4, 4.2, 42.2, 414.3 mg/kg-day (F); 10 rats per sex per group; GLP)	13 weeks	LOAEL = 414.3 mg/kg-day; NOAEL = 42.2 mg/kg-day	Significant decrease in aspartate cholesterol (F; $P < 0.05$)	Poon *et al.*,1997; ATSDR, 2002	Clinical chemistry
Humans (97 M/4 F	Inhalation /Dermal (epi)	Background (0.001-0.004 ppm ~ 0.016-0.064 mg/m^3) Higher levels – 0.01 ppm (0.16 mg/m^3)	12 years average exposure period (4 months to 35 years)	NS	Serum lipids normal	Thiess *et al.*,1978b	Clinical chemistry
Wistar rats	Oral feeding	N/A	12 weeks (90 days)	NOAEL = 1900 mg/kg-day	No toxicologically significant effects on hematology	Shaffer *et al.*,1945; ATSDR, 2002	Hematology
Marmoset monkeys	Oral gavage	N/A	Once daily for 13 weeks	NOAEL = 2500 mg/kg-day	No toxicologically significant effects on hematology	Kurata *et al.*,1998; ATSDR, 2002	Hematology
Sprague-Dawley rats (M&F)	Oral feeding	0, 5, 50, 500, 5000 mg/kg (0, 0.4, 3.7, 37.6, 375.2 mg/kg-day (M); 0, 0.4, 4.2, 42.2, 414.3 mg/kg-day (F); 10 rats per sex per group; GLP)	13 weeks	LOAEL = 375.2 – 414.3mg/kg-day; NOAEL = 37.6 – 42.2 mg/kg-day	Significant dose-dependent decrease in red blood cells and hemoglobin (M; $P < 0.01$)	Poon *et al.*,1997; ATSDR, 2002	Hematology
Sprague-Dawley rats (M&F)	Oral feeding	0, 5, 50, 500, 5000 mg/kg (0, 0.4, 3.7, 37.6, 375.2 mg/kg-day (M); 0, 0.4, 4.2, 42.2, 414.3 mg/kg-day (F); 10 rats per sex per group; GLP)	13 weeks	LOAEL = 345.0 – 411.0 mg/kg-day	Significant increase in white blood cells (F: $P < 0.05$), platelet count (M&F; $P < 0.05$); Significant decrease in mean corpuscular hemoglobin, mean corpuscular volume (F; $P < 0.05$)	Poon *et al.*,1997; ATSDR, 2002	Hematology
Sprague-Dawley rats (M&F)	Oral feeding	0, 0.2, 1.0, and 2.0% (0, 143, 737, 1440 mg/kg-day; M; 0, 154, 797, 1414 mg/kg-day; F) (15 rats per group): 0, 1.0, and 2.0% (0, 737, 1440 mg/kg-day; M; 0, 797, 1414 mg/kg-day; F): 0 and 2.0% (0, 1440 mg/kg-day; M; 0, 1414 mg/kg-day; F) (10 rats per group)	17 weeks: 2 or 6 weeks:	LOAEL = 737-797 mg/kg-day; NOAEL = 143-154 mg/kg-day	Significant dose-dependent decrease in hemoglobin (M), packed cell volume (M&F) by 17 weeks of age, and packed cell volume (F) by 6 weeks of age ($P < 0.001$-0.05); Substantial decrease in hemoglobin by 17 weeks of age (F)	Gray *et al.*, 1977	Hematology
Sherman rats	Oral feeding	N/A	52 weeks	NOAEL = 200 mg/kg-day	No toxicologically significant effects on hematology	Carpenter *et al.*,1953; ATSDR, 2002	Hematology
Sherman rats (M)	Oral feeding	N/A	104 weeks	NOAEL = 190 mg/kg-day	No toxicologically significant effects on hematology	Carpenter *et al.*,1953; ATSDR, 2002	Hematology
Fischer 344 rats	Oral feeding	N/A	104 weeks	NOAEL = 939 mg/kg-day	No toxicologically significant effects on hematology (F)	David *et al.*,1999, 2000a; ATSDR, 2002	Hematology
B6C3F$_1$ mice (M&F)	Oral feeding	0, 100, 500, 1500, or 6000 mg DEHP/kg feed (19.2, 98.5, 292.2, 1266.1 mg/kg-day for M; 23.8, 116.8, 354.2, 1458.2 mg/kg-day for F; 10-15 mice per sex per group)	79 or 105 weeks	LOAEL = 1266.1 – 1458.2 mg/kg-day; NOAEL = 292.2 – 354.2 mg/kg-day	Significant decrease in the mean corpuscular hemoglobin (ng/dL; M&F; $P < 0.05$)	David *et al.*, 2000b	Hematology

B6C3F$_1$ mice (M&F)	Oral feeding	0, 100, 500, 1500, or 6000 mg DEHP/kg feed (19.2, 98.5, 292.2, 1266.1 mg/kg-day for M; 23.8, 116.8, 354.2, 1458.2 mg/kg-day for F; 10-15 mice per sex per group)	79 or 105 weeks	LOAEL = 19.2 mg/kg-day	Significant decrease in the mean corpuscular hemoglobin (pg; M; $P < 0.05$)	David *et al.*, 2000b	Hematology
B6C3F$_1$ mice (M&F)	Oral feeding	0, 100, 500, 1500, or 6000 mg DEHP/kg feed (19.2, 98.5, 292.2, 1266.1 mg/kg-day for M; 23.8, 116.8, 354.2, 1458.2 mg/kg-day for F; 10-15 mice per sex per group)	79 or 105 weeks	NOAEL = 1458.2 mg/kg-day	No toxicologically significant change in the mean corpuscular hemoglobin (pg; F); WBC, reticulocytes, platelets, MCV, Myeloid/erythroid ratio, Hct, HgH, RBC (M&F)	David *et al.*, 2000b	Hematology
Human (M)	Inhalation /dermal (epi)	0.1, 0.2, 0.7 mg/m^3 DEHP and BBP (54 workers)	N/A	N/A	Dose-related changes in hemoglobin, α-1-antitrypsin, and immunoglobulin A	Nielson *et al.*,1985	Hematology
Humans (97 M/4 F	Inhalation /Dermal (epi)	Background (0.001-0.004 ppm ~ 0.016-0.064 mg/m^3) Higher levels – 0.01 ppm (0.16 mg/m^3)	12 years average exposure period (4 months to 35 years)	NS	Hematological tests normal	Thiess *et al.*,1978b	Hematology
Immune/Lymph nodes/Thymus/Spleen							
Fischer 344 rats	Oral gavage	N/A	Once	NOAEL = 5000 mg/kg-day	No toxicologically significant effect on the immune system	Berman *et al.*,1995; ATSDR, 2002	Immune
Fischer 344 rats	Oral gavage	N/A	Once daily for 14 days	NOAEL = 1500 mg/kg-day	No toxicologically significant effect on the immune system	Berman *et al.*,1995; ATSDR, 2002	Immune
BALB/cj mice (F; 6-7 wk old)	Intraperitoneal	1µg model allergen hen egg plus 100µg DEHP; Boosters 10 or 100 µg DEHP	Primary immunization followed by booster at 14 and 21 days	LOAEL = 10µg DEHP	100µg - DEHP adjuvant factor for IgG1 of 33 after 1 boost and 61 after 2 10µg – DEHP adjuvant factor for IgG1 of 1 after 1 boost and 13 after 2 when comared to OVA control; no adjvant effects with IgE and IgG2a	Larsen and Nielsen, 2008	Immune
Wistar rats (M)	Oral feeding	N/A	12 weeks (90 days)	NOAEL = 1900 mg/kg-day	No toxicologically significant effect on the immune system (M)	Shaffer *et al.*,1945; ATSDR, 2002	Immune
Guinea pig (NS)	Oral feeding	N/A	52 weeks	NOAEL = 64 mg/kg-day	No toxicologically significant effect on the immune system	Carpenter *et al.*,1953; ATSDR, 2002	Immune
Sherman rats	Oral feeding	N/A	104 weeks	NOAEL = 190 mg/kg-day	No toxicologically significant effect on the immune system	Carpenter *et al.*,1953; ATSDR, 2002	Immune
Fischer 344 rats (M)	Oral feeding	0, 12,500 mg/kg	79 weeks	LOAEL = 12,500 mg/kg	Increased incidence of mononuclear cell leukemia (tumor frequency = 0, 10%, respectively)	David *et al.*, 2001; Ito and Nakajima, 2008	Immune
Fischer 344 rats (M&F)	Oral feeding	0, 6000, 12,000 mg/kg (0, 322, 674 mg/kg-day (M); 0, 394, 774 mg/kg-day (F); 50 rats per sex per group; GLP)	103 weeks	LOAEL = 674 mg/kg-day; NOAEL = 322 mg/kg-day	Increased incidence of mononuclear cell leukemia (F; 10/50, 20%; 14/50, 28%; 17/50, 34%)	NTP, 1982	Immune
Fischer 344 rats (M&F)	Oral feeding	0, 100, 500, 2500, 12,500 mg/kg (0, 6, 28.9, 146.6, 789 mg/kg-day (M); 0, 7, 36, 182, 939 mg/kg-day (F); 70-85 rats per sex per group	104 weeks	LOAEL = 146.6 mg/kg-day; NOAEL = 28.9 mg/kg-day	Significant irreversible increase in the incidence of mononuclear cell leukemia (M; $P < 0.05$ from concurrent and historical control)	Moore, 1996; ECB, 2008	Immune

Fischer 344 rats (M)	Oral feeding	0, 100, 500, 2500, 12,500 mg/kg	104 weeks	LOAEL = 2500 mg/kg	Increased incidence of mononuclear cell leukemia (tumor frequency = 23, 26, 29, 49, 42%, respectively)	David *et al.*, 2000; Ito and Nakajima, 2008	Immune
Fischer 344 rats (F)	Oral feeding	0, 100, 500, 2500, 12,500 mg/kg	104 weeks	LOAEL = 12,500 mg/kg	Increased incidence of mononuclear cell leukemia (tumor frequency = 22, 34, 20, 25, 26%, respectively)	David *et al.*, 2000; Ito and Nakajima, 2008	Immune
Fischer 344 rats (M)	Oral feeding	0, 12,500 mg/kg, recovery	105 weeks	LOAEL = 12,500 mg/kg	Increased incidence of mononuclear cell leukemia (tumor frequency = 23, 42, 53%, respectively)	David *et al.*, 2001; Ito and Nakajima, 2008	Immune
Wistar rats (F)	Oral gavage	0, 0.015, 0.045, 0.135, 0.405, 1.215, 5, 15, 45, 135, 405 mg/kg-day; ? rats per group)	Once daily for 27 days during Gd6 to Ld 21, recovery til 144 days old	NOAEL = 405 mg/kg-day	No toxicologically significant effects on thymus (M)	Andrade *et al.*, 2006b	Thymus
$B6C3F_1$ mice (M&F)	Oral feeding	0, 1000, 5000, 10,000, 25,000 mg/kg (0, 250, 1210, 2580, 6990 mg/kg-day(M); 0, 270, 1430, 2890, 7900 mg/kg-day (F); 10 mice per sex per group; GLP)	28 days	LOAEL = 6990 mg/kg-day; NOAEL = 2580 mg/kg-day	Thymus atrophy	Eastman Kodak, 1992b; ECB, 2008	Thymus
Wistar rats (F)	Oral gavage	0, 0.015, 0.045, 0.135, 0.405, 1.215, 5, 15, 45, 135, 405 mg/kg-day; ? rats per group)	Once daily for 27 days during Gd6 to Ld 21, recovery til 144 days old	NOAEL = 405 mg/kg-day	No toxicologically significant effects on spleen (M)	Andrade *et al.*, 2006b	Spleen
Sprague-Dawley rats (M&F)	Oral feeding	0, 0.2, 1.0, and 2.0% (0, 143, 737, 1440 mg/kg-day; M; 0, 154, 797, 1414 mg/kg-day; F) (15 rats per group): 0, 1.0, and 2.0% (0, 737, 1440 mg/kg-day; M; 0, 797, 1414 mg/kg-day; F): 0 and 2.0% (0, 1440 mg/kg-day; M; 0, 1414 mg/kg-day; F) (10 rats per group)	17 weeks: 2 or 6 weeks:	LOAEL = 737-797 mg/kg-day; NOAEL = 143-154 mg/kg-day	Significant dose-dependent decrease in the absolute spleen weight (M, 17 weeks; $P < 0.05$)	Gray *et al.*, 1977	Spleen
Sprague-Dawley rats (M&F)	Oral feeding	0, 0.2, 1.0, and 2.0% (0, 143, 737, 1440 mg/kg-day; M; 0, 154, 797, 1414 mg/kg-day; F) (15 rats per group): 0, 1.0, and 2.0% (0, 737, 1440 mg/kg-day; M; 0, 797, 1414 mg/kg-day; F): 0 and 2.0% (0, 1440 mg/kg-day; M; 0, 1414 mg/kg-day; F) (10 rats per group)	17 weeks: 2 or 6 weeks:	LOAEL = 1414-1440 mg/kg-day; NOAEL = 737-797 mg/kg-day	Significant dose-dependent decrease in the absolute spleen weight (M&F, 2, 6, 17 weeks; $P < 0.001$-0.05)	Gray *et al.*, 1977	Spleen

Sprague-Dawley rats (M&F)	Oral feeding	0, 0.2, 1.0, and 2.0% (0, 143, 737, 1440 mg/kg-day; M; 0, 154, 797, 1414 mg/kg-day; F) (15 rats per group): 0, 1.0, and 2.0% (0, 737, 1440 mg/kg-day; M; 0, 797, 1414 mg/kg-day; F): 0 and 2.0% (0, 1440 mg/kg-day; M; 0, 1414 mg/kg-day; F) (10 rats per group)	17 weeks: 2 or 6 weeks:	LOAEL = 1414 mg/kg-day; NOAEL = 797 mg/kg-day	Significant dose-dependent increase in the relative spleen weight (F, 17 weeks; $P < 0.05$)	Gray *et al.*, 1977	Spleen
Sprague-Dawley rats (M&F)	Oral feeding	0, 0.2, 1.0, and 2.0% (0, 143, 737, 1440 mg/kg-day; M; 0, 154, 797, 1414 mg/kg-day; F) (15 rats per group): 0, 1.0, and 2.0% (0, 737, 1440 mg/kg-day; M; 0, 797, 1414 mg/kg-day; F): 0 and 2.0% (0, 1440 mg/kg-day; M; 0, 1414 mg/kg-day; F) (10 rats per group)	17 weeks: 2 or 6 weeks:	LOAEL = 1414-1440 mg/kg-day; NOAEL = 737-797 mg/kg-day	Significant dose-dependent decrease in the relative spleen weight (M&F, 2 weeks; $P < 0.05$)	Gray *et al.*, 1977	Spleen
Musculoskeletal							
Marmoset monkey	Oral gavage	N/A	Once daily for 13 weeks	NOAEL = 2500 mg/kg-day	No toxicologically significant effect on the musculoskeletal system	Kurata *et al.*,1998; ATSDR, 2002	Musculoskeletal
Fischer 344 rats	Oral feeding	N/A	104 weeks	NOAEL = 939 mg/kg-day	No toxicologically significant effect on the musculoskeletal system (F)	David *et al.*,1999, 2000a; ATSDR, 2002	Musculoskeletal
$B6C3F_1$ mice (F)	Oral feeding	N/A	104 weeks	NOAEL = 1458 mg/kg-day	No toxicologically significant effect on the musculoskeletal system	David *et al.*,1999, 2000b; ATSDR, 2002	Musculoskeletal
Kidney/Adrenal glands							
CRL:CD Sprague-Dawley rats (F)	Oral gavage	0, 11, 33, 100, 300 mg/kg-day (12-14 litters per group)	Once daily during Gd 8-17; recovery til maturity; continued dosing from 18 til 63-65	LOAEL = 300 mg/kg-day; NOAEL = 100 mg/kg-day	In pups dosed from Gd 8 to PNd 64; Dose-dependent significant decrease in adrenal weight ($P < 0.05$)	Gray *et al.*, 2009	Adrenal
CRL:CD Sprague-Dawley rats (F)	Oral gavage	0, 11, 33, 100, 300 mg/kg-day (12-14 litters per group)	Once daily during Gd 8-17; recovery til maturity; continued dosing from 18 til 63-65	NOAEL = 300 mg/kg-day	In pups dosed from Gd 8 to Ld 17 then recovery; No toxicologically significant change in adrenal weight	Gray *et al.*, 2009	Adrenal

Sprague-Dawley rats (M&F)	Oral feeding	0, 0.2, 1.0, and 2.0% (0, 143, 737, 1440 mg/kg-day; M; 0, 154, 797, 1414 mg/kg-day; F) (15 rats per group): 0, 1.0, and 2.0% (0, 737, 1440 mg/kg-day; M; 0, 797, 1414 mg/kg-day; F): 0 and 2.0% (0, 1440 mg/kg-day; M; 0, 1414 mg/kg-day; F) (10 rats per group)	17 weeks: 2 or 6 weeks:	LOAEL = 1440 mg/kg-day; NOAEL = 797 mg/kg-day	Significant dose-dependent decrease in the absolute adrenal weight (F, 2, 6, 17 weeks; $P < 0.001$)	Gray *et al.*, 1977	Adrenal
Sprague-Dawley rats (M&F)	Oral feeding	0, 0.2, 1.0, and 2.0% (0, 143, 737, 1440 mg/kg-day; M; 0, 154, 797, 1414 mg/kg-day; F) (15 rats per group): 0, 1.0, and 2.0% (0, 737, 1440 mg/kg-day; M; 0, 797, 1414 mg/kg-day; F): 0 and 2.0% (0, 1440 mg/kg-day; M; 0, 1414 mg/kg-day; F) (10 rats per group)	17 weeks: 2 or 6 weeks:	LOAEL = 797 mg/kg-day; NOAEL = 154 mg/kg-day	Significant dose-dependent decrease in the absolute adrenal weight (F, 2 weeks; $P < 0.01$)	Gray *et al.*, 1977	Adrenal
Sprague-Dawley rats (M&F)	Oral feeding	0, 0.2, 1.0, and 2.0% (0, 143, 737, 1440 mg/kg-day; M; 0, 154, 797, 1414 mg/kg-day; F) (15 rats per group): 0, 1.0, and 2.0% (0, 737, 1440 mg/kg-day; M; 0, 797, 1414 mg/kg-day; F): 0 and 2.0% (0, 1440 mg/kg-day; M; 0, 1414 mg/kg-day; F) (10 rats per group)	17 weeks: 2 or 6 weeks:	LOAEL = 1440 mg/kg-day; NOAEL = 737 mg/kg-day	Significant dose-dependent increase in the relative adrenal weight (M, 2, 6 weeks; $P < 0.05$)	Gray *et al.*, 1977	Adrenal
Rats (NS)	Oral gavage	0, 1200 mg/kg-day	Once daily for 3 days	LOAEL = 1200 mg/kg-day	2-3 fold increase in kidney microsomal lauric acid omega-hydroxylation activity	Sharma *et al.*,1989	Kidney
Sprague-Dawley rats (M)	Oral gavage	0, 10, 100, 1000, 2000 mg/kg-day (10 rats per group; from day 6, 14-16, 21, 42, 86 of age; GL)	Once daily for 5 days	LOAEL = 1000 mg/kg-day; NOAEL = 100 mg/kg-day	Increased kidney weight	Dostal *et al.*,1987a; ATSDR, 2002	Kidney
Fischer 344 rats (M)	Oral feeding	0, 2% (1600 mg/kg-day; 8 rats per group; GL)	7 days	LOAEL = 1600 mg/kg-day	Increased absolute and relative kidney weight	Exxon, 1982a,b; ECB, 2008	Kidney
Fischer 344 rats (M)	Oral feeding	0, 2% (1600 mg/kg-day; 8 rats per group; GL)	7 days	NOAEL = 1600 mg/kg-day	No histopathological findings in kidney	Exxon, 1982a,b; ECB, 2008	Kidney
Wistar rats (F)	Oral gavage	0, 40, 200, 1000 mg/kg-day (9-10 litters per group)	Once daily during Gd 6-15	LOAEL = 1000 mg/kg-day; NOAEL = 200 mg/kg-day	Dose-dependent significant increase in the relative kidney weight ($P \leq 0.05$)	Hellwig *et al.*, 1997	Kidney
Cynomolgous monkeys (M)	Oral gavage	N/A	Once daily for 14 days	NOAEL = 500 mg/kg-day	No toxicologically significant kidney effects	Pugh *et al.*,2000; ATSDR, 2002	Kidney
Marmoset Monkey (M&F; 250-400g)	Oral gavage	0, 2000 mg/kg-day (5 monkeys per sex per group)	Once daily for 14 days	NOAEL {LOAEL} = 2000 mg/kg-day	{Significant decrease in relative kidney weight (F)}	Rhodes *et al.*,1986; ATSDR, 2002	Kidney

Wistar - Alderley Park rats (M&F)	Oral gavage	0 and 2000 mg/kg-day (10 rats per sex per group; GLP)	Once daily for 14 days	LOAEL = 2000 mg/kg-day	Increased kidney weight (F) and increased peroxisomal proliferation in kidney	ICI, 1982b; Rhodes *et al.*,1986; ECB, 2008	Kidney
Wistar - Alderley Park rats (M&F)	Oral gavage	0 and 2000 mg/kg-day (10 rats per sex per group; GLP)	Once daily for 14 days	NOAEL = 2000 mg/kg-day	No toxicologically significant kidney effects	Rhodes *et al.*,1986; ECB, 2008; ATSDR, 2002	Kidney
Fischer 344 rats	Oral feeding	N/A	14 days	LOAEL = 1200 mg/kg-day	Increased kidney weight	Takagi *et al.*,1990; ATSDR, 2002	Kidney
Sprague-Dawley rats (M)	Oral feeding	0, 2% (900 mg/kg-day; 4 rats per group)	21 days	LOAEL = 900 mg/kg-day	Increased absolute and relative kidney weight	General Motors, 1982; ECB, 2008	Kidney
Fischer 344 rats (M&F)	Oral feeding	0, 0.01, 0.1, 0.6, 1.2, 2.5% (0, 11, 105, 667, 1224, 2101 mg/kg-day (M); 0, 12, 109, 643, 1197, 1892 mg/kg-day (F); 5 rats per sex per group; GLP)	21 days	LOAEL = 1892 mg/kg-day; NOAEL = 1197 mg/kg-day	Increased relative kidney weight	CMA, 1984b; Barber *et al.*,1987; ECB, 2008	Kidney
Wistar rats (F)	Oral gavage	0, 0.015, 0.045, 0.135, 0.405, 1.215, 5, 15, 45, 135, 405 mg/kg-day; ? rats per group)	Once daily for 27 days during Gd6 to Ld 21, recovery til 144 days old	NOAEL = 405 mg/kg-day	No toxicologically significant effects on kidneys (M)	Andrade *et al.*, 2006b	Kidney
B6C3F$_1$ mice (M&F)	Oral feeding	0, 1000, 5000, 10,000, 25,000 mg/kg (0, 250, 1210, 2580, 6990 mg/kg-day(M); 0, 270, 1430, 2890, 7900 mg/kg-day (F); 10 mice per sex per group; GLP)	28 days	LOAEL = 1210 mg/kg-day; NOAEL = 250 mg/kg-day	Decreased absolute kidney weight (M), and inflammation of kidney	Eastman Kodak, 1992b; ECB, 2008	Kidney
CRL:CD Sprague-Dawley rats (F)	Oral gavage	0, 11, 33, 100, 300 mg/kg-day (12-14 litters per group)	Once daily during Gd 8-17; recovery til maturity; continued dosing from 18 til 63-65	LOAEL = 300 mg/kg-day; NOAEL = 100 mg/kg-day	In pups dosed from Gd 8 to PNd 64; Significant decrease in kidney weight ($P < 0.05$) In pups dosed from Gd 8 to Ld 17 then recovery; Significant decrease in kidney weight ($P < 0.01$)	Gray *et al.*, 2009	Kidney
Wistar rats	Oral feeding	N/A	12 weeks (90 days)	NOAEL = 1900 mg/kg-day	No toxicologically significant kidney effects	Shaffer *et al.*,1945; ATSDR, 2002	Kidney
Marmoset monkeys	Oral gavage	N/A	Once daily for 13 weeks	NOAEL = 2500 mg/kg-day	No toxicologically significant kidney effects	Kurata *et al.*,1998; ATSDR, 2002	Kidney
Fischer 344 rats (M&F)	Oral feeding	0, 1000, 4000, 12,500, 25,000 mg/kg (0, 63, 261, 859, 1724 mg/kg-day (M); 0, 73, 302, 918, 1858 mg/kg-day (F); 10 rats per sex per group; GLP)	13 weeks	LOAEL = 261 mg/kg-day; NOAEL = 63 mg/kg-day	Increased relative kidney weight (M)	Eastman Kodak, 1992a; ECB, 2008	Kidney
Fischer 344 rats (M&F)	Oral feeding	0, 1000, 4000, 12,500, 25,000 mg/kg (0, 63, 261, 859, 1724 mg/kg-day (M); 0, 73, 302, 918, 1858 mg/kg-day (F); 10 rats per sex per group; GLP)	13 weeks	LOAEL = 918 mg/kg-day; NOAEL = 302 mg/kg-day	Increased relative kidney weight (F)	Eastman Kodak, 1992a; ECB, 2008	Kidney

Sprague-Dawley rats (M&F)	Oral feeding	0, 5, 50, 500, 5000 mg/kg (0, 0.4, 3.7, 37.6, 375.2 mg/kg-day (M); 0, 0.4, 4.2, 42.2, 419.3 mg/kg-day (F); 10 rats per sex per group; GLP)	13 weeks	LOAEL = 375.2 mg/kg-day; NOAEL = 37.6 mg/kg-day	Sinificant increase in relative kidney weight (M&F; P < 0.01-0.05)	Poon *et al.*,1997; ECB, 2008; ATSDR, 2002	Kidney
Sprague-Dawley rats (M&F)	Oral feeding	0, 0.2, 1.0, and 2.0% (0, 143, 737, 1440 mg/kg-day; M; 0, 154, 797, 1414 mg/kg-day; F) (15 rats per group): 0, 1.0, and 2.0% (0, 737, 1440 mg/kg-day; M; 0, 797, 1414 mg/kg-day; F): 0 and 2.0% (0, 1440 mg/kg-day; M; 0, 1414 mg/kg-day; F) (10 rats per group)	17 weeks: 2 or 6 weeks:	NOAEL = 1414-1440 mg/kg-day	No toxicologically significant change in the microscopic urine composition	Gray *et al.*, 1977	Kidney
Sprague-Dawley rats (M&F)	Oral feeding	0, 0.2, 1.0, and 2.0% (0, 143, 737, 1440 mg/kg-day; M; 0, 154, 797, 1414 mg/kg-day; F) (15 rats per group): 0, 1.0, and 2.0% (0, 737, 1440 mg/kg-day; M; 0, 797, 1414 mg/kg-day; F): 0 and 2.0% (0, 1440 mg/kg-day; M; 0, 1414 mg/kg-day; F) (10 rats per group)	17 weeks: 2 or 6 weeks:	LOAEL = 1414-1440 mg/kg-day; NOAEL = 737-797 mg/kg-day	Significant increase in the 0-6 hour urine specific gravity at 2 and 6 weeks (M, P < 0.05); Significant decrease in the 0-6 hour volume and number of cells in the urine (M; P < 0.01-0.05); Significant decrease in the urine specific gravity at week 6 and 17 in the concentration test, the urine cellularity, and urine volume in the dilution test (F; P < 0.01-0.05); Significant increase in the urine volume at 17 weeks (F; P < 0.05) and the specific gravity in the dilution test (P < 0.01)	Gray *et al.*, 1977	Kidney
Sprague-Dawley rats (M&F)	Oral feeding	0, 0.2, 1.0, and 2.0% (0, 143, 737, 1440 mg/kg-day; M; 0, 154, 797, 1414 mg/kg-day; F) (15 rats per group): 0, 1.0, and 2.0% (0, 737, 1440 mg/kg-day; M; 0, 797, 1414 mg/kg-day; F): 0 and 2.0% (0, 1440 mg/kg-day; M; 0, 1414 mg/kg-day; F) (10 rats per group)	17 weeks: 2 or 6 weeks:	LOAEL = 737 mg/kg-day; NOAEL = 143 mg/kg-day	Significant dose-dependent decrease in the absolute kidney weight (M, 17 weeks; P < 0.05)	Gray *et al.*, 1977	Kidney
Sprague-Dawley rats (M&F)	Oral feeding	0, 0.2, 1.0, and 2.0% (0, 143, 737, 1440 mg/kg-day; M; 0, 154, 797, 1414 mg/kg-day; F) (15 rats per group): 0, 1.0, and 2.0% (0, 737, 1440 mg/kg-day; M; 0, 797, 1414 mg/kg-day; F): 0 and 2.0% (0, 1440 mg/kg-day; M; 0, 1414 mg/kg-day; F) (10 rats per group)	17 weeks: 2 or 6 weeks:	LOAEL = 1414-1440 mg/kg-day; NOAEL = 737-797 mg/kg-day	Significant dose-dependent decrease in the absolute kidney weight (M,&F 2, 6, 17 weeks; P < 0.05)	Gray *et al.*, 1977	Kidney

Sprague-Dawley rats (M&F)	Oral feeding	0, 0.2, 1.0, and 2.0% (0, 143, 737, 1440 mg/kg-day; M; 0, 154, 797, 1414 mg/kg-day; F) (15 rats per group): 0, 1.0, and 2.0% (0, 737, 1440 mg/kg-day; M; 0, 797, 1414 mg/kg-day; F): 0 and 2.0% (0, 1440 mg/kg-day; M; 0, 1414 mg/kg-day; F) (10 rats per group)	17 weeks: 2 or 6 weeks:	LOAEL = 737-797 mg/kg-day; NOAEL = 143-154 mg/kg-day	Significant dose-dependent increase in the relative kidney weight (M&F, 17 weeks; $P < 0.001$-0.01)	Gray *et al.*, 1977	Kidney
Sv/129 mice (M)	Oral feeding	N/A	24 weeks	LOAEL = 2400 mg/kg-day	Degenerative kidney lesions (M)	Ward *et al.*,1998; ATSDR, 2002	Kidney
Syrian golden hamster	Oral feeding	N/A	30 weeks	LOAEL = 1436 mg/kg-day	Increase in relative kidney weight	Maruyama *et al.*,1994; ATSDR, 2002	Kidney
Rats (M)	Oral gavage	150 mg DEHP/70 kg-day (0.9 {2.1} mg/kg-day); 150 mg artificial kidney leachate/70 kg-day; controls (20-25 rats per dose group, 4-8 rats per group per sacrifice time)	Once daily, 3 times a week for 3, 6, 9, or 12 months	LOAEL = 0.9 {2.1} mg/kg-day	Significantly increased incidence of focal cystic changes in the kidneys of rats receiving DEHP or artificial kidney leachate at 12 months ($P < 0.04$), significantly decreased creatinine clearance in rats receiving DEHP for 12 months ($P < 0.01$).	Crocker *et al.*,1988; ECB, 2008	Kidney
Dog (NS)	Oral Capsule	N/A	Once daily for 5 days/week for 52 weeks	NOAEL = 59 mg/kg-day	No toxicologically significant kidney effects	Carpenter *et al.*,1953; ATSDR, 2002	Kidney
Sherman rats	Oral feeding	N/A	52 weeks	LOAEL = 200 mg/kg-day; NOAEL = 60 mg/kg-day	Increased kidney weight at 52 weeks	Carpenter *et al.*,1953; ATSDR, 2002	Kidney
Guinea pig (NS)	Oral feeding	N/A	52 weeks	NOAEL = 64 mg/kg-day	No toxicologically significant kidney effects	Carpenter *et al.*,1953; ATSDR, 2002	Kidney
B6C3F$_1$ mice (M&F)	Oral feeding	0, 3000, 6000 mg/kg (0, 672, 1325 mg/kg-day (M); 0, 799, 1821 mg/kg-day (F); 50 mice per sex per group; GLP)	103 weeks	LOAEL = 1325 mg/kg-day; NOAEL = 672 mg/kg-day	Increased kidney inflammation (M)	NTP, 1982; ECB, 2008	Kidney
Sherman rats (M)	Oral feeding	N/A	104 weeks	NOAEL = 190 mg/kg-day	No toxicologically significant kidney effects	Carpenter *et al.*,1953; ATSDR, 2002	Kidney
Fischer 344 rats (M&F)	Oral feeding	0, 100, 500, 2500, 12,500 mg/kg (5.8, 28.9, 146.6, 789.0 mg/kg-day (M); 7.3, 36.1, 181.7, 938.5 mg/kg-day (F); 50-80 rats per sex per group)	78 or 104 weeks	LOAEL = 146.6 mg/kg-day; NOAEL = 28.9 mg/kg-day	Significant increase in the absolute and relative kidney weight (M; $P < 0.05$)	David *et al.*, 2000a	Kidney
Fischer 344 rats (M&F)	Oral feeding	0, 100, 500, 2500, 12,500 mg/kg (5.8, 28.9, 146.6, 789.0 mg/kg-day (M); 7.3, 36.1, 181.7, 938.5 mg/kg-day (F); 50-80 rats per sex per group)	78 or 104 weeks	LOAEL = 938.5 mg/kg-day; NOAEL = 181.7 mg/kg-day	Significant increase in the absolute and relative kidney weight (F; $P < 0.05$)	David *et al.*, 2000a	Kidney

Fischer 344 rats (M&F; 6 wk old)	Oral feeding	0, 100, 500, 2500, 12,500 mg/kg (5.8, 28.9, 146.6, 789.0 mg/kg-day (M); 7.3, 36.1, 181.7, 938.5 mg/kg-day (F); 50-80 rats per sex per group)	78 or 104 weeks	NOAEL = 789-938.5 mg/kg-day (F)	No toxicologically significant change in urine volume, urine creatinine concentration, creatinine clearance, or other urinalysis parameters	David *et al.*,2000a	Kidney
Fischer 344 rats (M&F)	Oral feeding	0, 100, 500, 2500, or 12,500 mg DEHP/kg feed (5.8, 28.9, 146.6, 789.0 mg/kg-day for M; 7.3, 36.1, 181.7, and 938.5 mg/kg-day for F; 10 rats per sex per group)	78 or 104 weeks	NOAEL = 789.0 – 938.5 mg/kg-day	No toxicologically significant change in the incidence of renal tubule pigmentation at 78 or 104 weeks (M&F)	David *et al.*, 2000a	Kidney
Fischer 344 rats (M&F)	Oral feeding	0, 100, 500, 2500, or 12,500 mg DEHP/kg feed (5.8, 28.9, 146.6, 789.0 mg/kg-day for M; 7.3, 36.1, 181.7, and 938.5 mg/kg-day for F; 10 rats per sex per group)	78 or 104 weeks	NOAEL = 938.5 mg/kg-day	No toxicologically significant change in the incidence or severity of mineralization of the renal papilla at 104 weeks (F); No toxicologically significant change in the incidence or severity of chronic progressive nephropathy at 78 or 104 weeks (F) or the incidence of chronic progressive nephropathy at 78 or 104 weeks (M)	David *et al.*, 2000a	Kidney
Fischer 344 rats (M&F; 6 wk old)	Oral feeding	0, 100, 500, 2500, 12,500 mg/kg (5.8, 28.9, 146.6, 789.0 mg/kg-day (M); 7.3, 36.1, 181.7, 938.5 mg/kg-day (F); 50-80 rats per sex per group)	78 or 104 weeks	LOAEL = 789-938.5 mg/kg-day (M&F); NOAEL = 146.6-181.7 mg/kg-day	Significant increased in the blood urea nitrogen (M&F; $P < 0.05$) at 78 weeks, increased incidence of mineralization of renal papilla (M&F at 78 weeks), increased severity of chronic progressive nephropathy at 78 and 104 weeks (M; $P < 0.05$), and renal tubule pigmentation at 78 and 104 weeks (M&F)	David *et al.*,2000a	Kidney
Fischer 344 rats (M&F; 6 wk old)	Oral feeding	0, 100, 500, 2500, 12,500 mg/kg (5.8, 28.9, 146.6, 789.0 mg/kg-day (M); 7.3, 36.1, 181.7, 938.5 mg/kg-day (F); 50-80 rats per sex per group)	78 or 104 weeks	LOAEL = 5.8 mg/kg-day (M)	Significant dose-dependent increase in the incidence and severity of mineralization of the renal papilla at 104 weeks (M; $P < 0.05$)	David *et al.*,2000a	Kidney
Fischer 344 rats (M&F)	Oral feeding	0, 100, 500, 1500, or 12,500 mg DEHP/kg feed (5.8, 28.9, 146.6,722-728 mg/kg-day for M; 7.3, 36.1, 181.7, 882-879 mg/kg-day for F; 10 rats per sex per group)	104 weeks + 78 weeks then 26 week recovery	LOAEL = 722-879 mg/kg-day	Significant recovery (decrease) in absolute (M) and relative (M&F) kidney weight at 104 weeks ($P \leq 0.05$); Marginal recovery (decrease) of absolute kidney weight (F), and the severity of renal tubule pigmentation (M&F)	David *et al.*, 2001	Kidney
Fischer 344 rats (M&F)	Oral feeding	0, 100, 500, 1500, or 12,500 mg DEHP/kg feed (5.8, 28.9, 146.6,722-728 mg/kg-day for M; 7.3, 36.1, 181.7, 882-879 mg/kg-day for F; 10 rats per sex per group)	104 weeks + 78 weeks then 26 week recovery	NOAEL = 722-879 mg/kg-day	No toxicologically significant change in significantly elevated blood urea nitrogen at 78 or 104 weeks following recovery (M&F)	David *et al.*, 2001	Kidney

Fischer 344 rats (M&F)	Oral feeding	0, 100, 500, 1500, or 12,500 mg DEHP/kg feed (5.8, 28.9, 146.6,722-728 mg/kg-day for M; 7.3, 36.1, 181.7, 882-879 mg/kg-day for F; 10 rats per sex per group)	104 weeks + 78 weeks then 26 week recovery	LOAEL = 722-879 mg/kg-day	Significant increase in incidence of mineralization of the renal papilla (M; P≤0.05); Marginally or substantially increased incidence and/or severity of mineralization of the renal papilla (M&F) and chronic progressive nephropathy (M&F) following recovery	David *et al.*, 2001	Kidney
$B6C3F_1$ mice (M&F; 6 wk old)	Oral feeding	0, 100, 500, 1500, or 6000 mg DEHP/kg feed (19.2, 98.5, 292.2, 1266.1 mg/kg-day for M; 23.8, 116.8, 354.2, 1458.2 mg/kg-day for F; 10-15 mice per sex per group)	79 or 105 weeks	LOAEL = 1266.1 mg/kg-day; NOAEL = 292.2 mg/kg-day	Dose-dependent increase in the blood urea nitrogen at 78 weeks (M)	David *et al.*, 2000b	Kidney
$B6C3F_1$ mice (M&F; 6 wk old)	Oral feeding	0, 100, 500, 1500, or 6000 mg DEHP/kg feed (19.2, 98.5, 292.2, 1266.1 mg/kg-day for M; 23.8, 116.8, 354.2, 1458.2 mg/kg-day for F; 10-15 mice per sex per group)	79 or 105 weeks	LOAEL = 292.2 - 354.2 mg/kg-day; NOAEL = 98.5 - 116.8 mg/kg-day	Significant dose-dependent increase in the incidence of chronic progressive nephropathy at 78 and 105 weeks (F; P≤0.05); substantial dose-dependent increase in the severity of chronic progressive nephropathy (F); significant dose-dependent decrease in the absolute kidney weight (M; P≤0.05)	David *et al.*, 2000b	Kidney
$B6C3F_1$ mice (M&F; 6 wk old)	Oral feeding	0, 100, 500, 1500, or 6000 mg DEHP/kg feed (19.2, 98.5, 292.2, 1266.1 mg/kg-day for M; 23.8, 116.8, 354.2, 1458.2 mg/kg-day for F; 10-15 mice per sex per group)	79 or 105 weeks	LOAEL = 1266.1 mg/kg-day; NOAEL = 292.2 mg/kg-day	Dose-dependent increase in the severity of chronic progressive nephropathy at 78 and 105 weeks (M)	David *et al.*, 2000b	Kidney
$B6C3F_1$ mice (M&F; 6 wk old)	Oral feeding	0, 100, 500, 1500, or 6000 mg DEHP/kg feed (19.2, 98.5, 292.2, 1266.1 mg/kg-day for M; 23.8, 116.8, 354.2, 1458.2 mg/kg-day for F; 10-15 mice per sex per group)	79 or 105 weeks	NOAEL = 1458.2 mg/kg-day	No toxicologically significant change in the relative kidney weights at 105 weeks (F)	David *et al.*, 2000b	Kidney
$B6C3F_1$ mice (M&F; 6 wk old)	Oral feeding	0, 100, 500, 1500, or 6000 mg DEHP/kg feed (19.2, 98.5, 292.2, 1266.1 mg/kg-day for M; 23.8, 116.8, 354.2, 1458.2 mg/kg-day for F; 10-15 mice per sex per group)	79 or 105 weeks	LOAEL = 98.5 mg/kg-day; NOAEL = 19.2 mg/kg-day	Signficant dose-dependent decrease in the relative kidney weight at 105 weeks (M; P≤0.05)	David *et al.*, 2000b	Kidney
$B6C3F_1$ mice (M&F; 6 wk old)	Oral feeding	0, 100, 500, 1500, or 6000 mg DEHP/kg feed (19.2, 98.5, 292.2, 1266.1 mg/kg-day for M; 23.8, 116.8, 354.2, 1458.2 mg/kg-day for F; 10-15 mice per sex per group)	79 or 105 weeks	LOAEL = 1458.2 mg/kg-day; NOAEL = 354.2 mg/kg-day	Signficant decrease in the absolute kidney weight at 105 weeks (F; P≤0.05)	David *et al.*, 2000b	Kidney

B6C3F$_1$ mice (M&F)	Oral feeding	0, 100, 500, 1500, or 6000 mg DEHP/kg feed (19.2, 98.5, 292.2, 1211.0 – 1227.0 mg/kg-day for M; 23.8, 116.8, 354.2, 1413.0 – 1408.0 mg/kg-day for F; 10-15 mice per sex per group)	105 weeks + 79 weeks then 26 week recovery	LOAEL = 1211.0 – 1408.0 mg/kg-day	Significant recovery (increase) in absolute and relative liver weight at 105 weeks (M; P≤0.05); Significant recovery (decrease) in the incidence (M; P≤0.05) of chronic progressive nephropathy; Substantial recovery (decrease) in the severity of chronic progressive nephropathy (M&F)	David *et al.*, 2001	Kidney
B6C3F$_1$ mice (M&F)	Oral feeding	0, 100, 500, 1500, or 6000 mg DEHP/kg feed (19.2, 98.5, 292.2, 1211.0 – 1227.0 mg/kg-day for M; 23.8, 116.8, 354.2, 1413.0 – 1408.0 mg/kg-day for F; 10-15 mice per sex per group)	105 weeks + 79 weeks then 26 week recovery	NOAEL = 1211.0 – 1408.0 mg/kg-day	No toxicologically significant change in absolute or relative kidney weights following recovery (F)	David *et al.*, 2001	Kidney
B6C3F$_1$ mice (M)	Oral feeding	N/A	104 weeks	LOAEL = 1325 mg/kg-day; NOAEL = 672 mg/kg-day	Chronic inflammation of the kidney (M)	Kluwe *et al.*,1982; ATSDR, 2002	Kidney
Fischer 344 rats (M&F)	Oral feeding	0, 100, 500, 2500, 12,500 mg/kg (0, 5.8, 28.9, 146.6, 789.0 mg/kg-day (M); 0, 7.3, 36.1, 181.7, 938.5 mg/kg-day (F), or 12,500 mg/kg for 78 weeks and a 26 week recovery period; 70-85 rats per sex per group; 55 rats per sex in recovery group)	104 weeks	LOAEL = 146.6 mg/kg-day; NOAEL = 28.9 mg/kg-day	Irreversibly increased kidney weight	Moore, 1996; ECB, 2008	Kidney
Fischer 344 rats (M&F)	Oral feeding	0, 100, 500, 2500, 12,500 mg/kg (0, 5.8, 28.9, 146.6, 789.0 mg/kg-day (M); 0, 7.3, 36.1, 181.7, 938.5 mg/kg-day (F), or 12,500 mg/kg for 78 weeks and a 26 week recovery period; 70-85 rats per sex per group; 55 rats per sex in recovery group)	104 weeks	LOAEL = 789.0 mg/kg-day; NOAEL = 146.6 mg/kg-day	Irreversibly increased mineralization of the renal papilla (M), tubule cell pigment (M&F), and chronic progressive nephropathy (M), irreversible	Moore, 1996; ECB, 2008	Kidney
B6C3F$_1$ mice (M&F)	Oral feeding	0, 100, 500, 1500, 6000 mg/kg (0, 19.2, 98.5, 292.2, 1266.1 mg/kg-day (M); 0, 23.8, 116.8, 354.2, 1458.2 mg/kg-day (F), or 6000 mg/kg for 78 weeks and a 26 week recovery period; 70-85 rats per sex per group; 55 rats per sex in recovery group)	104 weeks	LOAEL = 292.2 mg/kg-day; NOAEL = 98.5 mg/kg-day	Partially reversible decreased kidney weight (M), chronic progressive nephropathy (M&F) – partially reversible	Moore, 1997; ECB, 2008	Kidney
Fischer 344 rats	Oral feeding	N/A	108 weeks	LOAEL = 2000 mg/kg-day	Lipofuschin pigments in tubular epithelium	Rao *et al.*,1990; ATSDR, 2002	Kidney
Wistar rats (M&F)	Oral feeding	0, 0.1, 0.5% (0, 50-80, 300-400 mg/kg-day; 43 rats per sex per group	3, 6, 12, 24 months	LOAEL = 300-400 mg/kg-day; NOAEL = 50-80 mg/kg-day	Increased kidney weight at 3 - 6 months, but not 12 - 24 months	Harris *et al.*,1956; ECB, 2008	Kidney

Sprague-Dawley rats (M&F)	Oral feeding	0, 0.5% (0, 200 mg/kg-day; 10 rats per sex per group)	Lifetime	LOAEL = 200 mg/kg-day	Kidney nephroses in a few animals	BASF, 1960; ECB, 2008	Kidney
Opossum kidney epithelial cells	*In vitro*	0.1 - 500 µmol/L (for both MEHP and 2-ethylhexanoic acid	3 days incubation	MEHP - ED_{50} for viability = 25 µmol/L	MEHP – dose dependent decrease in cell viability (3 µmol/L; $P < 0.05$), moderate cell swelling up to 25 µmol/L, doses higher than 25 µmol/L induced a dose-dependent cell shrinkage (100 µmol/L; $P < 0.05$) and incre ased cell debris, reduced and altered F-actin organization. Ethylhexanoic acid - did not reduce viability or alter cell volume	Rothenbacher *et al.*,1998	Kidney epithelial cells
Fischer 344 rats (M)	Oral feeding	Promotion – 1.2% DEHP (~600 mg/kg-day; 20 rats per group; initiation, N-ethyl-N-hydroxyethylnitrosamine (EHEN))	24 weeks, no interval between initiation and promotion	N/A	A strong promoting activity noted (increased incidence of renal adenomas and adenocarcinomas and the number of tumors per kidney)	Kurokawa *et al.*,1988; IARC, 2000	Kidney
Fischer 344 rats (M)	Oral feeding	Promotion – 0.3, 0.6, 1.2% DEHP (~250, 300, 600 mg/kg; 15 rats per group; initiator, N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN))	During weeks 5-8 and 12-20, no interval between initiation and promotion	N/A	No promoting activity	Hagiwara *et al.*,1990; IARC, 2000	Kidney-Urinary Bladder
Testes/Ovary/Development/Reproduction/Endocrine/Pituitary							
Sprague-Dawley rats	Oral gavage	N/A	Once	LOAEL = 2800 mg/kg-day	Morphological changes in Sertoli cells	Saitoh *et al.*,1997; ATSDR, 2002	Testes
Sprague-Dawley rats	Oral gavage	0, 20, 100, 200, 500 mg/kg-day (5 rats per group); MEHP = 393 mg/kg-day (4 rats per group); 2-ethylhexanol = 167 mg/gk-day (4 rats per group)	Once during Gd 3 to 3 day old pups	LOAEL = 100 mg/kg-day; NOAEL = 20 mg/kg-day	Abnormal gonocytes and decreased Sertoli cell proliferation	Li *et al.*,2000; ATSDR, 2002	Testes
Wistar rats (M)	Oral gavage	0, 2000 mg/kg	Once daily for 2 days	LOAEL = 2000 mg/kg	Slight rarefaction" or vacoulation in a few seminiferous tubule Sertoli cells; Ultrastructural changes such as mitochondrial swelling (with matrix granule degradation) and focal dilatation and vesiculation of the smooth endoplasmic reticulum (SER) in Leydig cells, and increased interstitial macrophage activity in cells with large cytoplasmic alterations (on the surface of Leydig cells)	Jones *et al.*, 1993	Testes
Wistar rats (M) *in vitro*	Oral gavage	0, 10µM, 100µM, 1mM **MEHP**	2-3 hour exposures	LOAEL = 10µM	Ultrastructural change such as mitochondrial swelling with a loss of matrix granules, focal dilatation of the SER, and increased number and length of filopodia associated with basal lamellar processes; Dose- and time-dependent decrease in LH-induced testosterone secretion from Leydig cells	Jones *et al.*, 1993	Testes

Sprague-Dawley rats (M&F)	Oral gavage	0, 10, 100, 1000, 2000 mg DEHP/kg bw (7-10 rats per group); 0,100, 200, 500, 1000 mg/kg (16 rat pups per group); 0, 200, 500, 1000 mg/kg (50 rat pups per group)	Once daily during PPd 6-10, 14-18, 21-25, 42-46, 86-90; Once daily for 5 days during PPd 6-10 – recovery for 4 weeks; Once daily for 5 days during PPd 6-10	LOAEL = 1000 mg/kg; NOAEL = 100 mg/kg	Significant dose-dependent decrease in the absolute and relative testis weight in rats dosed during PPd 6-10, 14-18, 42-46, significant decrease in relative testis weight in rats dosed during PPd 21-25 ($P < 0.05$); Reduction in tubule size; Significant reduction in the number of Sertoli cells per tubule in rats dose during PPd 6-10; Significant reduction in the number of spermatocyes in the center of the tubule and tubular diameter in rats treated during PPd 14-18; Significant increase in the number of affected tubules with Sertoli cells void of germ cells or spermatocytes with pyknotic nuclei in rats dosed during PPd 21-25; Significant increase of affected tubules and decreased the number of germ cells by 10-20% in other tubules in rats dosed during PPd 42-46	Dostal *et al.*, 1988	Testes
Sprague-Dawley rats (M&F)	Oral gavage	0, 10, 100, 1000, 2000 mg DEHP/kg bw (7-10 rats per group); 0,100, 200, 500, 1000 mg/kg (16 rat pups per group); 0, 200, 500, 1000 mg/kg (50 rat pups per group)	Once daily during PPd 6-10, 14-18, 21-25, 42-46, 86-90; Once daily for 5 days during PPd 6-10 – recovery for 4 weeks; Once daily for 5 days during PPd 6-10	LOAEL = 100mg/kg; NOAEL = 10 mg/kg	Significant dose-dependent decrease in the absolute testis weight in rats dosed during PPd 21-25 ($P < 0.05$)	Dostal *et al.*, 1988	Testes
Sprague-Dawley rats (M&F)	Oral gavage	0, 10, 100, 1000, 2000 mg DEHP/kg bw (7-10 rats per group); 0,100, 200, 500, 1000 mg/kg (16 rat pups per group); 0, 200, 500, 1000 mg/kg (50 rat pups per group)	Once daily during PPd 6-10, 14-18, 21-25, 42-46, 86-90; Once daily for 5 days during PPd 6-10 – recovery for 4 weeks; Once daily for 5 days during PPd 6-10	LOAEL = 2000mg/kg; NOAEL = 1000 mg/kg	Significant dose-dependent decrease in the relative and absolute testis weight in rats dosed during PPd 86-90 ($P < 0.05$); Significant increase in the number of tubules affected and the severity of effects (i.e., no spermatids, few spermatogonia or spermatocytes) in rats treated during PPd 86-90	Dostal *et al.*, 1988	Testes
Sprague-Dawley rats (M&F)	Oral gavage	0, 10, 100, 1000, 2000 mg DEHP/kg bw (7-10 rats per group); 0,100, 200, 500, 1000 mg/kg (16 rat pups per group); 0, 200, 500, 1000 mg/kg (50 rat pups per group)	Once daily during PPd 6-10, 14-18, 21-25, 42-46, 86-90; Once daily for 5 days during PPd 6-10 – recovery for 4 weeks; Once daily for 5 days during PPd 6-10	LOAEL = 1000mg/kg; NOAEL = 100 mg/kg	Significant decrease in testicular zinc in rats dosed during PPd 86-90 ($P < 0.05$)	Dostal *et al.*, 1988	Testes

Sprague-Dawley rats (M&F)	Oral gavage	0, 10, 100, 1000, 2000 mg DEHP/kg bw (7-10 rats per group); 0,100, 200, 500, 1000 mg/kg (16 rat pups per group); 0, 200, 500, 1000 mg/kg (50 rat pups per group)	Once daily during PPd 6-10, 14-18, 21-25, 42-46, 86-90; Once daily for 5 days during PPd 6-10 – recovery for 4 weeks; Once daily for 5 days during PPd 6-10	NOAEL = 2000 mg/kg	No toxicologically significant change in testicular zinc concentration in rats dosed during PPd 42-46	Dostal *et al.*, 1988	Testes
Sprague-Dawley rats (M&F)	Oral gavage	0, 10, 100, 1000, 2000 mg DEHP/kg bw (7-10 rats per group); 0,100, 200, 500, 1000 mg/kg (16 rat pups per group); 0, 200, 500, 1000 mg/kg (50 rat pups per group)	Once daily during PPd 6-10, 14-18, 21-25, 42-46, 86-90; Once daily for 5 days during PPd 6-10 – recovery for 4 weeks; Once daily for 5 days during PPd 6-10	NOAEL = 1000 mg/kg	No toxicologically significant change in testicular zinc concentration in rats dosed during PPd 21-25	Dostal *et al.*, 1988	Testes
Sprague-Dawley rats (M&F)	Oral gavage	0, 10, 100, 1000, 2000 mg DEHP/kg bw (7-10 rats per group); 0,100, 200, 500, 1000 mg/kg (16 rat pups per group); 0, 200, 500, 1000 mg/kg (50 rat pups per group)	Once daily during PPd 6-10, 14-18, 21-25, 42-46, 86-90; Once daily for 5 days during PPd 6-10 – recovery for 4 weeks; Once daily for 5 days during PPd 6-10	LOAEL = 200mg/kg; NOAEL = 100 mg/kg	Significant dose-dependent decrease in absolute testis weight in rats dosed during PPd 6-10 and then allowed to recover for 4 weeks ($P < 0.05$)	Dostal *et al.*, 1988	Testes
Sprague-Dawley rats (M&F)	Oral gavage	0, 10, 100, 1000, 2000 mg DEHP/kg bw (7-10 rats per group); 0,100, 200, 500, 1000 mg/kg (16 rat pups per group); 0, 200, 500, 1000 mg/kg (50 rat pups per group)	Once daily during PPd 6-10, 14-18, 21-25, 42-46, 86-90; Once daily for 5 days during PPd 6-10 – recovery for 4 weeks; Once daily for 5 days during PPd 6-10	LOAEL = 1000 mg/kg; NOAEL = 500 mg/kg	Significant decrease in relative testis weight ($P < 0.05$) in rats dosed during PPd 6-10 and allowed to recover for 4 weeks	Dostal *et al.*, 1988	Testes

Sprague-Dawley rats (M&F)	Oral gavage	0, 10, 100, 1000, 2000 mg DEHP/kg bw (7-10 rats per group); 0,100, 200, 500, 1000 mg/kg (16 rat pups per group); 0, 200, 500, 1000 mg/kg (50 rat pups per group)	Once daily during PPd 6-10, 14-18, 21-25, 42-46, 86-90; Once daily for 5 days during PPd 6-10 – recovery for 4 weeks; Once daily for 5 days during PPd 6-10	NOAEL = 1000 mg/kg	No toxicologically significant change in the number of Sertoli cell nuclei per tubule of rats dosed during PPd 6-10 and allowed to recover for 4 weeks	Dostal *et al.*, 1988	Testes
Sprague-Dawley rats (M&F)	Oral gavage	0, 10, 100, 1000, 2000 mg DEHP/kg bw (7-10 rats per group); 0,100, 200, 500, 1000 mg/kg (16 rat pups per group); 0, 200, 500, 1000 mg/kg (50 rat pups per group)	Once daily for 5 days during PPd 6-10 – recovery for 11, 12, 13, 16, 19, 23 weeks	LOAEL = 1000 mg/kg; NOAEL = 500 mg/kg	Significant dose-dependent decrease in testis weight at recovery week 13 (14%); Substantial dose-dependent decrease in testis weight at recovery week 12 (8%), 16 (6%), 23 (7%) weeks; Significant dose-dependent decrease in testicular spermatid heads per testis at recovery week 19 (16%); Substantial dose-dependent decrease in testicular spermatid heads per testis at 12 (13%) and 23 (8%) weeks	Dostal *et al.*, 1988	Testes
Sprague-Dawley rats (M&F)	Oral gavage	0, 10, 100, 1000, 2000 mg DEHP/kg bw (7-10 rats per group); 0,100, 200, 500, 1000 mg/kg (16 rat pups per group); 0, 200, 500, 1000 mg/kg (50 rat pups per group)	Once daily for 5 days during PPd 6-10 – recovery for 11, 12, 13, 16, 19, 23 weeks	LOAEL = 200 mg/kg	Significant dose-dependent decrease in testis weight at recovery week 19 (14%); Significant dose-dependent decrease in testicular spermatid heads per testis at recovery week 13 (20%)	Dostal *et al.*, 1988	Testes
Sprague-Dawley rats (M&F)	Oral gavage	0, 10, 100, 1000, 2000 mg DEHP/kg bw (7-10 rats per group); 0,100, 200, 500, 1000 mg/kg (16 rat pups per group); 0, 200, 500, 1000 mg/kg (50 rat pups per group)	Once daily during PPd 6-10, 14-18, 21-25, 42-46, 86-90; Once daily for 5 days during PPd 6-10 – recovery for 4 weeks; Once daily for 5 days during PPd 6-10	LOAEL = 500mg/kg; NOAEL = 200 mg/kg	Significant decrease in absolute and relative testis weight in rats dosed during PPd 6-10; Substantial dose-dependent delay in spermatid maturation in rats dosed during PPd 6-10 and allowed to recover for 4 weeks; Significant dose-dependent decrease in the relative testicular weight and number of Sertoli cells per tubule of rats dosed during PPd 6-10 ($P < 0.05$);	Dostal *et al.*, 1988	Testes
Wistar rats	Oral gavage	N/A	Once daily for 7 days	LOAEL = 2000 mg/kg-day	Decreased testes weight (38%); shrunken seminiferous tubules with necrotic debris and aspermatogenesis	Oishi, 1994; ATSDR, 2002	Testes
Fischer 344 rats (M)	Oral feeding	0, 2% (1600 mg/kg-day; 8 rats per group; GL)	7 days	NOAEL = 1600 mg/kg-day	No histopathological findings in testes	Exxon, 1982a,b; ECB, 2008	Testes
Sprague-Dawley rats (M)	Oral feeding	N/A	10 days	LOAEL = 1740 mg/kg-day)	Changes in testicular xenobiotic enzyme activity (20-25%)	Mehrotra *et al.*,1997; ATSDR, 2002	Testes

Wistar rats (M; 4, 10, 15 week old)	Oral gavage; Oral feeding	0, 2800 mg/kg-day ± testosterone proprionate (TP; 200 µg/kg/day or FSH (100 U); 0, 2% (0, ~ 1200 mg/kg-day	10 days; 10 or 42 days and then recovery to 4 week old rats	LOAEL = 2800 mg/kg-day	Age-dependent decrease in the testis, seminal vesicle, and prostate weight (4 > 10 > 15 weeks old) following 10 days of dosing – testicular pathologies; uniform tubular atrophy in 4 week rats, 5-50% tubule atrophy in 10 week old rats, no testicular effect in 15 week old rats– administration of TP or FSH mitigated decrements in accessory gland, but not testis in 4 week old rats	Gray and Butterworth, 1980	Testes
Wistar rats	Oral gavage	N/A	Once daily for 10 days on PPd 30-39	LOAEL = 2000 mg/kg-day	Aspermatogenesis with reduced testes, seminal vesicle, and ventral prostate weights, decreased testicular zinc	Oishi, 1986; ATSDR, 2002	Testes
Fischer 344 rats (M)	Oral gavage	0, 330, 1000, 3000 mg/kg and zinc at 0, 2, 20, 20 mg/kg (48 rats per group)	Once daily for 13 days	LOAEL = 1000 mg/kg-day; NOAEL = 330 mg/kg-day	Dose-dependent tubular degeneration and atrophy in testes combined with low zinc diet (2 mg/kg)	Agarwal *et al.*,1986a; ECB, 2008	Testes
Wistar - Alderley Park rats (M&F)	Oral gavage	0 and 2000 mg/kg-day (10 rats per sex per group; GLP)	Once daily for 14 days	LOAEL = 2000 mg/kg-day	Decreased testes weight and atrophy	ICI, 1982b; Rhodes *et al.*,1986; ECB, 2008	Testes
Sprague-Dawley rats	Oral gavage	N/A	Once daily for 14 days during PPd 25-38	LOAEL = 1000 mg/kg-day	Testicular damage	Sjoberg *et al.*,1986a; ATSDR, 2002	Testes
Sprague-Dawley rats	Oral feeding	N/A	14 days during PPd 40-53	LOAEL = 1700 mg/kg-day; NOAEL = 1000 mg/kg-day	Decreased testicular weight (43%) and severe seminiferous tubule damage	Sjoberg *et al.*,1986a; ATSDR, 2002	Testes
Sprague-Dawley rats	Oral feeding	N/A	14 days during PPd 25-38	LOAEL = 1700 mg/kg-day	Decreased testicular weight (79%) and severe testicular damage	Sjoberg *et al.*,1986a; ATSDR, 2002	Testes
Sprague-Dawley rats	Oral feeding	N/A	14 days during PPd 25-38	LOAEL = 1000 mg/kg-day	Decreased testicular weight (21%) and tubular damage	Sjoberg *et al.*,1986a; ATSDR, 2002	Testes
Fischer 344 rats	Oral feeding	0, 6300, 12,500, 25,000, 50,000, 100,000 mg/kg (0, 630, 1250, 2500, 5000, 10,000 mg/kg-day; 5 rats per sex per group)	14 days	LOAEL = 1250 mg/kg-day; NOAEL = 630 mg/kg-day	Testes atrophy	NTP, 1982; ECB, 2008	Testes
Wistar rats (M, 28 days old)	*In vitro*	0.1, 1, 10, 100 µM **MEHP**	3, 5, or 24 hours	LOAEL = 0.1 (24 hr), 1 (5 hr), 100 (3 hr)	Cultured rat Sertoli cells: Pretreatment with MEHP reduced the FSH-stimulated production of cAMP in a dose- and time-dependent fashion	Lloyd and Foster, 1988	Testes
Wistar rats	Oral gavage	0, 2000 mg/kg-day	Once daily for 15 days	LOAEL = 2000 mg/kg-day	Significant decrease in absolute and relative testicular weight ($P < 0.05$), Significant increase in testis GGT, LDH, β-glucuronidase ($P < 0.05$), Significant increase in SDH and acid phosphatase ($P < 0.05$; all enzymatic changes mitigated by admin of testosterone), histopathologic changes in tubules and damaged spermatogenic cells (mitigated by admin of testosterone), Significant reduction in sperm count ($P < 0.05$; mitigated by admin of testosterone)	Parmar *et al.*,1987; ATSDR, 2002	Testes
Wistar rats	Oral gavage	0, 2000 mg/kg-day	Once daily for 15 days	NOAEL = 2000 mg/kg-day	No toxicologically significant changes in Leydig cells	Parmar *et al.*,1987	Testes

Fischer 344 rats (M&F)	Oral feeding	0, 0.01, 0.1, 0.6, 1.2, 2.5% (0, 11, 105, 667, 1224, 2101 mg/kg-day (M); 0, 12, 109, 643, 1197, 1892 mg/kg-day (F); 5 rats per sex per group; GLP)	21 days	LOAEL = 2101 mg/kg-day; NOAEL = 1224 mg/kg-day	Decreased testes weight	CMA, 1984b; Barber *et al.*,1987; ECB, 2008	Testes
Wistar rats (M&F)	Oral gavage	0, 2500 mg/kg-day (6 rats per sex per group)	Once daily for 7 or 21 days	LOAEL = 2500 mg/kg-day	Significant time-dependent decrease in relative testes weight (M; $P < 0.001$)	Mangham *et al.*,1981	Testes
Wistar rats (F)	Oral gavage	0, 0.015, 0.045, 0.135, 0.405, 1.215, 5, 15, 45, 135, 405 mg/kg-day; ? dams per group; ? rats per group)	Once daily for 27 days during Gd6 to Ld 21, recovery til 144 days old	NOAEL = 405 mg/kg-day	No toxicologically significant effects on weight of grossly normal testes	Andrade *et al.*, 2006b	Testes
Wistar rats (F)	Oral gavage	0, 0.015, 0.045, 0.135, 0.405, 1.215, 5, 15, 45, 135, 405 mg/kg-day; ? dams per group; ? rats per group)	Once daily for 27 days during Gd6 to Ld 21, recovery til 144 days old	LOAEL = 405 mg/kg-day; NOAEL = 135 mg/kg-day	Histopathologic abnormalities in 3/9 grossly normal testes (in 2 testes, reduced grem cell layers, loss of stratification in seminiferous tubules; in other testis, marginal focal Leydig cell hyperplasia with 6.2-fold increased testosterone level)	Andrade *et al.*, 2006b	Testes
Wistar rats (F)	Oral gavage	0, 0.015, 0.045, 0.135, 0.405, 1.215, 5, 15, 45, 135, 405 mg/kg-day; ? dams per group; ? rats per group)	Once daily for 27 days during Gd6 to Ld 21, recovery til 144 days old	LOAEL = 405 mg/kg-day; NOAEL = 135 mg/kg-day	Increased unilateral or bilateral small scrotal testes (1 in control versus 3 in high dose group; tubular atrophy and reduction in germ cell layers; high dose group - multifocal Leydig cell hyperplasia	Andrade *et al.*, 2006b	Testes
Wistar rats (F)	Oral gavage	0, 0.015, 0.045, 0.135, 0.405, 1.215, 5, 15, 45, 135, 405 mg/kg-day; ? dams per group; ? rats per group)	Once daily for 27 days during Gd6 to Ld 21, recovery til 144 days old	LOAEL = 5 mg/kg-day; NOAEL = 1.215 mg/kg-day	One male each in 5, 135, and 405 mg/kg-day small undescended or ectopic testis (with histologically reduced spermatogenesis)	Andrade *et al.*, 2006b	Testes
Wistar rats (F)	Oral gavage	0, 0.015, 0.045, 0.135, 0.405, 1.215, 5, 15, 45, 135, 405 mg/kg-day; ? dams per group; ? rats per group)	Once daily for 27 days during Gd6 to Ld 21, recovery til 144 days old	NOAEL = 405 mg/kg-day	No evidence of hypospadias or preputial separation	Andrade *et al.*, 2006b	Testes
Wistar rats (F)	Oral gavage	0, 0.015, 0.045, 0.135, 0.405, 1.215, 5, 15, 45, 135, 405 mg/kg-day; ? dams per group; ? rats per group)	Once daily for 27 days during Gd6 to Ld 21, recovery til 144 days old	LOAEL = 15 mg/kg-day; NOAEL = 5 mg/kg-day (biologically significant reduction~ 20%)	Significantly decreased mean daily sperm production ($P < 0.05$; 0.045, 0.135, 0.405, 1.215, 5, 15, 45, 135, 405 mg/kg-day from concurrent control; 1.215, 15, 45, 135, 405 mg/kg-day from historic control)	Andrade *et al.*, 2006b	Testes
Wistar rats (F)	Oral gavage	0, 0.015, 0.045, 0.135, 0.405, 1.215, 5, 15, 45, 135, 405 mg/kg-day; ? dams per group; ? rats per group)	Once daily for 27 days during Gd6 to Ld 21, recovery til 144 days old	NOAEL = 405 mg/kg-day	No toxicologically significant effects on morphometry and cell counts of morphologically of normal testes, relative and absolute volume of seminiferous tubules, total tubular length, number of Sertoli cells	Andrade *et al.*, 2006b	Testes
B6C3F$_1$ mice (M&F)	Oral feeding	0, 1000, 5000, 10000, 25000 mg/kg (0, 250, 1210, 2580, 6990 mg/kg-day(M); 0, 270, 1430, 2890, 7900 mg/kg-day (F); 10 mice per sex per group; GLP)	28 days	LOAEL = 2580 mg/kg-day; NOAEL = 1210 mg/kg-day	Decreased absolute and relative testes weight	Eastman Kodak, 1992b; ECB, 2008	Testes

B6C3F$_1$ mice (M&F)	Oral feeding	0, 1000, 5000, 10,000, 25,000 mg/kg (0, 250, 1210, 2580, 6990 mg/kg-day(M); 0, 270, 1430, 2890, 7900 mg/kg-day (F); 10 mice per sex per group; GLP)	28 days	LOAEL = 6990 mg/kg-day; NOAEL = 2580 mg/kg-day	Testes atrophy	Eastman Kodak, 1992b; ECB, 2008	Testes
Fischer 344 rats (M)	Oral feeding	0, 0.02, 0.05, 0.1, 0.5, 1.0, 2.5% (0, 24, 52, 115, 559, 1093, 2496 mg/kg-day; 5 rats per group, 10 rats in control; GLP)	28 days	LOAEL = 2496 mg/kg-day; NOAEL = 1093 mg/kg-day	Decreased absolute and relative testes weights and atrophy of testes	BIBRA, 1990; ECB, 2008	Testes
Wistar rats (M)	Inhalation	0, 10, 50, 1000 mg/m^3 (10 rats per group)	28 days	NOAEC = 1000 mg/m^3	No toxicologically significant testicular effects	Klimische *et al.*,1992; ECB, 2008	Testes
Wistar rats (M; 25 days old)	Oral gavage	0, 50, 100, 250, 500 mg/kg (6 rats per dose group)	Once daily for 30 days	LOAEL = 250 mg/kg; NOAEL = 100 mg/kg	Destruction of advanced germ cell layers and vacuolar degeneration in testes	Parmar *et al.*,1995; ECB, 2008	Testes
Wistar rats (M; 25 days old)	Oral gavage	0, 50, 100, 250, 500 mg/kg (6 rats per dose group)	Once daily for 30 days	LOAEL = 100 mg/kg; NOAEL = 50 mg/kg	Significant dose-dependent decrease in relative testicular weight (31%; $P < 0.05$), testicular germ cell damage	Parmar *et al.*,1995; ATSDR, 2002	Testes
Wistar rats (M; 25 days old)	Oral gavage	0, 50, 100, 250, 500 mg/kg (6 rats per dose group)	Once daily for 30 days	LOAEL = 50 mg/kg	Significant dose-dependent decrease in absolute testicular weight (33%; $P < 0.05$)	Parmar *et al.*,1995; ATSDR, 2002	Testes
Wistar rats (M; 25 day old)	Oral gavage	0, 50, 100, 250, 500 mg/kg (6 rats per dose group)	Once daily for 30 days	LOAEL = 50 mg/kg	Dose-dependent significant increase in lactate dehydrogenase and gamma glutamyl transpeptidase, and decrease in SDH ($P < 0.05$)	Parmar *et al.*,1995; ECB, 2008	Testis
Wistar rats (M; 25 day old)	Oral gavage	0, 50, 100, 250, 500 mg/kg (6 rats per dose group)	Once daily for 30 days	LOAEL = 250 mg/kg; NOAEL = 100 mg/kg	Dose-dependent significant increase in β-glucuronidase, decrease in acid phosphatase ($P < 0.05$)	Parmar *et al.*,1995; ECB, 2008	Testis
Sprague-Dawley rats (M)	Oral gavage	N/A	Once daily for 6 weeks (40 days) during Gd 3-21 and PPd 1-21	LOAEL = 375 mg/kg-day	Decreased testes and anterior prostate weights	Moore *et al.*,2001; ATSDR, 2002	Testes
Wistar rats	Oral feeding	N/A	6 weeks (42 days)	LOAEL = 1200 mg/kg-day	Decreased testicular weight, decreased seminal vesicle and ventral prostate weight with gradual post-exposure recovery	Gray and Butterworth, 1980; ATSDR, 2002	Testes
CRL:CD Sprague-Dawley rats (F)	Oral gavage	0, 11, 33, 100, 300 mg/kg-day (12-14 litters per group)	Once daily during Gd 8-17; recovery til maturity; continued dosing from 18 til 63-65	LOAEL = 300 mg/kg-day; NOAEL = 100 mg/kg-day	In pups dosed from Gd 8 to PNd 64; marginal decrease in paired testes weight In pups dosed from Gd 8 to Ld 17 then recovery; Significant decrease in testis weight ($P < 0.01$)	Gray *et al.*, 2009	Testes
CRL:CD Sprague-Dawley rats (F)	Oral gavage	0, 11, 33, 100, 300 mg/kg-day (12-14 litters per group)	Once daily during Gd 8-17; recovery til maturity; continued dosing from 18 til 63-65	LOAEL = 100 mg/kg-day; NOAEL = 33 mg/kg-day	Dose-dependent significant decrease in seminal vesicle weight ($P < 0.05$)	Gray *et al.*, 2009	Testes
Fischer 344 rats (M)	Oral feeding	0, 320, 1250, 5000, 20,000 mg/kg (0, 18, 69, 284, 1156 mg/kg-day; 24 rats per group)	8 weeks (60 days)	LOAEL = 284 mg/kg-day; NOAEL = 69 mg/kg-day	Dose-dependent decrease in testes, epididymis, and prostate weight	Agarwal *et al.*,1986a/b; ECB, 2008	Testes
Fischer 344 rats (M)	Oral feeding	0, 320, 1250, 5000, 20,000 mg/kg (0, 18, 69, 284, 1156 mg/kg-day; 24 rats per group)	8 weeks (60 days)	LOAEL = 69 mg/kg-day; NOAEL = 18 mg/kg-day	Decreased testicular zinc content, decreased epididymal sperm density and motility, increased number of abnormal sperm cells	Agarwal *et al.*,1986a/b; ECB, 2008	Testes

Wistar rats	Oral feeding	N/A	12 weeks (90 days)	LOAEL = 900 mg/kg-day; NOAEL = 400 mg/kg-day	Tubular atrophy and degeneration	Shaffer *et al.*,1945; ATSDR, 2002	Testes
Unspecified rats (M)	Oral Feeding	Review – 0, 0.2, 1.0, 2.0% (0, 150, 750, 1500 mg/kg-day; 15 rats per group)	12 weeks (90 days)	LOAEL = 750 mg/kg-day; NOAEL = 150 mg/kg-day	Significant dose-dependent decrease in relative testis weight ($P < 0.001$)	Gangolli, 1982	Testes
Unspecified rats (M)	Oral Feeding	Review – 0, 0.2, 1.0, 2.0% (0, 150, 750, 1500 mg/kg-day; 15 rats per group)	12 weeks (90 days)	LOAEL = 150 mg/kg-day	Significant dose-dependent increase in the number and severity of histological evidence of testicular injury	Gangolli, 1982	Testes
Fischer 344 rats (M&F)	Oral feeding	0, 1000, 4000, 12,500, 25,000 mg/kg (0, 63, 261, 859, 1724 mg/kg-day (M); 0, 73, 302, 918, 1858 mg/kg-day (F); 10 rats per sex per group; GLP)	13 weeks	LOAEL = 859 mg/kg-day; NOAEL = 261 mg/kg-day	Decreased testes weight	Eastman Kodak, 1992a; ECB, 2008	Testes
Sprague-Dawley rats (M&F)	Oral feeding	0, 5, 50, 500, 5000 mg/kg (0, 0.4, 3.7, 37.6, 375.2 mg/kg-day (M); 0, 0.4, 4.2, 42.2, 419.3 mg/kg-day (F); 10 rats per sex per group; GLP)	13 weeks	LOAEL = 345.0-411.0 mg/kg-day	Significant decrease in absolute and relative testes weight ($P < 0.05$), Substantial increase in the incidence and severity of seminiferous tubule atrophy; testicular atrophy, complete loss of spermatogenesis (9/10),	Poon *et al.*,1997; ECB, 2008; ATSDR, 2002	Testes
Sprague-Dawley rats (M&F)	Oral feeding	0, 5, 50, 500, 5000 mg/kg (0, 0.4, 3.7, 37.6, 375.2 mg/kg-day (M); 0, 0.4, 4.2, 42.2, 419.3 mg/kg-day (F); 10 rats per sex per group; GLP)	13 weeks	LOAEL = 37.6 mg/kg-day; NOAEL = 3.7 mg/kg-day	Dose-dependent increase in incidence and severity of vacuolation of Sertoli cells (M)	Poon *et al.*,1997; ECB, 2008; ATSDR, 2002	Testes
Fischer 344 rats (M&F)	Oral feeding	0, 1600, 3100, 6300, 12,500, 25,000 mg/kg (0, 80, 160, 320, 630, 1250 mg/kg-day; 10 rats per sex per group)	13 weeks	LOAEL = 630 mg/kg-day; NOAEL = 320 mg/kg-day	Testicular atrophy	NTP, 1982; ECB, 2008	Testes
Fischer 344 rats (M&F)	Oral feeding	0, 1600, 3100, 6300, 12,500, 25,000 mg/kg (0, 80, 160, 320, 630, 1250 mg/kg-day; 10 rats per sex per group)	13 weeks	LOAEL = 1250 mg/kg-day	Testicular atrophy (10/10)	NTP, 1982; ECB, 2008	Testes
B6C3F$_1$ mice (M&F)	Oral feeding	0, 800, 1600, 3100, 6300, 12,500 mg/kg (0, 100, 200, 400, 800, 1600 mg/kg-day; 10 mice per sex per group)	13 weeks	NOAEL = 1600 mg/kg-day	No toxicologically significant effects on reproduction (M&F)	NTP, 1982; ECB, 2008	Testes
ICR mice (M&F)	Oral feeding	0, 0.01, 0.1, 0.3% (0, 20, 200, 600 mg/kg-day)	14 weeks	LOAEL = 600 mg/kg-day; NOAEL = 200 mg/kg-day	Decreased reproductive organ weight, atrophy of seminiferous tubules	Lamb *et al.*,1987	Testes
Sprague-Dawley rats (M&F)	Oral feeding	0, 0.2, 1.0, and 2.0% (0, 143, 737, 1440 mg/kg-day; M; 0, 154, 797, 1414 mg/kg-day; F) (15 rats per group): 0, 1.0, and 2.0% (0, 737, 1440 mg/kg-day; M; 0, 797, 1414 mg/kg-day; F): 0 and 2.0% (0, 1440 mg/kg-day; M; 0, 1414 mg/kg-day; F) (10 rats per group)	17 weeks: 2 or 6 weeks:	LOAEL = 737 mg/kg-day; NOAEL = 143 mg/kg-day	Significant dose-dependent decrease in the absolute testes weight (M, 6, 17 weeks; $P < 0.001$)	Gray *et al.*, 1977	Testes

Sprague-Dawley rats (M&F)	Oral feeding	0, 0.2, 1.0, and 2.0% (0, 143, 737, 1440 mg/kg-day; M; 0, 154, 797, 1414 mg/kg-day; F) (15 rats per group): 0, 1.0, and 2.0% (0, 737, 1440 mg/kg-day; M; 0, 797, 1414 mg/kg-day; F): 0 and 2.0% (0, 1440 mg/kg-day; M; 0, 1414 mg/kg-day; F) (10 rats per group)	17 weeks: 2 or 6 weeks:	LOAEL = 1414 mg/kg-day; NOAEL = 737 mg/kg-day	Significant dose-dependent decrease in the absolute testes weight (M, 2, 6, 17 weeks; $P < 0.001$)	Gray *et al.*, 1977	Testes
Sprague-Dawley rats (M&F)	Oral feeding	0, 0.2, 1.0, and 2.0% (0, 143, 737, 1440 mg/kg-day; M; 0, 154, 797, 1414 mg/kg-day; F) (15 rats per group): 0, 1.0, and 2.0% (0, 737, 1440 mg/kg-day; M; 0, 797, 1414 mg/kg-day; F): 0 and 2.0% (0, 1440 mg/kg-day; M; 0, 1414 mg/kg-day; F) (10 rats per group)	17 weeks: 2 or 6 weeks:	LOAEL = 737 mg/kg-day; NOAEL = 143 mg/kg-day	Significant dose-dependent decrease in the relative testes weight (M, 6, 17 weeks; $P < 0.001$)	Gray *et al.*, 1977	Testes
Sprague-Dawley rats (M&F)	Oral feeding	0, 0.2, 1.0, and 2.0% (0, 143, 737, 1440 mg/kg-day; M; 0, 154, 797, 1414 mg/kg-day; F) (15 rats per group): 0, 1.0, and 2.0% (0, 737, 1440 mg/kg-day; M; 0, 797, 1414 mg/kg-day; F): 0 and 2.0% (0, 1440 mg/kg-day; M; 0, 1414 mg/kg-day; F) (10 rats per group)	17 weeks: 2 or 6 weeks:	LOAEL = 737 mg/kg-day; NOAEL = 143 mg/kg-day	Significant dose-dependent increase in the total incidence and severity of testicular damage (M, 2, 6, 17 weeks; $P < 0.001$-0.05)	Gray *et al.*, 1977	Testes
Sprague-Dawley rats (M)	Oral feeding	0, 0.2, 1.0, and 2.0% (0, 143, 737, 1440 mg/kg-day; M; 0, 154, 797, 1414 mg/kg-day; F) (15 rats per group): 0, 1.0, and 2.0% (0, 737, 1440 mg/kg-day; M; 0, 797, 1414 mg/kg-day; F): 0 and 2.0% (0, 1440 mg/kg-day; M; 0, 1414 mg/kg-day; F) (10 rats per group)	17 weeks: 2 or 6 weeks:	LOAEL = 1440 mg/kg-day; NOAEL = 737 mg/kg-day	Dose-dependent flaccid appearance with reduced testicular size by week 6	Gray *et al.*, 1977	Testes
Sv/129 mice (M)	Oral feeding	N/A	24 weeks	LOAEL = 2400 mg/kg-day	Degenerative testicular lesions	Ward *et al.*,1998; ATSDR, 2002	Testes
Wistar rats	Oral feeding	N/A	78 weeks (18 months)	LOAEL = 2000 mg/kg-day	Testicular atrophy	Price *et al.*,1987; ATSDR, 2002	Testes
Sprague-Dawley rats (M)	Oral feeding	0, 0.02, 0.2, 2.0% (0, 7, 70, 700 mg/kg-day; 520 total rats; GL)	102 weeks	LOAEL = 7 {14} mg/kg-day	Testicular tubule atrophy and inhibition of spermatogenesis	Ganning *et al.*,1987, 1991; ECB, 2008; ATSDR, 2002	Testes

Fischer 344 rats (M&F)	Oral feeding	0, 6000, 12,000 mg/kg (0, 322, 674 mg/kg-day (M); 0, 394, 774 mg/kg-day (F); 50 rats per sex per group; GLP)	103 weeks	LOAEL = 322 mg/kg-day	Increased seminiferous tubule degeneration (5%, 2/44)	NTP, 1982; ECB, 2008	Testes
Fischer 344 rats (M&F)	Oral feeding	0, 6000, 12,000 mg/kg (0, 322, 674 mg/kg-day (M); 0, 394, 774 mg/kg-day (F); 50 rats per sex per group; GLP)	103 weeks	LOAEL = 674 mg/kg-day; NOAEL = 322 mg/kg-day	Increased bilateral seminiferous tubule degeneration (43/48, 90%; low dose 2/44, 5%; control 1/49, 2%), seminiferous tubules histologically devoid of germinal epithelium and spermatocytes, testicular atrophy	NTP, 1982; ECB, 2008	Testes
$B6C3F_1$ mice (M&F)	Oral feeding	0, 3000, 6000 mg/kg (0, 672, 1325 mg/kg-day (M); 0, 799, 1821 mg/kg-day (F); 50 mice per sex per group; GLP)	103 weeks	LOAEL = 1325 mg/kg-day; NOAEL = 672 mg/kg-day	Increased bilateral seminiferous tubule degeneration (7/49, 14%; low dose 2/48, 4%; control 1/49, 2%), testicular atrophy	NTP, 1982; ECB, 2008	Testes
Fischer 344 rats	Oral feeding	N/A	104 weeks	LOAEL = 674 mg/kg-day; NOAEL = 322 mg/kg-day	Severe seminiferous tubular degeneration and testicular atrophy	Kluwe *et al.*,1982; ATSDR, 2002	Testes
Fischer 344 rats (M&F)	Oral feeding	0, 100, 500, 2500, or 12,500 mg DEHP/kg feed (5.8, 28.9, 146.6, 789.0 mg/kg-day for M; 7.3, 36.1, 181.7, and 938.5 mg/kg-day for F; 10 rats per sex per group)	78 or 104 weeks	LOAEL = 789.0 mg/kg-day; NOAEL = 146.6 mg/kg-day	Significant decrease in the absolute and relative testis weight (M; $P < 0.05$); Significant increase in the incidence of aspermatogenesis and pituitary castration cells at 78 weeks (M; $P < 0.05$) and incidence and severity of pituitary castration cells at 104 weeks (M; 0.0 control versus 1.1 treated; $P < 0.05$)	David *et al.*, 2000a	Testes
Fischer 344 rats (M&F)	Oral feeding	0, 100, 500, 2500, or 12,500 mg DEHP/kg feed (5.8, 28.9, 146.6, 789.0 mg/kg-day for M; 7.3, 36.1, 181.7, and 938.5 mg/kg-day for F; 10 rats per sex per group)	78 or 104 weeks	LOAEL = 5.8 mg/kg-day	Substantial dose-dependent increase in the incidence of aspermatogenesis at 104 weeks (M; +6%)	David *et al.*, 2000a	Testes
Fischer 344 rats (M&F)	Oral feeding	0, 100, 500, 1500, or 12,500 mg DEHP/kg feed (5.8, 28.9, 146.6,722-728 mg/kg-day for M; 7.3, 36.1, 181.7, 882-879 mg/kg-day for F; 10 rats per sex per group)	104 weeks + 78 weeks then 26 week recovery	NOAEL = 722-879 mg/kg-day	No toxicologically significant change in the incidence of aspermatogenesis following recovery (M)	David *et al.*, 2001	Testes
Fischer 344 rats (M&F)	Oral feeding	0, 100, 500, 1500, or 12,500 mg DEHP/kg feed (5.8, 28.9, 146.6,722-728 mg/kg-day for M; 7.3, 36.1, 181.7, 882-879 mg/kg-day for F; 10 rats per sex per group)	104 weeks + 78 weeks then 26 week recovery	LOAEL = 722-728 mg/kg-day	Marginal recovery (increase) in absolute and relative testes weight at 104 weeks (M); Marginal recovery (decrease) in the incidence and severity of pituitary castration cells (M)	David *et al.*, 2001	Testes

Fischer 344 rats (M&F)	Oral feeding	0, 100, 500, 2500, 12,500 mg/kg (0, 5.8, 28.9, 146.6, 789.0 mg/kg-day (M); 0, 7.3, 36.1, 181.7, 938.5 mg/kg-day (F), or 12,500 mg/kg for 78 weeks and a 26 week recovery period; 70-85 rats per sex per group; 55 rats per sex in recovery group)	104 weeks	LOAEL = 789.0 mg/kg-day; NOAEL = 146.6 mg/kg-day	Irreversibly decreased testes weight, increased incidence and severity of bilateral hypospermia or aspermatogenesis	Moore, 1996; ECB, 2008	Testes
Fischer 344 rats (M&F)	Oral feeding	0, 100, 500, 2500, 12,500 mg/kg (0, 5.8, 28.9, 146.6, 789.0 mg/kg-day (M); 0, 7.3, 36.1, 181.7, 938.5 mg/kg-day (F), or 12,500 mg/kg for 78 weeks and a 26 week recovery period; 70-85 rats per sex per group; 55 rats per sex in recovery group)	104 weeks	LOAEL = 789.0 mg/kg-day; NOAEL = 146.6 mg/kg-day	Increased immature or abnormal sperm forms and hypospermia in epididymis	Moore, 1996; ECB, 2008	Testes
B6C3F$_1$ mice (M&F; 6 wk old)	Oral feeding	0, 100, 500, 1500, or 6000 mg DEHP/kg feed (19.2, 98.5, 292.2, 1266.1 mg/kg-day for M; 23.8, 116.8, 354.2, 1458.2 mg/kg-day for F; 10-15 mice per sex per group)	105 weeks	LOAEL = 292.2 mg/kg-day; NOAEL = 98.5 mg/kg-day	Significant dose-dependent decrease in the absolute testes weight at 105 weeks (M; P≤0.05); Significant dose-dependent increase in the incidence of bilateral hypospermia at 105 weeks (M; P≤0.05)	David *et al.*, 2000b	Testes
B6C3F$_1$ mice (M&F; 6 wk old)	Oral feeding	0, 100, 500, 1500, or 6000 mg DEHP/kg feed (19.2, 98.5, 292.2, 1266.1 mg/kg-day for M; 23.8, 116.8, 354.2, 1458.2 mg/kg-day for F; 10-15 mice per sex per group)	105 weeks	LOAEL = 1266.1 mg/kg-day; NOAEL = 292.2 mg/kg-day	Significant increase in the incidence of bilateral hypospermia in the testes at 105 weeks (M; P≤0.05)	David *et al.*, 2000b	Testes
B6C3F$_1$ mice (M&F; 6 wk old)	Oral feeding	0, 100, 500, 1500, or 6000 mg DEHP/kg feed (19.2, 98.5, 292.2, 1266.1 mg/kg-day for M; 23.8, 116.8, 354.2, 1458.2 mg/kg-day for F; 10-15 mice per sex per group)	105 weeks	LOAEL = 98.5 mg/kg-day; NOAEL = 19.2 mg/kg-day	Significant decrease in the relative testes weights at 105 weeks (M; P≤0.05)	David *et al.*, 2000b	Testes
B6C3F$_1$ mice (M&F)	Oral feeding	0, 100, 500, 1500, or 6000 mg DEHP/kg feed (19.2, 98.5, 292.2, 1211.0 – 1227.0 mg/kg-day for M; 23.8, 116.8, 354.2, 1413.0 – 1408.0 mg/kg-day for F; 10-15 mice per sex per group)	105 weeks + 79 weeks then 26 week recovery	LOAEL = 1211.0 – 1227.0 mg/kg-day	Significant recovery (increase) in the absolute and relative testes weight (M; P≤0.05); Significant recovery (decrease) in the incidence of bilateral hypospermia at 105 weeks (M; P≤0.05)	David *et al.*, 2001	Testes
B6C3F$_1$ mice	Oral feeding	N/A	104 weeks	LOAEL = 1325 mg/kg-day; NOAEL = 672 mg/kg-day	Seminiferous tubule degeneration	Kluwe *et al.*,1982; ATSDR, 2002	Testes

B6C3F$_1$ mice (M&F)	Oral feeding	0, 100, 500, 1500, 6000 mg/kg (0, 19.2, 98.5, 292.2, 1266.1 mg/kg-day (M); 0, 23.8, 116.8, 354.2, 1458.2 mg/kg-day (F), or 6000 mg/kg for 78 weeks and a 26 week recovery period; 70-85 rats per sex per group; 55 rats per sex in recovery group)	104 weeks	LOAEL = 292.2 mg/kg-day; NOAEL = 98.5 mg/kg-day	Partially reversible decreased testes weight, increased incidence and severity of bilateral hypospermia	Moore, 1997; ECB, 2008	Testes
B6C3F$_1$ mice (M&F)	Oral feeding	0, 100, 500, 1500, 6000 mg/kg (0, 19.2, 98.5, 292.2, 1266.1 mg/kg-day (M); 0, 23.8, 116.8, 354.2, 1458.2 mg/kg-day (F), or 6000 mg/kg for 78 weeks and a 26 week recovery period; 70-85 rats per sex per group; 55 rats per sex in recovery group)	104 weeks	LOAEL = 292.2 mg/kg-day; NOAEL = 98.5 mg/kg-day	Increased immature or abnormal sperm forms and hypospermia	Moore, 1997; ECB, 2008	Testes
Wistar rats (M&F)	Oral feeding	0, 1000, 3000, 9000 mg/kg (0, 113, 340, 1088 mg/kg-day; 25 rats per sex per group)	3 generations	LOAEL = 339 mg/kg-day; NOAEL = 110 mg/kg-day	Loss of spermatocytes in F$_1$ pups (2/10)	Schilling *et al.*,2001; CERHR, 2006; ECB, 2008	Testes
Wistar rats (M&F)	Oral feeding	0, 1000, 3000, 9000 mg/kg (0, 113, 340, 1088 mg/kg-day; 25 rats per sex per group)	3 generations	LOAEL = 1060 mg/kg-day; NOAEL = 339 mg/kg-day	Loss of spermatocytes in F$_1$ pups (7/9)	Schilling *et al.*,2001; CERHR, 2006; ECB, 2008	Testes
Wistar rats (M&F)	Oral feeding	0, 1000, 3000, 9000 mg/kg (0, 113, 340, 1088 mg/kg-day; 25 rats per sex per group)	3 generations	LOAEL = 1060 mg/kg-day; NOAEL = 339 mg/kg-day	Decreased testicular and epidiymal weight and size, testes atrophy, Leydig cell hyperplasia, interstitial edema and altered spermatogenesis in F$_1$ parents	Schilling *et al.*,2001; CERHR, 2006; ECB, 2008	Testes
Wistar rats (M&F)	Oral feeding	0, 1000, 3000, 9000 mg/kg (0, 113, 340, 1088 mg/kg-day; 25 rats per sex per group)	3 generations	LOAEL = 110 mg/kg-day	Dose-dependent decrease in prostate weight in F$_1$ parents	Schilling *et al.*,2001; CERHR, 2006; ECB, 2008	Testes
Sprague-Dawley rats (M&F)	Oral feeding	0.1, 0.5, 1.4, 4.8, 14, 46, 359, 775 mg/kg-day	3 generations	LOAEL = 14 mg/kg-day; NOAEL = 4.8 mg/kg-day	Dose-dependent adverse effects on testes parameters	Wolfe *et al.*,2003; ECB, 2008	Testes
Wistar rats (M&F)	Oral feeding	0, 1000, 3000, 9000 mg/kg (0, 113, 340, 1088 mg/kg-day; 25 rats per sex per group)	3 generations	LOAEL = 113 mg/kg-day	Marginal focal tubular atrophy	Schilling *et al.*,2001; CERHR, 2006; ECB, 2008	Testes
Wistar rats (M&F)	Oral feeding	0, 1000, 3000, 9000 mg/kg (0, 113, 340, 1088 mg/kg-day; 25 rats per sex per group)	3 generations	LOAEL = 340 mg/kg-day; NOAEL = 113 mg/kg-day	Decreased testes weight in F$_2$ generation, focal tubular atrophy, feminization of 49% of male offspring	Schilling *et al.*,2001; CERHR, 2006; ECB, 2008	Testes
Fischer 344 rats (M)	Oral feeding	0, 2500, 12,500 mg/kg	78 weeks	NOAEL = 12,500 mg/kg	Decreased incidence of testicular interstitial cell tumors (tumor frequency = 90, 100, 30%, respectively)	David *et al.*, 2000; Ito and Nakajima, 2008	Testes
Fischer 344 rats (M)	Oral feeding	0, 12,500 mg/kg	79 weeks	NOAEL = 12,500 mg/kg	Decreased incidence of testicular interstitial cell tumors (tumor frequency = 90, 30%, respectively)	David *et al.*, 2001; Ito and Nakajima, 2008	Testes
Fischer 344 rats (M)	Oral feeding	0, 100, 500, 2500, 12,500 mg/kg	104 weeks	NOAEL = 12,500 mg/kg	Decreased incidence of testicular interstitial cell tumors (tumor frequency = 92, 90, 91, 92, 31%, respectively)	David *et al.*, 2000a; Ito and Nakajima, 2008	Testes

Fischer 344 rats (M&F)	Oral feeding	0, 6000, 12,000 mg/kg (0, 322, 674 mg/kg-day (M); 0, 394, 774 mg/kg-day (F); 50 rats per sex per group; GLP)	103 weeks	LOAEL = 674 mg/kg-day; NOAEL = 322 mg/kg-day	Decreased incidence of testicular interstitial cell tumors ($P < 0.001$)	NTP, 1982; ECB, 2008	Testes
Fischer 344 rats (M&F)	Oral feeding	0, 100, 500, 2500, or 12,500 mg DEHP/kg feed (5.8, 28.9, 146.6, 789.0 mg/kg-day for M; 7.3, 36.1, 181.7, and 938.5 mg/kg-day for F; 10 rats per sex per group)	78 or 104 weeks	LOAEL = 789.0 mg/kg-day; NOAEL = 146.6 mg/kg-day	Significant decrease in the incidence of interstitial cell tumors of the testes at 78 and 104 weeks (M; $P < 0.05$)	David *et al.*, 2000a	Testes
Fischer 344 rats (M&F)	Oral feeding	0, 100, 500, 1500, or 12,500 mg DEHP/kg feed (5.8, 28.9, 146.6,722-728 mg/kg-day for M; 7.3, 36.1, 181.7, 882-879 mg/kg-day for F; 10 rats per sex per group)	104 weeks + 78 weeks then 26 week recovery	NOAEL = 722-879 mg/kg-day	No toxicologically significant change in the incidence of testicular interstitial cell tumors	David *et al.*, 2001	Testes
Fischer 344 rats (M&F)	Oral feeding	0, 100, 500, 2500, 12,500 mg/kg (0, 5.8, 28.9, 146.6, 789.0 mg/kg-day (M); 0, 7.3, 36.1, 181.7, 938.5 mg/kg-day (F), or 12,500 mg/kg for 78 weeks and a 26 week recovery period; 70-85 rats per sex per group; 55 rats per sex in recovery group)	104 weeks	NOAEL = 789.0 mg/kg-day	Decreased incidence of interstitial cell neoplasms	Moore, 1996; ECB, 2008	Testes
Fischer 344 rats (M)	Oral feeding	0, 6000, 12,000 mg/kg	104 weeks	NOAEL = 12,000 mg/kg	Decreased testis interstitial cell tumors	Kluwe *et al.*, 1985; Ito and Nakajima, 2008	Testes
Fischer 344 rats (M)	Oral feeding	0, 12,500 mg/kg, recovery	105 weeks	NOAEL = 12,500 mg/kg	Decreased testis interstitial cell tumors (tumor frequency = 92, 31, 32%, respectively)	David *et al.*, 2001; Ito and Nakajima, 2008	Testes
Sprague-Dawley rats (M)	Oral feeding	30, 95, 300 mg/kg-day	Lifetime	LOAEL = 300 mg/kg-day; NOAEL = 90 mg/kg-day	Increased incidence of Leydig cell tumors (abstract)	Berger, 1995; ECB, 2008	Testes
Sprague-Dawley rats (M&F)	Oral gavage	0, 10, 100, 1000, 2000 mg DEHP/kg bw (7-10 rats per group); 0,100, 200, 500, 1000 mg/kg (16 rat pups per group); 0, 200, 500, 1000 mg/kg (50 rat pups per group)	Once daily during PPd 6-10, 14-18, 21-25, 42-46, 86-90; Once daily for 5 days during PPd 6-10 – recovery for 4 weeks; Once daily for 5 days during PPd 6-10	NOAEL = 1000 mg/kg	No toxicologically significant change in the epididymal weight of rats dosed during PPd 6-10 and allowed to recover for 4, 11, 12, 16 weeks or relative epididymal weight in 13 week old rats	Dostal *et al.*, 1988	Epididymides
Sprague-Dawley rats (M&F)	Oral gavage	0, 10, 100, 1000, 2000 mg DEHP/kg bw (7-10 rats per group); 0,100, 200, 500, 1000 mg/kg (16 rat pups per group); 0, 200, 500, 1000 mg/kg (50 rat pups per group)	Once daily for 5 days during PPd 6-10 – recovery for 11, 12, 13, 16, 19, 23 weeks	LOAEL = 1000 mg/kg; NOAEL = 500 mg/kg-day	Significant decrease in absolute epididymal weight in 13 week old rats	Dostal *et al.*, 1988	Epididymides

Wistar rats (F)	Oral gavage	0, 0.015, 0.045, 0.135, 0.405, 1.215, 5, 15, 45, 135, 405 mg/kg-day; ? dams per group; ? rats per group)	Once daily for 27 days during Gd6 to Ld 21, recovery til 144 days old	NOAEL = 405 mg/kg-day	No toxicologically significant effects on weight of grossly normal epididymides	Andrade *et al.*, 2006b	Epididymides
CRL:CD Sprague-Dawley rats (F)	Oral gavage	0, 11, 33, 100, 300 mg/kg-day (12-14 litters per group)	Once daily during Gd 8-17; recovery til maturity; continued dosing from 18 til 63-65	LOAEL = 300 mg/kg-day; NOAEL = 100 mg/kg-day	In pups dosed from Gd 8 to PNd 64; Significant decrease in epididymal weight ($P < 0.01$); Dose-dependent decrease in epididymal sperm count ($P < 0.01$) In pups dosed from Gd 8 to Ld 17 then recovery; Significant dose-dependent decrease in epididymal weight ($P < 0.01$)	Gray *et al.*, 2009	Epididymides
Sprague-Dawley rats (M&F)	Oral feeding	0, 5, 50, 500, 5000 mg/kg (0, 0.4, 3.7, 37.6, 375.2 mg/kg-day (M); 0, 0.4, 4.2, 42.2, 419.3 mg/kg-day (F); 10 rats per sex per group; GLP)	13 weeks	LOAEL = 345.0-411.0 mg/kg-day	Substantial increase in the incidence and severity of bilateral reduction of sperm density in epididymides	Poon *et al.*,1997; ECB, 2008; ATSDR, 2002	Epididymides
B6C3F$_1$ mice (M&F; 6 wk old)	Oral feeding	0, 100, 500, 1500, or 6000 mg DEHP/kg feed (19.2, 98.5, 292.2, 1266.1 mg/kg-day for M; 23.8, 116.8, 354.2, 1458.2 mg/kg-day for F; 10-15 mice per sex per group)	105 weeks	LOAEL = 292.2 mg/kg-day; NOAEL = 98.5 mg/kg-day	Significant dose-dependent increase in the incidence of immature/abnormal epididymal sperm at 105 weeks (M; $P \leq 0.05$)	David *et al.*, 2000b	Epididymides
B6C3F$_1$ mice (M&F; 6 wk old)	Oral feeding	0, 100, 500, 1500, or 6000 mg DEHP/kg feed (19.2, 98.5, 292.2, 1266.1 mg/kg-day for M; 23.8, 116.8, 354.2, 1458.2 mg/kg-day for F; 10-15 mice per sex per group)	105 weeks	LOAEL = 1266.1 mg/kg-day; NOAEL = 292.2 mg/kg-day	Significant increase in the incidence of immature/abnormal epididymal sperm at 78 weeks and hypospermia of the epididymides at 105 weeks (M; $P \leq 0.05$)	David *et al.*, 2000b	Epididymides
B6C3F$_1$ mice (M&F)	Oral feeding	0, 100, 500, 1500, or 6000 mg DEHP/kg feed (19.2, 98.5, 292.2, 1211.0 – 1227.0 mg/kg-day for M; 23.8, 116.8, 354.2, 1413.0 – 1408.0 mg/kg-day for F; 10-15 mice per sex per group)	105 weeks + 79 weeks then 26 week recovery	LOAEL = 1211.0 – 1227.0 mg/kg-day	Significant recovery (decrease) in the incidence of hypospermia of the epididymides at 105 weeks (M; $P \leq 0.05$)	David *et al.*, 2001	Epididymides
B6C3F$_1$ mice (M&F)	Oral feeding	0, 100, 500, 1500, or 6000 mg DEHP/kg feed (19.2, 98.5, 292.2, 1211.0 – 1227.0 mg/kg-day for M; 23.8, 116.8, 354.2, 1413.0 – 1408.0 mg/kg-day for F; 10-15 mice per sex per group)	105 weeks + 79 weeks then 26 week recovery	NOAEL = 1211.0 – 1227.0 mg/kg-day	No toxicologically significant change in the incidence of immature/abnormal epididymal sperm following recovery (M)	David *et al.*, 2001	Epididymides
Wistar rats (F)	Oral gavage	0, 0.015, 0.045, 0.135, 0.405, 1.215, 5, 15, 45, 135, 405 mg/kg-day; ? dams per group; ? rats per group)	Once daily for 27 days during Gd6 to Ld 21, recovery til 144 days old	LOAEL = 405 mg/kg-day; NOAEL = 135 mg/kg-day	Seminal vesicle (plus coagulating gland) weight significantly decreased at high dose ($P < 0.05$)	Andrade *et al.*, 2006b	Seminal vesicle plus coagulating gland

Wistar rats (F)	Oral gavage	0, 0.015, 0.045, 0.135, 0.405, 1.215, 5, 15, 45, 135, 405 mg/kg-day; ? dams per group; ? rats per group)	Once daily for 27 days during Gd6 to Ld 21, recovery til 144 days old	LOAEL = 405 mg/kg-day; NOAEL = 135 mg/kg-day	Ventral prostate weight significantly reduced at high dose	Andrade *et al.*, 2006b	Ventral prostate
Sprague-Dawley rats (F)	Oral gavage	N/A	Once daily for 1-10 days	LOAEL = 2000 mg/kg-day	Suppressed ovulation with 25% decrease in preovulatory follicle granulosa cells and decreased serum estradiol	Davis *et al.*,1994a; ATSDR, 2002	Ovary
Marmoset monkeys	Oral feeding	0, 100, 500, 2500 mg/kg-day	N/A	LOAEL = 500 mg/kg-day; NOAEL = 100 mg/kg-day (BMD_{10} = 507-677, $BMDL_{10}$ = 258-303)	Significant increase in absolute and relative ovary and uterus weights ($P < 0.05$)	CERHR, 2006	Ovary/Uterus
Wistar rats (F)	Oral gavage	0, 40, 200, 1000 mg/kg-day (9-10 litters per group)	Once daily during Gd 6-15	LOAEL = 1000 mg/kg-day; NOAEL = 200 mg/kg-day	Significant decrease in the uterus weight ($P \leq 0.05$)	Hellwig *et al.*, 1997	Uterus
$B6C3F_1$ mice (M&F)	Oral feeding	0, 1000, 5000, 10,000, 25,000 mg/kg (0, 250, 1210, 2580, 6990 mg/kg-day(M); 0, 270, 1430, 2890, 7900 mg/kg-day (F); 10 mice per sex per group; GLP)	28 days	LOAEL = 7900 mg/kg-day; NOAEL = 2890 mg/kg-day	Absence of corpora lutea in ovary	Eastman Kodak, 1992b; ECB, 2008	Ovary
Fischer 344 rats (M&F)	Oral feeding	0, 1600, 3100, 6300, 12,500, 25,000 mg/kg (0, 80, 160, 320, 630, 1250 mg/kg-day; 10 rats per sex per group)	13 weeks	NOAEL = 1250 mg/kg-day	No toxicologically significant effects on reproduction (F)	NTP, 1982; ECB, 2008	Ovary
Sprague-Dawley rats (M&F)	Oral feeding	0, 0.2, 1.0, and 2.0% (0, 143, 737, 1440 mg/kg-day; M; 0, 154, 797, 1414 mg/kg-day; F) (15 rats per group): 0, 1.0, and 2.0% (0, 737, 1440 mg/kg-day; M; 0, 797, 1414 mg/kg-day; F): 0 and 2.0% (0, 1440 mg/kg-day; M; 0, 1414 mg/kg-day; F) (10 rats per group)	17 weeks: 2 or 6 weeks:	LOAEL = 1440mg/kg-day; NOAEL = 797 mg/kg-day	Significant dose-dependent decrease in the absolute ovary weight (F, 2, 6, 17 weeks; $P < 0.01$)	Gray *et al.*, 1977	Ovary
Sprague-Dawley rats (M&F)	Oral feeding	0, 0.2, 1.0, 2.0% (0, 143, 737, 1440 mg/kg-day (M); 0, 154, 797, 1414 mg/kg-day (F); 15 rats per sex per group	17 weeks	NOAEL = 1414 mg/kg-day	No histopathological changes in ovary or pituitary	Gray *et al.*,1977	Ovary
Mice (M&F)	Oral feeding	0.3%	18 weeks	LOAEL = 0.3%	Significant decrease in ovary and uterus weight	Reel *et al.*,1985	Ovary/uterus
Marmoset monkeys (M&F; 90-115 days old)	Oral gavage	0, 100, 500, 2500 mg/kg-day (9M and 6F per group)	Once daily for 65 weeks	LOAEL = 500 mg/kg-day; NOAEL = 100 mg/kg-day	Significant increase in absolute and relative ovary and uterine weight	Mitsubishi Chem Safety Inst, 2003; CERHR, 2006	Ovary/Uterus
$B6C3F_1$ mice (M&F)	Oral feeding	0, 3000, 6000 mg/kg (0, 672, 1325 mg/kg-day (M); 0, 799, 1821 mg/kg-day (F); 50 mice per sex per group)	103 weeks	LOAEL = 799 mg/kg-day	Inflammation, suppuration of uterus/endometrium (control 0/48; low dose 2/48, 4%; high dose 6/50, 12%)	NTP, 1982	Ovary/Uterus

Fischer 344 rats (M&F)	Oral feeding	0, 100, 500, 2500, or 12,500 mg DEHP/kg feed (5.8, 28.9, 146.6, 789.0 mg/kg-day for M; 7.3, 36.1, 181.7, and 938.5 mg/kg-day for F; 10 rats per sex per group)	78 or 104 weeks	NOAEL = 938.5 mg/kg-day	No toxicologically significant change in the absolute or relative uterus weight (F)	David *et al.*, 2000a	Uterus
B6C3F$_1$ mice (M&F)	Oral feeding	0, 100, 500, 1500, or 6000 mg DEHP/kg feed (19.2, 98.5, 292.2, 1266.1 mg/kg-day for M; 23.8, 116.8, 354.2, 1458.2 mg/kg-day for F; 10-15 mice per sex per group)	79 or 105 weeks	LOAEL = 1458.2 mg/kg-day; NOAEL = 354.2 mg/kg-day	Significant decrease in the absolute and relative uterus weight at 104 weeks (F; P≤0.05)	David *et al.*, 2000b	Uterus
B6C3F$_1$ mice (M&F)	Oral feeding	0, 100, 500, 1500, or 6000 mg DEHP/kg feed (19.2, 98.5, 292.2, 1211.0 – 1227.0 mg/kg-day for M; 23.8, 116.8, 354.2, 1413.0 – 1408.0 mg/kg-day for F; 10-15 mice per sex per group)	105 weeks + 79 weeks then 26 week recovery	LOAEL = 1413.0 – 1408.0 mg/kg-day	Significant recovery (increase) in the absolute and relative uterus weight (F; P≤0.05)	David *et al.*, 2001	Uterus
Wistar rats (M&F)	Oral feeding	0, 1000, 3000, 9000 mg/kg (0, 113, 340, 1088 mg/kg-day; 25 rats per sex per group)	3 generations	LOAEL = 1060 mg/kg-day; NOAEL = 339 mg/kg-day	Retarded preputial separation and vaginal opening and increased incidence of areolas/nipple analagen	Schilling *et al.*,2001; CERHR, 2006; ECB, 2008	Ovary/uterus
Sprague-Dawley rats (F)	Oral gavage	2000 mg DEHP/kg bw (7-8 dams dosed per group, 10 pups per dam)	Once daily during Ld 2-6, 6-10, 14-18, 15-17	LOAEL = 2000 mg/kg	Significant decrease in absolute mammary gland weight, the total RNA in mammary glands, and the RNA/DNA ratio ($P < 0.05$; Pair-fed controls had decreased absolute mammary gland weight, total RNA in mammary glands, and significantly decreased RNA/DNA ratio)	Dostal *et al.*, 1987	Mammary gland
Fischer 344 rats (F)	Oral feeding	0, 6000, 12,000 mg/kg	104 weeks	NOAEL = 12,000 mg/kg	Decreased incidence of mammary gland tumors	Kluwe *et al.*, 1985; Ito and Nakajima, 2008	Mammary glands
Sprague-Dawley rat	Oral gavage	N/A	Once daily for 3 days during Ld 15-17	LOAEL = 2000 mg/kg-day	Changes in milk composition	Dostal *et al.*,1987b; ATSDR, 2002	Reproduction
Sprague-Dawley rats	Oral gavage	N/A	Once daily for 4 days	NOAEL = 2000 mg/kg-day	No toxicologically significant effects on reproduction	Zacharewski *et al.*,1998; ATSDR, 2002	Reproduction
Sprague-Dawley rats (M&F)	Oral gavage	0, 10, 100, 1000, 2000 mg DEHP/kg bw (7-10 rats per group); 0,100, 200, 500, 1000 mg/kg (16 rat pups per group); 0, 200, 500, 1000 mg/kg (50 rat pups per group)	Once daily during PPd 6-10, 14-18, 21-25, 42-46, 86-90; Once daily for 5 days during PPd 6-10 – recovery for 4 weeks; Once daily for 5 days during PPd 6-10	NOAEL = 1000 mg/kg	No toxicologically significant change in fertility	Dostal *et al.*, 1988	Reproduction

Sprague-Dawley rats	Oral gavage	N/A	Once daily for 10 days on PPd 105-114	NOAEL = 2800 mg/kg-day	No toxicologically significant effects on reproduction	Gray and Butterworth, 1980; ATSDR, 2002	Reproduction
Marmoset Monkey (M&F; 250-400 g)	Oral gavage	0, 2000 mg/kg (5 marmosets per sex per group)	Once daily for 14 days	NOAEL = 2000 mg/kg-day	No toxicologically significant effects on reproduction	Rhodes *et al.*,1986; ATSDR, 2002	Reproduction
Cynomolgous monkeys (M)	Oral gavage	N/A	Once daily for 14 days	NOAEL = 500 mg/kg-day	No toxicologically significant effects on reproduction	Pugh *et al.*,2000; ATSDR, 2002	Reproduction
Sprague-Dawley rats	Oral feeding	N/A	14 days during PPd 60-73	NOAEL = 1700 mg/kg-day	No toxicologically significant effects on reproduction	Sjoberg *et al.*,1986a; ATSDR, 2002	Reproduction
Sprague-Dawley rats	Oral gavage	N/A	Once daily for 14 days during PPd 40-53, 60-73	NOAEL = 1000 mg/kg-day	No toxicologically significant effects on reproduction	Sjoberg *et al.*,1986a; ATSDR, 2002	Reproduction
Wistar rat	Inhalation	0, 50, 1000 mg/m^3	6 hours/day for 5 days/week for 28 days	NOAEC = 1000 mg/m^3	N/A	Klimisch *et al.*,1991	Reproduction
Wistar rats (F)	Oral gavage	0, 0.015, 0.045, 0.135, 0.405, 1.215, 5, 15, 45, 135, 405 mg/kg-day; ? dams per group; ? rats per group)	Once daily for 27 days during Gd6 to Ld 21, recovery til 144 days old	NOAEL = 405 mg/kg-day	No toxicologically significant effects on reproduction (time to mating, mating index, pregnancy index, fetal weights, total resorptions, viable fetuses, implantation sites, mating behavior)	Andrade *et al.*, 2006b	Reproduction
Wistar rats (M)	Inhalation	0, 10, 50, 1000 mg/m^3 (10 rats per group)	28 days	NOAEC = 1000 mg/m^3	No fertility effects	Klimische *et al.*,1992; ECB, 2008	Reproduction
CRL:CD Sprague-Dawley rats (F)	Oral gavage	0, 11, 33, 100, 300 mg/kg-day (12-14 litters per group)	Once daily during Gd 8-17; recovery til maturity; continued dosing from 18 til 63-65	NOAEL = 300 mg/kg-day	No toxicologically significant change in litter size	Gray *et al.*, 2009	Reproduction
CD-1 mice (5 wk of age)	Oral feeding	0.01, 0.03, 0.09% (F_0M premate 16, 47, 142; F_0F premate 20, 56, 168; mating 15, 40, 126; gestation 17, 47, 140; lactation 60, 172, 493; F_1M 16, 48, 145; F_1F 19, 56, 171 mg/kg-day	8-9 weeks	LOAEL = 493 mg/kg-day; NOAEL = 172 mg/kg-day	Significant decrease in F_1F offspring survival during PNd 4-14, significant decrease in total offspring of F_0 mice during PNd 4-21	Tanaka *et al.*,2002; CERHR, 2006	Reproduction
CD-1 mice (5 wk of age)	Oral feeding	0.01, 0.03, 0.09% (F_0M premate 16, 47, 142; F_0F premate 20, 56, 168; mating 15, 40, 126; gestation 17, 47, 140; lactation 60, 172, 493; F_1M 16, 48, 145; F_1F 19, 56, 171 mg/kg-day	8-9 weeks	NOAEL = 493 mg/kg-day	No toxicologically significant effects on sex ratio, litter size, or weight at birth	Tanaka *et al.*,2002; CERHR, 2006	Reproduction
Mice (M&F)	Oral feeding	0.3%	18 weeks	LOAEL = 0.3%	Complete suppression of fertility in females when bred to unexposed males	Reel *et al.*,1985	Reproduction

Cr1:CD-1 mice {ICR} (M&F)	Oral feeding	0, 0.01, 0.1, 0.3% (0, 14, 140, 420 mg/kg-day){0, 20, 200, 600 mg/kg-day}; 20 mice per sex per treatment group; 40 control mice)	18 weeks (126 days {98 days}); continuous breeding	LOAEL = 420 {600} mg/kg-day; NOAEL = 140 {200} mg/kg-day	Significant dose-dependent decrease in the number of litters per mated pair, the mean live pups per litter, and proportion of pups born alive ($P < 0.05$); Significant increase in live pup weight ($P < 0.05$) In crossover mating: significant reduction in fertility when mating control females and treated males ($P < 0.05$); significant reduction in fertility when matng treated females and control males ($P < 0.05$); Significant dose-dependent decrease in the # fertile per # cohabitated mice	Lamb *et al.*,1987; ECB, 2008; ATSDR, 2002	Reproduction
Sherman rats	Oral feeding	N/A	52 weeks	NOAEL = 328 mg/kg-day	No toxicologically significant effects on reproduction	Carpenter *et al.*,1953; ATSDR, 2002	Reproduction
Humans (97 M/4 F	Inhalation /Dermal (epi)	Background (0.001-0.004 ppm ~ 0.016-0.064 mg/m^3) Higher levels – 0.01 ppm (0.16 mg/m^3)	12 years average exposure period (4 months to 35 years)	NS	Children fathered by exposed men normal	Thiess *et al.*,1978b	Reproduction
Sherman rats	Oral feeding	N/A	104 weeks	NOAEL = 190 mg/kg-day	No toxicologically significant change in reproduction	Carpenter *et al.*,1953; ATSDR, 2002	Reproduction
Unspecified rats (M&F)	Oral gavage	1.5, 10, 30, 100, 300, 1000, 7500, 10000 mg DEHP/kg bw (0.1, 0.5, 1.5, 5, 15, 50, 400, 500 mg/kg-day)	Multigeneration RACB protocol	LOAEL = 400 mg/kg-day; NOAEL = 50 mg/kg-day With Additional animals from non-bred cohort, LOAEL = 15 mg/kg-day; NOAEL = 5 mg/kg-day	Significant dose-dependent increase in "reproductive tract malformations"	Foster *et al.*, 2000b	Reproduction
Wistar rats (M&F)	Oral feeding	0, 1000, 3000, 9000 mg/kg (0, 113, 340, 1088 mg/kg-day; 25 rats per sex per group; GLP)	3 generations	LOAEL = 340 {1088} mg/kg-day; NOAEL = 110 {340} mg/kg-day	Increased number of stillborn F_2 pups (increased 4-fold), decreased number of F_1 pups surviving (4%)	Schilling *et al.*,2001; CERHR, 2006; ECB, 2008	Reproduction
Wistar rats (M&F)	Oral feeding	0, 1000, 3000, 9000 mg/kg (0, 113, 340, 1088 mg/kg-day; 25 rats per sex per group; GLP)	3 generations	LOAEL = 1088 mg/kg-day; NOAEL = 340 mg/kg-day	Decreased number of delivered and liveborn F_1 pups, decreased viability index of neonatality in F_1 pups, decreased fertility in F_1 parents, increased post-implantation loss with F_0 adults (increased 2.1-fold), decreased F_1 male anogenital distance (14%), decreased number of F_0 M with confirmed fertility (12%)	Schilling *et al.*,2001; CERHR, 2006; ECB, 2008	Reproduction
Wistar rats (M&F)	Oral feeding	0, 1000, 3000, 9000 mg/kg (0, 113, 340, 1088 mg/kg-day; 25 rats per sex per group; GLP)	3 generations	LOAEL = 1088 mg/kg-day; NOAEL = 340 mg/kg-day	Decreased number of delivered pups and decreased mean number of pups per dam	Schilling *et al.*,2001; CERHR, 2006; ECB, 2008	Reproduction
Sprague-Dawley rats (M&F)	Oral feeding	0.1, 0.5, 1.4, 4.8, 14, 46, 359, 775 mg/kg-day	3 generations	LOAEL = 359 mg/kg-day; NOAEL = 46 mg/kg-day	Decreased fertility	Wolfe *et al.*,2003; ECB, 2008	Reproduction
CD-1 mice	Oral feeding	0, 0.01, 0.025, 0.05% (0, 19, 48, 95 mg/kg-day)	3 generations	LOAEL = 95 mg/kg-day; NOAEL = 48 mg/kg-day	Increased prenatal mortality in F1 litters, decreased number of viable pups in F1 litters	NTIS, 1988; ECB, 2008	Reproduction
CD-1 mice	Oral feeding	0, 0.01, 0.025, 0.05% (0, 19, 48, 95 mg/kg-day)	3 generations	NOAEL = 95 mg/kg-day	No change in parental toxicity or F2 generation	NTIS, 1988; ECB, 2008	Reproduction
Wistar rats	Oral gavage		Once during Gd 12	LOAEL = 4882 {9756} mg/kg-day	Slight increase in dead, resorbed, and malformed fetuses	Ritter *et al.*,1987	Development

C57BL/6NxSv/129 mice	Oral gavage	N/A	Once daily for 2 days during Gd 8-9	LOAEL = 1000 mg/kg-day	Decreased fetal viability, increased resorptions, and external malformations	Peters *et al.*,1997; ATSDR, 2002	Development
Sic-ICR mice	Oral gavage	N/A	Once daily for 3 days during Gd 7-9	LOAEL = 1000 mg/kg-day; NOAEL = 250 mg/kg-day	Decreased fetal viability, increased resorptions and external malformations	Shiota and Mima, 1985; ATSDR, 2002	Development
Sprague-Dawley rats (F)	Oral gavage	0, 750 or 1000 mg/kg	Once daily during Gd 14-18	LOAEL = 750 mg/kg-day	Significant reduction in the mRNA levels of insl3 in fetal testes ($P < 0.02$)	Wilson *et al.*, 2004	Development
Sprague-Dawley rats (F)	Oral gavage	0, 2000 mg/kg-day	Once daily for 5 days during Ld2-6, 6-10, 14-18	LOAEL = 2000 mg/kg-day	Decreased pup body weight (14-26%), biochemical indications of peroxisome proliferation in neonate livers	Dostal *et al.*,1987b; ATSDR, 2002	Development
Sprague-Dawley rats (M&F)	Oral gavage	0, 10, 100, 1000, 2000 mg DEHP/kg bw (7-10 rats per group); 0,100, 200, 500, 1000 mg/kg (16 rat pups per group); 0, 200, 500, 1000 mg/kg (50 rat pups per group)	Once daily during PPd 6-10, 14-18, 21-25, 42-46, 86-90; Once daily for 5 days during PPd 6-10 – recovery for 4 weeks; Once daily for 5 days during PPd 6-10	NOAEL = 1000 mg/kg	No toxicologically significant change in the mean number of uterine implants, the mean number of live fetuses per pregnant female, the number of resorptions, the ratio of total number of implants to the number of corpora lutea	Dostal *et al.*, 1988	Development
ddY-Sic mice	Oral gavage	N/A	Once daily for 5 days during Gd 6, 7, 8, 9, 10	LOAEL = 1000 mg/kg-day	Fetal lethality (60%)	Yagi *et al.*,1980; ATSDR, 2002	Development
ddY-Sic mice	Oral gavage	N/A	Once daily for 5 days during Gd 6, 7, 8, 9, 10	LOAEL = 100 mg/kg-day; NOAEL = 50 mg/kg-day	Fetal lethality (11.2%) versus control mice (2.0%)	Tomita *et al.*,1982a; ATSDR, 2002	Development
Wistar rats (F)	Oral gavage	0, 40, 200, 1000 mg/kg-day (9-10 litters per group)	Once daily during Gd 6-15	LOAEL = 1000 mg/kg-day; NOAEL = 200 mg/kg-day	Significant increase in the post-implantation loss ($P \leq 0.01$), in the total resorptions ($P \leq 0.01$), in the early and late resorptions ($P \leq 0.01$), in the number of litters with malformations ($P \leq 0.01$), in the number of affected fetuses with malformations per litter ($P \leq 0.01$), in the affected fetuses with variations per litter ($P \leq 0.01$), in the number of affected fetuses with retardations per litter ($P \leq 0.01$); Significant decrease in the fetal weights ($P \leq 0.01$); Substantial decrease in the live fetuses per dam (34%); Substantial dose-dependent increase in the number of fetuses with malformations, the number of fetuses with variations, the number of fetuses with retardations	Hellwig *et al.*, 1997	Development
Wistar rats (F)	Oral gavage	0, 40, 200, 1000 mg/kg-day (9-10 litters per group)	Once daily during Gd 6-15	NOAEL = 1000 mg/kg-day	No toxicologically significant change in the number of pregnant dams, maternal lethality, corpora lutea per dam, implantation sites per dam, dams with viable fetuses, and the number and percent of litters with variations or retardations	Hellwig *et al.*, 1997	Development

Wistar rats (F)	Inhalation (head nose)	0, 0.01, 0.05, 0.3mg/L (0, 10, 50, 300 mg/m^3)	6 hours per day for 9 days during Gd 6-15	$NOAEC_{maternal\ and\ devel}$ = 300 mg/m^3	Non-dose related decreased number of live fetuses per dams, non-dose-related increased percentage of resorptions per dam, no maternal toxicity	Merkle *et al.*,1988	Development
Sprague-Dawley rats	Oral gavage	N/A	Once daily for 10 days during Gd 14-21 and PPd 1-3	LOAEL = 750 mg/kg-day	Significant delay in male reproductive system maturation, reduced weight of sex organs in adult males	Gray *et al.*,1999; ATSDR, 2002	Development
Sprague-Dawley rats (M)	Oral gavage	N/A	Once daily for 10 days during Gd 14-21 and PPd 1-3	LOAEL = 750 mg/kg-day	Testicular degeneration and altered sexual differentiation in male offspring	Gray *et al.*,2000; ATSDR, 2002	Development
Sprague-Dawley rats (M)	Oral gavage	N/A	Once daily for 10 days during Gd 14-21 and PPd 1-3	LOAEL = 750 mg/kg-day	Decreased fetal testosterone synthesis during male sexual differentiation	Parks *et al.*,2000; ATSDR, 2002	Development
Fischer 344 rats	Oral gavage		Once daily for 14 days during PPd 1-21	LOAEL = 1000 mg/kg-day	Significant peroxisome proliferation in both liver and kidneys from pups	Stefanini *et al.*,1995	Development
CD-1 mice {1-CR}	Oral feeding	0, 0.025, 0.05, 0.10, 0.15% (0, 44, 91, 150.6, 292.5 mg/kg-day; 30-31 mice per group)	17 days during Gd 0-17	$LOAEL_{devel}$ = 91 mg/kg-day; $NOAEL_{devel}$ = 44 mg/kg-day	Decreased fetal body weight, decreased number of live fetuses per litter, increased number and percentage of resorptions, late fetal deaths, dead and malformed fetuses, and percent of malformed fetuses per litter (open eyes, exopthalmia, exencephaly, short, constricted or no tail, visceral malformations and skeletal defects (fused and branched ribs, misalignment, and fused thoracic vertebral centra)	Tyl *et al.*,1988; ECB, 2008; ATSDR, 2002	Development
ICR mice	Oral feeding	N/A	18 days during Gd 1-18	LOAEL = 170 mg/kg-day; NOAEL = 83 mg/kg-day	Increased percent resorptions and dead fetuses	Shiota *et al.*,1980; ATSDR, 2002	Development
CD-1 mice	Oral feeding	N/A	18 days during Gd 0-17	LOAEL = 95 mg/kg-day; NOAEL = 48 mg/kg-day	Increased prenatal and perinatal mortality	Price *et al.*,1988c; ATSDR, 2002	Development
Fischer 344 rats (CrlBr; F)	Oral feeding	0, 0.5, 1.0, 1.5, 2.0% (0, 357, 666, 856, 1055 mg/kg-day; 34-25 rats per group)	20 days during Gd 0-20	LOAEL = 1055 (2%) mg/kg-day); NOAEL = 856 (1.5%) mg/kg-day	Increased number and percentage of resorptions, non-live, and mean affected implants per litter	Tyl *et al.*,1988; ECB, 2008; ATSDR, 2002	Development
Fischer 344 rats (CrlBr; F)	Oral feeding	0, 0.5, 1.0, 1.5, 2.0% (0, 357, 666, 856, 1055 mg/kg-day; 34-25 rats per group)	20 days during Gd 0-20	$LOAEL_{maternal}$ = 666 mg/kg-day; $NOAEL_{maternal}$ = 357 mg/kg-day $LOAEL_{devel}$ = 357 mg/kg-day	Decreased mean fetal body weight per litter	Tyl *et al.*,1988; ECB, 2008; ATSDR, 2002	Development
Fischer 344 rats	Oral gavage	N/A	Once daily for 21 days during PPd 1-21	LOAEL = 500 mg/kg-day	Decreased pup body weight on PPd 21 (~ 24%)	Cimini *et al.*,1994; ATSDR, 2002	Development
Fischer 344 rats	Oral gavage	N/A	Once daily for 21 days during PPd 1-21	LOAEL = 1000 mg/kg-day	Significant peroxisome proliferation in both liver and kidneys from pups	Stefanini *et al.*,1995; ATSDR, 2002	Development
Fischer 344 rats	Oral feeding	N/A	21 days during Gd 0-20	LOAEL = 313 mg/kg-day; NOAEL = 164 mg/kg-day	Increased prenatal and perinatal mortality	Price *et al.*,1986; ATSDR, 2002	Development
Sprague-Dawley rats (M)	Oral gavage	N/A	Once daily for 6 weeks (40 days) during Gd 3-21 and PPd 1-21	LOAEL = 375 mg/kg-day	Altered sexual differentiation (M)	Moore *et al.*,2001; ATSDR, 2002	Development

CRL:CD Sprague-Dawley rats (F)	Oral gavage	0, 11, 33, 100, 300 mg/kg-day (12-14 litters per group)	Once daily during Gd 8-17; recovery til maturity; continued dosing from 18 til 63-65	LOAEL = 300 mg/kg-day; NOAEL = 100 mg/kg-day	In day 2 pups; Significant decrease in anogenital distance (M; P < 0.01); Significant increase in 13-day old males with areolae (P < 0.01); Significant increase in the number of areolae per male out of 12 (P < 0.01); Significant decrease in male pup weight (P < 0.01); Marginal decrease in female pup weight In pups dosed from Gd 8 to PNd 64; Significant decrease in ventral prostate, seminal vesicle, levator ani-bulbocavernsus weight (P<0.01); Substantial decrease in glans penis weight; Dose-dependent significant decrease in Cowper's gland weight (P < 0.01); Significant dose-dependent increase in the age at puberty (P < 0.05) In pups dosed from Gd 8 to Ld 17 then recovery; Significant decrease in glans penis, levator ani-bulbocavernosus weight (P < 0.01); Significant dose-dependent increase in the number of nipples per male (P < 0.01); Significant dose-dependent decrease in the weight of the Cowper's gland (P < 0.01) or the weight of the ventral prostate (P < 0.05)	Gray *et al.*, 2009	Development
CRL:CD Sprague-Dawley rats (F)	Oral gavage	0, 11, 33, 100, 300 mg/kg-day (12-14 litters per group)	Once daily during Gd 8-17; recovery til maturity; continued dosing from 18 til 63-65	NOAEL = 300 mg/kg-day	No toxicologically significant change in anogenital distance (F) or weight at puberty	Gray *et al.*, 2009	Development
CRL:CD Sprague-Dawley rats (F)	Oral gavage	0, 11, 33, 100, 300 mg/kg-day (12-14 litters per group)	Once daily during Gd 8-17; recovery til maturity; continued dosing from 18 til 63-65	LOAEL = 11 mg/kg-day	Significant dose-dependent increase in rats with combined "reproductive tract malformations" (phthalate syndrome; P < 0.01)	Gray *et al.*, 2009	Development
Fischer 344 rats (M)	Oral feeding	0, 320, 1250, 5000, 20,000 mg/kg (0, 18, 69, 284, 1156 mg/kg-day; 24 rats per group)	8 weeks (60 days)	LOAEL = 1156 mg/kg-day; NOAEL = 284 mg/kg-day	Decrease in mean litter size (correlated to degenerative testicular changes)	Agarwal *et al.*,1986a/b; ECB, 2008	Development
Wistar rats	Oral gavage	N/A	Once daily for 12 weeks (90 days) during preGd 90 – Gd 1	LOAEL = 1700 mg/kg-day; NOAEL = 340 mg/kg-day	Decreased fetal weight (10%), decrease in placental weight (8%)	Nikonorow *et al.*,1973; ATSDR, 2002	Development
CD-1 mice (F)	Oral gavage	0, 40, 200, 1000 mg/kg-day (15 mice pre treatment group; 30 control mice)	N/A	LOAEL = 200 mg/kg-day; NOAEL = 40 mg/kg-day	Decreased fetus viability	Huntingdon, 1997; ECB, 2008	Development
CD-1 mice (F)	Oral gavage	0, 40, 200, 1000 mg/kg-day (15 mice pre treatment group; 30 control mice)	N/A	LOAEL = 1000 mg/kg-day; NOAEL = 200 mg/kg-day	Increased number of resorptions and postimplantation losses	Huntingdon, 1997; ECB, 2008	Development
CD-1 mice (F)	Oral gavage	0, 40, 200, 1000 mg/kg-day (15 mice pre treatment group; 30 control mice)	N/A	LOAEL = 1000 mg/kg-day; NOAEL = 200 mg/kg-day	Increased cardiovascular abnormalities, tri-lobed left lungs, fused ribs, fused thoracic vertebral centers and arches, immature livers, kidney abnormalities	Huntingdon, 1997; ECB, 2008	Development

ICR mice (M&F)	Oral feeding	0, 0.01, 0.1, 0.3% (0, 20, 200, 600 mg/kg-day; 20 mice per sex per treatment group; 40 mice per control group;)	14 weeks (98 days), continuous breeding;	NOAEL = 600 mg/kg-day	No toxicologically significant developmental effects	ECB, 2008	Development
Wistar rats (M&F)	Oral feeding	0, 1000, 3000, 9000 mg/kg (0, 113, 340, 1088 mg/kg-day; 25 rats per sex per group; GLP)	3 generations	LOAEL = 1088 mg/kg-day; NOAEL = 340 mg/kg-day	Increased postimplantation losses in F_0 females	Schilling *et al.*,2001; CERHR, 2006; ECB, 2008	Development
Sprague-Dawley rats (M&F)	Oral feeding	0.1, 0.5, 1.4, 4.8, 14, 46, 359, 775 mg/kg-day	3 generations	LOAEL = 14 mg/kg-day; NOAEL = 4.8 mg/kg-day	N/A	Wolfe *et al.*,2003; ECB, 2008	Development
Fischer 344 rats	Oral gavage	N/A	Once	NOAEL = 5000 mg/kg-day	No toxicologically significant change in endocrine systems	Berman *et al.*,1995; ATSDR, 2002	Endocrine
Fischer 344 rats	Oral gavage	N/A	Once daily for 14 days	NOAEL = 1500 mg/kg-day	No toxicologically significant change in endocrine systems	Berman *et al.*,1995; ATSDR, 2002	Endocrine
Sprague-Dawley rats (F)	Oral gavage – *in vitro*	0, 750 or 1000 mg/kg	Once daily during Gd 14-18	LOAEL = 1000 mg/kg-day	Significant reduction in the testosterone in *ex-vivo* media ($P < 0.01$)	Wilson *et al.*, 2004	Endocrine
Sprague-Dawley rats (F)	Oral gavage – *in vitro*	0, 750 or 1000 mg/kg	Once daily during Gd 14-18	NOAEL = 1000 mg/kg-day	No toxicologically significant effect on the level of progesterone in *ex-vivo* media	Wilson *et al.*, 2004	Endocrine
CRL:CD Sprague-Dawley rats (F)	Oral gavage	0, 11, 33, 100, 300 mg/kg-day (12-14 litters per group)	Once daily during Gd 8-17; recovery til maturity; continued dosing from 18 til 63-65	LOAEL = 300 mg/kg-day; NOAEL = 100 mg/kg-day	In pups dosed from Gd 8 to PNd 64; Marginal decrease in testosterone or estradiol In pups dosed from Gd 8 to Ld 17 then recovery; Marginal decrease in testosterone	Gray *et al.*, 2009	Endocrine
Wistar rats (F)	Oral gavage	0, 0.015, 0.045, 0.135, 0.405, 1.215, 5, 15, 45, 135, 405 mg/kg-day; ? dams per group; ? rats per group)	Once daily for 27 days during Gd6 to Ld 21, recovery til 144 days old	LOAEL = 405 mg/kg-day; NOAEL = 135 mg/kg-day	Testosterone production significantly increased at 0.045, 0.405, 405 mg/kg-day ($P < 0.05$)	Andrade *et al.*, 2006b	Endocrine
Marmoset monkeys	Oral gavage	N/A	Once daily for 13 weeks	NOAEL = 2500 mg/kg-day	No toxicologically significant change in endocrine systems (M)	Kurata *et al.*,1998; ATSDR, 2002	Endocrine
Fischer 344 rats	Oral feeding	N/A	4-16 weeks	LOAEL = 1054 mg/kg-day	Altered metabolism of estradiol and estrogen receptor related functions	Eagon *et al.*,1994; ATSDR, 2002	Endocrine
Marmoset monkeys (M&F; 90-115 days old)	Oral gavage	0, 100, 500, 2500 mg/kg-day (9M and 6F per group)	Once daily for 65 weeks	LOAEL = 500 mg/kg-day; NOAEL = 100 mg/kg-day	Elevated serum 17β-estradiol	Mitsubishi Chem Safety Inst, 2003; CERHR, 2006	Endocrine
$B6C3F_1$ mice (M&F)	Oral feeding	N/A	104 weeks	NOAEL = 1458 mg/kg-day	No toxicologically significant change in endocrine systems (F)	David *et al.*,1999, 2000b; ATSDR, 2002	Endocrine
Fischer 344 rats (M&F)	Oral feeding	N/A	104 weeks	NOAEL = 939 mg/kg-day	No toxicologically significant change in endocrine systems (F)	David *et al.*,1999, 2000a; ATSDR, 2002	Endocrine
Unspecified rats (M)	Oral Feeding	Review – 0, 0.2, 1.0, 2.0% (0, 150, 750, 1500 mg/kg-day; 15 rats per group)	90 days	LOAEL = 150 mg/kg-day	Significant dose-dependent increase in the number castration cells in pituitary	Gangolli, 1982	Pituitary

Sprague-Dawley rats (M&F)	Oral feeding	0, 0.2, 1.0, and 2.0% (0, 143, 737, 1440 mg/kg-day; M; 0, 154, 797, 1414 mg/kg-day; F) (15 rats per group): 0, 1.0, and 2.0% (0, 737, 1440 mg/kg-day; M; 0, 797, 1414 mg/kg-day; F): 0 and 2.0% (0, 1440 mg/kg-day; M; 0, 1414 mg/kg-day; F) (10 rats per group)	17 weeks: 2 or 6 weeks:	LOAEL = 1414-1440 mg/kg-day; NOAEL = 737-797 mg/kg-day	Significant dose-dependent decrease in the absolute pituitary weight (M, 2 weeks; F, 6, 17 weeks; $P < 0.001$-0.05)	Gray *et al.*, 1977	Pituitary
Sprague-Dawley rats (M&F)	Oral feeding	0, 0.2, 1.0, and 2.0% (0, 143, 737, 1440 mg/kg-day; M; 0, 154, 797, 1414 mg/kg-day; F) (15 rats per group): 0, 1.0, and 2.0% (0, 737, 1440 mg/kg-day; M; 0, 797, 1414 mg/kg-day; F): 0 and 2.0% (0, 1440 mg/kg-day; M; 0, 1414 mg/kg-day; F) (10 rats per group)	17 weeks: 2 or 6 weeks:	LOAEL = 1440 mg/kg-day; NOAEL = 737 mg/kg-day	Significant dose-dependent increase in the relative pituitary weight (M, 17 weeks; $P < 0.001$)	Gray *et al.*, 1977	Pituitary
Sprague-Dawley rats (M&F)	Oral feeding	0, 0.2, 1.0, and 2.0% (0, 143, 737, 1440 mg/kg-day; M; 0, 154, 797, 1414 mg/kg-day; F) (15 rats per group): 0, 1.0, and 2.0% (0, 737, 1440 mg/kg-day; M; 0, 797, 1414 mg/kg-day; F): 0 and 2.0% (0, 1440 mg/kg-day; M; 0, 1414 mg/kg-day; F) (10 rats per group)	17 weeks: 2 or 6 weeks:	LOAEL = 737 mg/kg-day; NOAEL = 143 mg/kg-day	Dose-dependent increase in the total incidence of "castration cells" in pituitary (M, 17 weeks)	Gray *et al.*, 1977	Pituitary
Fischer 344 rats (M&F)	Oral feeding	0, 6000, 12,000 mg/kg (0, 322, 674 mg/kg-day (M); 0, 394, 774 mg/kg-day (F); 50 rats per sex per group; GLP)	103 weeks	LOAEL = 674 mg/kg-day; NOAEL = 322 mg/kg-day	Hypertrophy of cell in anterior pituitary (M; 22/49, 45%; controls 1/46, 2%); cytoplasmic enlargement and vacoulation	NTP, 1982; ECB, 2008	Anterior pituitary
Fischer 344 rats	Oral feeding	N/A	104 weeks	LOAEL = 674 mg/kg-day	Anterior pituitary cell hypertrophy	Kluwe *et al.*,1982; ATSDR, 2002	Pituitary
Fischer 344 rats (M)	Oral feeding	0, 6000, 12,000 mg/kg	104 weeks	LOAEL = 12,000 mg/kg	Decreased pituitary adenoma or carcinoma	Kluwe *et al.*, 1985; Ito and Nakajima, 2008	Pituitary
Fischer 344 rats (F)	Oral feeding	0, 6000, 12,000 mg/kg	104 weeks	LOAEL = 6000 mg/kg	Decreased pituitary adenoma or carcinoma	Kluwe *et al.*, 1985; Ito and Nakajima, 2008	Pituitary

Fischer 344 rats (M&F)	Oral feeding	0, 100, 500, 2500, 12,500 mg/kg (0, 5.8, 28.9, 146.6, 789.0 mg/kg-day (M); 0, 7.3, 36.1, 181.7, 938.5 mg/kg-day (F), or 12,500 mg/kg for 78 weeks and a 26 week recovery period; 70-85 rats per sex per group; 55 rats per sex in recovery group)	104 weeks	LOAEL = 789.0 mg/kg-day; NOAEL = 146.6 mg/kg-day	Irreversibly increased number of castration cells (30/60; M)	Moore, 1996; ECB, 2008	Pituitary
Fischer 344 rats (M&F)	Oral feeding	0, 6000, 12,000 mg/kg (0, 322, 674 mg/kg-day (M); 0, 394, 774 mg/kg-day (F); 50 rats per sex per group; GLP)	103 weeks	LOAEL = 674 mg/kg-day; NOAEL = 322 mg/kg-day	Decreased incidence of pituitary adenomas and carcinomas (M; P=0.012)	NTP, 1982; ECB, 2008	Pituitary
Gastrointestinal/Liver/Pancreas							
Human	Oral capsule	5 or 10 g (~71 or 143 mg/kg; based on 70 kg weight)	Once	LOAEL = 143 mg/kg-day; NOAEL = 71.4 mg/kg-day	Gastrointestinal distress	Shaffer *et al.*,1945; ECB, 2008; ATSDR, 2002	Gastrointestinal tract
Marmoset monkey	Oral gavage	N/A	Once daily for 13 weeks	NOAEL = 2500 mg/kg-day	No toxicologically significant gastrointestinal effects	Kurata *et al.*,1998; ATSDR, 2002	Gastrointestinal
Sherman rats	Oral feeding	N/A	52 weeks	NOAEL = 200 mg/kg-day	No toxicologically significant gastrointestinal effects	Carpenter *et al.*,1953; ATSDR, 2002	Gastrointestinal
Sherman rats	Oral feeding	N/A	104 weeks	NOAEL = 190 mg/kg-day	No toxicologically significant gastrointestinal effects	Carpenter *et al.*,1953; ATSDR, 2002	Gastrointestinal
Fischer 344 rats	Oral feeding	N/A	104 weeks	NOAEL = 939 mg/kg-day	No toxicologically significant gastrointestinal effects (F)	David *et al.*,1999, 2000a; ATSDR, 2002	Gastrointestinal
B6C3F$_1$ mice (F)	Oral feeding	N/A	104 weeks	NOAEL = 1458 mg/kg-day	No toxicologically significant gastrointestinal effects (F)	David *et al.*,1999, 2000b; ATSDR, 2002	Gastrointestinal
Sprague-Dawley rats (M&F)	Oral feeding	0, 0.2, 1.0, and 2.0% (0, 143, 737, 1440 mg/kg-day; M; 0, 154, 797, 1414 mg/kg-day; F) (15 rats per group): 0, 1.0, and 2.0% (0, 737, 1440 mg/kg-day; M; 0, 797, 1414 mg/kg-day; F): 0 and 2.0% (0, 1440 mg/kg-day; M; 0, 1414 mg/kg-day; F) (10 rats per group)	17 weeks: 2 or 6 weeks:	LOAEL = 1414-1440 mg/kg-day; NOAEL = 737-797 mg/kg-day	Significant dose-dependent decrease in the absolute stomach weight (M, 2, 6, 17 weeks; F 2, 6 weeks; $P < 0.01$-0.05)	Gray *et al.*, 1977	Stomach

Sprague-Dawley rats (M&F)	Oral feeding	0, 0.2, 1.0, and 2.0% (0, 143, 737, 1440 mg/kg-day; M; 0, 154, 797, 1414 mg/kg-day; F) (15 rats per group): 0, 1.0, and 2.0% (0, 737, 1440 mg/kg-day; M; 0, 797, 1414 mg/kg-day; F): 0 and 2.0% (0, 1440 mg/kg-day; M; 0, 1414 mg/kg-day; F) (10 rats per group)	17 weeks: 2 or 6 weeks:	LOAEL = 737 mg/kg-day; NOAEL = 143 mg/kg-day	Significant dose-dependent increase in the relative stomach weight (M, 17 weeks; $P < 0.01$)	Gray *et al.*, 1977	Stomach
Sprague-Dawley rats (M&F)	Oral feeding	0, 0.2, 1.0, and 2.0% (0, 143, 737, 1440 mg/kg-day; M; 0, 154, 797, 1414 mg/kg-day; F) (15 rats per group): 0, 1.0, and 2.0% (0, 737, 1440 mg/kg-day; M; 0, 797, 1414 mg/kg-day; F): 0 and 2.0% (0, 1440 mg/kg-day; M; 0, 1414 mg/kg-day; F) (10 rats per group)	17 weeks: 2 or 6 weeks:	LOAEL = 1414-1440 mg/kg-day; NOAEL = 737-797 mg/kg-day	Significant dose-dependent increase in the relative stomach weight (M&F, 2, 6, 17 weeks; $P < 0.001$-0.01)	Gray *et al.*, 1977	Stomach
Sprague-Dawley rats (M&F)	Oral feeding	0, 0.2, 1.0, and 2.0% (0, 143, 737, 1440 mg/kg-day; M; 0, 154, 797, 1414 mg/kg-day; F) (15 rats per group): 0, 1.0, and 2.0% (0, 737, 1440 mg/kg-day; M; 0, 797, 1414 mg/kg-day; F): 0 and 2.0% (0, 1440 mg/kg-day; M; 0, 1414 mg/kg-day; F) (10 rats per group)	17 weeks: 2 or 6 weeks:	LOAEL = 1440 mg/kg-day; NOAEL = 797 mg/kg-day	Significant dose-dependent decrease in the absolute small intestine weight (F, 2, 6 weeks; $P < 0.001$)	Gray *et al.*, 1977	Small intestine
Sprague-Dawley rats (M&F)	Oral feeding	0, 0.2, 1.0, and 2.0% (0, 143, 737, 1440 mg/kg-day; M; 0, 154, 797, 1414 mg/kg-day; F) (15 rats per group): 0, 1.0, and 2.0% (0, 737, 1440 mg/kg-day; M; 0, 797, 1414 mg/kg-day; F): 0 and 2.0% (0, 1440 mg/kg-day; M; 0, 1414 mg/kg-day; F) (10 rats per group)	17 weeks: 2 or 6 weeks:	LOAEL = 737 mg/kg-day; NOAEL = 143 mg/kg-day	Significant dose-dependent increase in the relative small intestine weight (M, 6, 17 weeks; $P < 0.05$)	Gray *et al.*, 1977	Small intestine

Sprague-Dawley rats (M&F)	Oral feeding	0, 0.2, 1.0, and 2.0% (0, 143, 737, 1440 mg/kg-day; M; 0, 154, 797, 1414 mg/kg-day; F) (15 rats per group): 0, 1.0, and 2.0% (0, 737, 1440 mg/kg-day; M; 0, 797, 1414 mg/kg-day; F): 0 and 2.0% (0, 1440 mg/kg-day; M; 0, 1414 mg/kg-day; F) (10 rats per group)	17 weeks: 2 or 6 weeks:	LOAEL = 1414-1440 mg/kg-day; NOAEL = 737-797 mg/kg-day	Significant dose-dependent increase in the relative small intestine weight (M, 2, 6, 17 weeks; F 6, 17 weeks; $P < 0.001$-0.05)	Gray *et al.*, 1977	Small intestine
Sprague-Dawley rats (M&F)	Oral feeding	0, 0.2, 1.0, and 2.0% (0, 143, 737, 1440 mg/kg-day; M; 0, 154, 797, 1414 mg/kg-day; F) (15 rats per group): 0, 1.0, and 2.0% (0, 737, 1440 mg/kg-day; M; 0, 797, 1414 mg/kg-day; F): 0 and 2.0% (0, 1440 mg/kg-day; M; 0, 1414 mg/kg-day; F) (10 rats per group)	17 weeks: 2 or 6 weeks:	LOAEL = 737 mg/kg-day; NOAEL = 143 mg/kg-day	Significant dose-dependent decrease in the absolute cecum weight (M, 17 weeks; $P < 0.05$)	Gray *et al.*, 1977	Cecum
Sprague-Dawley rats (M&F)	Oral feeding	0, 0.2, 1.0, and 2.0% (0, 143, 737, 1440 mg/kg-day; M; 0, 154, 797, 1414 mg/kg-day; F) (15 rats per group): 0, 1.0, and 2.0% (0, 737, 1440 mg/kg-day; M; 0, 797, 1414 mg/kg-day; F): 0 and 2.0% (0, 1440 mg/kg-day; M; 0, 1414 mg/kg-day; F) (10 rats per group)	17 weeks: 2 or 6 weeks:	LOAEL = 1414-1440 mg/kg-day; NOAEL = 737-797 mg/kg-day	Significant dose-dependent decrease in the absolute cecum weight (M, 6, 17 weeks; F, 2, 6 weeks; $P < 0.001$-0.05)	Gray *et al.*, 1977	Cecum
Sprague-Dawley rats (M&F)	Oral feeding	0, 0.2, 1.0, and 2.0% (0, 143, 737, 1440 mg/kg-day; M; 0, 154, 797, 1414 mg/kg-day; F) (15 rats per group): 0, 1.0, and 2.0% (0, 737, 1440 mg/kg-day; M; 0, 797, 1414 mg/kg-day; F): 0 and 2.0% (0, 1440 mg/kg-day; M; 0, 1414 mg/kg-day; F) (10 rats per group)	17 weeks: 2 or 6 weeks:	LOAEL = 797mg/kg-day; NOAEL = 154 mg/kg-day	Significant dose-dependent increase in the relative cecum weight (F, 17 weeks; $P < 0.05$)	Gray *et al.*, 1977	Cecum

Sprague-Dawley rats (M&F)	Oral feeding	0, 0.2, 1.0, and 2.0% (0, 143, 737, 1440 mg/kg-day; M; 0, 154, 797, 1414 mg/kg-day; F) (15 rats per group): 0, 1.0, and 2.0% (0, 737, 1440 mg/kg-day; M; 0, 797, 1414 mg/kg-day; F): 0 and 2.0% (0, 1440 mg/kg-day; M; 0, 1414 mg/kg-day; F) (10 rats per group)	17 weeks: 2 or 6 weeks:	LOAEL = 1414-1440 mg/kg-day; NOAEL = 737-797 mg/kg-day	Significant dose-dependent increase in the relative cecum weight (M, 2, 17 weeks; F 17 weeks; $P < 0.001$-0.05)	Gray *et al.*, 1977	Cecum
Fischer 344 rats	Oral feeding	N/A	108 weeks	LOAEL = 2000 mg/kg-day	Pseudoductular lesions in the pancreas	Rao *et al.*,1990; ATSDR, 2002	Pancreas
Fischer 344 rats (M)	Oral feeding	0, 100, 500, 2500, 12,500 mg/kg	104 weeks	LOAEL = 12,500 mg/kg-day	Pancreatic acinar cell adenoma (tumor frequency = 0, 0, 0, 0, 8%, respectively)	David *et al.*, 2000; Ito and Nakajima, 2008	Pancreas
Fischer 344 rats (F)	Oral feeding	0, 100, 500, 2500, 12,500 mg/kg	104 weeks	LOAEL = 12,500 mg/kg-day	Pancreatic acinar cell adenoma (tumor frequency = 0, 0, 0, 0, 3%, respectively)	David *et al.*, 2000; Ito and Nakajima, 2008	Pancreas
Fischer 344 rats	Oral gavage	N/A	Once	LOAEL = 1500 mg/kg-day	Centrilobular necrosis or inflammation	Berman *et al.*,1995; ATSDR, 2002	Liver
$B6C3F_1$ mouse	Oral gavage	N/A	Once daily for 2 days	LOAEL = 1150 mg/kg-day	Increase in absolute liver weight (9%), increased hepatocellular DNA synthesis (248%), decreased hepatocellular apoptosis (90%)	James *et al.*,1998; ATSDR, 2002	Liver
Fischer 344 rats	Oral gavage	N/A	Once daily for 2 days	LOAEL = 950 mg/kg-day	Increase in absolute liver weight (26%), increased hepatocellular DNA synthesis (1300%), decreased hepatocellular apoptosis (20%)	James *et al.*,1998; ATSDR, 2002	Liver
Fischer 344 rats	Oral gavage	N/A	Once daily for 3 days	LOAEL = 1200 mg/kg-day	Altered liver profile	Adinehzadeh and Reo, 1998; ATSDR, 2002	Liver
Sprague-Dawley rats (M)	Oral gavage	0, 10, 100, 1000, 2000 mg/kg-day (10 rats per group; from day 6, 14-16, 21, 42, 86 of age; GL)	Once daily for 5 days	LOAEL = 100 mg/kg-day; NOAEL = 10 mg/kg-day	Increased absolute and relative liver weight in 2, 3, 6, 12 week old rats, increased palmitoyl-CoA oxidase activity, increased carnitine acetyl transferase activity, increased peroxisomal proliferation, increased peroxisomal enzyme activity	Dostal *et al.*,1987a; ECB, 2008; ATSDR, 2002	Liver
$B6C3F_1$ mice (M)	Oral gavage	0, 1879, 2844, 4304, 6514, 9860 mg/kg-day (10 rats per group; GLP)	Once daily for 5 days	LOAEL = 1879 mg/kg-day	Enlarged liver with slight dose-response trend	Nuodex, 1981b; ECB, 2008	Liver
Sprague-Dawley rats (F)	Oral gavage	2000 mg DEHP/kg bw (7-8 dams dosed per group, 10 pups per dam)	Once daily during Ld 2-6, 6-10, 14-18, 15-17	LOAEL = 2000 mg/kg	Significant increase in relative liver weight in maternal rats dosed during Ld 2-6, 6-10, 14-18 ($P < 0.05$; Pair-fed controls for Ld 14-18 had significantly decreased maternal relative liver weight)	Dostal *et al.*, 1987	Liver
Sprague-Dawley rats (F)	Oral gavage	2000 mg DEHP/kg bw (7-8 dams dosed per group, 10 pups per dam)	Once daily during Ld 2-6, 6-10, 14-18, 15-17	NOAEL = 2000 mg/kg	No toxicologically significant change in suckling pup liver weights (Pair-fed controls for Ld 14-18 had significantly decreased suckling pup relative liver weight)	Dostal *et al.*, 1987	Liver
Sprague-Dawley rats (F)	Oral gavage	2000 mg DEHP/kg bw (7-8 dams dosed per group, 10 pups per dam)	Once daily during Ld 2-6, 6-10, 14-18, 15-17	LOAEL = 2000 mg/kg	Significant increase in palmitoyl-CoA oxidase in maternal and suckling pup rats dosed during Ld 2-6, 6-10, 14-18, 15-17 ($P < 0.05$)	Dostal *et al.*, 1987	Liver
Sprague-Dawley rats (F)	Oral gavage	2000 mg DEHP/kg bw (7-8 dams dosed per group, 10 pups per dam)	Once daily during Ld 2-6, 6-10, 14-18, 15-17	LOAEL = 2000 mg/kg	Significant increase in carnitine acetyltransferase in maternal and suckling pup rats dosed during Ld 2-6, 6-10, 14-18 ($P < 0.05$); Significant increase in carnitine acetyltransferase in maternal rats dosed during Ld 15-17 ($P < 0.05$)	Dostal *et al.*, 1987	Liver

Rabbit (N/A)	Oral gavage	N/A	Once daily for 7 days	LOAEL = 2000 mg/kg-day	Decreased liver weight, decreased liver enzyme acivity	Parmar *et al.*,1988; ATSDR, 2002	Liver
Mouse (N/A)	Oral gavage	N/A	Once daily for 7 days	LOAEL = 2000 mg/kg-day	Increased liver weight, increased liver enzyme activity	Parmar *et al.*,1988; ATSDR, 2002	Liver
Guinea pig (N/A)	Oral gavage	N/A	Once daily for 7 days	LOAEL = 2000 mg/kg-day	Increased liver weight, increased liver enzyme activity	Parmar *et al.*,1988; ATSDR, 2002	Liver
Albino rat	Oral gavage	N/A	Once daily for 7 days	LOAEL = 2000 mg/kg-day	Increased liver weight, increased liver enzyme activity	Parmar *et al.*,1988; ATSDR, 2002	Liver
Wistar rats	Oral gavage	N/A	Once daily for 7 days	LOAEL = 1000 mg/kg-day	Increased relative liver weight (36%)	Oishi, 1989; ATSDR, 2002	Liver
Wistar rats	Oral gavage	N/A	Once daily for 7 days	LOAEL = 2000 mg/kg-day	Increased relative liver weight (52%)	Oishi, 1994; ATSDR, 2002	Liver
Syrian golden hamster	Oral feeding	N/A	7 days	LOAEL = 2686 mg/kg-day	Increased relative liver weight (36%)	Hosokawa *et al.*,1994; ATSDR, 2002	Liver
Fischer 344 rat	Oral feeding	N/A	7 days	LOAEL = 53 mg/kg-day; NOAEL = 11 mg/kg-day	Increased relative liver weight (M)	David *et al.*,1999; ATSDR, 2002	Liver
Sprague-Dawley rats	Oral feeding	N/A	7 days	LOAEL = 2000 mg/kg-day	Increased relative liver weight (35%), induction of microsomal carboxylesterases	Hosokawa *et al.*,1994; ATSDR, 2002	Liver
Fischer 344 rats (M)	Oral feeding	0, 1.2% (670 mg/kg-day; 5 rats per group)	7 days	LOAEL = 670 mg/kg-day	Increased absolute and relative liver weight	Takagi *et al.*,1992; ECB, 2008	Liver
$B6C3F_1$ mouse	Oral feeding	N/A	7 days	LOAEL = 564 mg/kg-day; NOAEL = 188 mg/kg-day	Increased relative liver weight	David *et al.*,1999; ATSDR, 2002	Liver
C57BL/6 mouse	Oral feeding	N/A	7 days	LOAEL = 4000 mg/kg-day	Increased relative liver weight (88%), induction of microsomal carboxylesterases	Hosokawa *et al.*,1994; ATSDR, 2002	Liver
C57BL/6 mouse	Oral feeding	N/A	7 days	LOAEL = 385 mg/kg-day	Increased absolute and relative liver weight	Muhlenkamp and Gill, 1998; ATSDR, 2002	Liver
Fischer 344 rats (M)	Oral feeding	0, 2% (1600 mg/kg-day; 8 rats per group; GL)	7 days	LOAEL = 1600 mg/kg-day	Increased absolute and relative liver weight	Exxon, 1982a,b; ECB, 2008	Liver
Fischer 344 rats (M)	Oral feeding	0, 2% (0, 1600 mg/kg-day; 8 rats per group; GL)	7 days	NOAEL = 1600 mg/kg-day	No histopathological findings in liver	Exxon, 1982a, 1982b; ECB, 2008	Liver
Wistar rats (F)	Oral gavage	0, 40, 200, 1000 mg/kg-day (9-10 litters per group)	Once daily during Gd 6-15	LOAEL = 1000 mg/kg-day; NOAEL = 200 mg/kg-day	Dose-dependent significant increase in the relative liver weight ($P \leq 0.01$)	Hellwig *et al.*, 1997	Liver
Fischer 344 rats (M)	Oral gavage (*in vitro liver homogenate*)	0, 2000 mg/kg-day (5 rats per group)	Once daily for 14 days	LOAEL = 2000 mg/kg-day	Increased peroxisomal palmitoyl-CoA oxidase (9-fold), increased catalase activity (2-fold), decreased glutathione peroxidase activity (50%)	Tomaszewski *et al.*,1986; ECB, 2008	Liver
$B6C3F_1$ mice (F)	Oral gavage (*in vitro liver homogenate*)	0, 2000 mg/kg-day (5 mice per group)	Once daily for 14 days	LOAEL = 2000 mg/kg-day	Increased peroxisomal palmitoyl-CoA oxidase (21-fold), increased catalase activity (3-fold), decreased glutathione peroxidase activity (35%)	Tomaszewski *et al.*,1986; ECB, 2008	Liver
Wistar - Alderley Park rats (M&F)	Oral gavage	0, 2000 mg/kg-day (10 rats per sex per group; GLP)	Once daily for 14 days	LOAEL = 2000 mg/kg-day	Increased absolute and relative liver weights (40%), increased peroxisomal proliferation, proliferation of smooth endoplasmic reticulum (SER), mitochondrial changes	ICI, 1982b; Rhodes *et al.*,1986; ECB, 2008; ATSDR, 2002	Liver
Chinese hamsters	Oral gavage	N/A	Once daily for 14 days	LOAEL = 1000 mg/kg-day	Increased liver weight (55%), enzyme induction	Lake *et al.*,1986; ATSDR, 2002	Liver
Cynomolgous monkeys (M)	Oral gavage	N/A	Once daily for 14 days	NOAEL = 500 mg/kg-day	No toxicologically significant effects on the liver	Pugh *et al.*,2000; ATSDR, 2002	Liver

Marmoset Monkey (M&F; 250-400 g)	Oral gavage	0, 2000 mg/kg (5 marmosets per sex per group)	Once daily for 14 days	LOAEL = 2000 mg/kg-day	Increased liver weight (20%), increased catalase (25%)	Rhodes *et al.*,1986; ATSDR, 2002	Liver
Fischer 344 rats	Oral gavage	N/A	Once daily for 14 days	LOAEL = 150 mg/kg-day	Increased relative liver weight (18%), increased hepatocellular mitosis	Berman *et al.*,1995; ATSDR, 2002	Liver
Sprague-Dawley rats	Oral gavage	N/A	Once daily for 14 days	LOAEL = 1000 mg/kg-day	Increased relative liver weight (72%), increased peroxisomal and microsomal enzyme activity	Lake *et al.*,1986; ATSDR, 2002	Liver
Sprague-Dawley rats (M)	Oral gavage	0, 1000 mg/kg-day (6 rats per group)	Once daily for 14 days	LOAEL = 1000 mg/kg-day	Increased relative liver weight, increased peroxisomal enzyme activity	Lake *et al.*,1984a; ECB, 2008	Liver
Sprague-Dawley rats (M)	Oral gavage	0, 25, 100, 250, 1000 mg/kg-day (5 rats per group)	Once daily for 14 days	LOAEL = 25 mg/kg-day	Increased peroxisomal enzyme activity	Lake *et al.*,1984b; ECB, 2008	Liver
Sprague-Dawley rats (M)	Oral gavage	0, 25, 100, 250, 1000 mg/kg-day (5 rats per group)	Once daily for 14 days	LOAEL = 100 mg/kg-day; NOAEL = 25 mg/kg-day	Increased relative liver weight	Lake *et al.*,1984b; ECB, 2008	Liver
Wistar rats (M)	Oral gavage	0, 250, 500, 1000 or 2000 mg/kg-day (5 rats per group)	Once daily for 14 days	LOAEL = 1000 mg/kg-day; NOAEL = 500 mg/kg-day	Increased absolute liver weight	Khaliq and Srivastava, 1993; ECB, 2008	Liver
Wistar rats (M)	Oral gavage	0, 250, 500, 1000 or 2000 mg/kg-day (5 rats per group)	Once daily for 14 days	LOAEL = 500 mg/kg-day; NOAEL = 250 mg/kg-day	Increased relative liver weight	Khaliq and Srivastava, 1993; ECB, 2008	Liver
Sprague-Dawley rat	Oral feeding	N/A	14 days	LOAEL = 1905 mg/kg-day	Increased liver weight (87%), peroxisome proliferation, increased synthesis of NAD+ from trytophan	Shin *et al.*,1999; ATSDR, 2002	Liver
Fischer 344 rats	Oral feeding	N/A	14 days	LOAEL = 1200 mg/kg-day	Increased liver weight, increased oxidized deoxyguanosine in liver DNA	Takagi *et al.*,1990; ATSDR, 2002	Liver
Wistar rat	Oral feeding	N/A	14 days	LOAEL = 1894 mg/kg-day	Increased absolute liver weight (38%), peroxisomal proliferation	Van den Munckhof *et al.*,1998; ATSDR, 2002	Liver
BNL-CL.2 mouse liver epithelial cells	*In vitro*	0.1 or 1 mM (39 or 390 µg/mL)	1 or 4 hours	LOAEC = 390 µg/mL; NOAEC = 39 µg/mL	Strong induction of jun-B and jun-D expression. Small induction of c-fos and c-jun expression	Ledwith *et al.*,1993; ECB, 2008	Liver
Wistar rat hepatocytes	*In vitro*	200 or 300µM (57 or 84 µg/mL **MEHP**	Once	LOAEC = 57 µg/mL	Gap junction intercellular communication inhibited (may contribute to tumor promotion)	Leibold *et al.*,1994; ECB, 2008	Liver
Human hepatocytes	*In vitro*	0.2mM (56 µg/mL) **MEHP**	48 or 72 hours	NOAEC = 56 µg/mL	No induction of palmitoyl-CoA oxidase and carnitine acetyltransferase not induced	Butterworth *et al.*,1989; ECB, 2008	Liver
Sprague-Dawley rats (M; 6 weeks old)	*In vitro*	0, 20, 50, 100, 200, 500, 1000 µM **MEHP**	70 hours	LOAEL = 200 µM; NOAEL = 20 µM	Dose-dependent increase in the number of peroxisomes, palmitoyl-CoA oxidation, carnitine acetyltransferase	Gray *et al.*, 1977	Liver (hepatocytes)
Wisar rats (Surrey strain)	*In vitro*	0, 50, 100, 250µM **MEHP**	N/A	LOAEL = 50µM	Significant dose-dependent increase in palmitoyl-CoA oxidation ($P < 0.05$)	Hinton *et al.*, 1986	Liver
Guinea pig (N/A)	Oral gavage	N/A	Once daily for 15 days	LOAEL = 2000 mg/kg-day	Increased liver weight, decreased liver enzyme activity	Parmar *et al.*,1988; ATSDR, 2002	Liver
Albino rats	Oral gavage	N/A	Once daily for 15 days	LOAEL = 2000 mg/kg-day	Increased liver weight, changes in liver enzyme activity	Parmar *et al.*,1988; ATSDR, 2002	Liver
Mice (N/A)	Oral gavage	N/A	Once daily for 15 days	LOAEL = 2000 mg/kg-day	Increased liver weight, changes in liver enzyme activity	Parmar *et al.*,1988; ATSDR, 2002	Liver
Wistar rats (M)	Oral feeding	0, 0.01, 0.025, 0.05, 0.1, 0.5, 1.0% (0, 8, 22, 42, 88, 500, 900 mg/kg-day; 5-6 rats per group)	16 days	LOAEL = 88 mg/kg-day; NOAEL = 42 mg/kg-day	Increased relative liver weight	Fukuhara and Takabatake, 1977; ECB, 2008	Liver

CD-1 mice	Oral feeding	0, 0.025, 0.05, 0.10, 0.15% (0, 44, 91, 190.6, 292.5 mg/kg-day; 30-31 rats per group)	17 days during Gd 0-17	LOAEL = 190.6 mg/kg-day; NOAEL = 91 mg/kg-day	Increased relative liver weight	Tyl *et al.*,1988; ATSDR, 2002	Liver
Fischer 344 rats (CrlBr)	Oral feeding	0, 0.5, 1.0, 1.5, 2.0% (0, 357, 666, 856, 1055 mg/kg-day; 34-25 rats per group)	20 days during Gd 0-20	LOAEL = 666 (1%) mg/kg-day; NOAEL = 357 mg/kg-day	Increased absolute and relative liver weights	Tyl *et al.*,1988; ECB, 2008; ATSDR, 2002	Liver
Wistar rats (M)	Oral feeding	0, 2% (1830, 1650, and 1810 after 3, 10, 21 days; 4 rats per treatment group, 6 rats in control groups)	3, 10, and 21 days	LOAEL = 1650 mg/kg-day	Increased relative liver weight (M), increased peroxisome proliferation, increased proliferation of SER, increased peroxisomal enzyme activities, changed mitochondria on days 3, 10, and 21	Mann *et al.*,1985; ECB, 2008	Liver
Wistar rats (M&F)	Oral gavage	0, 2500 mg/kg-day (6 rats per sex per group)	Once daily for 7 or 21 days	LOAEL = 2500 mg/kg-day	Significant time-dependent increase in relative liver weight (M&F; $P < 0.001$), increased number of peroxisomes, increased proliferation of SER	Mangham *et al.*,1981; ECB, 2008	Liver
Wistar rats (M&F)	Oral gavage	0, 2500 mg/kg-day (6 rats per sex per group)	Once daily for 7 or 21 days	NOAEL = 2500 mg/kg-day	No histopathological findings in liver	Mangham *et al.*,1981; ECB, 2008	Liver
Fischer 344 rats (M&F)	Oral feeding	0, 0.1, 0.6, 1.2% (0, 80, 480, 960 mg/kg-day; 4-5 rats per sex per group; GLP)	7 or 21 days	LOAEL = 80 mg/kg-day	Increased liver weight and increased peroxisomal enzyme activity (M&F)	CMA, 1982c; ECB, 2008	Liver
Fischer 344 rats (M&F)	Oral feeding	0, 0.1, 0.6, 1.2% (0, 80, 480, 960 mg/kg-day; 4-5 rats per sex per group; GLP)	7 or 21 days	LOAEL = 480 mg/kg-day; NOAEL = 80 mg/kg-day	Increased hepatocellular hypertrophy (M)	CMA, 1982c; ECB, 2008	Liver
Fischer 344 rats (M&F)	Oral feeding	0, 0.1, 0.6, 1.2% (0, 80, 480, 960 mg/kg-day; 4-5 rats per sex per group; GLP)	7 or 21 days	LOAEL = 960 mg/kg-day; NOAEL = 480 mg/kg-day	Increased hepatocellular hypertrophy (F) and number of peroxisomes (M&F)	CMA, 1982c; ECB, 2008	Liver
Fischer 344 rats	Oral gavage	0, 700 mg/kg-day (5 rats per sex per group)	Once daily for 21days	LOAEL = 700 mg/kg-day	Increased relative liver weight (> 53%), morphological and biochemical evidence of peroxisome proliferation, and increased peroxisomal enzyme activation	Hodgson, 1987	Liver
Sprague-Dawley rats (M)	Oral feeding	0, 2% (900 mg/kg-day; 4 rats per group)	21 days	LOAEL = 900 mg/kg-day	Increased absolute and relative liver weight, increased number of peroxisomes, proliferation of SER, increased peroxisomal enzyme activity	General Motors, 1982; ECB, 2008	Liver
Fischer 344 rats (M)	Oral feeding	0, 2% (4-5 rats per group)	21 days	LOAEL = 2%	Increased relative liver weight, increased peroxisomal enzyme activity	Moody and Reddy, 1978; ECB, 2008	Liver
Fischer 344 rats	Oral feeding	N/A	21 days	LOAEL = 643 m/kg-day; NOAEL = 12 mg/kg-day	Increased relative liver weight (44%), increased enzyme activity indicative of peroxisome proliferation	Barber *et al.*,1987; ATSDR, 2002	Liver
Fischer 344 rats (M&F)	Oral feeding	0, 0.01, 0.1, 0.6, 1.2, 2.5% (0, 11, 105, 667, 1224, 2101 mg/kg-day (M); 0, 12, 109, 643, 1197, 1892 mg/kg-day (F); 5 rats per sex per group; GLP)	21 days	LOAEL = 643 mg/kg-day; NOAEL = 109 mg/kg-day	Increased absolute and relative liver weight, increased histopathology, increased number of peroxisomes (F), increased peroxisomal enzyme activity	CMA, 1984b; Barber *et al.*,1987; ECB, 2008	Liver
Fischer 344 rats (M&F)	Oral feeding	0, 0.01, 0.1, 0.6, 1.2, 2.5% (0, 11, 105, 667, 1224, 2101 mg/kg-day (M); 0, 12, 109, 643, 1197, 1892 mg/kg-day (F); 5 rats per sex per group; GLP)	21 days	LOAEL = 105 mg/kg-day; NOAEL = 11 mg/kg-day	Increased number of peroxisomes (M)	CMA, 1984b; Barber *et al.*,1987; ECB, 2008	Liver

Wistar rats	Oral feeding	N/A	21 days	LOAEL = 1730 mg/kg-day	Increased absolute liver weight (41%)	Mocchiutti and Bernal, 1997; ATSDR, 2002	Liver
Fischer 344 rats (M)	Oral feeding	0, 100, 1000, 6000, 12,000, 25,000 mg/kg (0, 11, 105, 667, 1223, 2100 mg/kg-day; 5 rats per group)	21 days	LOAEL = 105 mg/kg-day; NOAEL = 11 mg/kg-day	Biochemical and morphological evidence of peroxisome proliferation, increased peroxisomal enzyme activity	Short *et al.*,1987; ECB, 2008; ATSDR, 2002	Liver
Fischer 344 rats (M)	Oral feeding	0, 100, 1000, 6000, 12,000, 25,000 mg/kg (0, 11, 105, 667, 1223, 2100 mg/kg-day; 5 rats per group)	21 days	LOAEL = 667 mg/kg-day; NOAEL = 105 mg/kg-day	Increased relative liver weight and increased number of peroxisomes	Short *et al.*,1987; ECB, 2008	Liver
Cynomolgous monkey	Oral gavage	N/A	Once daily for 25 days	NOAEL = 500 mg/kg-day	No toxicologically significant effects on the liver	Short *et al.*,1987; ATSDR, 2002	Liver
Wistar rats (F)	Oral gavage	0, 0.015, 0.045, 0.135, 0.405, 1.215, 5, 15, 45, 135, 405 mg/kg-day; ? rat dams, ? rat pups per group)	Once daily for 27 days during Gd6 to Ld 21, recovery til 144 days old	NOAEL = 405 mg/kg-day	No toxicologically significant effects on liver (M)	Andrade *et al.*, 2006b	Liver
Wistar rat	Inhalation	0, 50, 1000 mg/m^3	6 hours/day for 5 days/week for 28 days	LOAEC = 1000 mg/m^3; NOAEC = 50 mg/m^3	Increased relative liver weight	Klimisch *et al.*,1991	Liver
Wistar rats (M)	Oral feeding	0, 60, 200, 600, 2000, 6000 mg/kg (0, 5, 18, 52, 182, 549 mg/kg-day; 6 rats per group)	14 or 28 days	LOAEL = 18 mg/kg-day; NOAEL = 5 mg/kg-day	Increased number of peroxisomes	RIVM, 1992; ECB, 2008	Liver
Wistar rats (M)	Oral feeding	0, 60, 200, 600, 2000, 6000 mg/kg (0, 5, 18, 52, 182, 549 mg/kg-day; 6 rats per group)	14 or 28 days	LOAEL = 5 mg/kg-day	Increased peroxisomal enzyme activity	RIVM, 1992; ECB, 2008	Liver
Wistar rats (M)	Oral feeding	0, 60, 200, 600, 2000, 6000 mg/kg (0, 5, 18, 52, 182, 549 mg/kg-day; 6 rats per group)	14 or 28 days	LOAEL = 182 mg/kg-day; NOAEL = 52 mg/kg-day	Dose-dependent increase in the absolute body weight at 2 or 4 weeks	RIVM, 1992; ECB, 2008	Liver
Alpk/AP rat	Oral gavage	N/A	Once daily for 28 days	LOAEL = 1000 mg/kg-day	Increased palmitoyl-CoA oxidase activity, decreased superoxide dismutase, decreased glutathione peroxidase activities	Elliot and Elcombe, 1987; ATSDR, 2002	Liver
Fischer 344 rats (M)	Oral gavage	0, 1000 mg/kg-day (5 rats per group)	Once daily for 28 days	LOAEL = 1000 mg/kg-day	Increased absolute and relative liver weight	Tenneco, 1981; ECB, 2008	Liver
B6C3F_1 mice (M&F)	Oral feeding	0, 1000, 5000, 10,000, 25,000 mg/kg (0, 250, 1210, 2580, 6990 mg/kg-day(M); 0, 270, 1430, 2890, 7900 mg/kg-day (F); 10 mice per sex per group; GLP)	28 days	LOAEL = 1210 mg/kg-day; NOAEL = 250 mg/kg-day	Increased absolute and relative liver weight	Eastman Kodak, 1992b; ECB, 2008	Liver
B6C3F_1 mice (M&F)	Oral feeding	0, 1000, 5000, 10,000, 25,000 mg/kg (0, 250, 1210, 2580, 6990 mg/kg-day(M); 0, 270, 1430, 2890, 7900 mg/kg-day (F); 10 mice per sex per group; GLP)	28 days	LOAEL = 6990 mg/kg-day; NOAEL = 2580 mg/kg-day	Hepatocellular hypertrophy	Eastman Kodak, 1992b; ECB, 2008	Liver

Fischer 344 rats (M)	Oral feeding	0, 0.02, 0.05, 0.1, 0.5, 1.0, 2.5% (0, 24, 52, 115, 559, 1093, 2496 mg/kg-day; 5 rats per group, 10 rats in control; GLP)	28 days	LOAEL = 559 mg/kg-day; NOAEL = 115 mg/kg-day	Increased absolute liver weight	BIBRA, 1990; ECB, 2008	Liver
Fischer 344 rats (M)	Oral feeding	0, 0.02, 0.05, 0.1, 0.5, 1.0, 2.5% (0, 24, 52, 115, 559, 1093, 2496 mg/kg-day; 5 rats per group, 10 rats in control; GLP)	28 days	LOAEL = 115 mg/kg-day; NOAEL = 52 mg/kg-day	Increased peroxisomal enzyme activity	BIBRA, 1990; ECB, 2008	Liver
Fischer 344 rats (M)	Oral feeding	0, 0.02, 0.05, 0.1, 0.5, 1.0, 2.5% (0, 24, 52, 115, 559, 1093, 2496 mg/kg-day; 5 rats per group, 10 rats in control; GLP)	28 days	LOAEL = 24 mg/kg-day	Increased relative liver weight	BIBRA, 1990; ECB, 2008	Liver
Fischer 344 rats (M&F)	Oral feeding	0, 0.2, 0.67, 2.0% (0, 150, 504, 1563 mg/kg-day (M); 0, 147, 490, 1416 mg/kg-day (F); 5 rats per sex per group; GLP)	28 days	LOAEL = 147 mg/kg-day	Increased absolute and relative liver weight, increased peroxisomal enzyme activity	Nuodex, 1981c; ECB, 2008	Liver
Fischer 344 rats	Oral feeding	N/A	28 days	LOAEL = 1200 mg/kg-day	Increased liver enzyme activities indicating peroxisome proliferation	Cattley *et al.*,1988; ATSDR, 2002	Liver
Fischer 344 rats, (M&F)	Oral feeding: Oral gavage	0, 0.67% (0, 350 mg/kg-day: 0, 700 mg/kg-day; 5 rats per sex per group)	28 days:21 days	LOAEL = 350 mg/kg-day: LOAEL = 700 {705} mg/kg-day	Increased relative liver weight (> 53%), increased number of peroxisomes, and increased peroxisomal enzyme activity	Hodgson, 1987; ECB, 2008	Liver
Wistar rats (M)	Oral feeding	0, 2%, 2%+0% (3 rats per group)	4 weeks, 2 weeks + 2 weeks control diet	LOAEL = 2%	Increased liver weight during treatment, decreased liver weight during recovery; Increased peroxisomal enzyme activity during treatment, decreased during recovery	Miyazawa *et al.*,1980; ECB, 2008	Liver
Wistar rats (M; 25 day old)	Oral gavage	0, 50, 100, 250, 500 mg/kg (6 rats per dose group)	30 days	LOAEL = 50 mg/kg	Significant dose-dependent decrease in aniline hydroxylase and ethylmorphine-N-demethylase ($P < 0.05$)	Parmar *et al.*,1995	Liver
Wistar rats (M; 25 day old)	Oral gavage	0, 50, 100, 250, 500 mg/kg (6 rats per dose group)	30 days	LOAEL = 100 mg/kg; NOAEL = 50 mg/kg	Significant dose-dependent decrease in P450s ($P < 0.05$)	Parmar *et al.*,1995	Liver
Fischer 344 rats	Oral gavage	N/A	Once daily for 54 days	LOAEL = 2000 mg/kg-day	Increased relative liver weight (89%), peroxisome proliferation	Tomaszewski *et al.*,1988; ATSDR, 2002	Liver
CRL:CD Sprague-Dawley rats (F)	Oral gavage	0, 11, 33, 100, 300 mg/kg-day (12-14 litters per group)	Once daily during Gd 8-17; recovery til maturity; continued dosing from 18 til 63-65	LOAEL = 100 mg/kg-day; NOAEL = 33 mg/kg-day	In pups dosed from Gd 8 to PNd 64; Dose-dependent significant increase in liver weight ($P < 0.01$)	Gray *et al.*, 2009	Liver
CRL:CD Sprague-Dawley rats (F)	Oral gavage	0, 11, 33, 100, 300 mg/kg-day (12-14 litters per group)	Once daily during Gd 8-17; recovery til maturity; continued dosing from 18 til 63-65	NOAEL = 300 mg/kg-day	In pups dosed from Gd 8 to Ld 17 then recovery; No toxicologically significant change in liver weight	Gray *et al.*, 2009	Liver
Wistar rats	Oral feeding	N/A	12 weeks (90 days)	NOAEL = 1900 mg/kg-day	No toxicologically significant effects on the liver	Shaffer *et al.*,1945; ATSDR, 2002	Liver
Fischer 344 rats	Oral feeding	N/A	2-13 weeks	LOAEL = 265 mg/kg-day; NOAEL = 53 mg/kg-day	Increased relative liver weight	David *et al.*,1999; ATSDR, 2002	Liver
B6C3F$_1$ mice (F)	Oral feeding	N/A	4-13 weeks	LOAEL = 188 mg/kg-day	Increased relative liver weight (F)	David *et al.*,1999; ATSDR, 2002	Liver

Marmoset monkey	Oral gavage	N/A	Once daily for 13 weeks	NOAEL = 2500 mg/kg-day	No toxicologically significant effects on the liver	Kurata *et al.*,1998; ATSDR, 2002	Liver
Fischer 344 rats (M&F)	Oral feeding	0, 1000, 4000, 12,500, 25,000 mg/kg (0, 63, 261, 859, 1724 mg/kg-day (M); 0, 73, 302, 918, 1858 mg/kg-day (F); 10 rats per sex per group; GLP)	13 weeks	LOAEL = 63 mg/kg-day	Increased absolute and relative liver weight	Eastman Kodak, 1992a; ECB, 2008	Liver
Fischer 344 rats (M&F)	Oral feeding	0, 1000, 4000, 12,500, 25,000 mg/kg (0, 63, 261, 859, 1724 mg/kg-day (M); 0, 73, 302, 918, 1858 mg/kg-day (F); 10 rats per sex per group; GLP)	13 weeks	LOAEL = 261 mg/kg-day; NOAEL = 63 mg/kg-day	Hepatocellular hypertrophy (M)	Eastman Kodak, 1992a; ECB, 2008	Liver
Fischer 344 rats (M&F)	Oral feeding	0, 1000, 4000, 12,500, 25,000 mg/kg (0, 63, 261, 859, 1724 mg/kg-day (M); 0, 73, 302, 918, 1858 mg/kg-day (F); 10 rats per sex per group; GLP)	13 weeks	LOAEL = 918 mg/kg-day; NOAEL = 302 mg/kg-day	Hepatocellular hypertrophy (F)	Eastman Kodak, 1992a; ECB, 2008	Liver
Sprague-Dawley rats (M&F)	Oral feeding	0, 5, 50, 500, 5000 mg/kg (0, 0.4, 3.7, 37.6, 375.2 mg/kg-day (M); 0, 0.4, 4.2, 42.2, 419.3 mg/kg-day (F); 10 rats per sex per group; GLP)	13 weeks	LOAEL = 375.2-419.3; 345.0-411.0 mg/kg-day; NOAEL = 37.6-42.2 mg/kg-day	Significant enlargement of liver (M&F), increase in absolute and relative liver weights (M&F; $P < 0.01$); Substantially increased incidence and severity of hepatocellular hypertrophy (M&F); Marginally increased focal necrosis (M&F), and increased number of peroxisomes M; proliferation	Poon *et al.*,1997; ECB, 2008; ATSDR, 2002	Liver
Sprague-Dawley rats (M&F)	Oral feeding	0, 5, 50, 500, 5000 mg/kg (0, 0.4, 3.7, 37.6, 375.2 mg/kg-day (M); 0, 0.4, 4.2, 42.2, 419.3 mg/kg-day (F); 10 rats per sex per group; GLP)	13 weeks	LOAEL = 345.0-411.0 mg/kg-day	Substantial increase in incidence and severity of liver anioskaryosis (M&F), nuclear hyperchromicity (M&F), endothelial prominence (M&F), Significant increase in aniline hydroxylase and aminopyrine-N-demethylase (M&F; $P < 0.05$)	Poon *et al.*,1997; ECB, 2008; ATSDR, 2002	Liver
Sprague-Dawley rats (M&F)	Oral feeding	0, 5, 50, 500, 5000 mg/kg (0, 0.4, 3.7, 37.6, 375.2 mg/kg-day (M); 0, 0.4, 4.2, 42.2, 419.3 mg/kg-day (F); 10 rats per sex per group; GLP)	13 weeks	NOAEL = 345.0-411.0 mg/kg-day	No toxicologically significant effect on ethoxyresorufin-O-deethylase activity	Poon *et al.*,1997; ECB, 2008; ATSDR, 2002	Liver
Fischer 344 rats	Oral feeding	N/A	4-16 weeks	LOAEL = 1054 mg/kg-day	Increased relative liver weight and biochemical evidence of cell proliferation	Eagon *et al.*,1994; ATSDR, 2002	Liver
Sprague-Dawley rats (M&F)	Oral feeding	0, 0.2, 1.0, and 2.0% (0, 143, 737, 1440 mg/kg-day; M; 0, 154, 797, 1414 mg/kg-day; F) (15 rats per group): 0, 1.0, and 2.0% (0, 737, 1440 mg/kg-day; M; 0, 797, 1414 mg/kg-day; F): 0 and 2.0% (0, 1440 mg/kg-day; M; 0, 1414 mg/kg-day; F) (10 rats per group)	17 weeks: 2 or 6 weeks:	LOAEL = 143-154 mg/kg-day	Significant dose-dependent increase in the absolute liver weight (M&F, 17 weeks; $P < 0.05$)	Gray *et al.*, 1977	Liver

Sprague-Dawley rats (M&F)	Oral feeding	0, 0.2, 1.0, and 2.0% (0, 143, 737, 1440 mg/kg-day; M; 0, 154, 797, 1414 mg/kg-day; F) (15 rats per group): 0, 1.0, and 2.0% (0, 737, 1440 mg/kg-day; M; 0, 797, 1414 mg/kg-day; F): 0 and 2.0% (0, 1440 mg/kg-day; M; 0, 1414 mg/kg-day; F) (10 rats per group)	17 weeks: 2 or 6 weeks:	LOAEL = 737-797 mg/kg-day; NOAEL = 143-154 mg/kg-day	Significant dose-dependent increase in the absolute liver weight (M&F, 2 and 6 weeks; P < 0.001-0.01)	Gray *et al.*, 1977	Liver
Sprague-Dawley rats (M&F)	Oral feeding	0, 0.2, 1.0, and 2.0% (0, 143, 737, 1440 mg/kg-day; M; 0, 154, 797, 1414 mg/kg-day; F) (15 rats per group): 0, 1.0, and 2.0% (0, 737, 1440 mg/kg-day; M; 0, 797, 1414 mg/kg-day; F): 0 and 2.0% (0, 1440 mg/kg-day; M; 0, 1414 mg/kg-day; F) (10 rats per group)	17 weeks: 2 or 6 weeks:	LOAEL = 143-154 mg/kg-day	Significant dose-dependent increase in the relative liver weight (M&F, 17 weeks; P < 0.001-0.05)	Gray *et al.*, 1977	Liver
Sprague-Dawley rats (M&F)	Oral feeding	0, 0.2, 1.0, and 2.0% (0, 143, 737, 1440 mg/kg-day; M; 0, 154, 797, 1414 mg/kg-day; F) (15 rats per group): 0, 1.0, and 2.0% (0, 737, 1440 mg/kg-day; M; 0, 797, 1414 mg/kg-day; F): 0 and 2.0% (0, 1440 mg/kg-day; M; 0, 1414 mg/kg-day; F) (10 rats per group)	17 weeks: 2 or 6 weeks:	LOAEL = 737-797 mg/kg-day; NOAEL = 143-154 mg/kg-day	Significant dose-dependent increase in the relative liver weight (M&F, 2, 6, 17 weeks; P < 0.001-0.01)	Gray *et al.*, 1977	Liver
Cr1:CD-1 mice {ICR} (M&F)	Oral feeding	0, 0.01, 0.1, 0.3% (0, 20, 200, 600 mg/kg-day; 20 mice per sex per treatment group; 40 control mice)	18 weeks (126 days; {98 days}; continuous breeding)	LOAEL = 420 {600} mg/kg-day; NOAEL = 200 mg/kg-day	Increased absolute and relative liver weight (M&F)	Lamb *et al.*,1987; ECB, 2008; ATSDR, 2002	Liver
Sv/129 mice (M)	Oral feeding	N/A	24 weeks	LOAEL = 2400 {420} mg/kg-day	Degenerative liver lesions (M)	Ward *et al.*,1998; ATSDR, 2002	Liver
CH3/HeNCr mice	Oral feeding	N/A	24 weeks	LOAEL = 1953 mg/kg-day	Significant increase in relative liver weight	Weghorst *et al.*,1994; ATSDR, 2002	Liver
Syrian Golden hamster	Oral feeding	N/A	30 weeks	NOAEL = 1436 mg/kg-day	No toxicologically significant effects on the liver	Maruyama *et al.*,1994; ATSDR, 2002	Liver
Albino Alderley Park rats (M&F)	Oral feeding	0, 50, 200, 1000 mg/kg-day (20 rats per sex per group; 30 rats per sex in control)	3, 7, 14, 28 days or 36 weeks (9 months)	LOAEL = 50 mg/kg-day	Increased liver weight, morphological changes in bile ducts, peroxisome proliferation, proliferation of SER, increased peroxisomal enzyme activity, lipid filled lysosomes, glycogen depletion, induction of cytochrome P-450 system, and mitochondrial changes (M)	CEFIC, 1982; Mitchell *et al.*,1985a; ECB, 2008; ATSDR, 2002	Liver

Wisar rats (ICI strain, ~200g)	Oral feeding	0.05%, 0.2%, or 1.0% DEHP (50, 200, 1000 mg/kg-day; 4 rats of each sex per group)	3, 7, 14, 28, and 270 days (36 weeks; 9 months)	LOAEL = 50 mg/kg-day	Significant dose-dependent increase in liver weight (M; $P < 0.05$), palmitoyl-CoA oxidation (M; $P < 0.05$), α-glycerophosphate dehydrogenase activity (M; $P < 0.05$), laurate hydroxylase activity (M&F; $P < 0.05$), ethoxycoumarin deethylase activity (F; $P < 0.05$); Significant decrease in uricase activity (M; $P < 0.05$)	Hinton *et al.*, 1986	Liver
Wisar rats (ICI strain, ~200g)	Oral feeding	0.05%, 0.2%, or 1.0% DEHP (50, 200, 1000 mg/kg-day; 4 rats of each sex per group)	3, 7, 14, 28, and 270 days (36 weeks; 9 months)	LOAEL = 200 mg/kg-day; NOAEL = 50 mg/kg-day	Significant dose-dependent increase in palmitoyl-CoA oxidation (F; $P < 0.05$), α-glycerophosphate dehydrogenase activity (F; $P < 0.05$), β-D-galactosidase activity (F; $P < 0.05$); ethoxycoumarin deethylase activity (M; $P < 0.05$); Significant dose-dependent decrease in nonprotein SH, Glucose-6 phosphatase activity (M&F; $P < 0.05$)	Hinton *et al.*, 1986	Liver
Wisar rats (ICI strain, ~200g)	Oral feeding	0.05%, 0.2%, or 1.0% DEHP (50, 200, 1000 mg/kg-day; 4 rats of each sex per group)	3, 7, 14, 28, and 270 days (36 weeks; 9 months)	LOAEL = 1000 mg/kg-day; NOAEL = 200 mg/kg-day	Significant dose-dependent increase in the liver weight (F; $P < 0.05$), catalase activity (M&F; $P < 0.05$), cytochrome p-450 (F; $P < 0.05$), β-D-galactosidase activity (M; $P < 0.05$)	Hinton *et al.*, 1986	Liver
Dog (NS)	Oral Capsule	N/A	Once daily for 5 days/week for 52 weeks	NOAEL = 59 mg/kg-day	No toxicologically significant effects on the liver	Carpenter *et al.*,1953; ATSDR, 2002	Liver
Fischer 344 rats (M)	Oral feeding	0, 1.2% (0, 600 mg/kg-day; 5-10 rats per group)	1, 2, 4, 8, 18, 39, 77, 151, 365 days	LOAEL = 600 mg/kg-day	Increased peroxisomal enzyme activation in liver	Conway *et al.*,1989; ECB, 2008	Liver
Sherman rats	Oral feeding	N/A	52 weeks	LOAEL = 200 mg/kg-day; NOAEL = 60 mg/kg-day	Increased liver weight at 52 weeks	Carpenter *et al.*,1953; ATSDR, 2002	Liver
Fischer 344 rats	Oral feeding	N/A	52 weeks	LOAEL = 947 mg/kg-day	Increased relative liver weight (50%) and DNA synthesis, morphological and biochemical evidence of peroxisome proliferation	Marsman *et al.*,1988; ATSDR, 2002	Liver
Guinea pig (NS)	Oral feeding	N/A	52 weeks	LOAEL = 64 mg/kg-day; NOAEL = 19 mg/kg-day	Increase in liver weight	Carpenter *et al.*,1953; ATSDR, 2002	Liver
Wistar rats	Oral feeding	N/A	79 weeks	LOAEL = 867 mg/kg-day	Changes in peroxisomal enzymes, increased liver weights	Tamura *et al.*,1990; ATSDR, 2002	Liver
Fischer 344 rats	Oral feeding	N/A	95 weeks	LOAEL = 2444 mg/kg-day	Peroxisome proliferation, decreased catalase activity, increased fatty acid oxidase activity	Rao *et al.*,1987; ATSDR, 2002	Liver
Sprague-Dawley rats (M)	Oral feeding	0, 0.02, 0.2, 2.0% (0, 7, 70, 700 mg/kg-day; 520 total rats; GL)	102 weeks	NOAEL = 700 mg/kg-day	No liver tumors	Ganning *et al.*,1987, 1991; ECB, 2008	Liver
Sprague-Dawley rats (M)	Oral feeding	0, 0.02, 0.2, 2.0% (0, 7, 70, 700 mg/kg-day; 520 total rats; GL)	102 weeks	LOAEL = 70 {140} mg/kg-day; NOAEL = 7 mg/kg-day	Increased peroxisome proliferation and number of mitochondria in liver	Ganning *et al.*,1987, 1991; ECB, 2008; ATSDR, 2002	Liver
Sprague-Dawley rats (M)	Oral feeding	0, 0.02, 0.2, 2.0% (0, 7, 70, 700 mg/kg-day; 520 total rats; GL)	102 weeks	LOAEL = 7 {140} mg/kg-day	Increased peroxisomal enzyme activity in liver	Ganning *et al.*,1987, 1991; ECB, 2008; ATSDR, 2002	Liver
Fischer 344 rats (M&F)	Oral feeding	0, 6000, 12,000 mg/kg (0, 322, 674 mg/kg-day (M); 0, 394, 774 mg/kg-day (F); 50 rats per sex per group; GLP)	103 weeks	LOAEL = 322 mg/kg-day	Increased incidence of clear cell change in liver (M; 4/50, 8%; 10/49, 20%; 11/49, 22%)	NTP, 1982; ECB, 2008	Liver

Sherman rats (M)	Oral feeding	N/A	104 weeks	LOAEL = 190 mg/kg-day; NOAEL = 60 mg/kg-day	Increased liver weight	Carpenter *et al.*,1953; ATSDR, 2002	Liver
Fischer 344 rats	Oral feeding	N/A	104 weeks	LOAEL = 92 mg/kg-day	Induced peroxisomal enzyme activity	Cattley *et al.*,1987; ATSDR, 2002	Liver
Fischer 344 rats	Oral feeding	N/A	104 weeks	LOAEL = 322 mg/kg-day	Increased incidence of foci of clear cell changes in liver	Kluwe *et al.*,1982	Liver
Sprague-Dawley rats (M)	Oral feeding	0, 2% (0, 1000 mg/kg-day; 5 rats per treatment group, 8 control rats)	104 weeks	LOAEL = 1000 {1377} mg/kg-day	Increased relative liver weight, increased mitochondrial number, increased peroxisome number, lipofuscin deposits, conjugated dienes, increased peroxisomal enzyme activity, increased lipid peroxidation	Lake *et al.*,1987; ECB, 2008; ATSDR, 2002	Liver
Fischer 344 rats (M&F)	Oral feeding	0, 100, 500, 2500, or 12,500 mg DEHP/kg feed (5.8, 28.9, 146.6, 789.0 mg/kg-day for M; 7.3, 36.1, 181.7, and 938.5 mg/kg-day for F; 10 rats per sex per group)	104 weeks	LOAEL = 28.9 mg/kg-day; NOAEL = 5.8 mg/kg-day	Substantial dose-dependent increase in absolute (+10%) and relative (+ 14%) liver weights (M)	David *et al.*, 2000a	Liver
Fischer 344 rats (M&F)	Oral feeding	0, 100, 500, 2500, or 12,500 mg DEHP/kg feed (5.8, 28.9, 146.6, 789.0 mg/kg-day for M; 7.3, 36.1, 181.7, and 938.5 mg/kg-day for F; 10 rats per sex per group)	104 weeks	LOAEL = 181.7 mg/kg-day; NOAEL = 36.1 mg/kg-day	Significant dose-dependent increase in absolute and relative liver weights (F; $P < 0.05$)	David *et al.*, 2000a	Liver
Fischer 344 rats (M&F)	Oral feeding	0, 100, 500, 2500, or 12,500 mg DEHP/kg feed (5.8, 28.9, 146.6, 789.0 mg/kg-day for M; 7.3, 36.1, 181.7, and 938.5 mg/kg-day for F; 10 rats per sex per group)	104 weeks	LOAEL = 789.0 - 938.5 mg/kg-day; NOAEL = 146.6 – 181.7 mg/kg-day	Significant increase in the incidence and severity of Kupffer cell/hepatocyte pigmentation (M&F; $P < 0.05$)	David *et al.*, 2000a	Liver
Fischer 344 rats (M&F)	Oral feeding	0, 100, 500, 2500, or 12,500 mg DEHP/kg feed (5.8, 28.9, 146.6, 789.0 mg/kg-day for M; 7.3, 36.1, 181.7, and 938.5 mg/kg-day for F; 10 rats per sex per group)	104 weeks	LOAEL = 146.6 mg/kg-day; NOAEL = 28.9 mg/kg-day	Significant increase in the incidence of Spongiosis hepatis (M; $P < 0.05$)	David *et al.*, 2000a	Liver
Fischer 344 rats (M&F)	Oral feeding	0, 100, 500, 2500, or 12,500 mg DEHP/kg feed (5.8, 28.9, 146.6, 789.0 mg/kg-day for M; 7.3, 36.1, 181.7, and 938.5 mg/kg-day for F; 10 rats per sex per group)	104 weeks	NOAEL = 938.5 mg/kg-day	No toxicologically significant change in the incidence of Spongiosis hepatis (F)	David *et al.*, 2000a	Liver

Fischer 344 rats (M&F)	Oral feeding	0, 100, 500, 1500, or 12,500 mg DEHP/kg feed (5.8, 28.9, 146.6,722-728 mg/kg-day for M; 7.3, 36.1, 181.7, 882-879 mg/kg-day for F; 10 rats per sex per group)	104 weeks + 78 weeks then 26 week recovery	LOAEL = 722 - 879 mg/kg-day	Significant recovery (decrease) in absolute (F) and relative (M&F) liver weight at 104 weeks (P≤0.05); Significant recovery (decrease) in the incidence of Kuppfer cell/hepatocyte pigmentation at 78 weeks (M; P≤0.05).Substantial recovery (decrease) in absolute liver weight (M) and the severity of Kuppfer cell/hepatocyte pigmentation at 104 weeks (M&F); Substantial recover (decrease) in the incidence of spongiosis hepatis at 104 weeks (M)	David *et al.*, 2001	Liver
$B6C3F_1$ mice (M&F)	Oral feeding	0, 100, 500, 1500, or 6000 mg DEHP/kg feed (19.2, 98.5, 292.2, 1211.0 – 1227.0 mg/kg-day for M; 23.8, 116.8, 354.2, 1413.0 – 1408.0 mg/kg-day for F; 10-15 mice per sex per group)	105 weeks	LOAEL = 1211.0 – 1408.0 mg/kg-day	Significant recovery (decrease) in absolute and relative liver weight at 105 weeks (M&F; P≤0.05); Marginal recovery (decrease) in the incidence and severity of chronic hepatic inflammation (M&F)	David *et al.*, 2001	Liver
$B6C3F_1$ mice (M&F)	Oral feeding	0, 100, 500, 1500, or 6000 mg DEHP/kg feed (19.2, 98.5, 292.2, 1266.1 mg/kg-day for M; 23.8, 116.8, 354.2, 1458.2 mg/kg-day for F; 10-15 mice per sex per group)	79 or 105 weeks	LOAEL = 1266.1 – 1458.2 mg/kg-day	Significant increase in the incidence of mice with hepatocyte pigmentation, increased cytoplasmic eosinophilia, and chronic hepatic inflammation at 79 weeks (M&F; P≤0.05)	David *et al.*, 2000b	Liver
$B6C3F_1$ mice (M&F)	Oral feeding	0, 100, 500, 1500, or 6000 mg DEHP/kg feed (19.2, 98.5, 292.2, 1266.1 mg/kg-day for M; 23.8, 116.8, 354.2, 1458.2 mg/kg-day for F; 10-15 mice per sex per group)	79 or 105 weeks	LOAEL = 1266.1 – 1458.2 mg/kg-day; NOAEL = 292.2 – 354.2 mg/kg-day	Significant increase in the incidence of mice with hepatocyte pigmentation (M&F), increased cytoplasmic eosinophilia (M&F), and chronic hepatic inflammation (M) at 105 weeks (M&F; P≤0.05), substantial increase in the severity of chronic hepatic inflammation at 105 weeks (M&F), and increased absolute liver weight at 105 weeks (F; P≤0.05)	David *et al.*, 2000b	Liver
$B6C3F_1$ mice (M&F)	Oral feeding	0, 100, 500, 1500, or 6000 mg DEHP/kg feed (19.2, 98.5, 292.2, 1266.1 mg/kg-day for M; 23.8, 116.8, 354.2, 1458.2 mg/kg-day for F; 10-15 mice per sex per group)	79 or 105 weeks	LOAEL = 98.5 mg/kg-day; NOAEL = 19.2 mg/kg-day	Significant increase in the absolute liver weight at 105 weeks (M; P≤0.05)	David *et al.*, 2000b	Liver
$B6C3F_1$ mice (M&F)	Oral feeding	0, 100, 500, 1500, or 6000 mg DEHP/kg feed (19.2, 98.5, 292.2, 1266.1 mg/kg-day for M; 23.8, 116.8, 354.2, 1458.2 mg/kg-day for F; 10-15 mice per sex per group)	79 or 105 weeks	LOAEL = 292.2 – 354.2 mg/kg-day; NOAEL = 98.5 – 116.8 mg/kg-day	Significant increase in the relative liver weight at 105 weeks (M&F; P≤0.05)	David *et al.*, 2000b	Liver

Fischer 344 rats (M&F)	Oral feeding	0, 100, 500, 2500, 12,500 mg/kg (0, 5.8, 28.9, 146.6, 789.0 mg/kg-day (M); 0, 7.3, 36.1, 181.7, 938.5 mg/kg-day (F), or 12,500 mg/kg for 78 weeks and a 26 week recovery period; 70-85 rats per sex per group; 55 rats per sex in recovery group)	104 weeks	LOAEL = 28.9 mg/kg-day; NOAEL = 5.8 mg/kg-day	Increased liver weight (M) and peroxisome proliferation	Moore, 1996; ECB, 2008	Liver
$B6C3F_1$ mice (M&F)	Oral feeding	0, 100, 500, 1500, 6000 mg/kg (0, 19.2, 98.5, 292.2, 1266.1 mg/kg-day (M); 0, 23.8, 116.8, 354.2, 1458.2 mg/kg-day (F), or 6000 mg/kg for 78 weeks and a 26 week recovery period; 70-85 rats per sex per group; 55 rats per sex in recovery group)	104 weeks	LOAEL = 98.5 mg/kg-day; NOAEL = 19.2 mg/kg-day	Partially reversible increased liver weight (M) and peroxisome proliferation	Moore, 1997; ECB, 2008	Liver
Fischer 344 rats	Oral feeding	N/A	108 weeks	LOAEL = 2000 mg/kg-day	Increase in liver weight (100%)	Rao *et al.*,1990; ATSDR, 2002	Liver
Wistar rats (M&F)	Oral feeding	0, 0.1, 0.5% (0, 50-80, 300-400 mg/kg-day; 43 rats per sex per group	3, 6, 12, 24 months	LOAEL = 300-400 mg/kg-day; NOAEL = 50-80 mg/kg-day	Increased liver weight at 3 - 6 months, but not 12 - 24 months	Harris *et al.*,1956; ECB, 2008	Liver
Wistar rats (M&F)	Oral feeding	0, 0.1, 0.5% (0, 50, 80, 300-400 mg/kg-day; 43 rats per sex per group)	3, 6, 12, 24 months	NOAEL = 300-400 mg/kg-day	No histological findings in the liver	Harris *et al.*,1956; ECB, 2008	Liver
Sprague-Dawley rats (M&F)	Oral feeding	0, 0.5% (0, 200 mg/kg-day; 10 rats per sex per group)	Lifetime	LOAEL = 200 mg/kg-day	Liver necroses and fat infiltration in a few animals	BASF, 1960; ECB, 2008	Liver
Humans (97 M/4 F	Inhalation /Dermal (epi)	Background (0.001-0.004 ppm ~ 0.016-0.064 mg/m^3) Higher levels – 0.01 ppm (0.16 mg/m^3)	12 years average exposure period (4 months to 35 years)	NS	Serum activities of liver enzymes normal	Thiess *et al.*,1978b	Liver
Wistar rats (M&F)	Oral feeding	0, 1000, 3000, 9000 mg/kg (0, 113, 340, 1088 mg/kg-day; 25 rats per sex per group; GLP)	3 generations	LOAEL = 113 mg/kg-day	Increased relative liver weight in F0 females	Schilling *et al.*,2001; CERHR, 2006; ECB, 2008	Liver
Wistar rats (M&F)	Oral feeding	0, 1000, 3000, 9000 mg/kg (0, 113, 340, 1088 mg/kg-day; 25 rats per sex per group; GLP)	3 generations	LOAEL = 340 mg/kg-day; NOAEL = 113mg/kg-day	Increased relative liver weight in F0 males	Schilling *et al.*,2001; CERHR, 2006; ECB, 2008	Liver
Wistar rats (M&F)	Oral feeding	0, 1000, 3000, 9000 mg/kg (0, 113, 340, 1088 mg/kg-day; 25 rats per sex per group; GLP)	3 generations	NOAEL = 1088 mg/kg-day	No histopathological changes in liver	Schilling *et al.*,2001; CERHR, 2006; ECB, 2008	Liver

Fischer 344 rats (F)	Oral gavage Oral feeding	Initiation – 10g DEHP/kg at 6, 12, or 24 hours (10 rats per group; positive promoter, 2-acetyl-aminofluorene (AAF); positive initiator, diethylnitrosamine (DEN)) Initiation – 1.2% (~600 mg/kg-day; 10 rats per group; promoter, Phenobarbital (PB); positive initiator, DEN)	Once, promoter 2 weeks after initiator 12 weeks, promoter 2 weeks after initiator	N/A	No tumor initiating activity of DEHP. Positive control group had increased number and volume of preneoplastic foci No tumor initiating activity of DEHP. Positive control group had increased number and volume of preneoplastic foci	Garvey *et al.*,1987; IARC, 2000	Liver
Fischer 344 rats (M)	Oral feeding	Promotion – 3000 mg/kg DEHP (~150 mg/kg; 18-20 rats per group; initiator, DEN; rats partially hepectomized)	6 weeks, promoter 2 weeks after initiator	N/A	No promotion	Ito *et al.*,1988; IARC, 2000	Liver
Sprague-Dawley rats (F)	Oral gavage	Promotion – 10, 100, 200, or 500 mgDEHP/kg; 5 rats per group; initiator, DEN)	Once daily for 3 times a week for 11 weeks, promoter 1 week after initiator	N/A	A weak promoting effect noted (2-fold increase in the number and area of ATPase-deficient foci) at 200 and 500 mg/kg	Oesterle and Deml, 1988; IARC, 2000	Liver
Sprague-Dawley rats (M&F)	Oral gavage	Promotion – 50, 200, 500, 1000, 2000 mgDEHP/kg; 8-10 rats per group; initiator, DEN)	Once daily for 3 times a week for 7-11 weeks	N/A	A weak promoting effect noted (increase in the number and area of ATPase-deficient foci and GGTase-positive foci in the lower dose groups, but were decreased in the high dose grooups	Gerbracht *et al.*,1990	Liver
Fischer 344 rats (M)	Oral feeding	Promotion – 12,000 mgDEHP/kg; initiator, N-nitrosodiethylamine Initiation – 12,000 mgDEHP/kg; promoter, PB	14 weeks, promoter 2 weeks after initiator 26 or 78 weeks(6 or 18 months), promoter 2 weeks after initiators	N/A	No promotion No initiating activity	Ward *et al.*,1986; IARC, 2000	Liver
Fischer 344 rats (F)	Oral feeding	Promotion – 1.2% DEHP (~600 mg/kg; 10 rats per group; positive promoter, PB; initiator, DEN)	12 or 26 weeks (3 or 6 months), promoter 3 weeks after initiator	N/A	No promotion	Popp *et al.*,1985; IARC, 2000	Liver
$B6C3F_1$ mice (M&F)	Oral gavage Oral feeding	Initiation – 25,000, 50,000 mg DEHP/kg (30 rats per sex per group; promoter, PB) Promotion – 3000, 6000, 12000 mg DEHP/kg (~600, 1200, 2400 mg/kg; 30 mice per sex per group; initiator, DEN)	Once, promoter 1-2 weeks after initiator 8, 16, 26 weeks (2, 4, or 6 months), promoter 1-2 weeks after initiator	N/A	No initiating activity A strong promoting effect noted (numerous foci and neoplasms)	Ward *et al.*,1983; IARC, 2000	Liver

$B6C3F_1$ mice (M)	Oral feeding	Promotion – 3000 mgDEHP/kg (~1200 mg/kg; 29 mice per group; initiator, DEN)	4, 12, 24 weeks (28, 84, 168 days), promoter 1-2 weeks after initiator	N/A	A strong promoting activity noted after exposure for 28 days only (significant and time-dependent increase in liver focal proliferative lesions); Significant increase in incidence of liver tumors at 168 days)	Ward *et al.*,1984; IARC, 2000	Liver
Fischer 344 rats (M)	Oral feeding	Initiation and promotion – 12,000 mgDEHP/kg (~550 mg/kg-day; 6-18 rats per group; positive promoter, PB; positive initiator, N-2-fluorenylacetamide (FFA))	24 {31} weeks, promoter 4 weeks after initiator	N/A	No initiating, promoting, or sequential syncarcinogenic effect	Williams *et al.*,1987; IARC, 2000	Liver
Fischer 344 rats (M)	Oral feeding	Promotion	24 weeks, promoter 4 weeks after initiator	N/A	No promotion	Maruyama *et al.*,1990, IARC, 2000	Liver
C3H/HeNCr mice (M&F)	Oral feeding	Promotion – 12,000 mgDEHP/kg (~2400 mg/kg; initiation, N-nitrosodiethylamine (NDEA))	26 weeks, promoter 2 weeks after initiator	N/A	A strong promoting activity noted (increased incidence of liver tumors)	Weghorst *et al.*,1993, 1994; IARC, 2000	Liver
$B6C3F_1$ mice (M)	Oral feeding	Promotion – 12,000 mgDEHP/kg (~2400 mg/kg; 2 mice per group; initiator, DEN; Positive promoter, PB)	26 weeks (6 months)	N/A	A strong promoting activity noted (liver tumors); A pronounced peroxisome proliferation in non-tumor livers in DEN- and DEHP-treated mice and in liver tumors in DEHP-treated mice, but not in tumors after DEN initiation and DEHP promotion	Schuller and Ward, 1984	Liver
$B6C3F_1$ mice (M)	Oral feeding	Promotion – 6000 mgDEHP/kg (~1200 mg/kg; 30 mice per group; initiator, DEN)	29 weeks	N/A	A strong promoting activity (increased incidence and area of liver focal proliferative lesions); 4 mice dosed with DEN and DEHP had hepatic adenomas	Hagiwara *et al.*,1986	Liver
$B6C3F_1$ mice (M&F 6 wk old offspring)	Oral feeding	Promotion – 6000 mgDEHP/kg (~1200 mg/kg; 48-55 mice per sex per group) Transplacental initiation: N-nitrosoethylurea (NEU) i.p. to pregnant mice DNA synthesis: 6 M dosed with 200 mg/kg 5-bromo-2'-deoxyuridine (Brdu) i.p.	78 weeks	N/A	A stong promoting activity noted (increase in liver focal proliferative lesions including hyperplastic foci, hepatocellular adenomas and carcinomas) Tumor promotion in liver may be result of increased DNA synthesis in initiated or focus cells rather than in non-proliferative cells	Ward *et al.*,1990	Liver
Fischer 344 rats	Oral feeding	N/A	78 weeks	LOAEL = 1579 mg/kg-day	Increased hepatocarcinomas by week 78 (43%; controls = 0%)	Hayashi *et al.*,1994; ATSDR, 2002	Liver
$B6C3F_1$ mice (M)	Oral gavage	Water, vehicle, 50, 200, 750 mg/kg 2-ethylhexanol	78 weeks	LOAEL = 200 mg/kg	Liver carcinoma (tumor frequency = 8, 12, 12, 14, 18%, respectively)	Astill *et al.*, 1996; Ito and Nakajima, 2008	Liver
$B6C3F_1$ mice (M)	Oral gavage	Water, vehicle, 50, 200, 750 mg/kg 2-ethylhexanol	78 weeks	LOAEL = 750 mg/kg	Liver adenoma (tumor frequency = 0, 0, 0, 0, 2%, respectively)	Astill *et al.*, 1996; Ito and Nakajima, 2008	Liver
$B6C3F_1$ mice (F)	Oral gavage	Water, vehicle, 50, 200, 750 mg/kg 2-ethylhexanol	78 weeks	LOAEL = 200 mg/kg	Liver carcinoma (tumor frequency = 2, 0, 2, 6, 10%, respectively)	Astill *et al.*, 1996; Ito and Nakajima, 2008	Liver
Fischer 344 rats (M)	Oral feeding	0, 2500, 12,500 mg/kg	79 weeks	LOAEL = 12,500 mg/kg	Hepatocellular carcinoma (tumor frequency = 10, 0, 40%, respectively)	David *et al.*, 1999; Ito and Nakajima, 2008	Liver

Fischer 344 rats (M)	Oral feeding	0, 2500, 12,500 mg/kg	79 weeks	NOAEL = 12,500 mg/kg	Hepatocellular adenoma (tumor frequency = 10, 10, 10%, respectively)	David *et al.*, 1999; Ito and Nakajima, 2008	Liver
Fischer 344 rats (F)	Oral feeding	0, 2500, 12,500 mg/kg	79 weeks	LOAEL = 12,500 mg/kg	Hepatocellular carcinoma (tumor frequency = 0, 0, 20%, respectively)	David *et al.*, 1999; Ito and Nakajima, 2008	Liver
Fischer 344 rats (F)	Oral feeding	0, 2500, 12,500 mg/kg	79 weeks	LOAEL = 12,500 mg/kg	Hepatocellular adenoma (tumor frequency = 0, 0, 10%, respectively)	David *et al.*, 1999; Ito and Nakajima, 2008	Liver
$B6C3F_1$ mice (M)	Oral feeding	0, 100, 500, 1500, 6000 mg/kg	79 weeks	LOAEL = 6000 mg/kg	Hepatocellular carcinoma (tumor frequency = 0, 0, 10, 0, 7%, respectively)	David *et al.*, 1999; Ito and Nakajima, 2008	Liver
$B6C3F_1$ mice (M)	Oral feeding	0, 100, 500, 1500, 6000 mg/kg	79 weeks	LOAEL = 500 mg/kg	Hepatocellular adenoma (tumor frequency = 7, 10, 20, 10, 7%, respectively)	David *et al.*, 1999; Ito and Nakajima, 2008	Liver
$B6C3F_1$ mice (F)	Oral feeding	0, 100, 500, 1500, 6000 mg/kg	79 weeks	LOAEL = 6000 mg/kg	Hepatocellular carcinoma (tumor frequency = 0, 0, 0, 0, 13%, respectively)	David *et al.*, 1999; Ito and Nakajima, 2008	Liver
$B6C3F_1$ mice (F)	Oral feeding	0, 100, 500, 1500, 6000 mg/kg	79 weeks	LOAEL = 100 mg/kg	Hepatocellular adenoma (tumor frequency = 0, 10, 10, 10, 27%, respectively)	David *et al.*, 1999; Ito and Nakajima, 2008	Liver
Fischer 344 rats (M)	Oral feeding	0, 2% (4-6 rats per group)	52 or 79 weeks	LOAEL = 2%	Increased hepatocellular carcinomas in 1/4 rats or 2/4 rats by 52 or 78 weeks, respectively	Tamura *et al.*,1990 a,b; ECB, 2008	Liver
Wistar rats (M)	Oral feeding	0, 2% (4-6 rats per group)	52 or 79 weeks	NOAEL = 2%	No neoplastic lesions	Tamura *et al.*,1990 a,b; ECB, 2008	Liver
129/Sv, PPARα-null mice (M)	Oral feeding	0, 0.01, 0.05% (0, 100, 500 mg/kg)	84 weeks	LOAEL = 500 mg/kg	Increased liver tumors (hepatocellular adenoma/ hepatocellular carcinoma/cholangiocellular carcinoma)tumors in PPARα-null mice (0/4, 9/4, 10/25.8% in control/treated mice, respectively)	Ito *et al.*, 2007; Ito and Nakajima, 2008	Liver
Fischer 344 rats (M)	Oral feeding	0, 2% (~1000 mg/kg-day; 10 treated rats, 8 controls)	95 weeks	LOAEL = 2% (~1000 {2444} mg/kg-day)	Increased liver tumors in treated rats (6/10) when compared to controls (0/8)	Rao *et al.*,1987; ECB, 2008; ATSDR, 2002	Liver
$B6C3F_1$ mice (M&F)	Oral feeding	0, 3000, 6000 mg/kg (0, 672, 1325 mg/kg-day (M); 0, 799, 1821 mg/kg-day (F); 50 mice per sex per group; GLP)	103 weeks	LOAEL = 672 {1325} mg/kg-day; {NOAEL = 672 mg/kg-day}	Increased hepatocellular carcinomas (M; 9/50, 18%; 14/48, 29%; 19/50, 38% ($P < 0.05$); F; 0/50; 7/50, 14%; 17/50, 34% ($P < 0.0001$)) and adenomas (M; 6/50, 12%; 11/48, 23%; 10/50, 20%) and combined carcinomas and adenomas (M; 15/50, 30%; 25/48, 52%; 29/50, 58%; F; 1/50, 2%; 12/50, 24%; 18/50, 36%)	NTP, 1982; ECB, 2008	Liver
Fischer 344 rats (M&F)	Oral feeding	0, 6000, 12,000 mg/kg (0, 322, 674 mg/kg-day (M); 0, 394, 774 mg/kg-day (F); 50 rats per sex per group; GLP)	103 weeks	LOAEL = 322 mg/kg-day	Increased incidence of neoplastic nodules (M; 2/50, 4%; 5/49, 10%; 7/49, 14%; F; 0/50; 4/49, 8%; 5/50, 10%), and combined neoplastic nodules and hepatocellular carcinomas (M; 3/50, 6%; 6/49, 12%; 12/49, 24%; F; 0/50; 6/49, 12%; 13/50, 26%)	NTP, 1982; ECB, 2008	Liver
Fischer 344 rats (M&F)	Oral feeding	0, 6000, 12,000 mg/kg (0, 322, 674 mg/kg-day (M); 0, 394, 774 mg/kg-day (F); 50 rats per sex per group; GLP)	103 weeks	LOAEL = 674 mg/kg-day; NOAEL = 322 mg/kg-day	Increased incidence of hepatic carcinomas (M; 1/50, 2%; 1/49, 2%; 5/49, 10%; F; 0/50; 2/49, 4%; 8/50, 16%)	NTP, 1982; ECB, 2008	Liver
Fischer 344 rats (M&F)	Oral feeding	0, 100, 500, 2500, 12,500 mg/kg (0, 6, 28.9, 146.6, 789 mg/kg-day (M); 0, 7, 36, 182, 939 mg/kg-day (F); 70-85 rats per sex per group	104 weeks	LOAEL = 789-939 mg/kg-day; NOAEL = 146.6-182 mg/kg-day	Dose-dependent partially reversible increase in incidence of liver adenomas (F) and carcinomas (M&F) and total number with hepatocellular tumors (F; $P < 0.05$)	Moore, 1996; ECB, 2008	Liver

Fischer 344 rats (M&F)	Oral feeding	0, 100, 500, 2500, 12,500 mg/kg (0, 6, 28.9, 146.6, 789 mg/kg-day (M); 0, 7, 36, 182, 939 mg/kg-day (F); 70-85 rats per sex per group	104 weeks	LOAEL = 146.6-182 mg/kg-day; NOAEL = 28.9-36 mg/kg-day	Dose-dependent partially reversible increase in incidence of liver adenomas (M) and total number with hepatocellular tumors (M; $P < 0.05$)	Moore, 1996; ECB, 2008	Liver
$B6C3F_1$ mice (M&F)	Oral feeding	0, 100, 500, 1500, 6000 mg/kg (0, 6, 19.2, 98.5, 292.2, 1266.1 mg/kg-day (M); 0, 23.8, 116.8, 354.2, 1458.2 mg/kg-day (F), or 6000 mg/kg for 78 weeks and a 26 week recovery period; 70 rats per sex per group; 55 rats per sex in recovery group)	104 weeks	LOAEL = 354.2 mg/kg-day; NOAEL = 116.8 mg/kg-day	Substantial increase in the hepatocellular adenomas, carcinomas, and significant increase in total number of hepatocellular tumors (F; $P < 0.05$)	Moore, 1997; ECB, 2008	Liver
$B6C3F_1$ mice (M&F)	Oral feeding	0, 100, 500, 1500, 6000 mg/kg (0, 6, 19.2, 98.5, 292.2, 1266.1 mg/kg-day (M); 0, 23.8, 116.8, 354.2, 1458.2 mg/kg-day (F), or 6000 mg/kg for 78 weeks and a 26 week recovery period; 70 rats per sex per group; 55 rats per sex in recovery group)	104 weeks	LOAEL = 98.5-116.8 mg/kg-day; NOAEL = 19.2-23.8 mg/kg-day	Partially reversible increased liver weight (M&F); Substantial increase in the hepatocellular adenomas, carcinomas, and significant increase in total number of hepatocellular tumors (M; $P < 0.05$)	Moore, 1997; ECB, 2008	Liver
Fischer 344 rats (F)	Oral feeding	0, 0.03, 0.1, 1.2% (~0, 15, 50, 550 mg/kg-day; 20 rats per group)	104 weeks	LOAEL = 550mg/kg-{1100} day; NOAEL = 50 mg/kg-day	Increased incidence of hepatocellular carcinomas and neoplastic nodules (tumor frequency = 0, 6, 5, 30%, respectively)	Cattley *et al.*,1987; ECB, 2008; ATSDR, 2002; Ito and Nakajima, 2008	Liver
Fischer 344 rats	Oral feeding	N/A	104 weeks	LOAEL = 147 mg/kg-day (M)	Increased hepatocellular tumors (M; 11/65)	David *et al.*,1999, 2000a; ATSDR, 2002	Liver
Fischer 344 rats	Oral feeding	N/A	104 weeks	LOAEL = 939 mg/kg-day (F)	Increased hepatocellular tumors (F; 22/80)	David *et al.*,1999, 2000a; ATSDR, 2002	Liver
Fischer 344 rats	Oral feeding	N/A	104 weeks	LOAEL = 322 mg/kg-day	Increased hepatocellular carcinoma	Kluwe *et al.*,1982; ATSDR, 2002	Liver
Fischer 344 rats (M)	Oral feeding	0, 6000, 12,000 mg/kg	104 weeks	LOAEL = 12,000 mg/kg	Increased hepatocellular carcinoma (tumor frequency = 2, 2, 10%, respectively)	Kluwe *et al.*, 1985; Ito and Nakajima, 2008	Liver
Fischer 344 rats (M)	Oral feeding	0, 6000, 12,000 mg/kg	104 weeks	LOAEL = 6000 mg/kg	Increased hepatocellular neoplastic nodule (tumor frequency = 4, 10, 14%, respectively)	Kluwe *et al.*, 1985; Ito and Nakajima, 2008	Liver
Fischer 344 rats (F)	Oral feeding	0, 6000, 12,000 mg/kg	104 weeks	LOAEL = 6000 mg/kg	Increased hepatocellular carcinoma (tumor frequency = 0, 4, 16%, respectively)	Kluwe *et al.*, 1985; Ito and Nakajima, 2008	Liver
Fischer 344 rats (F)	Oral feeding	0, 6000, 12,000 mg/kg	104 weeks	LOAEL = 6000 mg/kg	Increased hepatocellular neoplastic nodule (tumor frequency = 0, 8, 10%, respectively)	Kluwe *et al.*, 1985; Ito and Nakajima, 2008	Liver
Sprague-Dawley rats	Oral feeding	N/A	104 weeks	LOAEL = 1377 mg/kg-day	Increased hepatocellular carcinoma	Lake *et al.*,1987; ATSDR, 2002	Liver
$B6C3F_1$ mice	Oral feeding	N/A	104 weeks	LOAEL = 292 mg/kg-day (M)	Increased hepatocellular tumors (M; 27/65)	David *et al.*,1999, 2000b; ATSDR, 2002	Liver
$B6C3F_1$ mice	Oral feeding	N/A	104 weeks	LOAEL = 354 mg/kg-day (F)	Increased hepatocellular tumors (F; 19/65)	David *et al.*,1999, 2000b; ATSDR, 2002	Liver

$B6C3F_1$ mice	Oral feeding	N/A	104 weeks	LOAEL = 672 mg/kg-day	Increased hepatocellular carcinoma	Kluwe *et al.*,1982; ATSDR, 2002	Liver
$B6C3F_1$ mice (M)	Oral feeding	0, 3000, 6000 mg/kg	104 weeks	LOAEL = 3000 mg/kg	Increased hepatocellular carcinoma (tumor frequency = 18, 29, 38%, respectively)	Kluwe *et al.*,1985; Ito and Nakajima, 2008	Liver
$B6C3F_1$ mice (M)	Oral feeding	0, 3000, 6000 mg/kg	104 weeks	LOAEL = 3000 mg/kg	Increased hepatocellular adenoma (tumor frequency = 10, 23, 20%, respectively)	Kluwe *et al.*,1985; Ito and Nakajima, 2008	Liver
$B6C3F_1$ mice (F)	Oral feeding	0, 3000, 6000 mg/kg	104 weeks	LOAEL = 3000 mg/kg	Increased hepatocellular carcinoma (tumor frequency = 0, 14, 34%, respectively)	Kluwe *et al.*,1985; Ito and Nakajima, 2008	Liver
$B6C3F_1$ mice (F)	Oral feeding	0, 3000, 6000 mg/kg	104 weeks	LOAEL = 3000 mg/kg	Increased hepatocellular adenoma (tumor frequency = 2, 10, 2%, respectively)	Kluwe *et al.*,1985; Ito and Nakajima, 2008	Liver
Fischer 344 rats (M)	Oral gavage	Water, vehicle, 50, 150, 500 mg/kg 2-ethylhexanol	104 weeks	NOAEL = 500 mg/kg	Liver carcinoma (4, 12, 6, 6, 2% tumor frequency, respectively)	Astill *et al.*, 1996; Ito and Nakajima, 2008	Liver
Fischer 344 rats (M)	Oral gavage	Water, vehicle, 50, 150, 500 mg/kg 2-ethylhexanol	104 weeks	NOAEL = 500 mg/kg	Liver adenoma (0, 0, 0, 2, 0% tumor frequency, respectively)	Astill *et al.*, 1996; Ito and Nakajima, 2008	Liver
Fischer 344 rats (F)	Oral gavage	Water, vehicle, 50, 150, 500 mg/kg 2-ethylhexanol	104 weeks	NOAEL = 500 mg/kg	Liver carcinoma (0, 2, 2, 4, 0% tumor frequency, respectively)	Astill *et al.*, 1996; Ito and Nakajima, 2008	Liver
Fischer 344 rats (M)	Oral feeding	0, 100, 500, 2500, 12,500 mg/kg, recovery	105 weeks	LOAEL = 500 mg/kg	Hepatocellular carcinoma (tumor frequency = 1, 0, 2, 5, 30, 13%, respectively)	David *et al.*, 1999; Ito and Nakajima, 2008	Liver
Fischer 344 rats (M)	Oral feeding	0, 100, 500, 2500, 12,500 mg/kg, recovery	105 weeks	LOAEL = 100 mg/kg	Hepatocellular adenoma (tumor frequency = 5, 10, 5, 12, 26, 22%, respectively)	David *et al.*, 1999; Ito and Nakajima, 2008	Liver
Fischer 344 rats (M)	Oral feeding	0, 100, 500, 2500, 12,500 mg/kg, recovery	105 weeks	LOAEL = 12,500 mg/kg	Hepatocellular carcinoma (tumor frequency = 0, 2, 0, 2, 18%, respectively)	David *et al.*, 1999; Ito and Nakajima, 2008	Liver
Fischer 344 rats (F)	Oral feeding	0, 100, 500, 2500, 12,500 mg/kg, recovery	105 weeks	LOAEL = 12,500 mg/kg	Hepatocellular adenoma (tumor frequency = 0, 6, 2, 3, 10%, respectively)	David *et al.*, 1999; Ito and Nakajima, 2008	Liver
$B6C3F_1$ mice (M)	Oral feeding	0, 100, 500, 1500, 6000 mg/kg, recovery	105 weeks	LOAEL = 500 mg/kg	Hepatocellular carcinoma (tumor frequency = 6, 8, 14, 22, 31, 22%, respectively)	David *et al.*, 1999; Ito and Nakajima, 2008	Liver
$B6C3F_1$ mice (M)	Oral feeding	0, 100, 500, 1500, 6000 mg/kg, recovery	105 weeks	LOAEL = 100 mg/kg	Hepatocellular adenoma (tumor frequency = 6, 17, 20, 22, 27, 5%, respectively)	David *et al.*, 1999; Ito and Nakajima, 2008	Liver
$B6C3F_1$ mice (F)	Oral feeding	0, 100, 500, 1500, 6000 mg/kg, recovery	105 weeks	LOAEL = 1500 mg/kg	Hepatocellular carcinoma (tumor frequency = 4, 3, 5, 15, 23, 42%, respectively)	David *et al.*, 1999; Ito and Nakajima, 2008	Liver
$B6C3F_1$ mice (F)	Oral feeding	0, 100, 500, 1500, 6000 mg/kg, recovery	105 weeks	LOAEL = 100 mg/kg	Hepatocellular adenoma (tumor frequency = 0, 3, 6, 14, 49, 24%, respectively)	David *et al.*, 1999; Ito and Nakajima, 2008	Liver
Fischer 344 rats (M)	Oral feeding	0, 2% (~1000 mg/kg-day; 14 treated rats, 10 controls)	108 weeks	LOAEL = 2% (~1000 mg/kg-day)	Increased hepatocellular carcinomas in treated rats (11/14) when compared to controls (1/10)	Rao *et al.*,1990; ECB, 2008	Liver
Syrian golden hamsters (M&F)	Inhalation	15µg/m^3 vapor for a total exposure of 7-10 mg/kg-bw (65 hamsters per sex per treated group; 80 controls per sex)	Lifetime	NOAEC = 15µg/m^3	No significant differences in the tumor incidence in treated groups when compared with the control group	Schmezer *et al.*,1988; ECB, 2008	Liver
Syrian golden hamsters (M&F)	Intraperitoneal	3000 mg/kg injections (25 hamsters per sex per group)	Lifetime, injections once every 1, 2, or 4 weeks	NOAEL = 3000 mg/kg-day	No differences in the tumor incidence in the treated groups when compared with the control group	Schmezer *et al.*,1988; ECB, 2008	Liver
Thyroid							

Wistar rats	Oral feeding	N/A	3 days	LOAEL = 2000 mg/kg-day	Decreased serum T_4 and ultrastructural changes consistent with thyroid hyperactivity	Hinton *et al.*,1986; ATSDR, 2002	Thyroid
Wistar rats (F)	Intraperitoneal injection	0, 7.5 mg/kg (16 rats per group)	Once every other day for 14 days (7 injections) – recovery for 7 days	LOAEL = 7.5 mg/kg	Significant dose-dependent increase in T_3 and T_4 ($P < 0.01$) – Increase mitigated to control levels after 7 days recovery	Gayathri *et al.*, 2004	Thyroid
Wistar rats (F)	Intraperitoneal injection	0, 7.5 mg/kg (16 rats per group)	Once every other day for 14 days (7 injections) – recovery for 7 days	NOAEL = 7.5 mg/kg	No toxicologically significant change in TSH	Gayathri *et al.*, 2004	Thyroid
Wisar rats (Surrey strain)	Oral feeding	0, 1.0% DEHP (0, 1000 mg/kg-day; 6 rats per group)	3, 10, 21 days	LOAEL = 1000 mg/kg-day;	Increased number and size of lysosomes, enlarged Golgi, damaged mitochondira in rats treated for 21 days; Dose-dependent increase in T_3 at 21 days; Decrease in T_4 at 3, 10, and 21 days	Hinton *et al.*, 1986	Thyroid
Wistar rats	Oral feeding	N/A	12 weeks (3 months)	LOAEL = 1000 mg/kg-day	Ultrastructural changes consistent with thyroid hyperactivity	Price *et al.*,1988a; ATSDR, 2002	Thyroid
Sprague-Dawley rats (M&F)	Oral feeding	0, 5, 50, 500, 5000 mg/kg (0, 0.4, 3.7, 37.6, 375.2 mg/kg-day (M); 0, 0.4, 4.2, 42.2, 419.3 mg/kg-day (F); 10 rats per sex per group; GLP)	13 weeks	LOAEL = 345.0 mg/kg-day	Substantial increase in the incidence and severity of reduced follicle size (M&F) and colloid density (M)	Poon *et al.*,1997; ECB, 2008; ATSDR, 2002	Thyroid
Sprague-Dawley rats (M&F)	Oral feeding	0, 0.2, 1.0, and 2.0% (0, 143, 737, 1440 mg/kg-day; M; 0, 154, 797, 1414 mg/kg-day; F) (15 rats per group): 0, 1.0, and 2.0% (0, 737, 1440 mg/kg-day; M; 0, 797, 1414 mg/kg-day; F): 0 and 2.0% (0, 1440 mg/kg-day; M; 0, 1414 mg/kg-day; F) (10 rats per group)	17 weeks: 2 or 6 weeks:	LOAEL = 1440 mg/kg-day; NOAEL = 797 mg/kg-day	Significant dose-dependent decrease in the absolute thyroid weight (F, 6, 17 weeks; $P < 0.05$)	Gray *et al.*, 1977	Thyroid
Sprague-Dawley rats (M&F)	Oral feeding	0, 0.2, 1.0, and 2.0% (0, 143, 737, 1440 mg/kg-day; M; 0, 154, 797, 1414 mg/kg-day; F) (15 rats per group): 0, 1.0, and 2.0% (0, 737, 1440 mg/kg-day; M; 0, 797, 1414 mg/kg-day; F): 0 and 2.0% (0, 1440 mg/kg-day; M; 0, 1414 mg/kg-day; F) (10 rats per group)	17 weeks: 2 or 6 weeks:	LOAEL = 1414-1440 mg/kg-day; NOAEL = 737-797 mg/kg-day	Significant dose-dependent increase in the relative thyroid weight (M, 2 weeks; F, 6 weeks; $P < 0.05$)	Gray *et al.*, 1977	Thyroid
Fischer 344 rats (M&F)	Oral feeding	0, 6000, 12,000 mg/kg (0, 322, 674 mg/kg-day (M); 0, 394, 774 mg/kg-day (F); 50 rats per sex per group; GLP)	103 weeks	LOAEL = 674 mg/kg-day; NOAEL = 322 mg/kg-day	Decreased incidence of thyroid C-cell tumors ($P = 0.031$)	NTP, 1982; ECB, 2008	Thyroid
Fischer 344 rats (M)	Oral feeding	0, 6000, 12,000 mg/kg	104 weeks	LOAEL = 12,000 mg/kg	Thyroid C-cell adenoma or carcinoma (decrease)	Kluwe *et al.*, 1985; Ito and Nakajima, 2008	Thyroid

Cardiovascular							
Rats	Intravenous	58, 72, 95, 75, 95 mg (MEHP)	Increasing rate from 0.46 mg/min to 4.6 mg/min until death	LOAEL = 57 mg/kg; NOAEL = 28.5 mg/kg	Decrease in heart rate	Rock *et al.*,1987	Heart
Rats	Intravenous	58, 72, 95, 75, 95 mg (MEHP)	Increasing rate from 0.46 mg/min to 4.6 mg/min until death	LOAEL = 214 mg/kg; NOAEL = 157 mg/kg	Decrease in blood pressure	Rock *et al.*,1987	Heart
Wistar rats	Oral feeding	N/A	12 weeks (90 days)	NOAEL = 1900 mg/kg-day	No toxicologically significant effects on the cardiovascular system	Shaffer *et al.*,1945; ATSDR, 2002	Cardiovascular
Marmoset monkeys	Oral gavage	N/A	Once daily for 13 weeks	NOAEL = 2500 mg/kg-day	No toxicologically significant effects on the cardiovascular system	Kurata *et al.*,1998; ATSDR, 2002	Cardiovascular
Sprague-Dawley rats (M&F)	Oral feeding	0, 0.2, 1.0, and 2.0% (0, 143, 737, 1440 mg/kg-day; M; 0, 154, 797, 1414 mg/kg-day; F) (15 rats per group): 0, 1.0, and 2.0% (0, 737, 1440 mg/kg-day; M; 0, 797, 1414 mg/kg-day; F): 0 and 2.0% (0, 1440 mg/kg-day; M; 0, 1414 mg/kg-day; F) (10 rats per group)	17 weeks: 2 or 6 weeks:	LOAEL = 737 mg/kg-day; NOAEL = 143 mg/kg-day	Significant dose-dependent decrease in the absolute heart weight (M, 17 weeks; $P < 0.05$)	Gray *et al.*, 1977	Heart
Sprague-Dawley rats (M&F)	Oral feeding	0, 0.2, 1.0, and 2.0% (0, 143, 737, 1440 mg/kg-day; M; 0, 154, 797, 1414 mg/kg-day; F) (15 rats per group): 0, 1.0, and 2.0% (0, 737, 1440 mg/kg-day; M; 0, 797, 1414 mg/kg-day; F): 0 and 2.0% (0, 1440 mg/kg-day; M; 0, 1414 mg/kg-day; F) (10 rats per group)	17 weeks: 2 or 6 weeks:	LOAEL = 1414-1440 mg/kg-day; NOAEL = 737-797 mg/kg-day	Significant dose-dependent decrease in the absolute heart weight (M, 2 and 6 weeks; F, 2, 6, and 17 weeks; $P < 0.01$-0.001)	Gray *et al.*, 1977	Heart
Sprague-Dawley rats (M&F)	Oral feeding	0, 0.2, 1.0, and 2.0% (0, 143, 737, 1440 mg/kg-day; M; 0, 154, 797, 1414 mg/kg-day; F) (15 rats per group): 0, 1.0, and 2.0% (0, 737, 1440 mg/kg-day; M; 0, 797, 1414 mg/kg-day; F): 0 and 2.0% (0, 1440 mg/kg-day; M; 0, 1414 mg/kg-day; F) (10 rats per group)	17 weeks: 2 or 6 weeks:	LOAEL = 737-797 mg/kg-day; NOAEL = 143-154 mg/kg-day	Significant dose-dependent decrease in the relative heart weight (M&F, 2 weeks; $P < 0.01$-0.05)	Gray *et al.*, 1977	Heart

Sprague-Dawley rats (M&F)	Oral feeding	0, 0.2, 1.0, and 2.0% (0, 143, 737, 1440 mg/kg-day; M; 0, 154, 797, 1414 mg/kg-day; F) (15 rats per group): 0, 1.0, and 2.0% (0, 737, 1440 mg/kg-day; M; 0, 797, 1414 mg/kg-day; F): 0 and 2.0% (0, 1440 mg/kg-day; M; 0, 1414 mg/kg-day; F) (10 rats per group)	17 weeks: 2 or 6 weeks:	LOAEL = 737 mg/kg-day; NOAEL = 143 mg/kg-day	Significant dose-dependent increase in the relative heart weight (M, 6 weeks; $P < 0.05$)	Gray *et al.*, 1977	Heart
Sprague-Dawley rats (M&F)	Oral feeding	0, 0.2, 1.0, and 2.0% (0, 143, 737, 1440 mg/kg-day; M; 0, 154, 797, 1414 mg/kg-day; F) (15 rats per group): 0, 1.0, and 2.0% (0, 737, 1440 mg/kg-day; M; 0, 797, 1414 mg/kg-day; F): 0 and 2.0% (0, 1440 mg/kg-day; M; 0, 1414 mg/kg-day; F) (10 rats per group)	17 weeks: 2 or 6 weeks:	LOAEL = 1414-1440 mg/kg-day; NOAEL = 737-797 mg/kg-day	Significant dose-dependent increase in the relative heart weight (M&F, 6, 17 weeks; $P < 0.001$-0.05)	Gray *et al.*, 1977	Heart
Sprague-Dawley rats (M&F)	Oral feeding	0, 0.2, 1.0, and 2.0% (0, 143, 737, 1440 mg/kg-day; M; 0, 154, 797, 1414 mg/kg-day; F) (15 rats per group): 0, 1.0, and 2.0% (0, 737, 1440 mg/kg-day; M; 0, 797, 1414 mg/kg-day; F): 0 and 2.0% (0, 1440 mg/kg-day; M; 0, 1414 mg/kg-day; F) (10 rats per group)	17 weeks: 2 or 6 weeks:	LOAEL = 1414-1440 mg/kg-day; NOAEL = 737-797 mg/kg-day	Significant dose-dependent decrease in the relative heart weight (M&F, 2 weeks; $P < 0.05$)	Gray *et al.*, 1977	Heart
Sherman rats	Oral feeding	N/A	52 weeks	NOAEL = 200 mg/kg-day	No toxicologically significant effects on the cardiovascular system	Carpenter *et al.*,1953; ATSDR, 2002	Cardiovascular
Sherman rats (M)	Oral feeding	N/A	104 weeks	NOAEL = 190 mg/kg-day	No toxicologically significant effects on the cardiovascular system	Carpenter *et al.*,1953; ATSDR, 2002	Cardiovascular
Lung/Respiratory							
Rats	Intravenous	NS	Once	NS	Edema of the alveolar wall, infiltration by leukocytes, hemorrhage	Schulz *et al.*,1975; Rubin and Chang, 1978	Lung
Rats	Inhalation	0, 3.39, 6.82, 10.62 mg/L (5 male and 5 female per group)	Once for 4 hours, nose only	LOAEC = 3.39 mg/L	Dark red foci and patches in all groups, but more in DEHP-treated animals (19 out of 30 DEHP-exposed rats vs. 2 out of 10 control rats)	Huls, 1981	Lung
Rats	Inhalation	0, 3.39, 6.82, 10.62 mg/L (5 male and 5 female per group)	Once for 4 hours, nose only	NOAEC = 10.62 mg/L	No change in lung-to-body weight ratios	Huls, 1981	Lung
Human (0-4 weeks)	Inhalation (epi)	Inhalation exposure estimated at 1-4200 µg/hour	PVC respiratory tubes for an unknown period	N/A	Unusual lung disorders (hyaline membrane disease?) at 4 weeks in two infants artificially ventilated with PVC respiratory tubes, DEHP, not MEHP, found in urine	Roth *et al.*,1988	Lung

Rats (F)	Oral dietary	N/A	9 days, during last week of pregnancy and first 2 post-natal days	N/A	In pup lungs, substantial decrease in the number of parenchymal airspaces, significant increase in the airspace mean size, and increases in the number of type II pneumocytes	Magliozzi *et al.*,2003	Lung
Rats (F)	Oral dietary	N/A	9 days, during last week of pregnancy and first 2 post-natal days	N/A	In pup distal lung parenchyma, "alveolar simplification" (increased alveolar volume and decreased number/septation) and increased epithelial and mesenchymal cell proliferation	Rosicarelli and Stefanini, 2009	Lung
Wistar rats	Inhalation	0, 50, 1000 mg/m^3	6 hours/day for 5 days/week for 28 days	LOAEC = 1000 mg/m^3; NOAEC = 50 mg/m^3	Increased lung weight, foam cell proliferation, thickening of the alveolar septa	Klimisch *et al.*,1991	Lung
Marmoset monkeys	Oral gavage	N/A	Once daily for 13 weeks	NOAEL = 2500 mg/kg-day	No toxicologically significant effects on the respiratory system	Kurata *et al.*,1998; ATSDR, 2002	Lung
Sherman rats	Oral feeding	N/A	52 weeks	NOAEL = 200 mg/kg-day	No toxicologically significant effects on the respiratory system	Carpenter *et al.*,1953; ATSDR, 2002	Lung
Sherman rats (M)	Oral feeding	N/A	104 weeks	NOAEL = 190 mg/kg-day	No toxicologically significant effects on the respiratory system	Carpenter *et al.*,1953; ATSDR, 2002	Lung
Fischer 344 rats	Oral feeding	N/A	108 weeks	NOAEL = 2000 mg/kg-day	No toxicologically significant effects on the respiratory system	Rao *et al.*,1990; ATSDR, 2002	Lung
Fischer 344 rats (M&F; 6 wk old)	Oral feeding	0, 100, 500, 2500, 12,500 mg/kg (5.8, 28.9, 146.6, 789.0 mg/kg-day (M); 7.3, 36.1, 181.7, 938.5 mg/kg-day (F); 50-80 rats per sex per group)	78 or 104 weeks	NOAEL = 789.0 – 938.5 mg/kg-day	No toxicologically significant change in the absolute lung weight (M&F)	David *et al.*,2000a	Lung
Fischer 344 rats (M&F; 6 wk old)	Oral feeding	0, 100, 500, 2500, 12,500 mg/kg (5.8, 28.9, 146.6, 789.0 mg/kg-day (M); 7.3, 36.1, 181.7, 938.5 mg/kg-day (F); 50-80 rats per sex per group)	78 or 104 weeks	LOAEL = 28.9 mg/kg-day; NOAEL = 5.8 mg/kg-day	Substantial dose-dependent increase (+10%) in the relative lung weight (M)	David *et al.*,2000a	Lung
Fischer 344 rats (M&F; 6 wk old)	Oral feeding	0, 100, 500, 2500, 12,500 mg/kg (5.8, 28.9, 146.6, 789.0 mg/kg-day (M); 7.3, 36.1, 181.7, 938.5 mg/kg-day (F); 50-80 rats per sex per group)	78 or 104 weeks	LOAEL = 938.5 mg/kg-day; NOAEL = 181.7 mg/kg-day	Substantial increase (+11%) in the relative lung weight (F)	David *et al.*,2000a	Lung
B6C3F$_1$ mice (M&F; 6 week old)	Oral feeding	0, 100, 500, 1500, 6000 mg/kg (0, 19.2, 98.5, 292.2, 1266.1 mg/kg-day (M); 0, 23.8, 116.8, 354.2, 1458.2 mg/kg-day (F); 60-70 rats per sex per group)	79 or 105 weeks	LOAEL = 1266.1 mg/kg-day; NOAEL = 292.2 mg/kg-day	Signficant dose-dependent increase in the relative lung weight at 105 weeks (M; P≤0.05)	David *et al.*,2000b	Lung
B6C3F$_1$ mice (M&F; 6 week old)	Oral feeding	0, 100, 500, 1500, 6000 mg/kg (0, 19.2, 98.5, 292.2, 1266.1 mg/kg-day (M); 0, 23.8, 116.8, 354.2, 1458.2 mg/kg-day (F); 60-70 rats per sex per group)	79 or 105 weeks	NOAEL = 1266.1 – 1458.2 mg/kg-day	No toxicologically significant change in the absolute (M&F) or relative lung (F) weights at 105 weeks	David *et al.*,2000b	Lung

Humans (age 0-2)	Inhalation	246 case subjects and 246 matched controls (Oslo birth cohort)	N/A	NS	Bronchial obstruction, an indicator for the development of asthma, was higher in the presence of PVC in the floors (compared to wood or parquet flooring and painted walls and ceilings (but not related to PVC wall materials). It was also related to the amount of plasticizer-emitting material in the house (DHEP predominant in sedimented dust samples (69% of total) and suspended particulate matter (52% of total)	Jaakkola *et al.*,1999 Øie *et al.*,1997	Lung
Human (M)	Inhalation /dermal (epi)	0.1, 0.2, 0.7 mg/m^3 DEHP and BBP (54 workers)	N/A	N/A	No obstructive lung disease, no dose-related changes in conventional lung function tests	Nielson *et al.*,1985	Lung
Rat	*In vitro*	NS	Incubation for 30 minutes	NS	DEHP did not alter the methacholine dose-response curves of rat tracheal tissue	Doelman *et al.*,1990	Tracheal tissue
Rat	*In vitro*	10^{-4}M MEHP	Incubation for 30 minutes	NS	MEHP induced a reversible dose-dependent increase in the methacholine EC_{50}, suggesting that only continuous exposure might cause bronchial hyperresponsiveness	Doelman *et al.*,1990	Tracheal tissue
Neurological							
Sprague-Dawley (F)	Oral gavage	0, 1500 mg/kg	Once daily for 19 days during Gd 0-20	LOAEL = 1500 mg/kg	Significant decrease in fetal rat brain free cholesterol and sphingomyelin ($P < 0.05$); Significnat reduction in the amounts of monounsaturated and polyunsaturated fatty acids ($P < 0.05$); Significnat reduction of docosahexaenoic acid in cholesterol esters (43% reduction), diacylglycerol (60%), phosphatidylserine (33%), lysophosphatidylcholine (35%), and sphingomyelin (40%; $P < 0.05$) and the levels of arachidonic acid in cholesterol esters and lysophosphatidylcholine (~33% reduction; $P < 0.05$)	Xu *et al.*, 2007	Brain lipids
Human (M)	Inhalation /dermal (epi)	0.1, 0.2, 0.7 mg/m^3 DEHP and BBP (54 workers)	N/A	N/A	Non-dose-related various peripheral nervous system symptoms and signs	Nielson *et al.*,1985	Neural
CD-1 mice (5 wk of age)	Oral feeding	0.01, 0.03, 0.09% (F_0M premate 16, 47, 142; F_0F premate 20, 56, 168; mating 15, 40, 126; gestation 17, 47, 140; lactation 60, 172, 493; F_1M 16, 48, 145; F_1F 19, 56, 171 mg/kg-day	8-9 weeks	LOAELs = 60 and 172, 493, 60 mg/kg-day	Delayed time for surface righting in F on PNd 4, M on PNd 7, and F on PNd 7	Tanaka *et al.*,2002; CERHR, 2006	Neural/behavioral
CD-1 mice (5 wk of age)	Oral feeding	0.01, 0.03, 0.09% (F_0M premate 16, 47, 142; F_0F premate 20, 56, 168; mating 15, 40, 126; gestation 17, 47, 140; lactation 60, 172, 493; F_1M 16, 48, 145; F_1F 19, 56, 171 mg/kg-day	8-9 weeks	NOAEL = 493 mg/kg-day	No toxicologically significant effect on negative geotaxis, cliff avoidance, swimming behavior, olfactory orientation, movement (F_0), exploratory behavior (F_0)	Tanaka *et al.*,2002; CERHR, 2006	Neural/behavioral

Sprague-Dawley rats (M&F)	Oral feeding	0, 0.2, 1.0, and 2.0% (0, 143, 737, 1440 mg/kg-day; M; 0, 154, 797, 1414 mg/kg-day; F) (15 rats per group): 0, 1.0, and 2.0% (0, 737, 1440 mg/kg-day; M; 0, 797, 1414 mg/kg-day; F): 0 and 2.0% (0, 1440 mg/kg-day; M; 0, 1414 mg/kg-day; F) (10 rats per group)	17 weeks: 2 or 6 weeks:	LOAEL = 1414-1440 mg/kg-day; NOAEL = 737-797 mg/kg-day	Significant dose-dependent decrease in the absolute brain weight (M, 17 weeks; F, 6 and 17 weeks; $P < 0.05$-0.001)	Gray *et al.*, 1977	Brain
Sprague-Dawley rats (M&F)	Oral feeding	0, 0.2, 1.0, and 2.0% (0, 143, 737, 1440 mg/kg-day; M; 0, 154, 797, 1414 mg/kg-day; F) (15 rats per group): 0, 1.0, and 2.0% (0, 737, 1440 mg/kg-day; M; 0, 797, 1414 mg/kg-day; F): 0 and 2.0% (0, 1440 mg/kg-day; M; 0, 1414 mg/kg-day; F) (10 rats per group)	17 weeks: 2 or 6 weeks:	LOAEL = 737 mg/kg-day; NOAEL = 143 mg/kg-day	Significant dose-dependent increase in the relative brain weight (M, 17 weeks; $P < 0.01$)	Gray *et al.*, 1977	Brain
Sprague-Dawley rats (M&F)	Oral feeding	0, 0.2, 1.0, and 2.0% (0, 143, 737, 1440 mg/kg-day; M; 0, 154, 797, 1414 mg/kg-day; F) (15 rats per group): 0, 1.0, and 2.0% (0, 737, 1440 mg/kg-day; M; 0, 797, 1414 mg/kg-day; F): 0 and 2.0% (0, 1440 mg/kg-day; M; 0, 1414 mg/kg-day; F) (10 rats per group)	17 weeks: 2 or 6 weeks:	LOAEL = 1414-1440 mg/kg-day; NOAEL = 737-797 mg/kg-day	Significant dose-dependent increase in the relative brain weight (M, 6, 17 weeks; F, 2, 17 weeks; $P < 0.001$-0.01)	Gray *et al.*, 1977	Brain
$B6C3F_1$ mice (M&F; 6 wk old)	Oral feeding	0, 100, 500, 1500, or 6000 mg DEHP/kg feed (19.2, 98.5, 292.2, 1266.1 mg/kg-day for M; 23.8, 116.8, 354.2, 1458.2 mg/kg-day for F; 10-15 mice per sex per group)	79 or 105 weeks	LOAEL = 292.2 mg/kg-day; NOAEL = 98.5 mg/kg-day	Significant decrease in the absolute brain weight at 105 weeks (M; $P \leq 0.05$)	David *et al.*, 2000b	Brain
$B6C3F_1$ mice (M&F; 6 wk old)	Oral feeding	0, 100, 500, 1500, or 6000 mg DEHP/kg feed (19.2, 98.5, 292.2, 1266.1 mg/kg-day for M; 23.8, 116.8, 354.2, 1458.2 mg/kg-day for F; 10-15 mice per sex per group)	105 weeks	NOAEL = 1266.1 mg/kg-day	No toxicologically significant change in the relative brain weight (M)	David *et al.*, 2000b	Brain

[1] Gray highlighted cells represent studies with single exposure doses. Clear cells represent studies with multiple exposure doses.

[2] Red text highlights studies that are acute in exposure duration (0 to 14 days). Blue text highlights studies that have subchronic exposure durations (14 to 364 days). Purple text highlights studies that have chronic exposure durations (365+ days). Yellow text highlights initiation/promotion studies. Green text highlights carcinogenicity studies of any duration.

[3] Yellow highlighted areas have been chosen as toxicological endpoints and used in the calculation of acute, subchronic, and chronic ADI's for the general population, children, and men and women of childbearing age.

[4] Unfilled cells in the table are instances in which study details were not described in the reviewed publications.

[5] Conflicting entries between reviewed materials were reported as {entry}.

[6] Ld = Lactation day

[7] PPd = Postpartum day

[8] Gd = Gestation day

[9] GL = Guideline study

[10] GLP = Good Laboratory Practices used in the study

[11] SER = Smooth endoplasmic reticulum

[12] N/A = Not available or specified

[13] NOAELs and LOAELs reported in carcinogenicity studies do not imply that threshold carcinogenic effects apply for this endpoint, but are the lowest dose level associated with a carcinogenic effect (similar to "cancer effect levels" of ATSDR)

Appendix 3. Critical Study Reviews

Andrade *et al.* (2006b) investigated the reproductive effects of DEHP on adult male offspring exposed while *in utero* or postnatally via lactation. Pregnant female Wistar rats were gavage dosed daily during Gd 6 to Ld 21 with 0.015, 0.045, 0.135, 0.405, 1.215, 5, 15, 45, 135, and 405 mg DEHP/kg-day. The low dose range was used to emulate the estimated median daily intake of DEHP in the general German population (0.0138 mg/kg-day). Following dosing, adult male rats were raised on DEHP-free feed until PNd 144 ± 7 days. At this time, the rats were sacrificed, trunk blood harvested for testosterone determination, and gross pathology of the external genitalia, testicular position, and the presence of small/large testes were performed. Weights were then measured for the testes, epidiymides, ventral prostate, seminal vesicle (without fluid), liver, kidneys, spleen, and thymus. The left testicle was then fixed and processed for histopathology using hematoxylin and eosin, testicular morphometry (using macroscopically normal testes), and cell counts (Sertoli cell number and leptotene spermatocyte to Sertoli cell ratio). The right testis was used to estimate sperm production and morphology. A reduction of 20% or more in daily sperm production was considered biologically relevant because the sperm production of humans is typically near the threshold for reproductive competency. A decrease in 20% (i.e., from 48 to 55 million sperm/mL to 40 million sperm/mL) could, therefore, change a substantial proportion of the man population from fertile to subfertile. A threshold of greater than 10% abnormal sperm was chosen as biologically relevant, since normal control rats rarely display more than 10% abnormal sperm. Sexual behavior, fertility, and reproduction were also judged by mating 110-day or 130-day old treated male rats with unexposed females. Indices for time-to-mating, mating, and pregnancy were calculated. Filmed matings were also scored for mount and intromission latencies, intromission frequency, ejaculatory and post-ejaculatory latencies, and the number of intromissions until ejaculation. Also, following a successful mating (with 110-day old males), the rat dams were sacrificed on Gd 21, the uterus excised, and the weight and status (live/dead) of any fetuses, implantation sites, and resorptions dertermined.

Body weight and grossly normal testes and epididymides weights of 144-day old adults were not affected by any DEHP dose (Table A3.1). The liver, kidney, spleen, and thymus weights were also not significantly different from controls at this time (data not shown). The seminal vesicle (plus coagulating gland) weight was significantly decreased at the highest dose ($P < 0.05$). The weight of the ventral prostate was also substantially, but not significantly, reduced at this dose. Testosterone production was significantly increased in the 0.045, 0.405, and 405 mg/kg-day dose groups ($P < 0.05$).

Table A3.1 Body and Organ Weights of Adult Male Rats Exposed to DEHP from Gd 6 to Ld 21 (Andrade *et al.*, 2006b)											
	Maternal DEHP dose (mg/kg-day)										
Parameter	Control	0.015	0.045	0.135	0.405	1.215	5.0	15.0	45.0	135.0	405.0
# rats (litters)	20 (16)	20 (11)	19 (13)	20 (13)	20 (15)	20 (16)	20 (13)	20 (12)	20 (11)	20 (14)	20 (12)
Mean body weight ± SE (g)	454 ± 8.84	441 ± 6.63	456 ± 11.0	435 ± 8.84	433 ± 7.78	453 ± 10.2	448 ± 6.84	442 ± 6.28	430 ± 7.25	445 ± 10.25	435 ± 6.96
Mean testis weight ± SE (g)[e]	1.82 ± 0.03a	1.90 ± 0.02[d]	1.85 ± 0.04	1.86 ± 0.02	1.84 ± 0.03	1.93 ± 0.03	1.83 ± 0.02	1.83 ± 0.04	1.82 ± 0.03	1.94 ± 0.04	1.94 ± 0.03b
Mean epididymis ± SE (mg)[e]	611 ± 10.2a	628 ± 10.5	620 ± 14.6	623 ± 10.5	613 ± 11.0	646 ± 12.2	590 ± 10.3	615 ± 12.6	617 ± 10.9	648 ± 11.9	616 ± 12.7
Mean semin. vesic. ± SE (mg)	853 ± 24.1	898 ± 20.9	897 ± 28.3	838 ± 23.5	835 ± 28.4	825 ± 19.6	813 ± 21.0	845 ± 24.6	840 ± 23.8	842 ± 22.1	770 ± 19.3*
Mean prostate ± SE (mg)	438 ± 16.8	415 ± 18.9	473 ± 28.1	449 ± 18.8	433 ± 22.6	451 ± 17.6	448 ± 15.1	410 ± 17.5	420 ± 30.0	410 ± 19.0	366 ± 13.5
Mean testostero. ± SE (ng/mL)	3.5 ± 0.46c	6.0 ± 0.92	6.4 ± 0.85*	4.2 ± 0.63	5.6 ± 0.71*	4.8 ± 0.57	3.9 ± 0.45	5.2 ± 0.69	3.8 ± 0.36	4.6 ± 0.86	7.4 ± 1.21*

* $P < 0.05$

[a] N = 19 (15), [b] N = 19 (12), [c] N = 19(16)

[d] one animal in this group had an enlarged testis (2.7 g)

[e] macroscopically abnormal organs (i.e., small < 1.3 g) were excluded from this Table and discussed below

Testicular and epididymal malformations were observed in a few rat groups. One animal in the control group had bilateral small scrotal testes. Two male rats in the high dose group (405 mg/kg-day) had small scrotal testes and one male had bilateral small scrotal testes. Both the control and high dose testis had tubular atrophy and a substantial reduction in germ cell layers. The testis from the high dose group also had multifocal Leydig cell hyperplasia and enlarged cells with large or multiple nuclei. The aberrant testis from the 0.015 mg/kg-day dose group had dilatation of the lumen of the tubule, a reduction in germ cell layering, and increased desquamation into the tubule lumen. One male each in the 5, 135, and 405 mg/kg-day dose groups had a small undescended or ectopic testis that was located unilaterally (right side located in an inguinal pouch). Histologically, undescended testes were characterized by reduced spermatogenesis (decreased frequency of spermatocytes and spermatids). The Ectopic testis in the 405 mg/kg-day male had a substantial reduction of germ cells in many tubules, and singular tubular layers or no cells at all. In this testis, enlarged cells with large or multiple nuclei also showed desquamation into the lumen. In males with small ectopic or scrotal testes, the associated ipsilateral epididymides were also grossly smaller. Hypospadias or preputial separation malformations were not observed at any dose.

In morphologically normal testis in the high dose group, three out of nine testes had histopathological abnormalities. In two of these testes, a reduction in germ cell layers and a loss of stratification were observed in the seminiferous tubules. In the other testis, a marginal case of focal Leydig cell hyperplasia was observed. This animals also had testosterone levels 6.2-fold higher than control animals. No histopathological alterations were observed in testis of any other dose group.

Both sperm production and morphology were adversely affected by DEHP administration. Macroscopically small testes were associated with low sperm production. Sperm production in the remaining "normal" testes was also observed in groups dosed with 0.045 mg/kg-day and higher when compared to the concurrent control (Table A3.2). A significant decrease was also reported in groups dosed with 1.215 mg/kg-day and higher (except 5.0 mg/kg-

day) when compared to a recent historical control. When comparing the number of rats with greater than 34 million sperm per testis to the number of rats with less than 34 million sperm per testis a similar pattern was discovered (Table A3.2). The number of animals with > 10% versus < 10% abnormal sperm was significantly different from controls only in the 0.045 and 0.135 mg/kg-day dose groups.

Table A3.2 Sperm Production and Morphology of Adult Male Rats Exposed to DEHP from Gd 6 to Ld 21 (Andrade *et al.*, 2006b)

Parameter	Maternal DEHP dose (mg/kg-day)											
	Concurrent Control	Historical Control	0.015	0.045	0.135	0.405	1.215	5.0	15.0	45.0	135.0	405.0
# rats (litters)	19 (15)	91 (58)	20 (11)	19 (13)	20 (13)	20 (15)	20 (16)	20 (13)	20 (12)	20 (11)	20 (14)	19 (12)
Mean daily sperm production ± SE ($x10^6$)	42.5 ± 1.12	37.6 ± 0.69	41.3 ± 0.96	36.8 ± 1.42*	38.8 ± 1.36*	37.2 ± 1.01*	35.8 ± 1.38*!	36.6 ± 0.90*	31.9 ± 0.64*!	34.5 ± 0.82*!	33.3 ± 0.87*!	31.9 ± 0.91*!
Percent of concurrent control	-	88	97	87	91	88	84	86	75	81	78	75
Rats with > 34/< 34 $x10^6$ sperm per testis	19/1	67/25	18/2	12/7*	17/3	15/4	12/8*	15/5	6/14*!	12/8*	9/11*!	5/15*!
Rats with > 10%/< 10% abnormal sperm	17/0	88/0	18/1	13/6*!	15/5!	19/1	18/2	18/2	19/1	18/2	19/1	15/2

* statistically different from the concurrent control group; $P < 0.05$

! statistically different from the historical control group; $P < 0.05$

Testicular morphometry and cell counts of morphologically normal testes were not changed substantially by exposure to DEHP.The relative and absolute volume of seminiferous tubules, the total tubular length, the number of Sertoli cells/testis, and the ratio of leptotene spermatocytes to Sertoli cells were not significantly different than control testes (data not shown). The mean diameter of seminiferous tubules was only significantly different from controls in the 15 mg/kg-day dose group ($P < 0.05$).

Reproductive performance in DEHP-exposed animals was not significantly different than controls. Time to mating, mating index, pregnancy index, fetal weights, total resorptions, viable fetuses, implantation sites, the body weight gain and the number of males cohabited/number of pregnant females were not different in control and treated rats. Similarly, no significant changes were observed in mating behavior, with the exception of a slightly significant decrease in mounting latency in the 5 and 15 mg/kg-day dose groups ($P < 0.05$; data not shown).

The author summarizes by noting that reductions in sperm production were observed at the 15 to 405 mg/kg-day (19 to 25% decrease) and 0.045 to 5 mg/kg-day (9 to 15% decrease) doses. The latter changes were deemed not biologically significant, since they were within the variation of the recent historical controls and did not reach the threshold of 20% reduction in daily sperm production that could result in subfertility in humans. The author also concluded that a lack of changes in reproductive performance was expected, since previous studies have demonstrated that decreases of up to 90% in sperm production did not affect mice and rat fertility. The authors further conclude that changes in sperm production are not associated with changes in the number of Sertoli cells or their supportive capacity. Reproductive tract malformations such as small scrotal testes (3 rats at the 405 mg/kg-day dose) and

cryptorchidism/undescended testes (at the 5, 135, and 405 mg/kg-day doses) were deemed to be biologically relevant. Cryptorchid tests were primary inguinal, in contrast the abdominal placement in other studies. Histological changes in the affected testes ranged from reduced spermatogenesis to severe atrophy. Increases in testosterone production were reported in 3 dose groups (0.045, 0.405, 405 mg/kg-day). The high dose increase in testosterone occurred coincidentally with histological hyperplasia of Leydig cells and has not been seen in other studies with *in utero* and lactational exposures. Increased Leydig cell hyperplasia and testosterone have been reported, however, in rats treated postnatally with DEHP. The lack of significant changes in male reproductive behavior did not reinforce previous changes demonstrated in male rat brain aromatase activity at the same dose levels, suggesting that changes in aromatase activity in newborn males did not impair male sexual behavior later during adulthood. The author further postulated that decreases in the seminal vesicle weight may be related to abnormal tissue organization. The author concluded by suggesting that many of the effects seen resembled testicular dysgenesis syndrome in humans, that the severity of effects observed in both species were directly related to the dose of DEHP, and that the respective LOAELs from sperm production and reproductive tract anomalies were 15 and 5 mg/kg-day, respectively (NOAEL = 1.215 mg/kg-day).

CERHR (2006) investigated the effects of exposure to DEHP on female reproductive organs in marmoset monkeys. DEHP-induced effects can be seen in Table A3.3.

Table A3.3 Marmoset Ovary and Uterine Effect Levels
(CERHR, 2006)

Organ Weight (% of control)		Treatment Dose (mg/kg-day)			Calculated Benchmark dose (mg/kg-day)			
		100	500	2500	[1]BMD_{10}	[2]$BMDL_{10}$	[3]$BMD_{1\ SD}$	[4]$BMDL_{1\ SD}$
Ovary	Absolute	100	180 ($P < 0.05$)	169 ($P < 0.05$)	507	259	2063	1196
	Relative	106	167 ($P < 0.05$)	162 ($P < 0.05$)	572	303	1999	1173
Uterus	Absolute	106	188 ($P < 0.05$)	168	562	258	2545	1356
	Relative	100	167 ($P < 0.05$)	150	677	296	2759	1374

[1]BMD_{10} – benchmark dose associated with a 10% effect
[2]$BMDL_{10}$ – benchmark dose associated with the lower 95% confidence interval around the BMD_{10} estimate
[3]$BMD_{1\ SD}$ – benchmark dose equivalent to one standard deviation of the control distribution
[4]$BMDL_{1\ SD}$ – benchmark dose equivalent to one standard deviation of the lower 95% confidence interval distribution

David *et al.* (2000a) determined the chronic effects of dietary DEHP administration on rats. Male and female Fischer 344 rats were fed 0, 100, 500, 2500, or 12,500 mg DEHP/kg feed (5.8, 28.9, 146.6, 789.0 mg/kg-day for M; 7.3, 36.1, 181.7, and 938.5 mg/kg-day for F) for up to 104 weeks. Rat mortality and morbidity was evaluated twice daily. Body weight and food consumption were determined weekly for 17 weeks following initial dosing and every month afterwards. Gross pathology and organ histopathology was assessed at week 78 and following termination at week 105. Organ weights (brain, lungs, liver, spleen, kidney, testes, and uterus)

and terminal body weight were also measured at necropsy. Blood and urine samples for clinical chemistry and hematology were collected on weeks 26, 52, 78, and 104.

Animals treated with high doses of DEHP had slightly reduced survival, significantly reduced dose-related body weights, food consumption, BUN (M, dose-related, 26 to 105 weeks; F, dose-related, 26 to 78 weeks), globulin (M, dose-related, 26 to 105 weeks; F, dose-related, 26, 78, 105 weeks), albumin (M, dose-related, 26 to 105 weeks; F, dose-related, 26 to 78 weeks), and testes weight (absolute and relative), and significantly increased liver (dose-related, absolute and relative), kidney (dose-related, absolute and relative), lung (relative), and brain (relative) weights (Table A3.4, A3.5, A3.6, A3.7). These alterations were associated with significantly increased incidence or severity of Kupffer cell pigmentation (M&F), spongiosis hepatis (dose-related, M), mineralization of the renal papilla (dose-related, M&F), chronic progressive nephropathy (M), renal tubule pigmentation (dose-related, M&F), aspermatogenesis (dose-related, M), pituitary castration cells (M), pancreatic acinar cell adenoma (5/59, 8% versus controls 0/60), mononuclear cell leukemia (27/65, 42% versus controls 15/65, 23%), and a decrease in testes interstitial cell tumors (M; Table A3.4, A3.5, A3.6, A3.7). Similar effects were observed at lower doses in some target organs. LOAELS for substantial effects in the liver (28.9 mg/kg-day for substantial absolute and relative weight increase of 10% and 14% in males; NOAEL = 5.8 mg/kg-day), kidney (5.8 mg/kg-day for increased incidence and severity of mineralization of the renal papilla), lung (146.6 mg/kg-day for relative weight increase; NOAEL = 28.9 mg/kg-day), and testes (5.8 mg/kg-day for substantially increased incidence [6%] of aspermatogenesis) have been determined.

Table A3.4 DEHP-induced Hepatic Alterations in Fischer 344 Rats (David *et al.,* 2000a)

mg/kg-day	Weights at 104 weeks		Lesions at 78 weeks	Lesions at 104 weeks	
Male rats	Mean absolute liver weights (g)	Mean relative liver weights (g)	Kupffer cell pigmentation (# animals with lesion/total examined)	Kupffer cell /hepatocyte pigmentation: incidence (% incidence) severity	Spongiosis hepatis: incidence (% incidence)
0.0	8.96 ± 1.19	2.701 ± 0.295	0/10	0/80 (0%) 0.0	3/80 (4%)
5.8	9.13 ± 1.36	2.737 ± 0.357	-	0/50 (0%) 0.0	3/50 (6%)
28.9	9.87 ± 2.84 (+10%)	3.086 ± 1.273 (+14%)	-	0/55 (0%) 0.0	3/55 (6%)
146.6	11.11 ± 2.43*	3.462 ± 0.716*	0/10	1/65 (0%) 0.0	11/65* (17%)
789.0	14.64 ± 2.76*	4.947 ± 0.874*	9/10*	44/80* (55%) 0.9	11/80* (14%)
Female rats					
0.0	6.54 ± 0.70	2.908 ± 0.444	0/10	0/80 (0%) 0.0	0/80 (0%)
7.3	7.03 ± 1.09	3.001 ± 0.568	1/10	0/50 (0%) 0.0	0/50 (0%)
36.1	6.80 ± 0.87	2.851 ± 0.303	-	0/55 (0%) 0.0	0/55 (0%)
181.7	8.27 ± 1.43*	3.575 ± 0.795*	-	1/65 (0%) 0.0	1/65 (2%)
938.5	10.84 ± 1.93*	5.227 ± 0.981*	7/10*	24/80* (30%) 0.3	1/80 (1%)

* and grayed cells indicate a statistical difference from controls at $P \leq 0.05$

** and grayed cells indicate a statistical difference from the highest treatment dose at $P \leq 0.05$

Table A3.5 DEHP-induced Kidney Alterations in Fischer 344 Rats (David *et al.*,2000a)									
mg/kg-day	Blood Urea Nitrogen at 78 weeks (mg/dL)	Weights at 104 weeks		Lesions at 78 weeks			Lesions at 104 weeks		
Male rats		Mean absolute kidney weights (g)	Mean relative kidney weights (g)	Mineralization of the Renal Papilla incidence	Chronic Progressive Nephropathy incidence (severity)	Renal Tubule Pigmentation incidence (severity)	Mineralization of the Renal Papilla (% incidence) severity	Chronic Progressive Nephropathy (% incidence) severity	Renal Tubule Pigmentation (% incidence) severity
0.0	16 ± 1.4	2.52 ± 0.20	0.768 ± 0.056	2/10	10/10 (1.3)	10/10 (1.0)	12/60 (20%) 0.2	60/60 (100%) 1.7	58/60 (97%) 1.1
5.8	15 ± 1.6	2.54 ± 0.19	0.767 ± 0.045	-	-	-	19/50 (38%)* 0.4	49/50 (98%) 1.6	49/50 (98%) 1.3
28.9	14 ± 0.9	2.53 ± 0.20	0.792 ± 0.107	-	-	-	27/51 (53%)* 0.5	51/51 (100%) 1.6	51/51 (100%) 1.5
146.6	15 ± 1.9	2.68 ± 0.26*	0.843 ± 0.087*	3/10	10/10 (1.1)	10/10 (1.2)	31/62 (50%)* 0.5	60/62 (97%) 1.5	62/62 (100%) 1.8
789.0	20 ± 1.9*	2.84 ± 0.25*	0.975 ± 0.088*	5/10	10/10 (1.8)	10/10 (2.4)	45/62 (76%)* 1.0	62/62 (100%) 2.2*	62/62 (100%) 2.3
Female rats									
0.0	15 ± 1.1	1.78 ± 0.11	0.803 ± 0.124	0/10	4/10 (1.0)	10/10 (1.0)	17/60 (28%) 0.3	53/60 (88%) 1.1	60/60 (100%) 1.4
7.3	16 ± 0.8	1.84 ± 0.17	0.796 ± 0.107	-	-	-	15/50 (30%) 0.3	47/50 (94%) 1.2	50/50 (100%) 1.4
36.1	17 ± 7.1	1.79 ± 0.12	0.758 ± 0.052	-	-	-	15/50 (30%) 0.3	48/50 (96%) 1.1	49/50 (98%) 1.3
181.7	16 ± 1.2	1.84 ± 0.12	0.812 ± 0.110	1/10	6/10 (1.0)	10/10 (1.0)	13/60 (22%) 0.2	54/60 (90%) 1.0	60/60 (100%) 1.5
938.5	19 ± 1.8*	1.91 ± 0.11*	0.934 ± 0.062*	2/10	5/10 (1.0)	10/10 (2.3)	20/61 (33%) 0.3	55/61 (90%) 1.1	61/61 (100%) 2.3

* and grayed cells indicate a significant difference from control at $P \leq 0.05$

Average weights are ± the standard deviation

Severity = the mean severity grade based on a range of 1 (minimal) to 4 (severe)

Table A3.6 DEHP-induced Lung Alterations in Fischer 344 Rats at 105 Weeks (David *et al.*,2000a)		
Male rats (mg/kg-day)	Mean absolute lung weights (g)	Mean relative lung weights (g)
0.0	1.56 ± 0.17	0.475 ± 0.053
5.8	1.56 ± 0.17	0.471 ± 0.054
28.9	1.67 ± 0.40	0.523 ± 0.141
146.6	1.77 ± 0.47	0.561 ± 0.163*
789.0	1.60 ± 0.23	0.553 ± 0.130*
Female rats (mg/kg-day)		
0.0	1.18 ± 0.17	0.534 ± 0.130
7.3	1.30 ± 0.29	0.567 ± 0.168
36.1	1.16 ± 0.10	0.492 ± 0.051
181.7	1.23 ± 0.26	0.548 ± 0.188
938.5	1.21 ± 0.18	0.594 ± 0.103

* and grayed cells indicate a significant difference from control at $P \leq 0.05$

Average weights are ± the standard deviation

Table A3.7 DEHP-induced Chronic Reproductive Alterations in Fischer 344 Rats (David *et al.*,2000a)								
mg/kg-day	Weights at 104 weeks		Lesions at 78 weeks			Lesions at 104 weeks		
Male rats	Mean absolute testes/uterus weights (g)	Mean relative testes/uterus weights (g)	Interstitial cell tumor of testes - incidence	Aspermato genesis - incidence	Pituitary castration cells - incidence	Interstitial cell tumor of testes - Incidence (% incidence)	Aspermatogenesis - Incidence (% incidence)	Pituitary castration cells - Incidence (% incidence) severity
0.0	5.92 ± 1.90	1.812 ± 0.596	9/10	0/10	0/10	59/64 (92%)	37/64 (58%)	1/60 (2%) 0.0
5.8	6.05 ± 1.79	1.830 ± 0.556	-	-	-	45/50 (90%)	34/50 (64%)	0/50 (0%) 0.0
28.9	5.77 ± 1.67	1.782 ± 0.507	-	-	-	50/55 (91%)	43/55* (78%)	0/51 (0%) 0.0
146.6	6.28 ± 2.38	1.957 ± 0.692	10/10	0/10	0/10	60/65 (92%)	48/65* (74%)	1/52 (2%) 0.0
789.0	2.19 ± 1.15*	0.741 ± 0.369*	3/10*	10/10*	7/10*	20/64* (31%)	62/64* (97%)	30/60* (50%) 1.1
Female rats								
0.0	0.99 ± 1.09	0.434 ± 0.442	-	-	-	-	-	-
7.3	1.46 ± 3.03	0.629 ± 1.310	-	-	-	-	-	-
36.1	0.93 ± 1.24	0.409 ± 0.633	-	-	-	-	-	-
181.7	1.02 ± 1.05	0.462 ± 0.538	-	-	-	-	-	-
938.5	0.90 ± 0.63	0.437 ± 0.297	-	-	-	-	-	-

* and grayed cells indicate a significant difference from control at $P \leq 0.05$

** and grayed cells indicate a significant difference from high dose at $P \leq 0.05$

Average weights are ± the standard deviation

Severity = the mean severity grade based on a range of 1 (minimal) to 4 (severe)

David *et al.* (2000b) determined the chronic effects of administration of DEHP in the diet on mice. Male and female $B6C3F_1$ mice were fed 0, 100, 500, 1500, or 6,000 mg DEHP/kg feed (19.2, 98.5, 292.2, 1266.1 mg/kg-day for M; 23.8, 116.8, 354.2, 1458.2 mg/kg-day for F) for up to 104 weeks. Mouse mortality and morbidity was evaluated twice daily. Clinical signs were and behavioral issues were determined daily. Body weight and food consumption were determined weekly for 17 weeks following initial dosing and every month afterwards. Gross pathology and organ histopathology was assessed at week 79 and following termination at week 105. Organ weights (brain, lungs, liver, spleen, kidney, testes, and uterus) and terminal body weight were also measured at necropsy. Blood and urine samples for clinical chemistry and hematology were collected on weeks 26, 52, 78, and 104.

Mice treated with high doses of DEHP had significantly reduced survival, primarily in male animals. The cause of death was attributed to hepatocellular neoplasia (10/30 mice in 292.2, and 15/56 mice in 1266.1 mg/kg-day). High dose male mice also had significantly reduced body weights and body weight gain. Similar reductions were noted occasionally in high dose females. Treatment-related patterns in food consumption were note demonstrated. Clinical chemistry and hematology were not significantly different than controls at 26, 52, and 78 weeks. At 104 weeks, however, serum potassium was significantly lower in treated male mice than controls in a dose-dependent fashion ($P \leq 0.05$; data not shown), BUN and calcium were non-significantly increased in a dose-dependent fashion, and phosphorous was non-significantly decreased in the high dose group. Urine potassium also decreased at higher dose levels, but was probably due to higher urine volumes in high dose animals. The author concluded that serum potassium was probably not biologically relevant for this reason. When observing hematology

data, a significant dose-dependent reduction in the mean corpuscular hemoglobin was reported in male mice (Table A3.8). The mean corpuscular hemoglobin was also significantly reduced in male and female high dose group animals. Non-significant increases in the myeloid/erythroid ratio were also seen in both male and female animals of the higher dose groups. Other changes in hematology were not significant or not consistent between genders.

Table A3.8 Average Hematology Results for Mice Exposed to DEHP for 105 weeks (David *et al.*, 2000b)

Hematology Parameter (±SD)	Male mice (mg/kg-day)					Female Mice (mg/kg-day)				
	0	19.2	98.5	292.2	1266.1	0	23.8	116.8	354.2	1458.2
RBC (10^6/μL)	8.82 ± 1.6	9.53 ± 0.3	9.32 ±0.5	9.52 ± 0.5	10.21 ± 1.8	9.82 ± 1.5	9.12 ± 0.4	9.31 ± 1.0	9.4 ± 0.7	9.2 ± 1.5
HgH (mg/dL)	14.5 ± 1.9	14.9 ± 0.4	14.6 ± 0.7	14.9 ± 0.9	15.3 ± 1.9	15.7 ± 2.3	14.8 ± 0.6	14.8 ± 1.3	14.9 ± 0.8	14.5 ± 1.5
Hct (%)	41.5 ± 5.5	43.0 ± 1.0	42.5 ± 1.9	42.9 ± 2.6	45.3 ± 6.6	44.7 ± 6.7	41.7 ± 1.3	42.0 ± 3.6	42.9 ± 2.3	42.5 ± 4.4
MCV (fL)	47.8 ± 4.3	45.1 ± 0.9	45.6 ± 0.7	45.0 ± 1.1	44.6 ± 2.4	45.5 ± 0.9	45.8 ± 0.9	45.3 ± 2.6	45.7 ± 1.4	46.8 ± 4.0
Mean Corp. Hemo. (pg)	16.7 ± 1.5	15.7 ± 0.3*	15.7 ± 0.3*	15.6 ± 0.3*	15.1 ± 0.9*	16.0 ± 0.3	16.2 ± 0.4	16.0 ± 1.2	15.9 ± 0.5	16.0 ± 1.4
Mean Corp. Hemo. Content (g/dL)	34.9 ± 0.4	34.7 ± 0.5	34.3 ± 0.5	34.6 ± 0.5	33.9 ± 0.7*	35.2 ± 0.4	35.4 ± 0.6	35.3 ± 0.9	34.9 ± 0.4	34.1 ± 0.6*
Reticulocytes (% RBC)	2.6 ± 3.7	1.4 ± 0.4	1.4 ± 0.9	1.8 ± 1.0	1.8 ± 1.2	1.5 ± 1.0	1.7 ± 1.5	2.0 ± 1.9	1.6 ± 0.6	2.7 ± 2.7
Platelets (10^3/μL)	1463 ± 304	1224 ± 128	1305 ± 143	1092 ± 378	1156 ± 332	848 ± 170	732 ± 166	851 ± 164	854 ± 169	737 ± 191
WBC (10^3/μL)	8.3 ± 8.6	5.9 ± 2.4	3.9 ± 0.8	6.3 ± 6.8	5.1 ± 2.4	3.5 ± 2.0	5.6 ± 6.0	4.0 ± 1.8	2.6 ± 0.9	5.0 ± 3.7
Myeloid/Erythroid ratio	2.56 ± 0.6	2.43 ± 0.7	3.54 ± 1.5	3.27 ± 1.3	3.01 ± 1.1	2.09 ± 0.6	2.48 ± 0.5	2.96 ± 1.8	2.55 ± 0.5	2.69 ± 1.0

* $P \leq 0.05$

Body weights at mouse termination were significantly reduced for both males and females of the high dose group (Table A3.9)

Table A3.9 DEHP-induced Alterations in $B6C3F_1$ Mice Terminal Body Weight at 105 Weeks (David *et al.*, 2000b)

Gender	mg/kg-day	Sample Size	Terminal Body Weight (g)
Male mice	0.0	42	28.6 ± 3.0
	19.2	43	29.9 ± 3.1
	98.5	39	29.3 ± 1.8
	292.2	41	28.2 ± 3.3
	1266.1	19	25.8 ± 3.1 ($P \leq 0.05$)
Female mice	0.0	36	26.5 ± 2.8
	23.8	37	26.7 ± 2.8
	116.8	40	26.2 ± 2.1
	354.2	39	26.4 ± 2.9
	1458.2	35	24.9 ± 2.7 ($P \leq 0.05$)

Absolute and relative liver weights were significantly increased in both male and female mice following exposure to DEHP when compared to controls ($P \leq 0.05$; Table A3.10). In the high dose group, significant increased in hepatocyte pigmentation, cytoplasmic eosinophilia, hepatic inflammation were observed both at 79 and 104 weeks. Hepatocellular enlargement was also reported for the high dose group for many mice (67/70, M; 68/70, F), but not in controls (0/70, M; 1/70, F; data not shown). Other liver pathologies (including spongiosis hepatis) were not observed following gross and histopathological analysis.

Table A3.10 DEHP-induced Hepatic Alterations in B6C3F$_1$ Mice (David *et al.,* 2000b)								
mg/kg-day	Weight at 105 weeks		Lesions at 79 weeks			Lesions at 104 weeks		
Male mice	Mean absolute liver weights (g)	Mean relative liver weights (g)	Hepatocyte pigmentation (# animals with lesion/total examined; % incidence)	Increased cytoplasmic eosinophilia (# animals with lesion/total examined; % incidence)	Chronic hepatic inflammation (# animals with lesion/total examined; % incidence)	Hepatocyte pigmentation (# animals with lesion/total examined; % incidence)	Increased cytoplasmic eosinophilia (# animals with lesion/total examined; % incidence)	Chronic hepatic inflammation (# animals with lesion/total examined; % incidence)
0.0	1.51 ± 0.24	5.334 ± 0.986	0/15	0/15	0/15	1/70 (1%)	0/70 (0%)	34/70 (49%) 0.5
19.2	1.60 ± 0.30	5.380 ± 1.035	0/10	0/10	0/10	0/60 (0%)	0/60 (0%)	28/60 (47%) 0.5
98.5	1.73 ± 0.69*	5.967 ± 2.754	0/10	0/10	0/10	0/65 (0%)	0/65 (0%)	27/65 (42%) 0.4
292.2	1.92 ± 0.54*	6.961 ± 2.491*	0/10	0/10	0/10	1/65 (0%)	0/65 (0%)	35/65 (54%) 0.6
1266.1	2.37 ± 0.59*	9.234 ± 2.522*	15/15*	15/15*	15/15*	67/70 (96%)*	69/70 (99%)*	51/70 *(73%) 0.9
Female mice								
0.0	1.63 ± 0.55	6.150 ± 1.891	0/15	0/15	0/15	0/70 (0%)	1/70 (0%)	50/70 (71%) 0.8
23.8	1.74 ± 0.97	6.437 ± 3.293	0/10	0/10	0/10	0/60 (0%)	0/60 (0%)	34/60 (57%) 0.6
116.8	1.61 ± 0.48	6.102 ± 1.608	0/10	0/10	0/10	0/65 (0%)	0/65 (0%)	53/65 (82%) 0.9
354.2	1.73 ± 0.32	6.566 ± 0.944*	0/10	0/10	0/10	0/65 (0%)	0/65 (0%)	49/65 (75%) 0.8
1458.2	2.58 ± 0.84*	10.279 ± 2.621*	15/15*	15/15*	15/15*	55/70 (79%)*	70/70 (100%)*	58/70 (83%) 1.1

* and grayed cells indicate a statistical difference from controls at P≤0.05

** and grayed cells indicate a statistical difference from the highest treatment dose at P≤0.05

Absolute and relative kidney weights were significantly decreased in both male and female mice following exposure to DEHP when compared to controls ($P \leq 0.05$; Table A3.11). Decreases in weight inversely paralleled blood urea nitrogen concentrations. In the 354.2 and 1458.2 mg/kg-day dose groups, the incidence of chronic progressive nephropathy (CPN) for female mice was significantly higher than in control groups ($P \leq 0.05$) in both 78 and 105 week specimens. The severity of lesion was also substantially increased in a dose-dependent fashion in these groups and in male mice following high-dose treatments. Other renal pathologies were not observed following gross and histopathological analysis.

Table A3.11 DEHP-induced Kidney Alterations in B6C3F$_1$ Mice (David *et al.,* 2000b)					
mg/kg-day	Blood Urea Nitrogen at 78 weeks (mg/dL)	Weights at 104 weeks		Lesions at 78 weeks	Lesions at 105 weeks
Male mice		Mean absolute kidney weights (g)	Mean relative kidney weights (g)	Chronic Progressive Nephropathy incidence (severity)	Chronic Progressive Nephropathy incidence (% incidence) severity
0.0	24 ± 4.9	0.72 ± 0.07	2.561 ± 0.236	10/10 (1.2)	54/60 (90%) 1.1
19.2	26 ± 4.5	0.71 ± 0.07	2.438 ± 0.189	10/10 (1.0)	56/60 (93%) 1.0
98.5	32 ± 20.9	0.68 ± 0.05	2.345 ± 0.163*	9/10 (1.0)	52/60 (87%) 0.9
292.2	30 ± 10.3	0.61 ± 0.08*	2.242 ± 0.336*	9/10 (0.9)	54/60 (90%) 1.2
1266.1	38 ± 19.8	0.49 ± 0.05*	1.992 ± 0.199*	10/10 (1.8)	59/61 (97%) 2.3
Female mice					
0.0	-	0.52 ± 0.05	2.016 ± 0.198	4/10 (0.4)	35/60 (58%) 0.7
23.8	-	0.53 ± 0.05	2.039 ± 0.117	7/10 (0.7)	32/60 (53%) 0.6
116.8	-	0.53 ± 0.05	2.084 ± 0.177	6/10 (0.6)	37/60 (62%) 0.6
354.2	-	0.51 ± 0.05	1.999 ± 0.160	8/10* (0.8)	51/60 (85%)* 1.0
1458.2	-	0.47 ± 0.05*	1.945 ± 0.225	10/10* (2.2)	60/60 (100%)* 2.5

* and grayed cells indicate a significant difference from control at $P \leq 0.05$

Average weights are ± the standard deviation

Severity = the mean severity grade based on a range of 1 (minimal) to 4 (severe)

In male mice, the mean relative lung weight was increased in the high dose group (P≤0.05) in a dose-dependent fashion (Table A3.12). A non-significant increase in the relative

lung weight in female mice was also reported. No differences from control were reported for mean absolute lung weights. No gross or histological pathologies were observed in affected or normal lungs.

Table A3.12 DEHP-induced Lung Alterations in B6C3F$_1$ Mice at 105 Weeks (David *et al.,* 2000b)		
Male mice (mg/kg-day)	Mean absolute lung weights (g)	Mean relative lung weights (g)
0.0	0.22 ± 0.03	0.802 ± 0.109
19.2	0.23 ± 0.04	0.805 ± 0.162
98.5	0.23 ± 0.03	0.804 ± 0.116
292.2	0.25 ± 0.08	0.905 ± 0.296
1266.1	0.23 ± 0.05	0.946 ± 0.222*
Female mice (mg/kg-day)		
0.0	0.25 ± 0.03	0.963 ± 0.118
23.8	0.25 ± 0.04	0.955 ± 0.155
116.8	0.25 ± 0.05	0.966 ± 0.171
354.2	0.25 ± 0.06	0.986 ± 0.254
1458.2	0.24 ± 0.03	0.982 ± 0.122

* and grayed cells indicate a significant difference from control at P ≤ 0.05
Average weights are ± the standard deviation

Absolute and relative testes and uterus weights were significantly decreased in both male and female mice following exposure to DEHP when compared to controls (P≤0.05; Table A3.13). In male mice, changes in weight paralled the significant development of immature/abnormal epididymal sperm, bilateral hypospermia of the testis and hypospermia of the epididymis. Uterine or other testicular pathologies were not observed following gross and histopathological analysis.

Table A3.13 DEHP-induced Chronic Reproductive Alterations in B6C3F$_1$ Mice (David *et al.,* 2000b)							
mg/kg-day	Weights at 104 weeks		Lesions at 78 weeks		Lesions at 104 weeks		
Male mice	Mean absolute testes/uterus weights (g)	Mean relative testes/uterus weights (g)	Immature/abnormal epididymal sperm - incidence	Bilateral hypospermia of the testes - incidence	Immature/abnormal epididymal sperm - incidence (% incidence	Bilateral hypospermia of the testes - incidence (% incidence	Hypospermia of the epididymis – incidence (% incidence)
0.0	0.35 ± 0.05	1.241 ± 0.143	0/10	0/10	10/60 (17%)	2/60 (3%)	3/60 (5%)
19.2	0.34 ± 0.05	1.172 ± 0.150	0/10	0/10	14/60 (23%)	2/60 (3%)	0/60 (0%)
98.5	0.34 ± 0.04	1.156 ± 0.129*	0/10	0/10	11/60 (18%)	1/60 (2%)	1/60 (2%)
292.2	0.30 ± 0.07*	1.091 ± 0.196*	0/10	0/10	29/60* (48%)	18/60* (30%)	3/60 (5%)
1266.1	0.15 ± 0.02*	0.623 ± 0.080*	10/10*	10/10*	48/60* (80%)	57/60* (95%)	36/60* (60%)
Female mice							
0.0	0.51 ± 0.21	1.995 ± 0.814	-	-	-	-	-
23.8	0.49 ± 0.17	1.909 ± 0.738	-	-	-	-	-
116.8	0.55 ± 0.28	2.147 ± 1.043	-	-	-	-	-
354.2	0.59 ± 0.46	2.310 ± 1.691	-	-	-	-	-
1458.2	0.30 ± 0.17*	1.214 ± 0.649*	-	-	-	-	-

* and grayed cells indicate a significant difference from control at P ≤ 0.05
** and grayed cells indicate a significant difference from high dose at P ≤ 0.05
Average weights are ± the standard deviation
Severity = the mean severity grade based on a range of 1 (minimal) to 4 (severe)

Absolute brain weights were also significantly reduced in the male 292.2 and 1266.1 mg/kg-day dose groups ($P \leq 0.05$; data not shown). Significantly reduced and increased male relative brain weights were also observed in the 19.2 and 1266.1 mg/kg-day dose groups, respectively ($P \leq 0.05$). The author commented that a brain weight relationship to treatment was questionable.

Treatment-related lesions in the ovaries, pituitary gland, thyroid, or pancreas were not observed following gross and histopathological analysis.

The author concluded by stating that high doses of DEHP induce pathological events in the liver and testes, and exacerbates chronic kidney effects such as inflammation and nephropathy (LOAEL = ~ 300 mg/kg-day). These effects did not correlate to increased peroxisome proliferation or hepatocellular neoplasia, since these effects were observed at lower doses (~100 mg/kg-day) in mice. Unlike the rat, significant changes in hematology, spongiosis hepatis, pituitary lesions, or pancreatic lesions were not seen in mice, even though mice received higher doses of DEHP than rats. The author also concluded that since hepatocyte pigmentation and eosinophilia were not seen at dose-levels that significantly induce peroxisome proliferation, a threshold existed for "peroxisome proliferator-induced noncarcinogenic liver effects". The author further postulated that peroxisome proliferation had a role in the onset of testicular toxicity, but not in the extent or severity of damage (testicular lesions and hypospermia correlate in a time- and dose-dependent fashion with peroxisome proliferation). The author continues to speculate that significant time- and dose-dependent kidney lesions must occur prior to week 78 and that nephropathy is influenced by peroxisome proliferation or PPARα. Overall, the author concluded that there were clear differences in the response to DEHP when considering rats and mice and that the mouse response may be more relevant to humans because many rat responses (i.e., hematologic changes, mononuclear cell leukemia, spongiosis hepatis, pancreatic acinar cell adenoma, renal tubule mineralization) were considered rat-specific. A LOAEL of 292.2 – 354.2 mg/kg-day (NOAEL = 98.5 to 116.8 mg/kg-day) was based on non-carcinogenic effects in the liver, kidney, and testes.

David *et al.* (2001) determined the chronic effects of administration of DEHP in the diet on rats and mice. Male and female Fischer 344 rats and B6C3F$_1$ mice were fed DEHP in the diet for 78 and 104 weeks (rats; 0, 5.8, 28.9, 146.6, 789.0 mg/kg-day for M; 0, 7.3, 36.1, 181.7, 938.5 mg/kg-day for F: mice; 0, 5.8, 28.9, 146.6, 789.0 mg/kg-day for M; 0, 7.3, 36.1, 181.7, 938.5 mg/kg-day for F:) for up to 104 weeks. Rat mortality and morbidity was evaluated twice daily. Body weight and food consumption were determined weekly for 17 weeks following initial dosing and every month afterwards. Gross pathology and organ histopathology was assessed at week 78 and following termination at week 105. Organ weights (brain, lungs, spleen, kidney, testes, and uterus) and terminal body weight were also measured at necropsy. Blood samples for clinical chemistry and hematology were collected on weeks 26, 52, 78, and 104.

DEHP-induce effects, including the reversibility of these effects following a 26 week recovery period, can be seen for the rat liver (Table A3.14), mouse liver (Table A3.15), mouse kidney (Table A3.16), rat kidney (Table A3.17), the rat reproductive system (Table A3.18), and the mouse reproductive system (Table A3.19).

Table A3.14 DEHP-induced Hepatic Alterations in Fischer 344 Rats (David *et al.*, 2000a, 2001)

mg/kg-day	Weights at 104 weeks		Lesions at 78 weeks	Lesions at 104 weeks	
Male rats	Mean absolute liver weights (g)	Mean relative liver weights (g)	Kupffer cell pigmentation (# animals with lesion/total examined)	Kupffer cell /hepatocyte pigmentation: incidence (% incidence) severity	Spongiosis hepatis: incidence (% incidence) severity
0.0	8.96 ± 1.19	2.701 ± 0.295	0/10	0/80 (0%) 0.0	3/80 (4%)
5.8	9.13 ± 1.36	2.737 ± 0.357	-	0/50 (0%) 0.0	3/50 (6%)
28.9	9.87 ± 2.84 (+10%)	3.086 ± 1.273 (+14%)	-	0/55 (0%) 0.0	3/55 (6%)
146.6	11.11 ± 2.43*	3.462 ± 0.716*	0/10	1/65 (0%) 0.0	11/65* (17%)
789.0	14.64 ± 2.76*	4.947 ± 0.874*	9/10*	44/80* (55%) 0.9	11/80* (14%)
722.0	14.64 ± 2.76*	4.947 ± 0.874*	9/10*	44/80* (55%) 0.9	11/80* (14%)
728.0 - recovery	9.90 ± 2.23	3.095 ± 0.766**	-	11/50*,**(22%) 0.2	3/55 (6%)
Female rats					
0.0	6.54 ± 0.70	2.908 ± 0.444	0/10	0/80 (0%) 0.0	0/80 (0%)
7.3	7.03 ± 1.09	3.001 ± 0.568	1/10	0/50 (0%) 0.0	0/50 (0%)
36.1	6.80 ± 0.87	2.851 ± 0.303	-	0/55 (0%) 0.0	0/55 (0%)
181.7	8.27 ± 1.43*	3.575 ± 0.795*	-	1/65 (0%) 0.0	1/65 (2%)
938.5	10.84 ± 1.93*	5.227 ± 0.981*	7/10*	24/80* (30%) 0.3	1/80 (1%)
882.0	10.84 ± 1.93*	5.227 ± 0.981*	7/10*	24/80* (30%) 0.3	-
879.0 - recovery	7.22 ± 1.44*,**	3.293 ± 0.883*,**	-	3/55** (5%) 0.1	-

* and grayed cells indicate a statistical difference from controls at $P \leq 0.05$

** and grayed cells indicate a statistical difference from the highest treatment dose at $P \leq 0.05$

Table A3.15 DEHP-induced Hepatic Alterations in $B6C3F_1$ Mice (David *et al.*, 2000b, 2001)

mg/kg-day	Weight at 105 weeks		Lesions at 79 weeks			Lesions at 105 weeks		
Male mice	Mean absolute liver weights (g)	Mean relative liver weights (g)	Hepatocyte pigmentation (# animals with lesion/total examined; % incidence)	Increased cytoplasmic eosinophilia (# animals with lesion/total examined; % incidence)	Chronic hepatic inflammation (# animals with lesion/total examined; % incidence)	Hepatocyte pigmentation (# animals with lesion/total examined; % incidence)	Increased cytoplasmic eosinophilia (# animals with lesion/total examined; % incidence)	Chronic hepatic inflammation (# animals with lesion/total examined; % incidence)
0.0	1.51 ± 0.24	5.334 ± 0.986	0/15	0/15	0/15	1/70 (1%)	0/70 (0%)	34/70 (49%) 0.5
19.2	1.60 ± 0.30	5.380 ± 1.035	0/10	0/10	0/10	0/60 (0%)	0/60 (0%)	28/60 (47%) 0.5
98.5	1.73 ± 0.69*	5.967 ± 2.754	0/10	0/10	0/10	0/65 (0%)	0/65 (0%)	27/65 (42%) 0.4
292.2	1.92 ± 0.54*	6.961 ± 2.491*	0/10	0/10	0/10	1/65 (0%)	0/65 (0%)	35/65 (54%) 0.6
1266.1	2.37 ± 0.59*	9.234 ± 2.522*	15/15*	15/15*	15/15*	67/70 (96%)*	69/70 (99%)*	51/70 *(73%) 0.9
1211.0	2.37 ± 0.59*	9.234 ± 2.522*	15/15*	15/15*	15/15*	67/70 (96%)*	69/70 (99%)*	51/70 *(73%) 0.9
1227.0 - recovery	1.82 ± 0.56**	6.742 ± 2.247*,**	-	-	-	27/55*,** (49%)	22/55*,** (40%)	34/55 (62%) 0.8
Female mice								
0.0	1.63 ± 0.55	6.150 ± 1.891	0/15	0/15	0/15	0/70 (0%)	1/70 (0%)	50/70 (71%) 0.8
23.8	1.74 ± 0.97	6.437 ± 3.293	0/10	0/10	0/10	0/60 (0%)	0/60 (0%)	34/60 (57%) 0.6
116.8	1.61 ± 0.48	6.102 ± 1.608	0/10	0/10	0/10	0/65 (0%)	0/65 (0%)	53/65 (82%) 0.9
354.2	1.73 ± 0.32	6.566 ± 0.944*	0/10	0/10	0/10	0/65 (0%)	0/65 (0%)	49/65 (75%) 0.8
1458.2	2.58 ± 0.84*	10.279 ± 2.621*	15/15*	15/15*	15/15*	55/70 (79%)*	70/70 (100%)*	58/70 (83%) 1.1
1413.0	2.58 ± 0.84*	10.279 ± 2.621*	15/15*	15/15*	15/15*	55/70 (79%)*	70/70 (100%)*	58/70 (83%) 1.1
1408.0 - recovery	1.79 ± 0.59**	7.158 ± 2.538**	-	-	-	13/55*,** (24%)	6/55*,**(11%)	42/55 (76%) 1.0

* and grayed cells indicate a statistical difference from controls at $P \leq 0.05$

** and grayed cells indicate a statistical difference from the highest treatment dose at $P \leq 0.05$

Table A3.16 Reversal of DEHP-induced Kidney Alterations in B6C3F$_1$ Mice (David *et al.*, 2001)				
mg/kg-day	Weights at 105 weeks		Lesions at 79 weeks	Lesions at 105 weeks
Male mice	Mean absolute kidney weights (g)	Mean relative kidney weights (g)	Chronic Progressive Nephropathy incidence (severity)	Chronic Progressive Nephropathy incidence (% incidence) severity
0.0	0.72 ± 0.07	2.561 ± 0.236	10/10 (1.2)	54/60 (90%) 1.1
1211	0.49 ± 0.05*	1.992 ± 0.199*	10/10 (1.8)	59/61 (97%) 2.3
1227 - recovery	0.58 ± 0.07*,**	2.227 ± 0.239*,**	-	42/50 (84%)** 1.3
Female mice				
0.0	0.52 ± 0.05	2.016 ± 0.198	4/10 (0.4)	35/60 (58%) 0.7
1413	0.47 ± 0.05*	1.945 ± 0.225	10/10* (2.2)	60/60 (100%)* 2.5
1408 - recovery	0.47 ± 0.05*	1.910 ± 0.194	-	50/50 (100%)*1.6

* and grayed cells indicate a significant difference from control at P ≤ 0.05

** and grayed cells indicate a significant difference from high dose at P ≤ 0.05

Average weights are ± the standard deviation

Severity = the mean severity grade based on a range of 1 (minimal) to 4 (severe)

Table A3.17 Reversal of DEHP-induced Kidney Alterations in Fischer 344 Rats (David *et al.*, 2001)										
mg/kg-day	Blood Urea Nitrogen at 78 weeks (mg/dL)	Blood Urea Nitrogen at 105 weeks (mg/dL)	Weights at 105 weeks		Lesions at 78 weeks			Lesions at 105 weeks		
Male rats			Mean absolute kidney weights (g)	Mean relative kidney weights (g)	Mineraliz-ation of the Renal Papilla incidence	Chronic Progressive Nephropathy incidence (severity)	Renal Tubule Pigmentation incidence (severity)	Mineralization of the Renal Papilla (% incidence) severity	Chronic Progressive Nephropathy (% incidence) severity	Renal Tubule Pigmentation (% incidence) severity
0.0	16 ± 1.4	23 ± 24.5	2.52 ± 0.20	0.768 ± 0.056	2/10	-	10/10 (1.0)	12/60 (20%) 0.2	60/60 (100%) 1.7	58/60 (97%) 1.1
722.0	20 ± 1.9*	19 ± 2.4*	2.84 ± 0.25*	0.975 ± 0.088*	5/10	10/10 (1.8)	10/10 (2.4)	45/62 (76%)* 1.0	62/62 (100%) 2.2	62/62 (100%) 2.3
728 - recovery	20 ± 1.9*	17 ± 3.5	2.70 ± 2.26*,**	0.854 ± 0.120*,**	-	10/10 (1.3)	-	45/51 (88%)*,** 1.2	51/51 (100%) 2.4	51/51 (100%) 2.2
Female rats										
0.0	15 ± 1.1	16 ± 2.3	1.78 ± 0.11	0.803 ± 0.124	0/10	4/10 (1.0)	10/10 (1.0)	17/60 (28%) 0.3	53/60 (88%) 1.1	60/60 (100%) 1.4
882.0	19 ± 1.8*	20 ± 3.1	1.91 ± 0.11*	0.934 ± 0.062*	2/10	5/10 (1.0)	10/10 (2.3)	20/61 (33%) 0.3	55/61 (90%) 1.1	61/61 (100%) 2.3
879.0 - recovery	20 ± 1.4*	15 ± 1.7	1.86 ± 0.18	0.847 ± 0.126**	-	-	-	24/52 (46%) 0.5	51/52 (98%)*1.6	52/52 (100%) 1.8

* and grayed cells indicate a significant difference from control at P ≤ 0.05

** and grayed cells indicate a significant difference from high dose at P ≤ 0.05

Average weights are ± the standard deviation

Severity = the mean severity grade based on a range of 1 (minimal) to 4 (severe)

Table A3.18 DEHP-induced Chronic Reproductive Alterations in Fischer 344 Rats (David *et al.*, 2000a, 2001)								
mg/kg-day	Weights at 104 weeks		Lesions at 78 weeks			Lesions at 104 weeks		
Male rats	Mean absolute testes/uterus weights (g)	Mean relative testes/uterus weights (g)	Interstitial cell tumor of testes - incidence	Aspermato genesis - incidence	Pituitary castration cells - incidence	Interstitial cell tumor of testes - Incidence (% incidence)	Aspermatogene sis - Incidence (% incidence)	Pituitary castration cells - Incidence (% incidence) severity
0.0	5.92 ± 1.90	1.812 ± 0.596	9/10	0/10	0/10	59/64 (92%)	37/64 (58%)	1/60 (2%) 0.0
5.8	6.05 ± 1.79	1.830 ± 0.556	-	-	-	45/50 (90%)	34/50 (64%)	0/50 (0%) 0.0
28.9	5.77 ± 1.67	1.782 ± 0.507	-	-	-	50/55 (91%)	43/55* (78%)	0/51 (0%) 0.0
146.6	6.28 ± 2.38	1.957 ± 0.692	10/10	0/10	0/10	60/65 (92%)	48/65* (74%)	1/52 (2%) 0.0
789.0	2.19 ± 1.15*	0.741 ± 0.369*	3/10*	10/10*	7/10*	20/64* (31%)	62/64* (97%)	30/60* (50%) 1.1
722.0	2.19 ± 1.15*	0.741 ± 0.369*	3/10*	10/10*	7/10*	20/64* (31%)	62/64* (97%)	30/60* (50%) 1.1
728.0 – 26 wk recovery	2.81 ± 1.90*	0.893 ± 0.632*	-	-	-	17/53* (32%)	53/53* (100%)	20/48* (42%) 0.7
Female rats								
0.0	0.99 ± 1.09	0.434 ± 0.442	-	-	-	-	-	-
7.3	1.46 ± 3.03	0.629 ± 1.310	-	-	-	-	-	-
36.1	0.93 ± 1.24	0.409 ± 0.633	-	-	-	-	-	-
181.7	1.02 ± 1.05	0.462 ± 0.538	-	-	-	-	-	-
938.5	0.90 ± 0.63	0.437 ± 0.297	-	-	-	-	-	-

* and grayed cells indicate a significant difference from control at $P \leq 0.05$

** and grayed cells indicate a significant difference from high dose at $P \leq 0.05$

Average weights are ± the standard deviation

Severity = the mean severity grade based on a range of 1 (minimal) to 4 (severe)

Table A3.19 DEHP-induced Chronic Reproductive Alterations in B6C3F$_1$ Mice (David *et al.*, 2000b, 2001)							
mg/kg-day	Weights at 105 weeks		Lesions at 79 weeks		Lesions at 105 weeks		
Male mice	Mean absolute testes/uterus weights (g)	Mean relative testes/uterus weights (g)	Immature/abnormal epididymal sperm - incidence	Bilateral hypospermia of the testes - incidence	Immature/abnormal epididymal sperm - incidence (% incidence	Bilateral hypospermia of the testes - incidence (% incidence	Hypospermia of the epididymis – incidence (% incidence)
0.0	0.35 ± 0.05	1.241 ± 0.143	0/10	0/10	10/60 (17%)	2/60 (3%)	3/60 (5%)
19.2	0.34 ± 0.05	1.172 ± 0.150	0/10	0/10	14/60 (23%)	2/60 (3%)	0/60 (0%)
98.5	0.34 ± 0.04	1.156 ± 0.129*	0/10	0/10	11/60 (18%)	1/60 (2%)	1/60 (2%)
292.2	0.30 ± 0.07*	1.091 ± 0.196*	0/10	0/10	29/60* (48%)	18/60* (30%)	3/60 (5%)
1266.1	0.15 ± 0.02*	0.623 ± 0.080*	10/10*	10/10*	48/60* (80%)	57/60* (95%)	36/60* (60%)
1211.0	0.15 ± 0.02*	0.623 ± 0.080*	10/10*	10/10*	48/60* (80%)	57/60* (95%)	36/60* (60%)
1227.0 – 26 wk recovery	0.24 ± 0.04*,**	0.919 ± 0.163*,**	-	-	41/50* (82%)	36/50*,**(72%)	12/50*,** (24%)
Female mice							
0.0	0.51 ± 0.21	1.995 ± 0.814	-	-	-	-	-
23.8	0.49 ± 0.17	1.909 ± 0.738	-	-	-	-	-
116.8	0.55 ± 0.28	2.147 ± 1.043	-	-	-	-	-
354.2	0.59 ± 0.46	2.310 ± 1.691	-	-	-	-	-
1458.2	0.30 ± 0.17*	1.214 ± 0.649*	-	-	-	-	-
1413.0	0.30 ± 0.17*	1.214 ± 0.649*	-	-	-	-	-
1408.0 – 26 wk recovery	0.42 ± 0.19**	1.716 ± 0.785**	-	-	-	-	-

* and grayed cells indicate a significant difference from control at $P \leq 0.05$

** and grayed cells indicate a significant difference from high dose at $P \leq 0.05$

Average weights are ± the standard deviation

Severity = the mean severity grade based on a range of 1 (minimal) to 4 (severe)

- = no data

Dostal *et al.* (1987a) determined the effects of DEHP on peroxisome proliferation and other biochemical markers in the liver. Male Sprague-Dawley rats aged 6, 14, 16, 21, 42, and 86 days were administered DEHP via gavage daily for 5 days (0, 10, 100, 1000, 2000 mg/kg-day). Rats were sacrificed 24 hours after the last dose. The liver and kidney weights, the activity of liver enzymes (palmitoyl-CoA oxidase, carnitine acetyltransferase), and the concentration of plasma cholesterol and triglycerides were determined following sacrifice.

Exposure to high concentrations of DEHP (2000 mg/kg) significantly increased mortality in younger rats (6 to 10, 14 to 18, 21 to 25 days old) and significantly decreased body weights in older rats (42 to 46, 86 to 90 days old), both in an age-dependent fashion. Exposure to 1000 mg/kg DEHP significantly decreased body weight in younger rats (6 to 10, 14 to 18, 21 to 25 days old), but not older rats (42 to 46, 86 to 90 days old) in an age-dependent fashion ($P < 0.05$; Table A3.20). Other dose levels had no toxicologically significant effects. Within each age group alteration in body weight were also dose-dependent. Absolute liver weight was significantly increased in a dose-dependent fashion in 14 to 18, 42 to 46, 86 to 90 (100 mg/kg) and 16 to 20 and 21 to 25 (1000 mg/kg) day old rats ($P < 0.05$). Relative liver weight was also significantly increased in a dose-dependent fashion in 6 to 10, 16 to 20 (1000 mg/kg) and 14 to 18, 21 to 25, 42 to 46, 86 to 90 (100 mg/kg) day old rats ($P < 0.05$). Absolute kidney weight was significantly decreased in younger rats (6 to 10, 14 to 18, 21 to 25 days old; $P < 0.05$). Relative kidney weight, in contrast, was significantly increased in 42 to 46 (1000 mg/kg) and 86 to 90 (2000 mg/kg) day old rats ($P < 0.05$).

Table A3.20 DEHP-induced Effects on Body Weight, Liver Weight, and Kidney Weight (Dostal *et al.*, 1987a)							
Parameter	Dose (mg/kg)	Age During Dosing (days)					
		6-10	14-18	16-20	21-25	42-46	86-90
Body weight (g; mean ± SE)	0	23.7 ± 0.5	35.9 ± 0.6	42.7 ± 2.5	73.7 ± 2.0	220 ± 4	435 ± 9
	10	21.8 ± 0.6	37.3 ± 0.5	Not tested	67.3 ± 1.7*	Not tested	Not tested
	100	23.2 ± 0.6	37.0 ± 1.1	Not tested	70.0 ± 1.0	224 ± 4	441 ± 7
	1000	16.4 ± 0.8*	30.3 ± 1.3*, 13 of 19 died	38.4 ± 1.7	58.7 ± 1.9*	213 ± 5	422 ± 9
	2000	NA, all died	NA, 7 of 8 died	Not tested	NA, all died	201 ± 6*	404 ± 11*
Absolute liver weight (g; mean ± SE)	0	0.73 ± 0.02	1.17 ± 0.02	1.70 ± 0.07	3.36 ± 0.11	11.3 ± 0.3	16.6 ± 0.7
	10	0.65 ± 0.04	1.25 ± 0.04	Not tested	3.00 ± 0.11*	Not tested	Not tested
	100	0.74 ± 0.02	1.37 ± 0.05*	Not tested	3.47 ± 0.10	12.4 ± 0.3*	18.9 ± 0.5*
	1000	0.73 ± 0.02	1.55 ± 0.07*, 13 of 19 died	2.31 ± 0.14*	3.94 ± 0.12*	15.2 ± 0.5*	21.0 ± 0.8*
	2000	NA, all died	NA	Not tested	NA	15.4 ± 0.8*	19.9 ± 1.1*
Relative liver weight (g/100g bw; mean ± SE)	0	3.09 ± 0.06	3.27 ± 0.05	4.03 ± 0.24	4.56 ± 0.05	5.13 ± 0.09	3.80 ± 0.09
	10	2.97 ± 0.09	3.34 ± 0.07	Not tested	4.45 ± 0.09	Not tested	Not tested
	100	3.20 ± 0.05	3.69 ± 0.08*	Not tested	4.95 ± 0.09*	5.55 ± 0.08*	4.30 ± 0.12*
	1000	4.44 ± 0.12*	5.13 ± 0.20*, 13 of 19 died	5.99±0.14*	6.74 ± 0.14*	7.14 ± 0.11*	4.98 ± 0.12*
	2000	NA, all died	NA	Not tested	NA	7.62 ± 0.02*	4.90 ± 0.13*
Absolute kidney weight (g; mean ± SE)	0	0.31 ± 0.01	0.41 ± 0.01	0.52 ± 0.02	0.80 ± 0.02	1.86 ± 0.06	2.91 ± 0.08
	10	0.29 ± 0.01*	0.42 ± 0.01	Not tested	0.74 ± 0.04	Not tested	Not tested
	100	0.31 ± 0.01	0.42 ± 0.01	Not tested	0.79 ± 0.02	1.94 ± 0.05	2.95 ± 0.10
	1000	0.22 ± 0.01*	0.36 ± 0.01*, 13 of 19 died	0.45 ± 0.03	0.68 ± 0.03*	1.99 ± 0.07	3.07 ± 0.14
	2000	NA, all died	NA	Not tested	NA	1.85 ± 0.04	2.93 ± 0.13
Relative kidney weight (g/100g bw; mean ± SE)	0	1.33 ± 0.03	1.15 ± 0.01	1.23 ± 0.06	1.09 ± 0.02	0.84 ± 0.02	0.67 ± 0.02
	10	1.33 ± 0.04	1.11 ± 0.03	Not tested	1.10 ± 0.04	Not tested	Not tested
	100	1.32 ± 0.03	1.14 ± 0.02	Not tested	1.13 ± 0.03	0.86 ± 0.01	0.67 ± 0.02
	1000	1.38 ± 0.05	1.18 ± 1.13, 13 of 19 died	1.16 ± 0.04	1.16 ± 0.02	0.93 ± 0.02*	0.73 ± 0.02
	2000	NA, all died	NA	Not tested	NA	0.93 ± 0.02*	0.72 ± 0.02*

* $P < 0.05$

Liver enzyme activities were altered by exposure to DEHP (Table A3.21). Palmitoyl-CoA oxidase activity was significantly increased in a dose-dependent fashion in the 6 to 10, 21 to 25, 42 to 46, and 86 to 90 day old rat groups (100 mg/kg; $P < 0.05$). Exposure to DHEP (10 mg/kg) also significantly increased the activity of Palmitoyl-CoA oxidase in 14 to 18 day old rats ($P < 0.05$). Carnitine acetyltransferase activity was also significantly increased in a dose-dependent manner in 6 to 10 day old rats following exposure to 10 mg/kg DEHP, in 14 to 18, 21 to 25, 42 to 46, 86 to 90 day old rats following exposure to 100 mg/kg DEHP, and in 16 to 20 day old rats following exposure to 1000 mg/kg DEHP ($P < 0.05$). Hepatic protein levels were significantly increased in 6 to 10 (10, 1000 mg/kg), 14 to 18 and 21 to 25 (1000 mg/kg), and 86 to 90 (2000 mg/kg) day old rats ($P < 0.05$).

Table A3.21 DEHP-induced Effects on Liver Enzyme Activities (Dostal *et al.*, 1987a)							
Parameter	Dose (mg/kg)	Age During Dosing (days)					
		6-10	14-18	16-20	21-25	42-46	86-90
Palmitoyl-CoA oxidase (nmol/min/mg protein; mean ± SE)	0	4.17 ± 0.37	3.61 ± 0.28	5.31 ± 0.59	4.29 ± 0.33	4.91 ± 0.12	4.80 ± 0.50
	10	4.68 ± 0.33	5.47 ± 0.21*	Not tested	4.55 ± 0.37	Not tested	Not tested
	100	12.3 ± 1.1*	25.0 ± 2.3*	Not tested	9.34 ± 0.94*	12.3 ± 1.2*	19.1 ± 1.3*
	1000	53.4 ± 2.4*	97.5 ± 6.6*, 13 of 19 died	58.9 ± 2.9*	65.0 ± 4.6*	57.8 ± 4.7*	70.0 ± 4.9*
	2000	NA, all died	NA, 7 of 8 died	Not tested	NA, all died	73.8 ± 5.1*	81.5 ± 8.5*
Carnitine acetyl transferase (nmol/min/mg protein; mean ± SE)	0	6.97 ± 0.06	5.85 ± 0.41	11.4 ± 1.0	8.10 ± 0.55	6.18 ± 0.41	4.98 ± 0.63
	10	9.65 ± 0.72*	6.03 ± 0.54	Not tested	10.4 ± 1.0	Not tested	Not tested
	100	18.6 ± 1.0*	45.6 ± 4.1*	Not tested	19.1 ± 1.5*	22.2 ± 1.5*	21.9 ± 1.6*
	1000	64.9 ± 3.8*	151.0 ± 36.0*, 13 of 19 died	87.6 ± 1.6	94.6 ± 6.4*	70.0 ± 3.7*	53.9 ± 2.8*
	2000	NA, all died	NA	Not tested	NA	83.9 ± 5.1*	54.6 ± 3.0*
Hepatic protein (mg/g tissue)	0	136 ± 2	132 ± 2	130 ± 4	119 ± 2	137 ± 2	144 ± 3
	10	142 ± 2*	123 ± 2	Not tested	118 ± 2	Not tested	Not tested
	100	141 ± 2	129 ± 2	Not tested	118 ± 2	130 ± 2	145 ± 2
	1000	148 ± 2*	147 ± 3*, 13 of 19 died	134 ± 2	132 ± 3*	132 ± 3	146 ± 2
	2000	NA, all died	NA	Not tested	NA	138 ± 3	155 ± 3*

* $P < 0.05$

Plasma cholesterol and triglycerides were also altered by exposure to DEHP (Table A3.22). Plasma cholesterol was significantly increased in 6 to 10, 14 to 18, and non-significantly increased in 16 to 20 day old rats (1000 mg/kg; $P < 0.05$). In constrast, DEHP exposure significantly decreased plasma cholesterol in 42 to 46 (100 mg/kg), 21 to 25 (1000 mg/kg), and 86 to 90 (2000 mg/kg) day old rats ($P < 0.05$). Decreased plasma triglycerides were also reported in 6 to 10, 14 to 18, 16 to 20, 42 to 46 (100 mg/kg; $P < 0.05$), 21 to 25 and 86 to 90 (1000 mg/kg; $P < 0.05$) day old rats.

Table A3.22 DEHP-induced Effects on Plasma Cholesterol and Triglycerides (Dostal *et al.*, 1987a)							
Parameter	Dose (mg/kg)	Age During Dosing (days)					
		6-10	14-18	16-20	21-25	42-46	86-90
Plasma cholesterol (mg/dL; mean ± SE)	0	127 ± 5	158 ± 6	96 ± 9	65 ± 3	72 ± 5	64 ± 6
	10	123 ± 3	149 ± 10	Not tested	71 ± 4	Not tested	Not tested
	100	114 ± 5	179 ± 12	Not tested	69 ± 4	59 ± 3*	61 ± 3
	1000	164 ± 15*	285 ± 27*, 13 of 19 died	130 ± 18	54 ± 2*	48 ± 2*	54 ± 4
	2000	NA, all died	NA, 7 of 8 died	Not tested	NA, all died	49 ± 2*	48 ± 4*
Plasma triglyceride (mg/dL; mean ± SE)	0	130 ± 16	231 ± 25	162 ± 13	142 ± 19	124 ± 16	135 ± 15
	10	105 ± 13	196 ± 19	Not tested	104 ± 14	Not tested	Not tested
	100	119 ± 5	162 ± 15	Not tested	122 ± 14	73 ± 6*	102 ± 9
	1000	111 ± 8	149 ± 21, 13 of 19 died	143 ± 16	79 ± 7*	73 ± 6*	75 ± 14*
	2000	NA, all died	NA	Not tested	NA	56 ± 7*	45 ± 5*

* $P < 0.05$

Overall, the authors suggested that the oral LD_{50} for DEHP was lower in younger suckling animals, that differences in body weight and mortality were not related to increases in liver weight and peroxisome proliferation, that suckling and adult rats had similar potentials for tumor formation, that high cholesterols levls in younger rats may be related to their milk consumption, and that a critical window for effects (i.e., 14 to 18 days old) may exist for rats.

Dostal *et al.* (1987b) investigated the transfer of DEHP through rat milk and its effect on milk composition and the mammary gland. Pregnant Sprague-Dawley rats were allowed to birth pups naturally. Litter sizes were then adjusted to 10 pups each and then randomly assigned to a dose-group. In the first experiment, gavage doses of 2000 mg DEHP/kg were administered daily on Ld 2 to 6, 6 to 10, or 14 to 18 to the rat dams. Rats were sacrificed 24 hours after the last dose, and the liver and blood was collected. In the second experiment, gavage doses of 2000 mg DEHP/kg were administered daily on Ld 15 to 17 between 7:30 and 9:30am to the rat dams. The pups were removed from the dam two hours after dosing. Six hours after dosing, milk was collected from the teats, and the liver, blood, and six abdominal-inguinal mammary glands were removed for analysis.

Body weight was significantly reduced in lactating rats and pups following the 5 dose, but not the 3 dose, regimen (Table A3.23). Food consumption was significantly reduced in dams dosed during Ld 14 to 18 (data not shown).

Table A3.23 Body Weights of DEHP-dosed Rat Dams and Their Suckling Pups (Dostal *et al.,* 1987b)

Ld	Dose Group	Lactating Rat Dam Weights (g; Average ± SE; n=7-8)	Suckling Rat Pup Weights (g; Average ± SE; n=70-80)
2-6	Control	271 ± 7	13.7 ± 0.4
	DEHP	224 ± 5 (P < 0.05)	10.1 ± 0.2 (P < 0.05)
6-10	Control	295 ± 3	21.4 ± 1.0
	DEHP	243 ± 5 (P < 0.05)	17.1 ± 0.7 (P < 0.05)
14-18	Control	288 ± 6	34.9 ± 1.0
	Pair-fed*	235 ± 6 (P < 0.05)	31.6 ± 1.1 (P < 0.05)
	DEHP	247 ± 5 (P < 0.05)	30.0 ± 0.8 (P < 0.05)
15-17	Control	341 ± 9	38.9 ± 1.7
	Pair-fed*	322 ± 11	37.5 ± 1.6
	DEHP	320 ± 9	40.5 ± 1.4

* Pair-fed rats are those control rats fed exactly the amount of food consumed by DEHP-treated rats during the previous 24 hours

The relative liver weight was increased in dams of all three treatment groups (Lds), but not suckling rats (Table A3.24). The relative liver weight in suckling rats of dams administered DEHP on Ld 14 to 18 was significantly decreased when compared to pair-fed controls, but not regular controls. Hepatic palmitoyl-CoA oxidase and carnitine acetyltransferase were also significantly increased following exposure to DEHP during Ld 2 to 6, 6 to 10, 14 to 18, and 15 to 17 (3 daily doses) in both dams and suckling pups (Table A3.24). Levels of these enzymes were

also significantly increased when compared to pair-fed dams exposed to DEHP on Ld 14 to 18 and 15 to 17.

Table A3.24 DEHP-induced Liver Effects in Rat Dams and Suckling Pups (Dostal *et al.,* 1987b)

Ld (with 5 daily doses)	Dose Group	Lactating Rat Dams (Average ± SE; n=7-8)			Suckling Rat Pups (Average ± SE; n=70-80)		
		Relative liver weight (g/100 g BW)	Palmitoyl-CoA oxidase (nmol/min/mg)	Carnitine acetyltransferase (nmol/min/mg)	Relative liver weight (g/100 g BW)	Palmitoyl-CoA oxidase (nmol/min/mg)	Carnitine acetyltransferase (nmol/min/mg)
2-6	Control	5.05 ± 0.12	6.63 ± 0.41	9.72 ± 0.68	2.98 ± 0.06	3.35 ± 0.16	7.6 ± 0.45
	DEHP	5.87 ± 0.13 (P < 0.05)	40.8 ± 3.3 (P < 0.05)	53.1 ± 3.4 (P < 0.05)	2.93 ± 0.05	7.22 ± 0.35 (P < 0.05)	13.6 ± 1.1 (P < 0.05)
6-10	Control	5.03±0.08	5.52 ± 0.58	7.16 ± 0.58	2.73 ± 0.04	2.47 ± 0.21	8.43 ± 0.47
	DEHP	5.49 ± 0.13 (P < 0.05)	37.5 ± 2.5 (P < 0.05)	58.0 ± 4.8 (P < 0.05)	2.60 ± 0.06	4.68 ± 0.26 (P < 0.05)	16.0 ± 1.6 (P < 0.05)
14-18	Control	5.21 ± 0.10	7.20 ± 0.43	8.29 ± 0.43	3.17 ± 0.06	2.74 ± 0.20	7.01 ± 0.43
	Pair-fed*	4.03 ± 0.12 (P < 0.05)	7.13 ± 0.89	10.4 ± 0.7 (P < 0.05)	2.84 ± 0.04 (P < 0.05)	3.40 ± 0.20 (P < 0.05)	6.62 ± 0.31
	DEHP	5.56 ± 0.07 (P < 0.05; PW)	37.3 ± 1.7 (P < 0.05; PW)	42.2 ± 1.9 (P < 0.05; PW)	3.10 ± 0.04 (PW)	5.75 ± 0.36 (P < 0.05; PW)	15.1 ± 1.0 (P < 0.05; PW)
15-17	Control	-	4.63 ± 0.28	4.91 ± 0.47	-	2.17 ± 0.32	5.49 ± 0.66
	Pair-fed*	-	3.57 ± 0.59	6.34 ± 0.50	-	2.34 ± 0.38	6.47 ± 0.63
	DEHP	-	18.09 ± 1.16 (P < 0.05; PW)	16.70 ± 1.77 (P < 0.05; PW)	-	5.08 ± 0.41 (P < 0.05; PW)	8.95 ± 1.49

* Pair-fed rats are those control rats fed exactly the amount of food consumed by DEHP-treated rats during the previous 24 hours

Note: P < 0.05 signifies a treatment difference from the concurrent control, PW signifies a significant difference (P < 0.05) from the pair-fed control

DEHP also decreased the levels of circulating plasma cholesterol and triglycerides in dams exposed during all Ld periods (Table A3.25)

Table A3.25 Lipid Effects of DEHP on Exposed Rat Dams (Dostal *et al.,* 1987b)

Ld	Dose Group	Plasma cholesterol (mg/100 mL; Average ± SE; n=7-8)	Plasma triglycerides (mg/100 mL; Average ± SE; n=7-8)
2-6	Control	94 ± 3	73 ± 3
	DEHP	44 ± 3 (P < 0.05)	33 ± 2 (P < 0.05)
6-10	Control	93 ± 5	57 ± 6
	DEHP	52 ± 3 (P < 0.05)	42 ± 4
14-18	Control	100 ± 5	46 ± 3
	Pair-fed*	86 ± 4 (P < 0.05)	49 ± 4
	DEHP	46 ± 4 (P < 0.05; PW)	27 ± 3 (P < 0.05; PW)

* Pair-fed rats are those control rats fed exactly the amount of food consumed by DEHP-treated rats during the previous 24 hours

Note: P < 0.05 signifies a treatment difference from the concurrent control, PW signifies a significant difference (P < 0.05) from the pair-fed control

In rats dosed three times with DEHP, the total milk solids, lipid, and protein in milk samples were increased when compared to controls, while the milk lactose was significantly decreased (Table A3.26). Mammary gland weight (absolute and relative) and the amount of RNA in mammary tissue were also reduced significantly in DEHP exposed dams (Table A3.27).

Table A3.26 Approximate DEHP-induced Changes in Rat Milk Composition** (Dostal *et al.,* 1987b)					
Ld	Dose Group	Milk Composition (Estimated average; n=8-9)			
		Solids (mg/g)	Protein (mg/mL)	Lipids (mg/mL)	Lactose (mg/mL)
15-17	Control	313	50	140	37
	Pair-fed*	317	66 (P < 0.05)	144	27 (P < 0.05)
	DEHP	408 (P < 0.05; PW)	62 (P < 0.05)	200 (P < 0.05; PW)	23 (P < 0.05)

* Pair-fed rats are those control rats fed exactly the amount of food consumed by DEHP-treated rats during the previous 24 hours

** Values estimated from graph bars

Note: P < 0.05 signifies a treatment difference from the concurrent control, PW signifies a significant difference (P < 0.05) from the pair-fed control

Table A3.27 DEHP-induced Changes in Rat Mammary Glands (Dostal *et al.,* 1987b)					
Ld	Dose Group	Mammary Gland Parameters (Average ± SE; n=9)			
		Absolute mammary gland weight (g)	Total DNA in mammary glands (mg)**	Total RNA in mammary glands (mg)**	RNA/DNA
15-17	Control	16.2 ± 1.1	42.3 ± 2.7	150 ± 13	3.51 ± 0.13
	Pair-fed*	13.6 ± 0.6	44.1 ± 1.6	124 ± 8	2.80 ± 0.17 (P < 0.05)
	DEHP	11.2 ± 0.4 (P < 0.05; PW)	38.3 ± 2.5	114 ± 10 (P < 0.05)	2.97 ± 0.13 (P < 0.05)

* Pair-fed rats are those control rats fed exactly the amount of food consumed by DEHP-treated rats during the previous 24 hours

** Total nucleic acids in the 6 abdominal/inguinal mammary glands

Note: P < 0.05 signifies a treatment difference from the concurrent control, PW signifies a significant difference (P < 0.05) from the pair-fed control

DEHP and MEHP were also discovered in rat dam milk and plasma (Table A3.28). Results suggested that DEHP partitioned into the milk from the blood at a faster pace than MEHP. *In vitro* radiolabeled experiments also revealed that DEHP primarily partitioned into the milk fat globule layer (94.4%) when compared to the whey (4.0%) and casein pellet (1.6%; data not shown).

Table A3.28 DEHP and MEHP in Rat Milk and Plasma (Dostal *et al.,* 1987b)									
Ld	Dose Group	DEHP				MEHP			
		Lactating Rat Dams (Average ± SE)			Suckling Rat Pups (Average ± SE)	Lactating Rat Dams (Average ± SE)			Suckling Rat Pups (Average ± SE)
		Plasma (µg/mL)	Milk (µg/mL)	Milk/plasma	Plasma (µg/mL)	Plasma (µg/mL)	Milk (µg/mL)	Milk/plasma	Plasma (µg/mL)
15-17	Control (number rats)	< 0.5 (9)	4.7 ± 0.9 (7)	-	< 0.5 (9)	< 0.5 (9)	< 0.5 (5) 1.8 (2)	-	< 0.5 (9)
	DEHP (number rats)	< 0.5 (4) 1.2 ± 0.1 (5)	216 ± 23 (7)	> 200	< 0.5 (9)	76 ± 12 (9)	25 ± 6 (8)	0.33	< 0.5 (8) 1.1 (1)

The author concluded that administration of DEHP to rat dams during lactation resulted in a reduction of suckling pup weight and that this reduction was due to decreased food consumption in the adult dam rats. Peroxisomal enzyme activities also suggested, however, that

DEHP was getting transferred to pups through the milk during lactation. This conclusion was confirmed by the demonstration of DEHP and MEHP in rat milk harvested during lactation from treated animals. Pup dose calculated from the milk concentrations of DEHP and MEHP were 25mg/kg and 3 mg/kg, respectively. The former concentration has been reported in previous studies to induce peroxisomal enzyme activities at least 2-fold over controls. Partitioning studies demonstrated that the majority of this dose is concentrated in the milk fat globules. This applies to human milk as well (data not shown). Binding relationships are different for MEHP, which equilibrates between being free and bound to albumin. In this study, changes in the pup weights were also due to changes in the milk composition and volume, and ultimately consumption by pups. Decreases in lactose concentration support the author's assumption because lactose synthesis is regulated by food supply. Lactose is also the primary regulator of the quantity of milk secreted. Changes in liver weight induced by decreased food consumption may have also affected milk production. This is because the synthetic capacity of the liver in terms of milk components is increased during lactation (as is the liver weight to compensate). Data also demonstrated that rats exposed to DEHP were hypolipidemic (in the plasma). Hypolipidemia undoubtedly affected milk production and composition.

Dostal *et al.* (1988) determined the testicular effects of DEHP on exposed-neonatal and recovered-adult rats. Neonatal and adult male Sprague-Dawley rats were gavage dosed with DEHP (0, 10, 100, 1000, 2000 mg/kg) daily for 5 days (PPd 6 to 10, 14 to 18, 21 to 25, 42 to 46, 86 to 90). Rats were sacrificed 24 hours following the last dose and the testes were excised, weighed, and fixed for histology or processed for zinc analysis. In another study, suckling male Sprague-Dawley rats were gavage dosed with DEHP (0, 100, 200, 500, 1000 mg/kg) daily beginning on PPd 6 for 5 days. Twenty-four hours or 4 weeks after the last dose, the rats were sacrificed, and the testes and epididymides were removed, weighed, and processed for histopathological examination. In an additional study assessing fertility, suckling male Sprague-Dawley rats were gavage dosed with DEHP (0, 200, 500, 2000 mg/kg) daily for 5 days beginning on PPd 6. Twenty-four hours after the last dose, 5 to 6 rats from each group were sacrificed for testicular examination and analysis. Rats not sacrificed were paired with 12 week old virgin Fischer 344 female rats at 8, 10, 11, 12, and 15 weeks of age and cohabitated for 7 days. Daily inspections of female rats were performed in order to assess mating effectiveness. The females were sacrificed eleven days after the last day of mating and examined for live implants, resorptions, and corpora lutea. Twenty-four hours following the 10-, 11-, 12-, and 15-week matings, 5 to 6 male rats were sacrificed, the testes and epididymides removed, weighed, and fixed for histopathology, and spermatid heads counted. Remaining male rats were sacrificed at 19 and 23 week old, the testis weight determined, and spermatid heads counted. Standard parameters for testicular analysis included the zinc concentration, histological changes in testes and epididymides, seminiferous tubule cell counts, Sertoli cell nuclei per tubule, the number of spermatocytes per tubule, and testicular spermatid head count.

DEHP (10 mg/kg) administered daily for 5 days to rats of various ages (6 to 10, 14 to 18, 21 to 25, 42 to 46, 86 to 90 days old) had no significant effect on the absolute or relative testis

weight (Table A3.29). Other doses of DEHP significantly reduced either absolute or relative testis weight in all age groups. The decrease in testis weight was observed even though many of the dose groups had significant decrements in body weight.

Table A3.29 Testis Weight in Sprague-Dawley Rats Exposed to DEHP Daily for Five Days (Dostal *et al.*, 1988)

Dose	Age of Dosing (days; n=6-10)				
	6-10	14-18	21-25	42-46	86-90
Pre-dosing weights (mg ± SE)	17.0 ± 0.2	69 ± 2	220 ± 5	1890 ± 40	3010 ± 110
0 mg/kg (mg ± SE)	41.3 ± 2.0	149 ± 3 (n=13-16)	466 ± 18 (n=13-16)	2090 ± 50	3060 ± 90
10 mg/kg (mg ± SE)	37.9 ± 1.8	156 ± 5	425 ± 22	Dose not tested	Dose not tested
100 mg/kg (mg ± SE)	36.3 ± 1.8	144 ± 6	397 ± 20 (P < 0.05)	2140 ± 50	3150 ± 70
1000 mg/kg (mg ± SE)	19.9 ± 1.1 (P < 0.05)	72 ± 7 (P < 0.05; n=6; 13 of 19 died)	218 ± 10 (P < 0.05; n=13-16)	1750 ± 120 (P < 0.05)	2970 ± 120
2000 mg/kg (mg ± SE)	Dose fatal	Dose fatal	Dose fatal	1320 ± 110 (P < 0.05)	2450 ± 230 (P < 0.05)
Pre-dosing weights (g/100g bw ± SE)	0.12 ± 0.01	0.25 ± 0.01	0.49 ± 0.01	1.03 ± 0.04	0.73 ± 0.04
0 mg/kg (g/100g bw ± SE)	0.18 ± 0.01	0.42 ± 0.01 (n=13-16)	0.63 ± 0.02 (n=13-16)	0.95 ± 0.02	0.71 ± 0.03
10 mg/kg (g/100g bw ± SE)	0.17 ± 0.01	0.42 ± 0.01	0.63 ± 0.02	Dose not tested	Dose not tested
100 mg/kg (g/100g bw ± SE)	0.15 ± 0.01	0.39 ± 0.02	0.56 ± 0.02	0.96 ± 0.03	0.72 ± 0.01
1000 mg/kg (g/100g bw ± SE) Percent change from control to treated	0.12 ± 0.01 (P < 0.05) -33.3%	0.24 ± 0.01 (P < 0.05; n=6; 13 of 19 died) -42.9%	0.37 ± 0.01 (P < 0.05; n=13-16) -41.3%	0.82 ± 0.05 (P < 0.05) -13.7%	0.70 ± 0.02 -1.4%
2000 mg/kg (g/100g bw ± SE) Percent change from control to treated	NA	NA	NA	0.66 ± 0.05 (P < 0.05) -30.5%	0.60 ± 0.04 (P < 0.05) -15.5%

Doses of 10 and 100 mg DEHP/kg did not adversely affect the testes of treated rats in any age group. Testicular structure was morphologically altered in 11-day old rats at 1000 mg/kg. In treated rats, the tubule size was reduced when compared to controls. The number of Sertoli cell nuclei per tubule was also significantly reduced in 11-day old rats treated with 1000 mg/kg DEHP when compared to controls (34%; 38.8 ± 3.0 nuclei in controls versus 25.5 ± 2.5 nuclei in dosed rats versus 23.9 ± 3.6 nuclei in untreated 6-day old rats; $P < 0.05$; mean ± SD). In 19-day old rats, doses of 1000 mg/kg reduced the tubular diameter, reduced the spermatocytes located in the center of the tubule by 80% (18 ± 4 in controls versus 4 ± 2 spermatocytes in treated versus 1.1 ± 0.4 spermatocytes in 14-day old untreated pups; mean ± SD), but did not reduce the number of Sertoli cell nuclei per tubule (38.9 ± 1.5 nuclei in controls versus 38.5 ± 2.2 nuclei in dosed rats; mean ± SD). In 26-day old rats, doses of 1000 mg/kg significantly affected the testicular tubules (27% severely affected, 70% moderately affected, 2% minimally affected or normal) as evaluated by the number of germ cells. In severely affected tubules, the Sertoli cytoplasm extended to the lumen but had no germ cells. In addition, many tubules had spermatocytes with pyknotic and/or karyorrhectic nuclei. In 47-day old rats, 1000 mg DEHP/kg administration severely affected 20% of the tubules and decreased the number of germ cells by 10 to 20% in the remaining 80% of the tubules. Treatment with 2000 mg DEHP/kg decreased

tubule diameter by 30 to 50% in 47-day old rats, and resulted in substantial germ cell loss (69% severely affected, 16% moderately affected, 10% minimally affected, 5% normal). In 91-day old rats, only one rat out of eight was affected by 1000 mg DEHP/kg treatments. The affected rat had approximately 75% of its tubules showing a substantial loss of spermatids and spermatocytes. At treatment doses of 2000 mg/kg, four of eight rats were also affected (42% tubules severely affected, 15% moderately affected, 13% minimally affected, and 30% normal). Moderately and severely damaged tubules had Sertoli cells, spermatocytes, and spermatogonia, but no spermatids.

Testicular zinc concentrations were not significantly altered in 26 or 47 day old rats dosed with DEHP at any dose. In 9-day old rats, however, doses of 1000 and 2000 mg DEHP/kg significantly decreased zinc in the testes (Table A3.30).

Table A3.30 DEHP-induced Changes in the Mean Zinc Testicular Concentration of Rats (Dostal *et al.,* 1988)

Dose	Age of Dosing (days; n=4-12)			
	14-18	21-25	42-46	86-90
0 mg/kg (µg/g bw ± SE)	21.0 ± 0.8	20.5 ± 1.0	23.8 ± 1.2	25.3 ± 0.20
10 mg/kg (µg/g bw ± SE)	-	25.4 ± 0.8	-	-
100 mg/kg (µg/g bw ± SE)	-	23.6 ± 1.2	22.1 ± 0.9	25.8 ± 0.5
1000 mg/kg (µg/g bw ± SE)	-	24.1 ± 2.2	22.9 ± 1.2	23.2 ± 0.4 (P < 0.05)
2000 mg/kg (µg/g bw ± SE)	Dose was fatal	Dose was fatal	22.8 ± 1.0	19.5 ± 1.5 (P < 0.05)

11-day old rats treated with DEHP had significantly decreased body weights (1000 mg/kg; data not shown) and absolute and relative testis weights (500 and 1000 mg/kg; data not shown). In DEHP-dosed 11-day old rats that were allowed to recover for 4 weeks, a small non-dose-dependent decrease in body weight (200 mg/kg) and significant decrements in absolute (200, 500, 1000 mg/kg; $P < 0.05$) and relative (1000 mg/kg; $P < 0.05$) testis weight were observed (Table A3.31). In the recovery group, the maturation of tubular spermatids was reduced in a dose-dependent fashion, even though the number of Sertoli cell nuclei per tubule was not affected at the highest dose (23.4 ± 3.0 nuclei in controls versus 21.6 ± 4.3 nuclei in dosed rats; mean ± SD).

Table A3.31 DEHP-induced Testicular Effects in Rats Allowed to Recover for 4 Weeks (Dostal *et al.,* 1988)

Dose	Body Weight (g ± SE)	Testis Weight (g ± SE)	Epididymal Weight (g ± SE)	Spermatid Step (maturation)			
				8-9	10-12	13-14	15-16
0 mg/kg	184 ± 4	1.63 0.05	0.18 ± 0.01	0	0	0	7
100 mg/kg	177 ± 5	1.51 ± 0.04	0.18 ± 0.01	-	-	-	-
200 mg/kg	169 ± 5 (P < 0.05)	1.42 ± 0.03 (P < 0.05)	0.17 ± 0.01	0	0	0	9
500 mg/kg	178 ± 5	1.47 ± 0.05 (P < 0.05)	0.18 ± 0.01	0	2	6	0
1000 mg/kg	168 ± 7	1.30 ± 0.07 (P < 0.05)	0.16 ± 0.01	3	3	1	0

An additional study exposing 6-day old rats daily for 5 days demonstrated that body weight was significantly reduced following exposure to 1000 mg/kg and relative testis weight was reduced following exposure to 500 and 1000 mg/kg. Unlike the previous study, Sertoli cell number was also significantly reduced following 500 and 1000 mg DEHP/kg doses (Table A3.32).

Table A3.32 DEHP-induced Testicular Effects in 6 day Old Rats (Dostal *et al.,* 1988)

Dose	Body Weight (g ± SE)	Testis Weight (g ± SE)	Sertoli Cells (nuclei/tubule)
0 mg/kg	24.0 ± 0.9	0.17 ± 0.01	33.9 ± 1.3
200 mg/kg	22.1 ± 1.6	0.16 ± 0.01	31.6 ± 2.2
500 mg/kg	23.3 ± 1.4	0.13 ± 0.01 ($P < 0.05$)	27.3 ± 2.1 ($P < 0.05$)
1000 mg/kg	18.7 ± 0.9 ($P < 0.05$)	0.12 ± 0.01 ($P < 0.05$)	22.9 ± 3.0 ($P < 0.05$)

Fertility of male rats was assessed at 8, 10, 11, 12, and 15 weeks of age. Fertility was reduced at 8 weeks of age when compared to rats at 11 weeks (62% versus 100% in controls). Overall, fertility, the mean number of uterine implants, the mean number of live fetuses per pregnant female, the number of resorptions, and the ratio of total number of implants to the number of corpora lutea were not altered in rats administered DEHP when compared to controls. The number of ovarian corpora lutea was also similar in all females. In week 10 rats, the ratio of males with two females pregnant versus males with female pregnant decreased overall. In addition, there was a non-significant dose-related increase in the resorptions for 12 week old rats (0.26, 0.26, 0.44, 0.63 resorptions for 0, 200, 500, and 1000 mg/kg respectively).

In a similar dosing scenario, the testis weight was significantly reduced in rats at 13 and 19 weeks old. Dose-dependent reductions were observed at 13 and 19 weeks of age, in addition to 12, 16, and 23 weeks of age (Table A3.33). The weight of the epididymides in 13 week old rats was also significantly reduced (12%) in the 1000 mg/kg treatment group (data not shown). These changes were not significant in any DEHP dose group when expressed relative to body weight. Dose-dependent reductions in the testicular sperm heads were noted in 12, 13, 19, and 23 week old rats. Significant differences, however, were only reported for 13 week old rats (200, 500, 1000 mg/kg) and 19 week old rats (1000 mg/kg). When expressed in terms of per gram of tissue, only 13 week old rats dosed with 200 and 500 mg/kg were significantly reduced when compared to controls (data not shown). Significant changes in the number of sperm heads were not accompanied by changes in the number of Sertoli cell nuclei (12.2 ± 1.5 nuclei in controls versus 11.9 ± 1.1 nuclei in dosed rats; mean ± SD). Epididymal weight was not affected by DEHP pretreatment for rats 11, 12, and 16 weeks of age (data not shown). In addition, treatment-related pathologies were not present in the testes of rats age 11 to 16 when dosed with DEHP.

Table A3.33 The Effects of Neonatal Exposure to DEHP on Adult Rat Parameters (Dostal *et al.*, 1988)				
Week	Dose (mg/kg)	Body Weight (g)	Testis Weight (g)	Testicular Spermatid Heads ($*10^6$ per testis)
11	0	430 ± 16	3.36 ± 0.10	252 ± 10
	200	442 ± 18	3.47 ± 0.10	265 ± 9
	500	451 ± 16	3.18 ± 0.05	233 ± 8
	1000	430 ± 6	3.33 ± 0.16	269 ± 14
12	0	469 ± 8	3.49 ± 0.08*	272 ± 12
	200	438 ± 16	3.26 ± 0.08	240 ± 5
	500	462 ± 9	3.27 ± 0.10	238 ± 11
	1000	456 ± 11	3.22 ± 0.13	238 ± 12
13	0	513 ± 25	3.67 ± 0.13*	305 ± 15*
	200	510 ± 6	3.49 ± 0.10	255 ± 12 ($P < 0.05$)
	500	493 ± 11	3.34 ± 0.09	254 ± 7 ($P < 0.05$)
	1000	489 ± 13	3.17 ±0.06 ($P < 0.05$)	245 ± 7 ($P < 0.05$)
16	0	571 ± 28	3.61 ± 0.06	285 ± 5
	200	572 ± 19	3.44 ± 0.12	256 ± 8
	500	557 ± 19	3.43 ± 0.11	264 ± 12
	1000	559 ± 27	3.40 ± 0.13	289 ± 13
19	0	633 ± 20	3.70 ± 0.07*	273 ± 8
	200	626 ± 18	3.50 ± 0.07 ($P < 0.05$)	262 ± 9
	500	608 ± 15	3.32 ± 0.16 ($P < 0.05$)	251 ± 13
	1000	587 ± 17	3.18 ± 0.25 ($P < 0.05$)	228 ± 26 ($P < 0.05$)
23	0	704 ± 17	3.88 ± 0.07	284 ± 8
	200	696 ± 29	3.68 ± 0.09	271 ± 14
	500	679 ± 22	3.66 ± 0.12	264 ± 8
	1000	704 ± 21	3.61 ± 0.12	261 ± 11

* a significant dose-related trend as assessed by Jonckheere's test; $P < 0.05$

The author notes that this study demonstrates that Sertoli cells are lost following neonatal DEHP exposures and postulates that this loss could be due to decreased proliferation (primarily) or cytotoxic loss. The author also notes that normal numbers of Sertoli cells were present in rats recovered for 4 weeks, and at 13 weeks when testicular weight and sperm head count were significantly reduced. Compensatory proliferation (rate of or duration of) were given as possible reasons for Sertoli cell repopulation. Four weeks of recovery following dosing of 6 to 10 day old rats did not return testes to normal weight or spermatid production, even though Sertoli cells were at essentially normal population levels. Sexual maturity in dosed rats was assessed in order to determine if DEHP delayed normal maturation. Fertility and other related parameters in 8 to 15 week old rats were not consistently affected by DEHP administration, suggesting that effects may be very subtle. DEHP-induced histological changes in testis support previous findings in which younger rats are more sensitive to testicular injury than older rats. An absence of changes

in testicular zinc concentration is also supported by studies identifying that previous estimates of zinc loss may be directly due to the loss of spermatids, since zinc and spermatids are co-localized structurally.

Foster *et al.* (2006a) reviewed the reproductive effects of phthalates following *in utero* exposures. The author noted that sentitivity to phthalate-induced reproductive toxicity was age- (fetal > neonatal > pubertal > adult), species- (rats/guinea pigs > mice/hamster), and generation-dependent ($F_1 > F_0$). The primary postnatal target was the Sertoli cell. Effects were also noted in Leydig cells, but these were secondary to tubular damage. Adverse reproductive effects were subsequently narrowed down to a critical *in utero* window of exposure. Exposure during this period produced a suite of effects in male rodents including; malformations of the epididymis, vas deferens, seminal vesicles, and prostate, hypospadias, cryptorchidism, retention of the nipples/areolae, and reduced anogenital distance. Severity of effects were dependent on the phthalate (DEHP > DBP > BBP) and increased with dose, with lower doses primarily affecting anogenital distance and areolae retention.

The earliest adverse effects of phthalates have been associated with the development and function of Leydig cells. Exposed fetal testes develop Leydig cell hyperplasia or large aggregates of fetal Leydig cells. This effect is preceded by by a large reduction in fetal testicular testosterone production. Lowered testosterone production probably accounts for malformations in the vas deferens, epididymis, and seminal vesicles (from an inability of the Wolffian duct to develop normally), malformations in the prostate, retained nipples and areolae, hypospadias, and decreased ano-genital distance (through reductions in dihydrotestosterone). Cryptorchidism is also probably mediated through lowered androgen levels in concert with another Leydig cell factor, insulin-like factor 3 (insl3), possibly a promoter found on insl3 (SF-1), or the insl3 receptor (LGR8). Phthalate-induced Leydig cell aggregates (Gd 19 cells) are also preceded by downregulation of steroid production and transport mRNA (steroid acute regulatory protein, 34% of control; P450 side chain cleavage enzyme, 5% of control; CYP 17 (P450c17), 59% of control, 3-β hydroxysteroid dehydrogenase, 52% of control) resulting in testosterone and insl3 levels 10% and 25% of control, respectively. AR, 17-β hydroxysteroid dehydrogenase, FSH and LH receptor mRNA were not affected by phthalate exposure.

Gonocyte proliferation is also impaired in the seminiferous cords in phthalate exposed rodents. Multi-nucleate gonocytes reported after exposures may be related to decreases in stem cell factor (SCF) and c-Kit. C-Kit gene expression in fetal tissues can be reduced significantly by as little as 0.1 mg/kg-day phthalate [undescribed].

Foster *et al.* (2006b) summarized the determination of a DEHP NOAEL for rat reproduction. In this SOT abstract, the RACB protocol was used in rats to evaluate DEHP-induced reproductive and developmental effects (1.5, 10, 30, 100, 300, 1000, 7500, 10000 mg/kg; 0.1, 0.5, 1.5, 5, 15, 50, 400, 500 mg/kg-day). The author assessed rats for phthalate syndrome (malformation of the testis, epididymides, prostate, seminal vesicles, and external

genitalia). Enhanced detection of phthalate syndrome was achieved by retaining extra non-breeding animals from each litter that would normally be removed. In rats, significant changes in reproductive tract malformations (RTM) were not observed below 400 mg/kg-day (Table A3.34). At and above that dose, dose-dependent changes were observed in all generations.

Table A3.34 Rat Reproductive Tract Malformations (RTM) Induced by DEHP (Foster *et al.,* 2006b)

	7500 mg/kg (400 mg/kg-day)	10,000 mg/kg (500 mg/kg-day)
F_0	1/10 prostate lesion	2/10 testis lesions
F_1 Bred	7/10 RTM	10/10 RTM
F_1 Non-bred	11/30 RTM	21/21 RTM
F_2 Bred	9/10 RTM	No F_2 offspring produced
F_2 Non-bred	11/20 RTM	No F_2 offspring produced

The author stated that the NOAEL should be 50 mg/kg-day (LOAEL = 400 mg/kg-day) if considering the breeding males. When examining additional animals in the non-bred cohort, reproductive tract malformations were observed at 15 and 50 mg/kg-day, effectively lowering the NOAEL to 5 mg/kg-day. The author summarized that by retaining additional F_1 and F_2 animals, there was an enhanced ability to detect reproductive tract malformations.

Gangolli (1982) reviewed the effects of DEHP on rat testes. The author noted that Shaffer *et al.* (1945) dosed rats for 90 days via the diet with DEHP (0, 0.075, 0.75, 1.5, 5.0%), resulting in tubular atrophy and testicular degeneration (at 1.5 and 5.0% doses). The author also cited Harris *et al.* (1956) in which dietary dosing of rats with 0.5% DEHP for 3 or 24 months led to occasional tubular atrophy. Calley *et al.* (1966) experiments were also reported. In these experiments, mice that were dosed i.p. daily with DEHP (250 mg/kg) for 6 weeks developed a significantly reduced testis weight. Subsequent work done by Gray *et al.* (1977) demonstrated that dietary administration of 0.2, 1.0 and 2.0% DEHP to rats for 90 days resulted in a dose-related relative liver weight increase, and a decrease in the in the relative testis weights (1.0 and 2.0%; Table A3.35). Histology in this study revealed evidence of testis injury (reduction in the diameter of seminiferous tubules, a germinal epithelium comprised of Sertoli cells, spermatogoinia, and a few spermatocytes. Pituitary castration cells were also increased in a dose-dependent fashion.

Table A3.35 Summary of Findings Following 90 day Dietary Dosing of DEHP to Rats (Gangolli 1982)					
DEHP Dietary Level (%, w/w)	DEHP Mean Daily Intake (mg/kg-day)	No. Rats	Relative Testis Weight (g/100 g body weight)	Histological Findings	
				Testicular Injury	Castration Cells in Pituitary
0.0	0	15	0.60	0	0
0.2	150	15	0.61	4 (+)*	1
1.0	750	15	0.41 ($P < 0.001$)	12 (+++)	4
2.0	1500	15	0.23 ($P < 0.001$)	15 (+++)	9

* Severity of damage: (+) slight, (++) moderate, (+++) severe

The author also commented on species sensitivity. The rat, mouse, guinea pig, and ferret, but not the hamster, were susceptible to testicular damage induced by gavage exposures with DEHP (2000 mg/kg for 10 days).

DEHP-induced testicular effects may be related to the disposition of its metabolites, since only MEHP significantly dissociated germ cells from Sertoli cells *in vitro* (Table A3.36) in a dose-dependent fashion (data not shown). This effect was not observed when dosing hamster preparations with MEHP.

Table A3.36 Effect of DEHP and Metabolites on the Dissociation of Germinal Cells from Sertoli Cells (Gangolli 1982)		
Treatment	Total Number of Germinal Cells Released ($*10^6$)	
	24 hr exposure	48 hr exposure
Control	5.8 ± 0.7	4.0 ± 0.5
DEHP (200µM)	5.8 ± 0.2	3.9 ± 0.9
2-ethylhexanol (200µM)	5.3 ± 0.7	3.6 ± 0.6
MEHP (200µM)	18.6 ± 2.0 ($P < 0.001$)	18.3 ± 3.0 ($P < 0.001$)

Gayathri *et al.* (2004) investigated the effects of DEHP on thyroid hormones in Wistar rats. Female wistar rats (150 g) were grouped into 2 groups (16 rats per group). The treatment group was administered DEHP at 7.5 mg/kg via intraperitoneal injection every other day for 14 days (7 injections).

A significant increase in serum T_3 and T_4 and a marginal decrease in TSH was observed in treated rats (Table A3.37). Increases in T_3 and decreases in TSH were mitigated following a recovery of 7 days. T_4 levels remained high, but were not significantly different than controls. Treated rats also displayed pathological evidence of "reactional hyperplasia" when compared to controls.

Table A3.37 Effects of DEHP on Thyroid Hormones after Intraperitoneal Exposures (Gayathri *et al.*, 2004)		
Group	Control	DEHP treatment (7.5 mg/kg)
T_3 (µg/L) ± SD	0.56 ± 0.02	0.89 ± 0.04 (P < 0.01)
T_4 (µg/L) ± SD	39.0 ± 1.98	46.8 ± 2.39 (P < 0.01)
TSH (µlU/mL) ± SD	0.34 ± 0.017	0.32 ± 0.016
7 day Recovery from DEHP Dosing		
Group	Control	DEHP treatment
T_3 (µg/L) ± SD	0.56 ± 0.03	0.50 ± 0.03
T_4 (µg/L) ± SD	39.3 ± 1.96	44.0 ± 2.20
TSH (µlU/mL) ± SD	0.34 ± 0.017	0.347 ± 0.0172

Gray and Butterworth (1980) determined the effects of gavage administration of DEHP on the development, age-dependency, and reversibility of testicular atrophy in male Wistar albino rats. Four, ten, or fifteen week old Wistar rats were gavage dosed daily with DEHP (2800 mg/kg-day) for 10 days. Testosterone proprionate (200 µg/kg-day) and follicle stimulating hormone (FSH; 100 units of pregnant mares serum gonadotrophin) were also administered in some experiments. In another experiment, DEHP was administered in the diet (2%, ~ 1200 mg/kg-day) to 4 week old rats for 10 or 42 days and then switched to a control diet until treatments end. Following treatments, the rats were sacrificed. The testes, seminal vesicles, and ventral prostate were then weighed, and fixed for subsequent histological exam.

Administration of DEHP for 10 days resulted in an age-dependent decrease in the relative testis, seminal vesicle, prostate weight, and body weights. These were associated with various pathologies seen on histologic exam (See Table A3.38). Spermatogonia, Sertoli cells, and some primary spermatocytes were remaining in affected testes. Interstitial tissue was not affected in testes. Administration of testosterone or FSH to DEHP-exposed rats prevented decrements in the accessory gland weights but did not affect testicular weights in 4 week old rats. DEHP-induced testicular, seminal vesicle, and prostate weight decrements and pathologies were reversible regardless if treatment was continued until or past puberty. If treatments were administered past puberty, however, recovery was comparatively slower. The author hypothesized that DEHP may injure Sertoli cells, since the germinal cells are located inside the Sertoli cell barrier.

Table A3.38 DEHP-induced Age-dependent Effects on Reproductive Organs in Wistar Rats (Gray and Butterworth, 1980)				
Rat age at start of treatment (weeks)	Relative organ weight (% control)			Body weight (% control)
	Testis	Seminal Vesicle	Prostate	
4	67 Uniform tubular atrophy (loss of advanced germinal cells)	63	68	78
10	107 5-50% of tubules atrophic (loss of advanced germinal cells)	63	78	79
15	109 No effect	102	103	89

Gray *et al.* (1977) determined the intermediate-term toxicity of DEHP in 2 to 4 week old Sprague-Dawley rats. Male and female rats were administered DEHP in the diet for 17 weeks (15 rats each group; 0, 0.2, 1.0, and 2.0%; [0, 143, 737, 1440 mg/kg-day (M); 0, 154, 797, 1414 mg/kg-day (F)] or 2 or 6 weeks (5 rats each group; 0, 1.0, and 2.0%). Rats were observed frequently for behavioral issues, weighed on days 0, 1, 2, 6, 9, 13, 16, 20 and thereafter on weekly intervals, and food and water weighed measured for a 24 hour period preceding the determination of body weights. Urine was collected on the last two days of treatment and analysed (microscopic particles, albumin, glucose, ketones, bile salts, blood). Urine specific gravity and volume were also measured at various intervals following water deprivation and loading. After incubation, rats were sacrificed, blood samples drawn for hematology (hemoglobin concentration, packed cell volume, erythrocyte, reticulocyte, and leucocyte counts, urea, glucose, total protein, glutamic-pyruvic transaminase activity, glutamic oxalacetic transaminase activity, and lactic dehydrogenase activity), observed for gross pathologies, and organs (brain, heart, liver, stomach, small intestine, cecum, spleen, kidneys, adrenal glands, gonads, pituitary, thyroid) weighed. Some of these tissues were preserved in neutral buffered formalin, sectioned, and stained with hematoxylin-eosin for microscopic analysis. A paired feeding study with male rats (10 per group) was also initiated. These rats were administered 0 or 2% DEHP in the diet, with control rats receiving the same amount of feed as consumed by treated rats.

Rats dosed with 2% DEHP lost hair in the head and ventral body surface from week 1 through the study end. By week 17 all of the 2% treated females, but only 2 of the males had widespread loss of fur. One of these females died on day 119 (week 17). No gross pathologies other than fur loss or body size were reported for female rats. In male rats, flaccid testes with reduced size were noticed in groups fed 2% (by week 2) or 1% (by week 6) DEHP.

Significant decrements in body weight, food consumption, and water consumption were seen in high dose treated rats (Table A3.39). Statistically significant differences from control were seen by day 2 (2% diet), day 6 (1% diet, males), and day 83 (1% diet, females). Body weight (at day 83 and on) and weight gain was also significantly reduced in rats fed the 2% diet treatment in the paired-feeding study, even though these rats consumed more feed than controls (data not shown). Because of this data, the author suggested that unpalatability was, therefore, only partially responsible for the reduced body weight gain.

Table A3.39 Mean Body Weight, Water Consumption, and Food Consumption of DEHP-exposed Rats (Gray *et al.*, 1977)							
Gender	Dietary Level % (mg/kg-day)	Body weight, water intake, and food consumption (g, ml, g) per rat at day					
		Day 0	Day 1	Day 27	Day 55	Day 90	Day 120
Males	0.0 (0)	96, 18.3, 12.3	105, 18.5, 13.7	340, 37.1, 28.7	478, 38.0, 29.7	569, 28.5, 25.2	628, 26.3, 24.1
	0.2 (143)	98, 17.6, 13.1*	105, 19.7, 14.0	325, 37.3, 26.2	455, 36.3, 24.9	539, 32.3, 23.4	588, 24.7, 21.2
	1.0 (737)	98, 18.0, 13.7*	100, 15.1, 10.9**	297**, 34.3, 26.7	417***, 27.9**, 24.5*	493***, 29.0, 24.6	546***, 27.7, 22.8
	2.0 (1440)	99, 18.4, 13.9	99, 15.7, 8.7**	187***, 24.9**, 16.7**	300***, 30.9, 23.1*	413***, 34.4**, 23.4**	447***, 26.9, 22.1
Females	0.0 (0)	85, 15.9, 11.5	92, 15.7, 10.1	214, 21.5, 18.3	273, 21.9, 15.9	309, 18.1, 15.6	329, 19.4, 15.4
	0.2 (154)	88, 17.9, 11.9	95, 18.9, 10.9	216, 24.9, 16.6	277, 34.5, 17.6	308, 26.5, 16.1	325, 22.1, 16.2
	1.0 (797)	87, 17.4, 11.6	90, 15.4, 8.9	210, 26.6*, 18.2	259, 24.5, 16.2	284*, 25.9*, 18.1	297**, 22.6, 17.3
	2.0 (1464)	88, 18.3, 11.9	87, 14.8, 5.8**	131***, 21.1, 12.1**	164***, 21.3, 11.6**	191***, 18.9, 11.5**	201***, 16.7, 7.5**

* P < 0.05, **P < 0.01, ***P < 0.001

Hematological parameters were also altered following DEHP exposures. Significant decrements in hemoglobin concentration (M) and packed cell volume (M&F) were observed by 17 weeks (Table A3.40). No differences in serum parameters were determined in treated or control rats.

Table A3.40 Mean Hematological Data for DEHP-exposed Rats (Gray *et al.*, 1977)							
Gender	Dietary Level % (mg/kg-day)	Study Factors (week 2, 6, and 17)					
		Number of rats	Hb (g/100ml)	PCV (%)	RBC (10^6/mm^3)	Reticulocytes (% of RBC)	WBC (10^3/mm^3)
Males	0.0 (0)	5, 5, 15	14.8, 15.1, 16.0	45, 48, 46	6.26, 7.08, 7.57	2.0, 1.4, 0.9	7.2, 6.2, 6.4
	0.2 (143)	15	., ., 15.4	., ., 45	., ., 7.44	., ., 0.6	., ., 7.5
	1.0 (737)	5, 5, 15	13.0**, 15.0, 14.5*	40, 45, 43**	5.77**, 6.96, 6.97	2.7, 1.6, 0.8	5.6, 6.0, 6.5
	2.0 (1440)	5, 5, 15	14.8, 14.9, 14.5*	42, 46, 43***	6.48, 6.86, 7.60	1.3, 1.3, 0.9	5.3, 5.0, 6.5
Females	0.0 (0)	5, 5, 15	15.1, 15.8, 14.9	43, 49, 45	6.50, 7.81, 7.14	1.6, 1.2, 0.9	4.9, 6.9, 4.7
	0.2 (154)	15	., ., 14.9	., ., 44	., ., 7.05	., ., 0.8	., ., 4.4
	1.0 (797)	5, 5, 15	14.4, 15.4, 14.4	38, 44***, 42*	6.24, 7.90, 7.26	2.2, 1.0, 1.0	4.2, 4.7, 5.4
	2.0 (1464)	5, 5, 15	14.8, 14.4, 13.8	41, 43***, 42**	6.82, 7.49, 6.78	0.8*, 1.2, 0.8	6.4, 4.5, 5.5

HB – hemoglobin, PCV – Packed Cell Volume, RBC – Red Blood Cells, WBC – White Blood Cells

* P < 0.05, **P < 0.01, ***P < 0.001

Urinary composition was normal when viewed microscopically. No consistent dose- or time-related changes were seen in the number of urinary cells, specific gravity, or volume following the concentration test, and specific gravity or volume during the 2 hour dilution test. Significant increases in 0 to 6 hour specific gravity in the concentration test were determined only for male rats at 2 weeks (2% diet, P < 0.05) and 6 weeks (1 and 2% diet, P < 0.01). A significant decrease in 0 to 6 hour volume (P < 0.01) and number of cells in the urine (P < 0.05) was also seen in response to this test (2% diet). Female rats had significantly reduced urine specific gravity (2% diet; weeks 6 and 17; P < 0.05 – 0.01) in the concentration test, reduced urine cellularity (2% diet; week 6; P < 0.05), increased urine volume (2% diet; 17 weeks; P < 0.05), increased specific gravity in the dilution test (P < 0.01), and decreased urine volume in the dilution test (P < 0.05).

Absolute and relative organ weights were significantly changed in the 1% and 2% treatment groups when compared to controls (Table A3.41, A3.42). Both the absolute (M&F) and relative (M) liver weights were also significantly increased in groups dosed with 0.2% DEHP for 17 weeks.

Table A3.41 Absolute Organ Weights in Rats Fed DEHP
(Gray *et al.*, 1977)

Gender	Dietary Level % (mg/kg-day)	Organ Weights (g; week 2, 6, and 17)							
		Number of rats	Brain	Heart	Liver	Spleen	Kidneys	Stomach	Body Weight
Male	0.0 (0)	5, 5, 15	1.95, 2.10, 2.26	0.81, 1.32, 1.65	5.54, 11.24, 14.16	0.52, 0.81, 0.87	1.65, 2.94, 3.40	1.23, 1.69, 1.84	176, 376, 615
	0.2 (143)	15	., ., 2.22	., ., 1.54	., ., 15.81*	., ., 0.86	., ., 3.39	., ., 1.81	., ., 579
	1.0 (737)	5, 5, 15	1.90, 1.98, 2.24	0.69, 1.29, 1.49*	10.16***, 15.12**, 18.38***	0.53, 0.62, 0.77*	1.66, 2.53*, 3.38	1.13, 1.50, 1.78	164, 320**, 532**
	2.0 (1440)	5, 5, 15	1.81, 1.88, 2.13*	0.44***, 0.81**, 1.31***	6.83, 12.57, 18.10***	0.26**, 0.52*, 0.71***	1.11***, 1.62***, 3.04**	1.04*, 1.42*, 1.67*	106**, 189***, 440***
Female	0.0 (0)	5, 5, 15	1.80, 1.93, 2.09	0.68, 0.86, 0.98	5.42, 6.92, 7.35	0.44, 0.63, 0.52	1.61, 1.91, 1.85	1.04, 1.40, 1.27	151, 241, 327
	0.2 (154)	15	., ., 2.07	., ., 1.00	., ., 8.55*	., ., 0.54	., ., 1.97	., ., 1.27	., ., 329
	1.0 (797)	5, 5, 15	1.77, 1.90, 2.06	0.61, 0.80, 0.93	7.76***, 9.46**, 10.32***	0.40, 0.52, 0.52	1.46, 1.76, 1.95	0.95, 1.29, 1.25	147, 215, 301
	2.0 (1464)	5, 5, 15	1.71, 1.70**, 1.96***	0.34***, 0.44***, 0.75***	5.67, 6.19, 8.67*	0.20***, 0.27***, 0.35***	0.90***, 1.01***, 1.33***	0.83*, 1.04**, 1.28	86***, 106***, 193***
Gender	Dietary Level % (mg/kg-day)	Organ Weights (g; week 2, 6, and 17)							
		Number of rats	Small Intestine	Cecum	Adrenals (mg)	Gonads (F=mg)	Pituitary (mg)	Thyroid (mg)	Body Weight
Male	0.0 (0)	5, 5, 15	5.97, 9.06, 8.84	0.95, 1.55, 1.54	35.0, 46.2, 53.9	1.90, 3.25, 3.65	7.2, 9.2, 11.5	11.7, 13.7, 23.1	176, 376, 615
	0.2 (143)	15	., ., 8.93	., ., 1.38	., ., 54.4	., ., 3.49	., ., 11.4	., ., 21.8	., ., 579
	1.0 (737)	5, 5, 15	6.09, 10.06, 8.80	0.90, 1.52, 1.31*	32.2, 35.6, 53.4	1.48, 0.95***, 2.17***	6.4, 8.6, 12.2	13.6, 15.0, 26.4	164, 320**, 532**
	2.0 (1440)	5, 5, 15	4.62, 8.14, 8.31	0.71, 1.06*, 1.27*	28.4, 35.7, 46.1	0.62***, 0.81***, 1.00***	4.4*, 6.3, 12.3	12.3, 14.6, 22.0	106**, 189***, 440***
Female	0.0 (0)	5, 5, 15	5.44, 6.79, 6.54	0.84, 1.04, 0.96	54.0, 54.2, 55.9	86, 133, 99	8.1, 10.7, 13.4	12.3, 15.0, 24.2	151, 241, 327
	0.2 (154)	15	., ., 6.82	., ., 1.05	., ., 61.1	., ., 95	., ., 12.9	., ., 21.1	., ., 329
	1.0 (797)	5, 5, 15	5.55, 6.78, 7.17	0.83, 1.10, 1.01	38.0**, 50.0, 65.3	73, 132, 108	7.1, 11.6, 13.8	13.7, 15.0, 23.1	147, 215, 301
	2.0 (1464)	5, 5, 15	3.68***, 4.03***, 5.82	0.55***, 0.55**, 0.83	28.2***, 29.2***, 37.5***	33**, 41***, 49***	3.5***, 4.9**, 7.6***	9.2, 10.6*, 16.6*	86***, 106***, 193***

* P < 0.05, **P < 0.01, ***P < 0.001

Table A3.42 Relative Organ Weights in Rats Fed DEHP
(Gray *et al.*, 1977)

Gender	Dietary Level % (mg/kg-day)	Organ Weights (g; week 2, 6, and 17)						
		Number of rats	Brain	Heart	Liver	Spleen	Kidneys	Stomach
Male	0.0 (0)	5, 5, 15	1.12, 0.56, 0.37	0.46, 0.35, 0.27	3.15, 2.99, 2.31	0.30, 0.21, 0.14	0.95, 0.78, 0.56	0.70, 0.45, 0.30
	0.2 (143)	15	., ., 0.39	., ., 0.27	., ., 2.72***	., ., 0.15	., ., 0.59	., ., 0.31
	1.0 (737)	5, 5, 15	1.17, 0.62, 0.42**	0.42**, 0.40*, 0.28	6.19***, 4.72***, 3.45***	0.32, 0.19, 0.14	1.01, 0.79, 0.64**	0.70, 0.47, 0.33**
	2.0 (1440)	5, 5, 15	1.75, 1.03**, 0.50***	0.42*, 0.43***, 0.30*	6.47***, 6.76***, 4.12***	0.24*, 0.28, 0.16	1.05, 0.86, 0.70***	1.01**, 0.78**, 0.38***
Female	0.0 (0)	5, 5, 15	1.19, 0.80, 0.64	0.45, 0.36, 0.30	3.61, 2.88, 2.25	0.29, 0.26, 0.16	1.07, 0.80, 0.57	0.69, 0.58, 0.39
	0.2 (154)	15	., ., 0.64	., ., 0.30	., ., 2.60*	., ., 0.17	., ., 0.60	., ., 0.39
	1.0 (797)	5, 5, 15	1.21, 0.89, 0.69	0.42*, 0.38, 0.31	5.28***, 4.40***, 3.46**	0.27, 0.24, 0.17	1.00, 0.82, 0.65***	0.65, 0.60, 0.42
	2.0 (1464)	5, 5, 15	2.04***, 1.63, 1.10***	0.40*, 0.41*, 0.39***	6.62***, 5.83***, 4.59***	0.24*, 0.25, 0.19*	1.06, 0.95**, 0.71***	0.98**, 0.98***, 0.72***
Gender	Dietary Level % (mg/kg-day)	Organ Weights (g; week 2, 6, and 17)						
		Number of rats	Small Intestine	Cecum	Adrenals (mg)	Gonads (F=mg)	Pituitary (mg)	Thyroid (mg)
Male	0.0 (0)	5, 5, 15	3.39, 2.42, 1.45	0.54, 0.42, 0.25	20.1, 12.3, 8.9	1.08, 0.88, 0.60	4.1, 2.6, 1.9	6.6, 3.6, 3.8
	0.2 (143)	15	., ., 1.55	., ., 0.24	., ., 9.5	., ., 0.61	., ., 1.9	., ., 3.4
	1.0 (737)	5, 5, 15	3.76, 3.15*, 1.66*	0.55, 0.47, 0.25	19.9, 11.1, 10.1	0.90, 0.30***, 0.41***	3.9, 2.4, 2.3	8.3, 4.7, 5.1
	2.0 (1440)	5, 5, 15	4.41**, 4.58*, 1.91***	0.67**, 0.54, 0.29*	26.9*, 20.0*, 10.9	0.59, 0.44***, 0.23***	4.0, 3.0, 2.8***	12.0**, 8.1, 5.1
Female	0.0 (0)	5, 5, 15	3.62, 2.80, 2.01	0.56, 0.43, 0.29	36.0, 22.5, 17.2	57, 55, 30	5.4, 4.5, 4.2	8.2, 6.2, 7.5
	0.2 (154)	15	., ., 2.09	., ., 0.32	., ., 19.0	., ., 29	., ., 4.0	., ., 6.5
	1.0 (797)	5, 5, 15	3.79, 3.16, 2.40	0.57, 0.51, 0.34*	25.8**, 23.0, 21.9**	50, 61, 37	4.8, 5.4, 4.6	9.3, 7.1, 7.8
	2.0 (1464)	5, 5, 15	4.39, 3.80***, 3.13***	0.65, 0.52, 0.44***	33.1, 27.7, 20.4	41, 39, 25	4.1, 4.7, 4.0	10.1, 10.1**, 9.1

* P < 0.05, **P < 0.01, ***P < 0.001

DEHP also induced histological changes in tissues. A dose-related increase in enlarged pituitary basophils (containing a clear central vacuole) of the pars distalis was reported after 17 weeks of dosing. Time- and dose-dependent induction of testicular lesions was also determined histopathologically (Table A3.43). Ultrastructurally, severly damages testes had reduced seminiferous tubule diameters and germinal epithelium comprised only of Sertoli cells, spermatogonia, or a few spermatocytes. In moderately damaged testes, approximately 50% of the tubules had similar pathologies. The remaining tubules had normal thickness epithelium and spermatids at all developmental stages, but were lacking many mature sperm. In slightly damages testes, a reduced number of spermatids and mature sperm were observed. Adverse effects were not seen histopathologically in the female gonads or pituitary or in the interstitial cells of the male testes in all dose groups.

Table A3.43 Incidence and Severity of Testicular and Pituitary Damage in DEHP-dosed Rats (Gray *et al.*, 1977)

Dietary Level % (mg/kg-day)	Number of rats (week 2, 6, and 17)	Severity of Testicular Damage (week 2, 6, and 17)			Total incidence of testicular damage	"Castration cells" in pituitary
		Slight	Moderate	Severe		
0.0 (0)	5, 5, 15	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0
0.2 (143)	15	., ., 4	., ., 0	., ., 0	., ., 4	., ., 1
1.0 (737)	5, 5, 15	3, 1, 5	1, 1, 5	0, 2, 2	4, 4, 12***	0, 0, 4
2.0 (1440)	5, 5, 15	0, 0, 0	0, 0, 5	5, 5, 10	5*, 5*, 15***	0, 0, 9

* P < 0.05, **P < 0.01, ***P < 0.001

The author postulated that even though pathological changes were not seen in the kidney, changes in urinary concentration and dilution suggested that a functional impairment was occurring. Additional changes in organ weights with no changes in histopathology were primarily attributed to decreases in body weight. Notable exceptions were cecal enlargement, which has been reported following exposures to di-*n*-butyl phthalate that do not induce weight changes, and increases in the relative heart weight, since it has been shown in other studies that the concentration of DEHP in the heart was four times higher than that in the liver. Data from additional studies demonstrating mitochondrial swelling and dilation of SER and RER, and an inhibition of drug-metabolizing activity also supported a DEHP-induced toxic effect to the liver. Testicular changes were supported histopathologically. The author suggested that damage reported following the administration of 0.2% DEHP in the diet was more than likely a treatment-related effect because of the dose-response relationship of the testicular data and the presence of castration cells.

Gray *et al.* (1982) investigated the biochemical effects of MEHP, 2-ethylhexanol, and other chemicals on primary cultures of rat hepatocytes. Adult male Wistar (Porton-derived) rats were used to generate primary hepatocyte cultures. These were seeded into culture dishes, incubated for 24 hours, and then dosed with MEHP (200 μM) for 2-ethylhexanol (200 μM and 1 mM) for 48 hours. Following incubation, whole cell homogenates were prepared for transmission electron microscopy or assayed for 7-ethoxycoumarin *o*-deethylase (a microsomal

marker), carnitine acetyltransferase activity (peroxisomal and mitochondrial marker), and protein.

Ultrastucturally, cells treated with 200 μM MEHP or 1 mM 2-ethylhexanol had increased numbers of peroxisomes. Biochemically, 200 μM MEHP and 1 mM 2-ethylhexanol strongly increased carnitine acetyltransferase activity (872% and 943%, respectively). 7-ethoxycoumarin *o*-deethylase activity was also increased (197%) following incubation with 1mM 2-ethylhexanol. Carnitine acetyltransferase activity was only minimally increased (121%) following incubations with 200 μM 2-ethylhexanol. The author commented that the demonstration of peroxisome proliferation ultrastructurally and biochemically by MEHP, 2-ethylhexanol, and clofibrate in this *in vitro* rat hepatocyte model mimics effects seen in *in vivo* experiments.

Gray *et al.* (1983) investigated the biochemical effects of MEHP and other phthalates on primary cultures of rat hepatocytes. Six week old male Sprague-Dawley rats were used to generate primary hepatocyte cultures. These were seeded into culture dishes, incubated for 2 hours, and then dosed with MEHP (20, 50, 100, 200, 500, 1000 μM) for 70 hours. Following incubation, whole cell homogenates were prepared for transmission electron microscopy or assayed for cyanide-insensitive palmitoyl-CoA oxidation (a peroxisome-specific marker), carnitine acetyltransferase activity (peroxisomal and mitochondrial marker), and protein.

Ultrastucturally, cells treated with 200 μM MEHP had increased numbers of peroxisomes, many lacking nucleoids. Higher doses of MEHP were cytotoxic. Biochemically, MEHP increased the cellular palmitoyl-CoA oxidation (150 to 550%) and carnitine acetyltransferase activity (250 to 950%) in a dose-related fashion (20 to 200 μM). Ultrastructural changes were not observed following similar treatment with di-*n*-octyl phthalate (DnOP). An increase (125 to 300%) in palmitoyl-CoA oxidation was demonstrated, however, following incubation with DnOP.

Gray *et al.* (2009) investigated the transgenerational effects of DEHP in male CRL:CD (Sprague-Dawley) rats. Sprague-Dawley rat dams were dosed via gavage with 0, 11, 33, 100, or 300 mg/kg-day DEHP during Gd 8 to Ld 17. Sprague-Dawley rats were selected because of their higher sensitivity to phthalate-induced reproductive effects than Wistar or Long Evans Hooded rats. A majority of male offspring were then allowed to recover on untreated feed from 18 to maturity. A number of other male offspring also continued dosing with DEHP from 18 to 63 to 65 days of age. Body weight, food consumption, and clinical signs of toxicity were assessed daily during and after dosing. Following recovery or dosing, rats were sacrificed, and the anogenital distance, sperm counts, reproductive organ weights, serum testosterone and estradiol, and other reproductive parameters were determined.

Administration of DEHP had no effect on the maternal weight or weight gain during gestation, birth, or lactation. Litter size was also not altered following DEHP exposure during gestation or lactation. Anogenital distance was significantly reduced in a dose-dependent fashion in two day old male offspring (but not females) following exposure to DEHP (Table A3.44). Day

two body weights were also significantly reduced in males and slightly reduced in female offspring. Both the percent of males with areolae and the number of areolae per male out of 12 were significantly increased following higher DEHP exposures. The author noted that areolar development was not affected by rat size, unlike the anogenital distance.

Table A3.44 Changes in Rat Reproductive Parameters in Day 2 Pups Following Gestational Exposure to DEHP (Gray *et al.,* 2009)

Reproductive Parameters	DEHP Dose (mg/kg-day)				
	0	11	33	100	300
Mean anogenital dist. in female pups (mm ± SE)	1.34 ± 0.04	1.26 ± 0.02	1.29 ± 0.03	1.36 ± 0.02	1.31 ± 0.03
Mean anogenital dist. in male pups (mm ± SE)	3.25 ± 0.11	3.21 ± 0.05	3.17 ± 0.09	3.17 ± 0.05	2.74 ± 0.08 (P < 0.01)
Female pup weights (g ± SE)	6.95 ± 0.17	7.04 ± 0.12	7.10 ± 0.13	7.27 ± 0.11	6.74 ± 0.08
Male pup weights (g ± SE)	7.51 ± 0.17	7.44 ± 0.11	7.42 ± 0.15	7.64 ± 0.12	6.98 ± 0.10 (P < 0.01)
13-day old males with areolae (% ± SE)	11 ± 5.5	21 ± 8.9	10 ± 4.7	16 ± 6.7	55 ± 10.1 (P < 0.01)
Number of areolae per male out of 12 (± SE)	0.7 ± 0.4	0.8 ± 0.3	0.3 ± 0.1	0.7 ± 0.3	2.9 ± 0.6 (P < 0.01)

Note: Pup values are litter means

DEHP administration during gestation, lactation, and then from PNd 18 to 64 did not affect the offspring weight, body weight gain or serum testosterone or estradiol concentrations (Table A3.45). Dose-dependent decreases in the weight of the Cowper's gland and adrenals, and the total epididymal sperm count were observed following dosing. In contrast, dose-dependent increases were observed in the liver weight and age of puberty. Significant decreases in weight were observed in the highest dose group for all organs except the glans penis, testes, and liver. A significant increase in weight was determined for the liver at the 100 and 300 mg/kg-day doses. Substantial non-significant decreases in weight were reported for the glans penis and testes in the high dose group.

DEHP administration during Gd 8 to Ld 17 and then recovery until maturity did not affect the body weight in a dose-dependent or biologically significant manner (Table A3.45). All reproductive organ weights were significantly decreased in the high dose group, however, and for seminal vesicle weights in the 100 and 300 mg/kg-day dose group. Adrenal and liver weights were not significantly different in treated and control groups. This suggested that effects on these organs were reversible, since weights were reduced and increased respectively for these two organs in rats with continuous DEHP treatment from gestation to maturity. As with rats dosed continuously from gestation to birth, kidney weights in the rats treated from Gd 8 to Ld 17 were significantly reduced in the high dose group. This suggested that DEHP-induced kidney effects were irreversible. The number of nipples per male was significantly increased in the high dose group. The concentration of MEHP in the amniotic fluid increased in a dose-dependent fashion.

The author also stated that the use of relative organ weights was not valid and was not done in this study because of two assumptions that are rarely met; 1) the use of relative weights assumes a linear relationship exists between organ weights and body weight, and 2) the intercept of the relationship passes throught zero. Further descriptive analysis of the data revealed that a standard litter-based analysis of the data did not describe the severity of DEHP-induced testicular effects in an accurate manner.

Table A3.45 Changes in Reproductive Parameters in Rat Pups Following Gestational (and Additional) Exposure to DEHP (Gray *et al.*, 2009)					
F_1 Rat Adults Necropsied After Exposure to DEHP During Gd 8 to Ld 17 and Then to PNd 64					
Reproductive Parameters	DEHP Dose (mg/kg-day)				
	0	11	33	100	300
Number males (litters)	20 (7)	16 (6)	19 (7)	17 (7)	20 (7)
Mean body weight (g ± SE)	371 ± 17	385 ± 7.5	373 ± 10.1	388 ± 12.7	356 ± 9.0
Mean glans penis weight (mg ± SE)	102 ± 3.2	103 ± 2.8	96.5 ± 3.3	103 ± 4.9	90.4 ± 3.8
Mean ventral prostate weight (mg ± SE)	361 ± 19	362 ± 21	374 ± 16	365 ± 15	303 ± 14 (P < 0.01)
Mean seminal vesicle weight (mg ± SE)	1015 ± 47	1104 ± 27	1078 ± 46	1063 ± 47	836 ± 54 (P < 0.01)
Mean levator ani-bulbocavernosus weight (mg ± SE)	913 ± 47	972 ± 27	900 ± 30	926 ± 23	756 ± 26 (P < 0.01)
Mean Cowper's gland weight (mg ± SE)	94.5 ± 3.7	91.2 ± 4.9	86.2 ± 4.5	88.3 ± 5.1	76.1 ± 3.8 (P < 0.01)
Mean epididymides weight (mg ± SE)	667 ± 17	674 ± 25	685 ± 15	659 ± 21	530 ± 22 (P < 0.01)
Mean whole epididymal sperm count * 10^6	92.3 ± 4.5	84.4 ± 3.4	91.6 ± 2.8	84.2 ± 4.1	53.3 ± 6.5 (P < 0.01)
Mean paired testes weight (mg ± SE)	3019 ± 72	2971 ± 139	3068 ± 77	3186 ± 78	2797 ± 93
Mean adrenal weight (mg ± SE)	50.6 ± 2.7	48.3 ± 2.4	47.2 ± 3.0	47.0 ± 2.1	42.7 ± 1.3 (P < 0.05)
Mean liver weight (g ± SE)	16.6 ± 0.72	18.3 ± 0.63	18.0 ± 0.81	20.3 ± 0.78 (P < 0.01)	19.8 ± 0.86 (P < 0.01)
Mean kidney weight (mg ± SE)	2936 ± 123	3066 ± 96	2956 ± 72	2945 ± 113	2755 ± 93 (P < 0.05)
Mean age at puberty (± SE)	45.7 ± 0.64	47.3 ± 1.6	47.6 ± 0.77	47.1 ± 0.98	49.1 ± 0.7 (P < 0.05)
Mean weight at puberty (g ± SE)	233 ± 7.3	251 ± 12.0	251 ± 11.4	252 ± 9.7	251 ± 6.3
Mean weight at 18 days (g ± SE)	34.7 ± 1.3	33.5 ± 0.8	36.0 ± 0.9	37.3 ± 1.0	34.6 ± 0.9
Mean body weight gain (g ± SE)	338 ± 9.2	349 ± 6.0	334 ± 6.1	351 ± 8.1	324 ± 5.5
Mean testosterone (ng/mL ± SE)	2.13 ± 0.28	2.81 ± 0.48	1.99 ± 0.25	2.16 ± 0.28	1.75 ± 0.19
Mean estradiol (pg/mL ± SE)	72.4 ± 23.1	48.0 ± 9.4	48.9 ± 13.6	37.0 ± 4.9	56.0 ± 20.0
Note: Pup values are litter means					
F_1 Rat Adults Necropsied After Exposure to DEHP During Gd 8 to Ld 17 and Then Recovered Until Maturity					
Number males (litters)	63 (13)	55 (12)	67 (14)	76 (14)	54 (13)
Mean body weight (g ± SE)	607 ± 14	664 ± 17 (P < 0.05)	637 ± 18	634 ± 16	616 ± 15
Mean glans penis weight (mg ± SE)	102 ± 1.9	102 ± 2.2	100 ± 1.5	100 ± 1.9	93.0 ± 1.6 (P < 0.01)
Mean ventral prostate weight (mg ± SE)	794 ± 35	781 ± 40	819 ± 21	734 ± 20	691 ± 33 (P < 0.05)
Mean seminal vesicle weight (mg ± SE)	2107 ± 66	2031 ± 68	2045 ± 47	1999 ± 62 (P < 0.05)	1720 ± 46 (P < 0.01)
Mean levator ani-bulbocavernosus weight (mg ± SE)	1309 ± 32	1368 ± 40	1352 ± 19	1319 ± 34	1162 ± 33 (P < 0.01)
Mean Cowper's gland weight (mg ± SE)	205 ± 11	194 ± 13	205 ± 11	198 ± 13	169 ± 5.4 (P < 0.01)
Mean epididymides weight (mg ± SE)	659 ± 11	637 ± 16	655 ± 8.1	630 ± 18	550 ± 48 (P < 0.01)
Mean testis weight (mg ± SE)	1797 ± 25	1767 ± 57	1841 ± 27	1800 ± 46	1660 ± 75 (P < 0.05)
Mean adrenal weight (mg ± SE)	45.0 ± 2.0	44.4 ± 3.4	46.8 ± 1.3	46.5 ± 1.9	44.9 ± 2.1
Mean liver weight (g ± SE)	19.0 ± 0.64	21.1 ± 0.63	20.1 ± 0.70	20.6 ± 0.8	19.2 ± 0.62
Mean kidney weight (mg ± SE)	1979 ± 57	2035 ± 39	1965 ± 58	1975 ± 48	1780 ± 42 (P < 0.01)
Number of nipples per male	0 ± 0	0.08 ± 0.08	0 ± 0	0.15 ± 0.12	1.22 ± 0.41 (P < 0.01)
Mean serum testosterone (ng/mL ± SE)	1.51 ± 0.21	1.29 ± 0.16	1.32 ± 0.16	1.36 ± 0.10	1.22 ± 0.16
Amniotic fluid MEHP (ng/mL; no. litters/no. pups)	7.2 ± 2.2 (2/24)	68.4 ± 17 (2/26)	168 ± 24 (2/28)	748 ± 236 (2/26)	2324 ± 430 (2/32)
Note: Pup values are litter means					

The author combined reproductive tract malformations and lesions of the testes and epididymides to segregate rat offspring into those with and without phthalate syndrome. In the lower dose groups, affected males had retained nipples, flaccid fluid-filled testes, hypoplastic or malformed epididymides, epididymal granuloma with small testes, seminiferous tubule degeneration, malformed seminal vesicles or coagulating glands, and hermaphroditism.

Abnormal gubernacular ligaments were identified in one rat each from the 100 and 300 mg/kg-day dose groups. When considering rats with phthalate syndrome, there was a significant increase in the percentage of affected males in the 11, 33, 100, and 300 mg/kg-day dose groups (Table A3.46 and Table A3.47).

Table A3.46 Specific Reproductive Tract Lesions (Percent) in Offspring Dosed during Gestation and Lactation, and Gestation, Lactation, and Until Maturity
(Gray *et al.,* 2009)

DEHP Dose (mg/kg-day)	0	11	33	100	300
Permanent nipples	0.0	1.4	0.0	2.2	27.0
Malformed seminal vesicle	0.0	0.0	0.0	1.1	5.4
Gross testis abnormality	0.0	5.6	0.0	1.1	17.6
Testis histopathology	0.0	4.2	11.6	4.3	24.3
Gross epididymal abnormality	0.0	1.4	0.0	1.1	20.3
Epididymal histopathology	0.0	1.4	0.0	2.2	14.9
Severe malformation of glans penis	0.0	0.0	0.0	1.1	1.4
Ovotestis/uterus present	0.0	0.0	0.0	1.1	0.0
Coagulating gland malformed	0.0	2.8	0.0	6.5	6.8
Hypospadias	0.0	0.0	0.0	1.1	1.4
Vaginal pouch	0.0	0.0	0.0	1.1	1.4
Cranial suspensory ligament-testis	0.0	0.0	0.0	0.0	0.0
Abnormal gubernaculum	0.0	0.0	0.0	1.1	1.4
Vas deferens agenesis	0.0	0.0	0.0	1.1	0.0
Total affected with phthalate syndrome	0.0	11.3	11.6	12.9	51.3

Table A3.47 DEHP-induced Gross and Histological Lesions in Rats with Phthalate Syndrome
(Gray *et al.,* 2009)

DEHP Dose (mg/kg-day)	0	11	33	100	300
Pups dosed Gd 8-PNd 17 and PNd 18-64 – cohort 1	0/20	2/16	0/19	2/17	7/20
Pups dosed Gd 8 – PNd 17 – cohort 1	0/23	3/25	6/31	5/25	17/23
Pooled 1	0/43 pups	5/41	6/50	7/42	24/43
Pups dosed Gd 8 – PNd 17 – cohort 2	0/40	3/30	4/36	5/51	14/31
Pooled 1 and 2 (% affected)	0/83	8/71 (11.3; $P < 0.01$)	10/86 (11.6; $P < 0.01$)	12/93 (12.9; $P < 0.01$)	38/74 (51.3; $P < 0.001$)

The author commented that the LOAEL generated from the summarized data (11mg/kg-day) was similar to that described in limited detail by Foster *et al.* (2006; 15 mg/kg-day; NOAEL = 5 mg/kg-day; Table A3.34) and the level of concern and critical effect discussed by the NTP CERHR Panel (2003; 10 to 113 mg/kg-day level of concern for exposures included in gestational or peripubertal periods; 14 to 23 mg/kg-day for critical effect including small reproductive organ size; NOAEL = 4.8 mg/kg-day). The author further described various calculations of statistical power derived from changes in the weights of various reproductive organs, anogenital distance, or age at puberty on a "pups per litter" basis. The author concluded that the most sensitive determinant of reproductive toxicity was an aggregate of endpoints such as those present in the

phthalate syndrome and that concentrations as low as 68 ng/mL could potentially induce reproductive tract malformations in 10% of the rats. The author also recognized that low doses of DEHP (10, 100 mg/kg-day) did not accelerate puberty in male rats or increase testosterone or estradiol concentrations, in contrast to human models proposed by others (Akingbemi *et al.,* 2004).

Hellwig *et al.* (1997) determined the rat prenatal toxicity of DEHP. Mature virgin Wistar rats were exposed to DEHP (0, 40, 200, 1000 mg/kg-day) daily from day 6 to 15 post-coitum. Clinical behavior, body weight, food consumption, maternal liver, kidney, and uterus weights, and various developmental changes (pup weights, malformations, variations, retardations, etc) were reported following rat sacrifice.

Administration of DEHP during gestation initially decreased food consumption and body weights. Significantly increased relative liver and kidney weights, reduced uterine weights, and substantial postimplantation loss were also reported. The number of live fetuses per dam and mean fetal body weights were reduced (Table A3.48). In contrast, the number of fetuses with developmental effects was significantly increased. The number of number of fetuses with external (9 fetuses/6 litters; 13.4%), soft tissue (22/9; 75.6%), and skeletal malformations (26/9; 67.4%) increased in the highest dose group. Dose-dependent increases in developmental alterations were reported for a few pathologies such as: dilated renal pelvis, thoracic vertebral body/bodies dumbbell shaped (asymmetric), and skeletal variations involving the accessory 14^{th} rib(s) (Table A3.49). No effects were seen on the number of pregnant dams, maternal lethality, corpora lutea per dam, implantation sites per dam, dams with viable fetuses, and the number and percent of litters with variations or retardations.

Table A3.48 Maternal and Developmental Effects After DEHP Administration to Rats During Gestation (Hellwig *et al.*, 1997)				
Parameters	Dose Levels (mg/kg-day)			
	0	40	200	1000
Litters investigated	10	9	10	9
Clinical signs	-	-	-	+ (2/9; vaginal hemorrhage on Gd 15)
Day 0 mean body weight (g)	216.2	219.6	216.1	215.1
Day 6 mean body weight (g)	249.8	258.5	251.0	247.8
Day 10 mean body weight (g)	265.6	279.4 ($P \leq 0.05$)	267.5	259.6
Day 15 mean body weight (g)	293.2	310.1	294.7	286.1
Day 20 mean body weight (g)	362.4	384.0	362.0	336.4
Mean relative liver weight (g)	4.411	4.443	4.624	5.074 ($P \leq 0.01$)
Mean relative kidney weight (g)	0.506	0.527	0.557	0.574 ($P \leq 0.05$)
Mean uterus weight (g)	74.0	82.5	72.8	45.4 ($P \leq 0.05$)
Percent mean pre-/post-implantation loss	4.5/10.4	7.6/3.6	18.6/4.3	7.8/40.1 ($P \leq 0.01$)
Mean total resorptions (%)	1.4 (9.8)	0.6 (3.6)	0.7 (4.3)	5.3 ($P \leq 0.01$)/40.1 ($P \leq 0.01$)
Mean early/late resorptions	1.1/0.3	0.3/0.2	0.6/0.1	4.2 ($P \leq 0.01$)/1.1
Mean live fetuses per dam	12.8	14.3	12.5	8.4
Mean fetal weights (g)	3.9	3.9	3.9	3.2 ($P \leq 0.01$)
Number fetuses with malformations (%)	2 (1.6)	4 (3.1)	5 (4.0)	48 (63)
Number litters with malformations (%)	1 (10)	3 (33)	4 (40)	9 ($P \leq 0.01$) (100)
- affected fetuses per litter (%)	1.7	2.9	8.1	70.1 ($P \leq 0.01$)
Number fetuses with variations (%)	32 (25)	39 (30)	33 (26)	61 (80)
Number litters with variations (%)	10 (100)	9 (100)	8 (80)	9 (100)
- affected fetuses per litter (%)	24.3	29.7	23.8	80.2 ($P \leq 0.01$)
Number fetuses with retardations (%)	50 (39)	40 (31)	36 (29)	43 (57)
Number litters with retardations (%)	10 (100)	9 (100)	9 (90)	9 (100)
- affected fetuses per litter (%)	37.5	32.0	29.5	58.3 ($P \leq 0.01$)

Table A3.49 Developmental Pathologies Following Administration of DEHP to Rat Dams During Gestation (Hellwig *et al.*, 1997)				
Parameters	Dose Levels (mg/kg-day)			
	0	40	200	1000
Number of fetuses (litters) observed	129 (10)	129 (10)	125 (10)	76 (9)
Menigocele	-	-	-	1 (1)
Cleft palate	-	-	-	1 (1)
Anopthalmia	-	-	-	2 (1)
Filiformed tail	-	-	-	5 (5)
Efferent urinary tract severely dilated	-	-	-	6 (4)
Situs inversus	1 (1)	-	-	-
Hydrocephaly	-	-	-	6 (5)
Truncus arteriosus commenus	-	-	-	1 (1)
Transposition (aorta é right ventricle)	-	-	-	1 (1)
Malformation of great vessels	-	-	-	1 (1)
Transposition of great vessels	-	-	-	1 (1)
Globular shaped heart (ventricular dilation)	-	-	2 (1)	1 (1)
Hernia diaphragmatica	-	1 (1)	-	1 (1)
Agenesia of kidney(s)	-	-	-	4 (2)
Agenesia of ureter(s)	-	-	-	4 (2)
Hyperplasia of kidney	-	-	-	1 (1)
Abnormal position of testis/testes	-	-	-	4 (3)
Abnormal position of ovaries	-	-	-	6 (3)
Hypoplasia of uterine horn(s)	-	-	-	7 (4)
Dilated renal pelvis	4 (4)	12 (5)	9 (7)	17 (7)
Hydroureter	-	2 (2)	1 (1)	17 (8)
Fetus with multiple malformations of ribs and vertebral column	-	-	-	1 (1)
Scoliosis	-	-	-	1 (1)
Thoracic vertebral arch and corr. rib missing	-	-	-	1 (1)
Thoracic vertebral body/bodies dumbbell shaped (asymmetric)	1 (1)	2 (2)	3 (3)	13 (7)
Thoracic vertebral body/bodies bipartite (asymmetric)	-	-	-	11 (6)
Cervical arches fused	-	-	-	3 (2)
Thoracic vertebral column severely malformed	-	-	-	1 (1)
Thoracic vertebrae fused	-	-	-	1 (1)
Lumbar vertebrae fused and/or irregularly shaped	-	-	-	6 (5)
Sacral vertebral column severely malformed	-	-	-	1 (1)
Sacral vertebra(e) absent	-	-	-	4 (4)
Sacral vertebral body bipartite (asymmetric)	-	-	-	1 (1)
Sacral vertebrae fused and/or irregularly shaped	-	-	-	5 (3)
Caudal vertebra(e) absent	-	-	-	5 (5)
Sternebrae bipartite, ossification center bipartite	-	1 (1)	-	14 (7)
Fused ribs	-	-	-	1 (1)
Supernumerary rib	-	-	-	1 (1)
Bifurcated rib(s)	-	-	-	2 (2)
Forelimb bent (including bony part)	-	-	-	1 (1)
Total skeletal variations	28 (10)	27 (9)	24 (8)	41 (9)
- accessory thoracic vertebra	-	-	-	10 (5)
- sternebrae bipartite (symmetrical)	1 (1)	2 (2)	-	9 (4)
- accessory 14^{th} rib(s)	1 (1)	1 (1)	2 (2)	21 (7)
Total skeletal retardations	50 (10)	40 (9)	36 (9)	43 (9)
- skull incompletely ossified	-	-	-	11 (6)
- sternebrae: only ossification center	13 (6)	4 (3)	2 (2)	22 (8)

The author noted that "no treatment-related" effects were observed in the 40 or 200 mg/kg-day treatment groups (dams or fetuses) and that the 1000 mg/kg-day dose was distinctly teratogenic. Further, it was reported that DEHP was a more potent teratogen than 2-ethylhexanol on a molar basis.

Hinton *et al.* (1986) administered DEHP in feed to rats in order to determine alterations in liver and thyroid structure and function. In the first experiment, male Wistar rats (University of Surrey strain; initially 85 to 115 g) were fed 0 and 2% dietary DEHP (2000 mg/kg-day), respectively, for 3, 10, and 21 days. Following experimental intervals, six control rats and four treatment rats were sacrificed for analysis. Treatment with DEHP for 21 days induced peroxisome proliferation, induction of ω-oxidation of fatty acids and an accumulation of fat in the centrilobular zone of the liver without hepatic fatty necrosis (Table A3.50)

Table A3.50 Morphological Changes in Livers of Rats Administered 2% DEHP (Hinton *et al.*, 1986)

Effect	**Comparative change**
Hepatomegaly	+++
General appearance	Dark
Centrilobular loss of glycogen	+
Total glycogen loss after 21 days texposure	+
Periportal fat accumulation	+
Peroxisome proliferation	+++
Smooth endoplasmic proliferation	++
Loss of rough endoplasmic reticulum	+
Increased density of inner mitochondrial matrix	++
Initial burst of mitosis	++
+ = degree of change when compared to controls	

In the second experiment, 4 male and 4 female Wistar rats (ICI strain; initially 200 g) were administered 0.05%, 0.2%, or 1.0% DEHP in feed (50, 200, 1000 mg/kg-day) for 3, 7, 14, 28 days, and 9 months prior to sacrifice and analysis. Six control rats of each sex were also sacrificed for each timepoint (Table A3.51).

Table A3.51 Hepatic Alterations in Wistar Rats (Hinton *et al.*, 1986)							
Site of Effect (percent of control)	DEHP (mg/kg-day)						Clofibrate (400 mg/kg-day)
	50 (M)	200 (M)	1000 (M)	50 (F)	200 (F)	1000 (F)	
Body weight, g	102	98	93	101	92	91	101
Liver weight, g	113 (P < 0.05)	129 (P < 0.05)	155 (P < 0.05)	111	106	127 (P < 0.05)	151
Palmitoyl-CoA oxidation	162 (P < 0.05)	257 (P < 0.05)	568 (P < 0.05)	137	205 (P < 0.05)	550 (P < 0.05)	> 300 (P < 0.05)
α-glycerophosphate dehydrogenase	215 (P < 0.05)	299 (P < 0.05)	336 (P < 0.05)	156	205 (P < 0.05)	310 (P < 0.05)	-
Catalase	100	109	122 (P < 0.05)	100	109	137 (P < 0.05)	162 (P < 0.05)
Uricase	94 (P < 0.05)	78 (P < 0.05)	89 (P < 0.05)	100	100	106	-
% Catalase sedimentable							
Glucose-6 phosphatase	91	77 (P < 0.05)	50 (P < 0.05)	80 (P < 0.05)	77 (P < 0.05)	60 (P < 0.05)	51 (P < 0.05)
Cytochrome p-450	110	120	135	111	102	117 (P < 0.05)	176 (P < 0.05)
Cytochrome b_5	130	115 (P < 0.05)	117	104	115	116	99
Laurate hydroxylase	220 (P < 0.05)	362 (P < 0.05)	479 (P < 0.05)	164 (P < 0.05)	193 (P < 0.05)	311 (P < 0.05)	795 (P < 0.05)
Ethoxycoumarin deethylase	102	114 (P < 0.05)	110 (P < 0.05)	148 (P < 0.05)	151 (P < 0.05)	193 (P < 0.05)	-
β-D-galactosidase	119	115	189 (P < 0.05)	126	133 (P < 0.05)	187 (P < 0.05)	216 (P < 0.05)
Nonprotein SH	125	87 (P < 0.05)	68 (P < 0.05)	68 (P < 0.05)	81 (P < 0.05)	88	66 (P < 0.05)

In the third experiment, male Wistar rats (ICI strain; initially 750 to 800 g) were fed control, clofibrate (400 mg/kg-day), fenofibrate (200 mg/kg-day), and DEHP (1000 mg/kg-day) diets. Four control and four treatment rats were sacrificed at 3 and 13 days following treatment initiation. Overall, the older rats did not consume the diets as readily as younger rats and had slight body weight decrements. In all treatments, absolute and relative liver weight increased after 3 days and through 90 days (Table A3.52).

Table A3.52 Dietary Effects of DEHP, Fenofibrate, and Clofibrate on Mature Rats (Hinton *et al.*, 1986)				
Parameter	Control	Fenofibrate	Clofibrate	DEHP
Food consumption (0-3 d; g/rat/day)	21.46	20.56	10.00	15.40
Food consumption (3-13 d; g/rat/day)	24.61	16.80	11.46	16.50
Additive consumption (0-3 d; g/rat/day)	-	191	101	446
Additive consumption (3-13 d; g/rat/day)	-	140	183	781
Body weight (3 d; g ± S.E.)	771 ± 19	93	85 (P < 0.05)	97
Body weight (13 d; g ± S.E.)	775 ± 31	87	73	88
Absolute liver weight (3 d; g ± S.E.)	20.06 ± 1.96	127	73	110
Absolute liver weight (13 d; g ± S.E.)	20.72 ± 1.22	106	74	111
Relative liver weight (3 d; % of body weight ± S.E.)	0.026 ± 0.003	136 (P < 0.05)	85	114
Relative liver weight (13 d; % of body weight ± S.E.)	0.029 ± 0.001	121 (P < 0.05)	101	126 (P < 0.05)

Histopathological investigation (H&E) of liver sections revealed slightly increased centrilobular eosinophilia, a marked glycogen loss from the centrilobular areas, and a periportal

accumulation of fat in all treatment groups by 13 days. The distribution of "neutral fat" was also affected by DEHP and other treatments. The number of mitotic figures was not affected by DEHP treatment for 13 days.

Ultrastructural observations with electron microscopy revealed compound-related peroxisomal increases, increased density in the inner mitochondrial matrix with swelling and internal structure loss, proliferation of the smooth endoplasmic reticulum (SER), and dilation and degranulation of the rough endoplasmic reticulum (RER). The number of lipid droplets was also increased in treated animals when compared to controls, with day 3 having more lipid droplets than day 13. Enzymes associated with peroxisomes and the endoplasmic reticulum were also induced or inhibited by treatments. KCN-insensitive palmitoyl-CoA oxidase (PCoA), total catalase, cytochrome P-450, and laurate hydroxylase activity were induced by treatments. In contrast, glucose-6-phosphatase and β-D-galactosidase activity were reduced in treated animals (Table A3.53).

Table A3.53 Dietary Effects of DEHP, Fenofibrate, and Clofibrate on Mature Rat Liver Enzymes (Hinton *et al.*, 1986)

Parameter	Control	Fenofibrate	Clofibrate	DEHP
Total catalase activity (3 d; U/mg h/min; ± SE)	1.53 ± 0.12	113	89	100
Total catalase activity (13 d; U/mg h/min ± SE)	1.71 ± 0.10	128 (P < 0.05)	112	121 (P < 0.05)
KCN-ins PCoA Ox activity (3 d; nmole NAD+reduced/min/kg protein ± SE)	0.60 ± 0.03	282	305 (P < 0.05)	121
KCN-ins PCoA Ox activity (13 d; nmole NAD+reduced/min/kg protein ± SE)	0.54 ± 0.06	712 (P < 0.05)	256 (P < 0.05)	424 (P < 0.05)
Glucose-6-phosphatase activity (3 d; µmole/min/mg homog protein ± SE)	27.4 ± 2.4	77	102	83
Glucose-6-phosphatase activity (13 d; µmole/min/mg homog protein)	31.4 ± 0.7	62 (P < 0.05)	92	67
Cytochrome P-450 activity (3 d; nmole/mg microsomal protein ± SE)	1.10 ± 0.03	118	85	118
Cytochrome P-450 activity (13 d; nmole/mg microsomal protein ± SE)	0.70 ± 0.01	150	130	145
Laurate hydroxylase activity (3d; units/mg microsomal protein ± SE)	2.79 ± 0.26	267 (P < 0.05)	132 (P < 0.05)	216 (P < 0.05)
Laurate hydroxylase activity (13d; units/mg microsomal protein ± SE)	4.70 ± 0.43	312 (P < 0.05)	292 (P < 0.05)	266 (P < 0.05)
β-D-galactosidase activity (3 d; µmole/min/mg homog protein ± SE)	3.31 ± 0.49	87	97	87
β-D-galactosidase activity (13 d; µmole/min/mg homog protein ± SE)	3.78 ± 0.25	81	79	98

In the fourth experiment, male Wistar rats (University of Surrey strain) were fed a control diet, 0.4% clofibrate, or 1% DEHP (~1000 mg/kg-day). At days 3, 10, and 21, six control rats and six treatment rats were sacrificed for analysis of thyroid structure and function. Overall,

ultrastructural observations revealed increased number and size of lysosomes, enlarged Golgi apparatus, and damaged mitochondria of thyroids in rats treated with DEHP for 21 days. These changes were more significant than slight changes seen in serum triiodothyronine (T_3) or thyroxine (T_4; Table A3.54). Ultrastructural damage was suggestive of thyroid hyperactivity.

Table A3.54 Effects of Dietary Administration of DEHP on the Rat Thyroid (Hinton *et al.*, 1986)			
Parameter	Control	20,000 mg/kg DEHP (% control)	4000 mg/kg Clofibrate (% control)
T_3 (µg/L) – 3 days	0.37 ± 0.07	89	-
T_3 (µg/L) - 10 days	0.33 ± 0.08	79	-
T_3 (µg/L) – 21 days	0.30 ± 0.05	140	-
T_4 (µg/L) – 3 days	30 ± 6	66	-
T_4 (µg/L) - 10 days	27 ± 3	85	-
T_4 (µg/L) – 21 days	33 ± 5	64	-
Parameter	Control	10,000 mg/kg DEHP (% control)	4000 mg/kg Clofibrate (% control)
T_3 (µg/L) – 7 days	0.37 ± 0.05	54	41
T_3 (µg/L) – 21 days	0.32 ± 0.03	119	125
T_4 (µg/L) – 7 days	33.3 ± 0.5	58	36 ($P < 0.05$)
T_4 (µg/L) – 21 days	31.0 ± 2.0	55 ($P < 0.05$)	65 ($P < 0.05$)

In vitro experiments involving rat hepatocytes and 0.05, 0.1, and 0.25 mM MEHP were also conducted in order to determine enzymatic and ultrastructural effects. *In vitro* experiments revealed MEHP-induced dose-dependent induction of palmitoyl-CoA oxidation (Table A3.55). The highest *in vitro* dose of MEHP also resulted in increased blebbing and vacoulation with no associated increase in lactate dehydrogenase (cell death). Increased lipid accumulation was also observed, with small droplet accumulation occurring at cellular margins. A rapid increase in the incorporation of palmitate into triglycerides and cholesterol esters and increase in fatty acid oxidation was also observed in cells collected from fasted rats. Fenofibric acid and clofibric acid responded similarly to clofibrate and MEHP.

Table A3.55 *In vitro* Effect of MEHP on Palmitoyl-CoA Oxidation (Hinton *et al.*, 1986)	
Treatment	CN- independent palmitoyl-CoA oxidation
Control	1.03 ± 0.03
0.05 mM MEHP	2.90 ± 0.57 ($P < 0.05$)
0.1 mM MEHP	5.85 ± 0.27 ($P < 0.05$)
0.25 mM MEHP	10.27 ± 0.30 ($P < 0.05$)

Jones *et al.* (1993) determined the effects of DEHP and MEHP on *in vitro* and *in vivo* Leydig cell structure. Male Wistar rats were gavage dose with DEHP (0, 2000 mg/kg) once daily for 2 days. Twenty-four hours following the last dose, rats were sacrificed and their testes

removed, fixed, and prepared for histopathological evaluation using toluidine blue and transmission electron microscopy. In *in vitro* experiments, normal untreated male Wistar rats were sacrificed and had their testes removed. Leydig cells were isolated from these testes by collagenase digestion. Isolated cells were exposed to MEHP (0, 10 µM, 100 µM, 1 mM) in the presence or absence of lutenizing hormone (100 ng/mL) for 2 to 3 hours. Lutenizing hormone (LH) was added to cultures to assess Leydig cell functional integrity (testosterone output). At the end of treatment, cells were either processed for testosterone determination or electron microscopy.

Exposure to DEHP resulted in a "slight rarefaction" or vacoulation in a few seminiferous tubule Sertoli cells when viewed via histopathology sections. Ultrastructurally, DEHP exposures also induced mitochondrial swelling (with matrix granule degradation) and focal dilatation and vesiculation of the smooth endoplasmic reticulum (SER) in Leydig cells, and increased interstitial macrophage activity in cells with large cytoplasmic alterations (on the surface of Leydig cells). *In vitro* exposure to MEHP also induced mitochondrial swelling with a loss of matrix granules, focal dilatation of the SER, and increased number and length of filopodia associated with basal lamellar processes. *In vitro* experiments also demonstrated that LH-induced testosterone secretion from Leydig cells was decreased up to 20 to 25% of controls in a dose- and time-dependent fashion.

The author concludes that exposure to DEHP or MEHP impacts Leydig cell structure and function and that changes induced by the two are relatively similar. Similar qualitative changes are also demonstrated between MEHP and MnOP, although quantitatively MnOP suppresses the secretion of testosterone from Leydig cell to a greater extent than MEHP. The author further postulated that macrophage accumulation might be related to Leydig cells with leaky membranes and that damage to Leydig cells is likely to affect Sertoli cells because of the relationship between androgen levels and spermatogenesis.

Lamb *et al.* (1987) investigated the reproductive effects of DEHP and three other phthalates (DEP, DBP, and DHP) in mice in a continuos breeding protocol. Male and female COBS Crl:CD-1 mice were dosed with DEHP in the diet (0, 0.01, 0.1, 0.3%; 0, 14 to 20, 140 to 200, 420 to 600 mg/kg-day) for 7 days in same sex cages, then randomly combined into breeding pairs and dosed via diet for another 98 days. Information (body weight, proportion of males, number of litters per pair, number of live pups) was collected on newborn pups within 12 hours of birth and then litters were discarded. Following 98 days, the pair was separated. Dosing was continued for 21 days. Litters produced during this period were kept for 21 days or more. At the end of dosing high dose mice were mated to control mice to determine the affected sex. Information was gathered on the offspring as above. Parental animals were necropsied following dosing and sacrifice. Organ weights and histology were assessed for both male and female parents. The percentage of motile sperm, sperm concentration, and percentage of abnormal sperm were also determined in male animals.

No treatment-related clinical signs of toxicity were observed during the breeding periods, even though one male died in the 0.1% group and two females died in the 0.3% group. No reductions in feed consumption were reported for dosed animals and only a slight decrement in male mean body weight was seen at week one (36.3 g, control vs. 34.6 g, high dose). Decrements in body weight were not observed in male mice at the termination of the study (week 13).

Fertility and reproductive performance was adversely affected in groups dosed with higher concentrations of DEHP (Table A3.56). The number of fetile pairs of mice were significantly decreased in a dose-dependent fashion ($P < 0.01$ at 0.3%). The number of litters per pair, live pups per litter, and proportion of pups born alive were also significantly decreased dose-dependently ($P < 0.01$ at 0.1%). The reproductive effects were not time-dependent.

Table A3.56 DEHP-induced Changes in Fertility or Reproduction in F_0 Generation Mice (Lamb *et al.*, 1987)

Reproductive Parameter	Control	0.01% (14-20 mg/kg-day)	0.1% (140-200 mg/kg-day)	0.3% (420-600 mg/kg-day)
# Fertile/# cohabitated (%)	40/40 (100)	20/20 (100)	14/19 (74)	0/18 (0) $P < 0.01$
Mean litters/pair ± SE (n)	4.65 ± 0.13 (40)	4.65 ± 0.18 (20)	3.07 ± 0.49 (14) $P < 0.01$	-
Mean live pups/litter ± SE (n)	10.62 ± 0.32 (40)	9.92 ± 0.50 (20)	5.16 ± 1.13 (14) $P < 0.01$	-
Mean proportion of pups born alive ± SE (n)	0.98 ± 0.01 (40)	0.99 ± 0.01 (20)	0.80 ± 0.09 (14) $P < 0.01$	-
Mean live pup weight in g ± SE (n)	1.57 ± 0.02 (40)	1.58 ± 0.03 (20)	1.62 ± 0.04 (14) $P < 0.01$	-

Crossover mating studies with F_0 mice revealed that there was a decrease in fertility for both males and females when compared to controls, but no change in libido (Table A3.57).

Table A3.57 Crossover Mating Trials to Determine the DEHP-affected Sex of Mouse (Lamb *et al.*, 1987)

Reproductive Parameter	Control male X control female	Control female X 0.3% DEHP male	Control male X 0.3% DEHP female
# with copulatory plugs/# cohabited (%)	18/20 (90)	16/20 (80)	13/16 (81)
# fertile/# cohabited (%) [1]	18/20 (90)	4/20 (20) $P < 0.05$	0/16 (0) $P < 0.05$
Mean live pups/litter ± SE (n)	8.56 ± 0.82 (18)	6.5 ± 2.36 (4)	-
Mean proportion of pups born alive ± SE (n)	0.91 ± 0.06 (18)	0.71 ± 0.24 (4) $P < 0.05$	-
Mean live pup weight in g ± SE (n)	1.64 ± 0.06 (17) $P < 0.05$ [2]	1.73 ± 0.09 (3) $P < 0.05$ [2]	-

[1] a fertile pair = a pair that produced a litter of one or more live or dead pups

[2] one litter had all dead pups

After crossover studies, the remaining mice were sacrificed. Select organs were then weighed. The high DEHP dose group significantly increased the absolute and relative liver weights, and significantly reduced testis, epididymis, and prostate weights ($P < 0.05$ to 0.01; Table A3.58). The percent of motile sperm and sperm concentration were also significantly reduced ($P < 0.01$). The percent of abnormal sperm was significantly increased ($P < 0.01$).

Table A3.58 DEHP-induced Changes in Organ Weight and Sperm Parameters in Mice (Lamb *et al.*, 1987)		
	Control	0.3% DEHP
Body weight ± SE (g)	38.25 ± 0.43 (35)	39.01 ± 0.62 (19)
Mean Liver ± SE (g)	2.23 ± 0.05 (36)	2.84 ± 0.07 (19) P < 0.01
Mean right testis ± SE (mg)	135 ± 4.4 (36)	55 ± 7.9 (19) P < 0.01
Mean right epididymis ± SE (mg)	58 ± 1.3 (36)	47 ± 1.9 (19) P < 0.01
Mean prostate ± SE (mg)	70 ± 2.7 (36)	62 ± 4.1 (19) P < 0.05
Mean seminal vesicle (mg)	369 ± 15.5 (36)	362 ± 15.1 (19)
Mean % motile sperm ± SE	87.47 ± 2.38 (36)	34.70 ± 13.41 (10) P < 0.01
Mean sperm concentration ± SE (# sperm X 10^3/mg caudal tissue)	473 ± 24 (36)	101 ± 50 (19) P < 0.01
Mean % abnormal sperm ± SE [1]	2.01 ± 0.42 (36)	15.37 ± 5.50 (8) P < 0.01

[1] Sperm without tails were not included when calculating percentage of abnormal sperm

No histologic pathologies were observed in female mice. Bilateral atrophy of the seminiferous tubules (testicular atrophy) was reported in male mice of the high dose group (0.3% DEHP; 420 to 600 mg/kg-day).

Lloyd and Foster (1988) determined the effects of MEHP on FSH responsiveness in cultured rat Sertoli cells. Sertoli cell cultures were prepared from the testes of juvenile Wistar rats (Alpk:AP; 28 days old) and incubated in a defined culture medium. Germ cells were removed from this culture by hypotonic shock. Twenty-four hours later cultures were treated with MEHP (0.1, 1, 10, 100 µM) for 3, 5, or 24 hours. Two or three hours prior to the incubations end, FSH, forskolin, or choleratoxin were added to the media. At the end of incubation, the medium was removed and assayed for cAMP.

Addition of FSH, forskolin, or choleratoxin to the culture media for 2 to 3 hours increased the production of cAMP from 0 to 11.2 pmol cAMP/mg protein (control cells) to 33.6 to 85.0, 728 to 1881, and 17.5 to 80.3 pmol cAMP/mg protein, respectively, in a dose-related fashion.

Pre-treatment with MEHP (0.1 to 100 µM) reduced the production of FSH-stimulated cAMP in a time- and dose-dependent manner. Significant reductions were seen at 3hr (100 µM), 5hr (1 µM), and 24 hr (0.1 µM). Pretreatment with MEHP did not reduce the forskolin or choleratoxin-iduced elevations in cAMP concentration. For both forskolin and choleratoxin, low doses (0.01-1 µM) potentiated cAMP production. ATP levels did not change after treatment with 1 to 100 µM MEHP. The author postulated that MEHP may be reducing the FSH-stimulated production of cAMP by interacting with the FSH receptor (and not adenyl cyclase or phosphodiesterase), since it is the only component of the FSH-stimulated adenyl cyclase pathway that is not involved also in forskolin and choleratoxin-mediated cAMP production. The

author further postulated that low doses of MEHP may be acting as a weak receptor agonist or is preventing the FSH receptor from interacting with the adenyl cyclase system.

Mangham *et al.* (1981) determined the hepatic and testicular effects of gavage-dosed DEHP in rats. Groups of male and female Wistar rats (6 each group; approximately 150g ea) were dosed via gavage with 0 or 2500 mg/kg-day DEHP daily for 7 or 21 days. Behavior and clinical sins of toxicity were assessed at periodic intervals. Individual body weights were assessed weekly. Following the final dose, animals were starved overnight and then sacrificed. Both the liver and testes were then surgically removed for biochemical or morphological investigation. Morphological alterations were determined by fixation and histological preparation of liver and testis slices. Hematoxylin and eosin, adenosine triphosphatase, Gomori-type acid phosphatase, and glucose-6-phosphate stains were used on each stain. Ultrastructure was also observed with a transmission electron microscope following different fixation and preparation techniques. DEHP-induced biochemical changes (succinate dehydrogenase activity, 7-ethoxycoumarin O-deethylase, aniline 4-hydroxylase, glucose-6-phosphatase, cytochrome p450 content, total protein, and alcohol dehydrogenase) were also investigated using whole liver homogenates.

Clinical signs of toxicity or behavioral abnormalities were not observed in dosed rats. The body weight of male rats was substantially decreased after 10 and 20 days of exposure. Body weight decrements were accompanied by decreases in food consumption (control, 22.6; DEHP, 13.4 g/rat-day), but not water intake. Exposure to DEHP for 7 or 21 days resulted in time-dependent increases in relative liver weights in both genders and decrements in relative testiscular weights in male rats (Table A3.59).

Table A3.59 Alterations in Relative Organ Weights Following DEHP Exposure (Mangham *et al.*, 1981)

Treatment	Relative organ weight (g / 100g body wt.)			
	Liver		Testes	
	7 days (M/F)	21 days (M/F)	7 days (M)	21 days (M)
Corn Oil	3.7 ± 0.1 / 3.6 ± 0.1	3.3 ± 0.1 / 3.2 ± 0.1	1.0 ± 0.04 / -	1.0 ± 0.03 / -
DEHP	7.2 ± 0.2* / 6.3 ± 0.2*	8.2 ± 0.2* / 7.1 ± 0.4*	0.64 ± 0.03* / -	0.56 ± 0.03* / -
DA79P	5.1 ± 0.6* / 4.7 ± 0.1*	5.0 ± 0.2* / 4.3 ± 0.2*	0.99 ± 0.08 / -	0.52 ± 0.11** / -

Note: results are mean of 6 animals ± SEM
* $P < 0.001$; ** $P < 0.01$

Biochemically, DEHP suppressed the activity of mitochondrial succinate dehydrogenase (7 and 21 days) in male rats, significantly increased the activity of 7-ethoxycoumarin *O*-deethylase and microsomal cytochrome P-450 (M&F; 7 and 21 days), increased (F) and decreased (M) the activity of aniline 4-hydroxylase, significantly decreased microsomal glucose-6-phosphatase activity (M&F; 7 and 21 days) without changing the microsomal protein content, and increased the activity of cytosolic alcohol dehydrogenase (M; Table A3.60).

Table A3.60 Alterations in Rat Biochemistry Following Exposure to DEHP (Mangham *et al.*, 1981)					
Biochemical Parameter	Treatment (values are expressed as percent of control)				
	Control (M/F)	DEHP		DA79P	
		7 days (M/F)	21 days (M/F)	7 days (M/F)	21 days (M/F)
Succinate dehydrogenase (µmol/min/g of liver)	9.1 / 8.5	75* / 90	60** / 115	95 / 120***	60** / 115
7-Ethoxycoumarin *O*-deethylase (µmol/hr/g of liver)	6.4 / 3.9	160** / 170**	170** / 194**	55** / 155**	45** / 100
Aniline 4-hydroxylase (µmol/hr/g of liver)	3.0 / 2.3	85* / 120*	75* / 130**	50** / 125*	45** / 105
Microsomal protein (mg/g of liver)	34.0 / 30.2	105 / 100	90 / 105	100 / 110	85*** / 95
Cytochrome P-450 (nmol/mg of microsomal protein)	0.98 / 0.84	135** / 120***	130** / 130**	90*** / 100	70** / 85*
Glucose-6-phosphatase (nmol/min/mg of microsomal protein)	525 / 540	65** / 65**	70** / 65**	75** / 80*	60** / 80
Alcohol dehdrogenase (µmol/min/g of liver)	1.3 / 3.3	155** / 110	155** / 105	80*** / 125***	75*** / 115***

* $P < 0.01$, ** $P < 0.001$, ***$P < 0.05$

Histochemically, the activity of glucose-6-phosphatase was reduced in the centrilobular areas of male rats. By 21 days, this depression of activity was uniform across hepatic areas. In female rats, the activity of glucose-6-phosphatase was reduced in portal areas after 21, but not 7 days of exposure. In contrast, acid phosphatase activity was increased in portal areas by day 7, and overall in hepatic lobules by 21 days following exposure (M&F). The activity of adenosine triphosphate was only slightly reduced in male rat livers by 21 days following treatment. In the testes, microscopic exam revealed that DEHP affected 50 to 80% of the tubules in each male animal. By 21 days, this progressed to bilateral tubular atrophy in 50 to 100% of the tubules. In this study, DEHP did not affect the interstitial or Sertoli cells.

Morphologically, the livers from DEHP-treated animals did not have any histological abnormalities when compared to control animals. Ultrastructural changes were present, however, in DEHP-treated rats. DEHP induced proliferation of the smooth endoplasmic reticulum, an increase in the number of microbodies, mitochondrial alterations (reduction of cristae, increase in number of dense bodies, crenation of the membrane), an increase in lysosomes (by 21 days) and peroxisome proliferation.

Overall, hepatotoxic effects were more severe in male rats. This difference may be due to intestinal hydrolysis, differences in absorption, and/or hepatic degradation.

Miller *et al.* (2001) determined the effects of DEHP on the thyroid.

Table A3.61 Total Serum T_3 and T_4 In Intact and Thyroidectomized Rats After Exposure to WY-14,643 (Miller *et al.*, 2001)

Serum Parameter	Control intact	WY intact	Control thyroidectomized	WY thyroidectomized
T_3 (µg/L) – 24 hr	0.583 ± 0.041	0.491 ± 0.067	0.221 ± 0.041	0.107 ± 0.036 (P ≤ 0.05)
T_3 (µg/L) – 48 hr	0.713 ± 0.095	0.442 ± 0.017 (P ≤ 0.05)	0.171 ± 0.075	0.172 ± 0.039
T_3 (µg/L) – 72 hr	0.576 ± 0.051	0.572 ± 0.069	0.348 ± 0.163	0.267 ± 0.083
T_4 (µg/L) – 24 hr	42 ± 3	31 ± 6	11 ± 5	4 ± 1
T_4 (µg/L) – 48 hr	40 ± 2	34 ± 3 (P ≤ 0.05)	9 ± 5	5 ± 1
T_4 (µg/L) – 72 hr	44 ± 3	30 ± 2 (P ≤ 0.05)	3 ± 1	3 ± 1

Moore (1996, 1997) determined the tumor incidence in rats and mice following chronic DEHP exposure. These can be seen in Table A3.62.

Table A3.62 Tumor Incidence in Rodents Following Chronic DEHP Exposure (Moore, 1996, 1997; ECB, 2008)

Rats						
mg/kg-day M/F	0.0/0.0 (n=80)	5.8/7.3 (n=50)	28.9/36.1 (n=55)	146.6/181.7 (n=65)	789.0/938.5 (n=80)	Recovery (n=55)
Hepatocellular carcinomas (M)	1/80 (1%)	0/50 (0%)	1/55 (2%)	3/65 (5%)	24/80 (30%)	7/55 (13%)
Hepatocellular carcinomas (F)	0/80 (0%)	1/50 (2%)	0/55 (0%)	1/65 (2%)	14/80 (18%)	4/55 (7%)
Hepatocellular adenomas (M)	4/80 (5%)	5/50 (10%)	3/55(6%)	8/65 (12%)	21/80 (26%)	12/55 (22%)
Hepatocellular adenomas (F)	0/80 (0%)	3/50 (6%)	1/55 (2%)	2/65 (3%)	8/80 (10%)	6/55 (11%)
Total # with Hepatocellular Tumors (M)	5/80 (6%)	5/50* (10%)	4/55 (7%)	11/65*,** (17%)	34/80*,** (43%)	18/55*,** (33%)
Total # with Hepatocellular Tumors (F)	0/80 (0%)	4/50* (8%)	1/55 (1%)	3/65 (5%)	21/80* (26%)	9/55* (16%)
Mononuclear Cell Leukemia (M)	15/80 (19%)	13/50 (26%)	16/55 (29%)	32/65*,** (49%)	27/80* (34%)	29/55*,** (53%)
Mononuclear Cell Leukemia (F)	14/80 (18%)	17/50 (34%)	11/55 (20%)	16/65 (25%)	17/80 (21%)	18/55 (33%)
Mice						
mg/kg-day M/F	0.0/0.0 (n=70)	19.2/23.8 (n=60)	98.5/116.8 (n=65)	292.2/354.2 (n=65)	1266.1/1458.2 (n=70)	Recovery (n=55)
Hepatocellular carcinomas (M)	4/70 (6%)	5/60 (8%)	9/65 (14%)	14/65 (22%)	22/70 (31%)	12/55 (22%)
Hepatocellular carcinomas (F)	3/70 (4%)	2/60 (3%)	3/65 (5%)	10/65 (15%)	16/70 (23%)	23/55 (42%)
Hepatocellular adenomas (M)	4/70 (6%)	10/60 (17%)	13/65 (29%)	14/65 (22%)	19/70 (27%)	3/55 (6%)
Hepatocellular adenomas (F)	0/70 (0%)	2/60 (3%)	4/65 (6%)	9/65 (14%)	34/70 (49%)	13/55 (24%)
Total # with Hepatocellular Tumors (M)	8/70 (11%)	14/60 (23%)	21/65* (32%)	27/65*,** (42%)	37/70*,** (53%)	14/55* (26%)
Total # with Hepatocellular Tumors (F)	3/70 (4%)	4/60 (7%)	7/65 (11%)	19/65* (29%)	44/70* (63%)	30/55* (55%)
Historical Controls for Mononuclear Cell Leukemia (from the Covance lab that performed the 1996 Moore study)						
Mononuclear Cell Leukemia (M)	12/50 (24%)	18/50 (36%)	22/59 (38%)	21/58 (36%)		
Mononuclear Cell Leukemia (M)	8/50 (16%)	15/50 (30%)	12/48 (25%)	20/55 (36%)		
Mononuclear Cell Leukemia (F)	5/50 (10%)	16/49 (33%)	8/60 (13%)	21/60 (35%)		
Mononuclear Cell Leukemia (F)	7/50 (14%)	10/50 (20%)	11/50 (22%)	14/55 (25%)		

* and grayed cells indicate a significant difference from experimental control at P ≤ 0.05

** and grayed cells indicate a significant difference from historical control at P ≤ 0.05

Moss *et al.* (1988) investigated the effects of DEHP and MEHP on Sertoli cell lactate production *in vitro*. Sertoli and germ cell mixed cell cultures were prepared from the testes of

juvenile Sprague-Dawley rats (28 days old) and incubated in a defined culture medium. Various ratios of the two cell types were created by removing germ cells with solution washes. Cultures were then incubated, treated with DEHP (200 μM) or MEHP (0.1, 1, 10, 100, 200 μM) for a set time, and the media removed to assay for lactate and pyruvate.

Treatment with DEHP slightly increased the lactate concentration, decreased the pyruvate concentration, and increased the lactate/pyruvate ratio in a nonsignificant fashion in cells. Treatment with MEHP significantly increased the lactate concentration (1 μM; 1.72x; $P < 0.01$), significantly decreased the pyruvate concentration (100 μM; 2.2x; $P < 0.001$), and increased the lactate/pyruvate ratio (1 μM; 1.40x; $P < 0.05$) in a dose-related fashion. The author reported that the lack of activity associated with DEHP was consistent with opinions that metabolites were responsible for toxic activities and that changes in energy substrates were not likely to have precipitated germ cell detachment.

NTP (1982) determined the tumor incidence in rats and mice following chronic exposure. These can be seen in Table A3.63.

Table A3.63 Tumor Incidence in Rodents Following Chronic DEHP Exposure (NTP, 1982)

Rats			
mg/kg-day M/F	0.0/0.0	322.0/399.0	674.0/774.0
Hepatocellular carcinomas (M)	1/50 (2%)	1/49 (2%)	5/49 (10%) P < 0.05
Hepatocellular carcinomas (F)	0/50 (0%)	2/49 (4%)	8/50 (16%) P < 0.005
Neoplastic nodules (M)	2/50 (4%)	5/49 (10%)	7/49 (14%)
Neoplastic nodules (F)	0/50 (0%)	4/49 (8%)	5/50 (10%) P < 0.05
Mononuclear Cell Leukemia (M)	13/50 (26%)	20/50 (40%)	17/50 (34%)
Mononuclear Cell Leukemia (F)	10/50 (20%)	14/50 (28%)	17/50 (34%)
Mice			
mg/kg-day M/F	0.0/0.0	672.0/799.0	1325.0/1821.0
Hepatocellular carcinomas (M)	9/50 (18%)	14/48 (29%)	19/50 (38%) P < 0.05
Hepatocellular carcinomas (F)	0/50 (0%)	7/50 (14%)	17/50 (34%) P < 0.0001
Hepatocellular adenomas (M)	6/50 (12%)	11/48 (23%)	10/50 (20%)
Hepatocellular adenomas (F)	1/50 (2%)	5/50 (10%)	1/50 (2%)

Grayed cells indicate a significant difference from experimental controls

Parmar *et al.* (1987) investigated the involvement of testosterone in the testicular effects induced by DEHP in rats. Adult male Wistar rats were dosed daily for 15 days with 2000 mg/kg DEHP (oral); 1 mg/kg testosterone (subcut.); 2000 mg/kg DEHP (oral) and 1 mg/kg testosterone (subcut.); or a solvent (subcut). After 15 days of dosing, the rats were sacrificed, the testes and epididymides removed and weighed, one testis processed for biochemical assays (SDH, GGT, and LDH, acid phosphatase, β-glucuronidase activity, and protein content), one testis fixed and processed for histopathology, and the epididymidal spermatozoa counted.

No overtly toxic reactions were observed with any treatment. Testicular weight was significantly reduced in the DEHP-only treatment, but not in solvent or DEHP + testosterone treatments (Table A3.64).

Table A3.64 DEHP- and Testosterone-induced Changes in Rat Testis Weight (Parmar *et al.,* 1987)

DEHP Dose	Absolute (g ± SE)	Relative (g/100g b.w. ± SE)
0 mg/kg	2.56 ± 0.06	1.21 ± 0.07
1mg/kg Testosterone	2.40 ± 0.18	1.22 ± 0.05
2000 mg/kg DEHP and 1 mg/kg testosterone	2.30 ± 0.20	1.11 ± 0.07
2000 mg/kg DEHP	1.42 ± 0.21 (P < 0.05)	0.72 ± 0.08 (P < 0.05)

Testicular enzymes also changed following DEHP administration. GGT, LDH, and β-glucuronidase significantly increased following DEHP exposure. SDH and acid phosphatase significantly decreased following exposure to DEHP. Addition of testosterone to DEHP doses mitigated DEHP-induced enzymatic effects (Table A3.65).

Table A3.65 Testicular Enzyme Activities Influenced by DEHP and Testosterone Exposure (Parmar *et al.,* 1987)

DEHP Dose	GGT (nmol p-nitroaniline formed/min/mg protein)	LDH (µmol NADH oxidized/min/mg protein)	SDH (nmol NADH oxidized/min/mg protein)	β-glucuronidase (nmol phenolphthalein lib/min/mg protein)	Acid phosphatase (nmol p-nitrophenol formed/min/mg protein)
0 mg/kg	27.03 ± 1.3	0.163 ± 0.02	3.33 ± 0.36	0.141 ± 0.006	5.42 ± 0.35
1mg/kg Testosterone	25.66 ± 1.6	0.183 ± 0.03	3.69 ± 0.39	0.165 ± 0.02	5.31 ± 0.13
2000 mg/kg DEHP and 1 mg/kg testosterone	33.36 ± 3.5	0.204 ± 0.02	2.52 ± 0.18	0.176 ± 0.02	5.04 ± 0.35
2000 mg/kg DEHP	54.97 ± 5.3 (P < 0.05)	0.287 ± 0.03 (P < 0.05)	1.29 ± 0.27 (P < 0.05)	0.342 ± 0.03 (P < 0.05)	4.11 ± 0.33 (P < 0.05)

Administration of DEHP also significantly reduced the overall sperm count. Addition of testosterone to DEHP doses mitigated the DEHP-induced reduction in sperm cell count (Table A3.66)

Table A3.66 DEHP- and Testosterone-induced Effects on Sperm Cell Number (Parmar *et al.,* 1987)

DEHP Dose	Estimated Sperm Cell Count (*10^6)
0 mg/kg	8.6
1mg/kg Testosterone	9.0
2000 mg/kg DEHP and 1 mg/kg testosterone	7.3
2000 mg/kg DEHP	2.3 (P < 0.05)

Administration of DEHP induced a disorganization of the normal testicular architecture when testes were viewed via histopathology. These changes were typified by irregular seminiferous tubules with reduced diameters, increased multinucleate giant cells and pyknotic spermatocytes in the tubule lumens, a reduced number of spermatocytes in the tubule lumens,

and an increased number of damaged spermatogenic cells (but no effects on Leydig cells or fibroblasts). Co-administration of DEHP with testosterone mitigated most of the DEHP-induced effects. Only a slight disturbance of normal spermatogenesis and some vacuolar degeneration was noted in the testes of these rats.

The author noted the higher dose of testosterone, lower dose of DEHP, and adult age of rats, probably accounted for testosterones ability to reverse DEHP-induced testicular decrements in this study, but not in a previous study by Gray and Butterworth (1980). The author also states that enzyme activities presented in the study are tied to Sertoli cell function and germ cell maturation. GGT activity normally parallels that of Sertoli cell replication and maturation. β-glucuronidase activity is inversely related to sperm maturation. Both activities were altered in this study, suggesting that DEHP interferes with Sertoli cell function. Co-administration of testosterone preserved normal enzyme activities. Increases in SDH and acid phosphatase activities are associated with the maturation of germ cells. LDH activity declines with testicular development. Decreased SDH and acid phosphatase and increased LDH activity correlate well to damage and loss of tubular spermatozoa seen upon histopathology. Similar effects are seen in hypophysectomy, azoospermia, and cryptorchidism.

Parmar *et al.* (1995) determined the testicular effects in developing rats following DEHP administration. Twenty-five day old male Wistar albino rats were gavage dosed daily with 0, 50, 100, 250, or 500 mg DEHP/kg for 30 days. Body weights were deermined on the first and last day of dosing. Animals were sacrificed 24 hours following the last dose. Immediately following death, the testes and liver were removed and weighed. One testis was then fixed for histochemical study and the other testis was processed for biochemistry (SDH, GGT, LDH, acid phosphatase, β-glucuronidase, and protein). The liver was also processed for the determination of hepatic cytochrome P-450 monoxygenases.

Dosed animals were weak and lethargic when compared to controls (data not shown). Testicular weight was significantly reduced in most animals at all doses (Table A3.67)

Table A3.67 Testicular Weight in Juvenile Wistar Rats After Gavage Dosing with DEHP for 30 Days (Parmar *et al.,* 1995)

DEHP Dose	Absolute (g ± SE)	Relative (g/100g b.w. ± SE)
0 mg/kg	1.80 ± 0.19	1.53 ± 0.16
50 mg/kg	1.20 ± 0.10 (P < 0.05)	1.15 ± 0.10
100 mg/kg	1.13 ± 0.10 (P < 0.05)	1.05 ± 0.04 (P < 0.05)
250 mg/kg	0.77 ± 0.09 (P < 0.05)	0.65 ± 0.07 (P < 0.05)
500 mg/kg	0.60 ± 0.01 (P < 0.05)	0.55 ± 0.05 (P < 0.05)

The activity of the testicular enzymes LDH and GGT were increased, and SDH decreased. Acid phosphatase activity was decreased significantly at the 250 and 500 mg/kg dose, while β-glucuronidase was significantly increased at these dose levels (Table A3.68).

Table A3.68 Testicular Enzyme Activities Influenced by DEHP Exposure (Parmar *et al.,* 1995)					
DEHP Dose	GGT (nmoles p-nitroaniline formed/min/mg protein)	LDH (µmoles NADH oxidized/min/mg protein)	SDH (nmoles NADH oxidized/min/mg protein)	β-glucuronidase (nmoles phenolphthalein lib/min/mg protein)	Acid phosphatase (nmoles p-nitrophenol formed/min/mg protein)
0 mg/kg	18.5 ± 0.5	0.22 ± 0.01	3.90 ± 0.2	0.121 ± 0.02	3.84 ± 0.2
50 mg/kg	23.1 ± 1.6 (P < 0.05)	0.26 ± 0.01 (P < 0.05)	2.70 ± 0.4 (P < 0.05)	0.166 ± 0.03	3.82 ± 0.2
100 mg/kg	27.3 ± 3.4 (P < 0.05)	0.28 ± 0.01 (P < 0.05)	2.61 ± 0.3 (P < 0.05)	0.198 ± 0.03	3.29 ± 0.3
250 mg/kg	33.4 ± 4.8 (P < 0.05)	0.32 ± 0.03 (P < 0.05)	2.19 ± 0.4 (P < 0.05)	0.223 ± 0.03 (P < 0.05)	3.04 ± 0.2 (P < 0.05)
500 mg/kg	39.3 ± 5.8 (P < 0.05)	0.36 ± 0.03 (P < 0.05)	1.91 ± 0.4 (P < 0.05)	0.278 ± 0.04 (P < 0.05)	2.84 ± 0.2 (P < 0.05)

The activity of liver p450 monoygenases, aniline hydroxylase and ethylmorphine N-demethylase were significantly reduced in a dose-dependent fashion (Table A3.69)

Table A3.69 Hepatic Parameters Affected by 30 Days of DEHP Exposure (Parmar *et al.,* 1995)			
DEHP Dose	P-450 (nmoles/mg protein)	Aniline hydroxylase (pmoles p-aminophenol formed/min/mg/protein)	Ethylmorphine N-demethylase (pmoles 3-OH benzo(a)pyrene formed/min/mg protein)
0 mg/kg	0.45 ± 0.05	45.2 ± 3.5	27.1 ± 1.2
50 mg/kg	0.36 ± 0.06	34.5 ± 2.6 (P < 0.05)	22.4 ± 1.0 (P < 0.05)
100 mg/kg	0.30 ± 0.04 (P < 0.05)	31.3 ± 2.8 (P < 0.05)	20.5 ± 1.6 (P < 0.05)
250 mg/kg	0.26 ± 0.06 (P < 0.05)	28.5 ± 2.6 (P < 0.05)	16.4 ± 1.2 (P < 0.05)
500 mg/kg	0.20 ± 0.06 (P < 0.05)	23.5 ± 3.4 (P < 0.05)	13.8 ± 1.1 (P < 0.05)

Histopathological exam of testis demonstrated that control treatments were normal, no significant changes were present in the structure of the capsule, seminiferous tubules, or interstitium of rats treated with 50 and 100 mg/kg, and significant disorganization and damage was occurring in the spermatogenic layers in 250 mg/kg treatment animals. In rats dosed with 500 mg/kg, the normal structure of the testes was completely disorganized, the seminiferous tubules were reduced in diameter and irregular in size and shape, the gametogenic layers were markedly destroyed while the basal layer was intact. Vacoular degeneration was also present in the testes of these animals, but Leydig cells and fibroblasts were normal.

The author states that GGT and β-glucuronidase activity normally correlate to that of Sertoli cell replication and maturation (maximum activity occurs during formation of inter-Sertoli cell junction formation n days 15 to 20). DEHP-induced increases in activity of these enzymes suggest that it may interfere with Sertoli cell function. SDH and acid phosphatase activity is associated with the appearance of spermatogonia and spermatocytes (low activity on

days 10 to 12, rise to adult levels with spermatocyte maturation by 40 days and on). LDH activity is high in neonates, but declines as spermatocytes differentiate into spermatids. Changes in these enzymes suggest that germ cell differentiation is also adversely affected by DEHP. Pathological changes in the germ cells (but not the basal layer containing spermatogonia) were seen at higher doses of DEHP, supporting this conclusion. The author suggested that disturbance in the Sertoli cell-germ cell interactions could account for these changes and testicular recovery following cessation of DEHP exposures (data not shown). The author also suggests that dose-dependent inhibition of P450's and other hepatic enzymes mean that DEHP or one of its metabolites (probably MEHP) are accumulating in the liver. Finally, the author noted that biochemical changes occurred at lower doses than histopathological changes.

Poon *et al.* (1997) investigated the effects of DEHP on liver, thyroid, and testicular structure and function. Male and female adult Sprague-Dawley rats were exposed to 0, 5, 50, 500, 5000 mg/kg DEHP (0, 0.4/0.4, 3.7/4.2, 37.6/42.2, 375.2/419.3 mg/kg-day for M/F, respectively, 10 rats per sex per group) in feed for 13 weeks.

In another experiment, adult rats were exposed to DEHP in feed at 5000 mg/kg (345/411 mg/kg-day for M/F, respectively, 10 rats per sex per group) for 13 weeks as a positive control. Following exposures, rats were sacrificed and exsanguinated. An alloquot of blood was preserved and assayed for hematological parameters and biochemistry and rats were then analyzed for gross pathologies. Particular organs were excised and fixed prior to analysis via histopathology and biochemistry.

In general, no changes to body weight (F), food consumption, or clinical toxicity were observed for the any of the dose levels. A marginal non-significant decrease was observed in male body weights at doses of 3.7 to 375.2 mg/kg-day.

In the primary study, DEHP increased the absolute and relative liver weights in all rats at the highest dose level and also enlarged the livers in males and females. Absolute and relative liver weights were also increased in males and females in a secondary study when DEHP was used as a positive control (Table A3.70). The number and severity of male and female rats with hepatocellular hypertrophy and focal necrosis was increased in the highest dose. Ultrastructurally, treated male and female rats had increased peroxisomal percent of cell area, liver anisokaryosis, nuclear hyperchromicity, and endothelial prominence. Significant non-dose-related increases were also seen in male and female aminopyrine-N-demethylase and aniline hydroxylase activity in the high dose group.

Relative kidney weight was significantly increased in males and females in the 13 week feeding study. Absolute and relative kidney weights were increased in males in the other study in which DEHP was utilized as the positive control.

Absolute and relative testes weights were significantly increased in males in the study in which DEHP was utilized as the positive control. Dose-related increases in the number of male

rats with Sertoli cell vacoulation and bilateral reduction in epididymal sperm density and a non-dose-related increase in seminiferous tubule atrophy were also observed in high dose groups.

The thyroid was also affected by DEHP treatment. Reduced thyroid follicle size and colloid density were described in male and female rats treated with DEHP in feed for 13 weeks as positive controls.

Significant dose-related decreases in male rat red blood cells and hemoglobin and female mean corpuscular hemoglobin and mean corpuscular volume were observed in the 5000 mg/kg dose group in the 13 week feeding study. Significant increases were also observed in the number of female white blood cells and male and female platelets in the high dose group of the same study.

Significant increases were seen in male and female albumin, albumin/globulin ratio, and inorganic phosphate, male aspartate cholesterol, potassium, and calcium, and female total protein. Significant decreases were observed in female aspartate cholesterol, aminotransferase, and alanine aminotransferase (@ 40.8 mg/kg-day), and male aminotransferase (LOAEL = 0.4 mg/kg-day).

Table A3.70 DEHP-induced Organ Effects in Male and Female Sprague-Dawley Rats (Poon *et al.*, 1997)

	DEHP Dose (mg/kg-day) in Feed (M-F ± SD) for 13 weeks						
Adverse Effect	0 (corn oil)	0.4	3.5 - 4.2	37.6 – 42.2	375.2 – 419.3	0 (corn oil)	345.0-411.0
Male Final Body Weight; g	548 ± 53	550 ± 45	534 ± 39	522 ± 31	529 ± 22	518 ± 36	533 ± 38
Male Liver Weight; g (% body weight)	18.0 ± 2.5 (3.28)	18.5 ± 2.2 (3.37)	18.1 ± 2.9 (3.37)	17.9 ± 1.5 (3.44)	25.4 ± 2.0; P < 0.01 (4.80; P < 0.01)	17.1 ± 1.7 (3.31)	24.2 ± 2.3; P < 0.05 (4.55; P < 0.05)
Female Liver Weight; g (% body weight)	9.17 ± 1.09 (3.23)	9.58 ± 0.99 (3.17)	8.76 ± 0.50 (3.13)	9.25 ± 0.54 (3.24)	11.0 ± 1.67; P < 0.01 (4.01; P < 0.01)	9.83 ± 1.04 (3.32)	12.20 ± 1.20; P < 0.05 (4.10; P < 0.05)
Number of male rats with hepatocellular hypertrophy	0/10	0/10	0/10	0/10	10/10 (average severity = 2.0, mild)	-	-
Number of male rats with liver focal necrosis	0/10	0/10	0/10	0/10	1/10 (average severity = 1.0, minimal)	-	-
Number of female rats with hepatocellular hypertrophy	0/10	0/10	0/10	0/10	10/10 (average severity = 1.1, minimal)	-	-
Number of female rats with liver focal necrosis	0/10	0/10	0/10	0/10	2/10 (average severity = 1.0, minimal)	-	-
Peroxisomes - % cell area in male rats	-	-	-	-	-	4.53	13.11
Peroxisomes - % cell area in female rats	-	-	-	-	-	3.69	11.32
Liver anioskaryosis in male rats	-	-	-	-	-	1/10 (average severity = 0.1, minimal)	10/10 (average severity = 2.3, mild)
Liver anioskaryosis in female rats	-	-	-	-	-	9/10 (average severity = 1.5, mild)	10/10 (average severity = 2.5, mild)
Liver nuclear hyperchromicity in male rats	-	-	-	-	-	0/10	9/10 (average severity = 1.7, mild)
Liver nuclear	-	-	-	-	-	3/10 (average	10/10 (average

hyperchromicity in female rats						severity = 0.6, minimal)	severity = 2.0, mild)
Liver endothelial prominence in male rats	-	-	-	-	-	0/10	7/10 (average severity = 0.8, minimal)
Liver endothelial prominence in female rats	-	-	-	-	-	0/10	6/10 (average severity = 0.7, minimal)
Male aminopyrine-N-demethylase (nmol/min/mg prot.)	-	-	-	-	-	~38	~47.5; P < 0.05
Female aminopyrine-N-demethylase (nmol/min/mg prot.)	52.6 ± 20.3	48.1 ± 8.9	52.7 ± 13.8	47.4 ± 10.7	88.4 ± 45.3; P < 0.01	~29	~47; P < 0.05
Male aniline hydroxylase (nmol/min/mg prot.)	-	-	-	-	-	~62.5	~78; P < 0.05
Female aniline hydroxylase (nmol/min/mg prot.)	-	-	-	-	-	~75	~93; P < 0.05
Male Ethoxyresorufin-O-deethylase activity (approx. nmol/min/mg protein)	-	-	-	-	-	0.120	0.137
Female Ethoxyresorufin-O-deethylase activity (approx. nmol/min/mg protein)	-	-	-	-	-	0.163	0.175
Male Kidney Weight; g (% body weight)	3.27 ± 0.33 (0.60)	3.35 ± 0.36 (0.61)	3.25 ± 0.31 (0.61)	3.32 ± 0.33 (0.64)	3.63 ± 0.34 (0.69; P < 0.01)	1.60 ± 0.10 (0.31)	1.88 ± 0.23; P < 0.05 (0.35; P < 0.05)
Female Kidney Weight; g (% body weight)	1.94 ± 0.16 (0.69)	2.01 ± 0.17 (0.67)	1.94 ± 0.12 (0.69)	1.95 ± 0.08 (0.68)	2.10 ± 0.24 (0.77; P < 0.05)	1.02 ± 0.06 (0.35)	1.07 ± 0.08 (0.35)
Male Testes Weight; g (% body weight)	-	-	-	-	-	3.46 ± 0.23 (0.67)	2.73 ± 1.13; P < 0.05 (0.51; P < 0.05)
Number of male rats with seminiferous tubule atrophy	1/10 (average severity = 0.1, minimal)	3/10 (average severity = 0.5, minimal)	1/10 (average severity = 0.4, minimal)	0/10	9/10 (average severity = 1.5, mild)	7/10 (average severity = 0.8, minimal)	9/10 (average severity = 2.8, moderate)
Number of male rats with Sertoli cell vacoulation	0/10	4/10 (average severity = 0.2, minimal)	4/10 (average severity = 0.5, minimal)	7/10 (average severity = 1.0, minimal)	9/10 (average severity = 2.4, mild)	-	-
Number of male rats with bilateral reduction in sperm density in epididymis	-	-	-	-	-	0/10	5/10 (average severity = 1.4, mild)
Reduced thyroid follicle size in male rats	-	-	-	-	-	4/10 (average severity = 0.4, minimal)	8/10 (average severity = 1.6, mild)
Reduced thyroid follicle size in female rats	-	-	-	-	-	4/10 (average severity = 0.4, minimal)	8/10 (average severity = 1.9, mild)
Reduced thyroid colloid density in male rats	-	-	-	-	-	0/10	8/10 (average severity = 0.8, minimal)
Reduced thyroid colloid density in female rats	-	-	-	-	-	2/10 (average severity = 0.1, minimal)	3/10 (average severity = 0.2, minimal)
Male red blood cells (10^6)	8.23 ± 0.31	8.07 ± 0.33	7.77 ± 0.32	7.83 ± 0.38	7.48 ± 0.63; P < 0.01	-	-
Male hemoglobin (g/dL)	14.9 ± 0.53	14.7 ± 0.81	14.3 ± 0.22	14.4 ± 0.72	13.7 ± 0.88; P < 0.01	-	-
Female white blood cells (10^3)	-	-	-	-	-	3.58 ± 1.51	5.84 ± 1.58; P < 0.05
Female mean corpuscular hemoglobin (pg)	-	-	-	-	-	18.66 ± 0.60	18.14 ± 0.46; P < 0.05
Female mean corpuscular volume ($\mu m/m^3$)	-	-	-	-	-	54.43 ± 1.30	53.00 ± 1.18; P < 0.05
Male platelet count (10^3)	-	-	-	-	-	911 ± 63	1028 ± 184; P < 0.05
Female platelet count (10^3)	-	-	-	-	-	836 ± 99	951 ± 112; P < 0.05

Male albumin (g/dL)	2.9 ± 0.13	2.9 ± 0.10	2.9 ± 0.09	2.9 ± 0.07	3.3 ± 0.20; P < 0.01	3.53 ± 0.22	3.83 ± 0.45; P < 0.05
Male albumin/globulin	0.92 ± 0.05	0.89 ± 0.08	0.88 ± 0.06	0.93 ± 0.07	1.07 ± 0.06; P < 0.01	-	-
Male aspartate cholesterol (mg/dL)	81.2 ± 19.6	80.6 ± 14.0	80.1 ± 13.0	84.9 ± 15.1	87.7 ± 15.7	-	-
Male aminotransferase (U/L)	64 ± 11	52 ± 12; P < 0.05	50 ± 8; P < 0.01	44 ± 4; P < 0.01	50 ± 5; P < 0.01	-	-
Male potassium (meq/L)	4.64 ± 0.24	4.82 ± 0.23	4.71 ± 0.34	4.82 ± 0.31	5.11 ± 0.48; P < 0.05	-	-
Male calcium (mg/dL)	-	-	-	-	-	8.09 ± 1.56	11.04 ± 1.43; P < 0.05
Male inorganic phosphate (mg/dL)	-	-	-	-	-	6.39 ± 0.59	8.21 ± 1.38; P < 0.05
Male total protein (mg/dL)	-	-	-	-	-	6.25 ± 0.53	6.60 ± 0.55
Female albumin (g/dL)	-	-	-	-	-	3.92 ± 0.41	4.32 ± 0.34; P < 0.05
Female albumin/globulin	1.05 ± 0.04	1.01 ± 0.06	1.03 ± 0.06	1.05 ± 0.06	1.14 ± 0.05; P < 0.01	-	-
Female aspartate cholesterol (mg/dL)	87.1 ± 14.4	85.8 ± 14.1	71.6 ± 13.6	83.9 ± 21.8	68.4 ± 8.3; P < 0.05	-	-
Female alanine aminotransferase (U/L)	98 ± 28	84 ± 11	88 ± 18	70 ± 14; P < 0.01	104 ± 21	-	-
Female aminotransferase (U/L)	50 ± 5	45 ± 9	42 ± 7	38 ± 4; P < 0.01	44 ± 8	-	-
Female inorganic phosphate (mg/dL)	-	-	-	-	-	7.22 ± 1.54	8.69 ± 0.95; P < 0.05
Female total protein (mg/dL)	-	-	-	-	-	6.39 ± 0.45	6.97 ± 0.40; P < 0.05

Grayed cells indicate a significant difference from control
Average weights are ± the standard deviation
Severity = the mean severity grade based on a range of 1 (minimal) to 4 (severe)

Pugh *et al.* (2000) investigated the adverse effects associated with DEHP exposure in cynomolgous monkeys. Cynomolgous monkeys were gavage dosed with 500 mg/kg-day DEHP and clofibrate (250 mg/kg-day) (4 per group) for 14 days. Exposure to DEHP resulted in a slight but non-significant decrease in the relative thyroid/parathyroid weights (and a significant decrease in clofibrate treatments; Table A3.71). No significant changes in serum calcium, or phosphorus were observed.

Table A3.71 DEHP and Clofibrate-induced Weight Changes in the Monkey Thyroid/parathyroid (Pugh *et al.*, 2000)

Treatment	Calculated absolute thyroid/parathyroid weight (g)	Relative thyroid/parathyroid weight (%)
Control	56.98	0.022 ± 0.006
DEHP (500 mg/kg-day)	42.8	0.018 ± 0.003
Clofibrate (250 mg/kg-day)	34.7	0.014 ± 0.001 (P < 0.05)

Sekiguchi *et al.* (2006) investigated the changes in thyroid hormones following exposure to DEHP. Immature intact and hypophysectomized Fischer 344 rats were injected with DEHP (0, 500, 2000 mg/kg) once a day for 3 days. The relative amounts of T_4 were then determined biochemically. High doses of DEHP significantly reduced the amount of T_4 in the blood (P <

0.05; Table A3.72). Hypophysectomized rats also had decreased T_4 levels at all doses, which weren't affected significantly following exposure to DEHP.

Table A3.72 DEHP-induced Effects on Serum T_4 Levels in Fischer 344 Rats (Sekiguchi *et al.*, 2006)			
Parameter	Subcutaneous DEHP Dose		
	0 mg/kg	500 mg/kg	2000 mg/kg
Intact female rat T_4 (µg/L)	67.4	61.5	41.3 ($P < 0.05$)
Hypophysectomized female rat T_4 (µg/L)	26.6	24.1	21.4

Sharpe *et al.* (2003) reviewed disorders of testicular function in adults with special emphasis on proliferation and functional maturation of Sertoli cells. Sertoli cells have a variety of roles in the development and function of the testis including: 1) being the first to differentiate in the fetal gonad (which allows development of the seminiferous cords, prevention of germ-cell entry into meiosis, and differentiation of Leydig cells), 2) secreting anti-Mullerian hormone (AMH; resulting in regression of the Mullerian ducts), and 3) supporting spermatogenesis following puberty. Functional maturation from a developmental role to a supporting role in spermatogenesis is characterized by a loss of proliferation and the formation of inter-Sertoli tight junctions. Tight junctions allow the formation of an adluminal compartment in which spermatogenesis can proceed.

Testis size and daily sperm production in the adult are dependent on the number of Sertoli cells, since each Sertoli cell can support only a certain number of germ cells. The number of Sertoli cells is determined prior to adulthood (i.e., when fetal, neonatal, or peripubertal), since only immature cells can proliferate. The relative timing of proliferation is different with different species. For example, in rodents Sertoli cell proliferation occurs primarily in fetal and neonatal periods, in humans it occurs more evenly in fetal/neonatal and peripubertal periods, while in rehesus monkeys it occurs more in peripubertal periods. A moderate amount of Sertoli cells are lost as individuals age.

The number of Sertoli cells is controlled by many factors. The fragile X and FMR-1 genes are two genes that control some aspects of Sertoli cell proliferation. The hormone FSH stimulates Sertoli cells to proliferate. Thyroid hormones decrease the proliferation of Sertoli cells by inducing the cells to mature. This mechanism probably occurs through interaction with androgens or FSH or may reflect a change in the thyroid receptor expression or sensitivity in cells. In immature Sertoli cells, both thyroid hormone (T_3) and FSH are able to increase expression of the androgen receptor (AR) and inhibit the expression of AMH alone and in an additive manner. In general, the expression of AR in Sertoli cells probably occurs late in puberty. Increased cell-to-cell contact may also reduce the proliferation of Sertoli cells. In humans, there is a large variation in the number of Sertoli cells (< 50 to 950 * 10^6 cells per testis). This variation is potentially due to fetal, neonatal, or peripubertal events.

The author postulated further that disorders that comprise Testicular Dysgenesis Syndrome (TDS; low sperm counts, hypospadias, cryptorchidism, testicular germ-cell cancer) may have an origin in the abnormal fetal development or function of Sertoli or Leydig cells.

The author noted that exposure to some phthalates *in utero* can induce disorders similar to that described by TDS. These disorders may be related to the permanent inhibition of Sertoli cell maturation, resulting in cells that can not support spermatogenesis. Treatment with phthalates during neonatal periods has also been reported to reduce the proliferation of Sertoli cells.

The maturation of Sertoli cells (whether it be rapid or through a cascade of steps) can be gauged by a variety of cell markers. The Sertoli-mediated secretion of inhibin B has been correlated to the number of Sertoli cells in a general way. Adult individuals may have large variations in the blood concentration of inhibin B, however, limiting its usefulness at the individual level. Markers of Sertoli cell immaturity include AMH, aromatase, neural cell adhesion molecule (NCAM), cytokeratin 18, and m2A antigen. Mature Sertoli cells express GATA-1, $p27^{Kip1}$, and AR (in humans). Constituitive expression of Wilms' tumor gene (WT1) also begins in early fetal life and continues throughout adulthood. A loss of maturation factors may occur in circumstances where Sertoli cells lose their germ cells. These changes are difficult to distinguish from cases in which Sertoli cells have failed to mature.

Wilson *et al.* (2004) determined the effects of gavage administration of DEHP on the production of fetal hormones in the testes and genetic regulation of insulin-like peptide 3 (Insl3) in Sprague-Dawley rats. In the first experiment, pregnant Sprague-Dawley rats were gavage dosed with 0 or 750 mg DEHP /kg rat daily from Gd 14 to 18. On Gd 18, dams were sacrificed, fetuses removed, anesthetized, and testes removed from male rats. All testes from animals within a litter were pooled, homogenized, frozen, and later assayed for total RNA, the quality of RNA, and the genetic regulation of insl3 using real-time rt-PCR. In the second experiment, pregnant Sprague-Dawley rats were gavage dosed with 0 or 1000 mg DEHP /kg rat daily from Gd 14 to 18. On Gd 18, dams were sacrificed, fetuses removed, anesthetized, and testes removed from male rats. Each testis was immediately transferred to the medium-filled wells of 24-well plates. Testes were incubated for 3 hours. The media was then removed and frozen for subsequent hormone analysis. Testes were then homogenized and assayed for total RNA, the quality of RNA, and the genetic regulation of insl3 using real-time rt-PCR.

Both treatments with DEHP significantly reduced the mRNA levels of insl3 in fetal testes ($P < 0.02$), suggesting a decrease in its gene expression. Treatment with 1000 mg/kg DEHP also significantly reduced the concentration of testosterone in media incubated with testes for 3 hours ($P < 0.01$), but did not change the overall amount of progesterone. The author postulated that these insl3-related effects probably cause agenesis of the gubernacular ligaments in exposed male rat offspring. Further, they postulated that these effects are most likely resultant from a maturational delay of fetal Leydig cells (hyperplasia) and may be associated with the production of Steroidogenic Factor (SF-1).

Wilson *et al.* (2008) reviewed the effects of phthalates on male rat reproductive tract development. In this publication, *in utero* DEHP exposure was reported to decrease fetal testis testosterone production and reduce the expression of steroidogenic genes (cyp17 [17α-hydroxylase C17,20-lyase]; cyp11a, and steroidogenic acute regulatory protein [StAR]). DEHP was also reported to decrease the production of insl3in other publications. The severity of changes was shown to be dependent on the exposed rat strain, with Wistar rats having higher incidence of gubernacular changes and lower incidence of epididymal changes. Sprague-Dawley rats, conversely, had higher incidences of epididymal changes when compared to gubernacular changes. In particular, testosterone production was more affected in Sprague-Dawley rats and insl3 production was more affected Wistar rats following exposures. The author goes further and describes multi-anti-androgen studies in which combinations of different phthalates induced adverse tissue effects in a dose-additive manner, irrespective of their specific cellular target.

Xu *et al.* (2007) determined the affects of DEHP on fetal rat lipid profiles in the brain. Pregnant female Sprague-Dawley rats were gavage dosed with DEHP (0, 1500 mg/kg) during Gd 0 to 19. Fetal brain tissue was isolated on Gd 20 and analyzed for total lipid concentrations, free fatty acids, free cholesterol, cholesterol esters, diacylglycerol, triacylglyceride, phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, lysophosphatidylcholine, cardiolipin, and sphingomyelin.

Free cholesterol and sphingomyelin were significantly decreased in fetal rat brains (33 and 54%, respectively; $P < 0.05$) following DEHP exposure. DEHP also reduced the concentration of free fatty acids, diacylglycerol, cholesterol esters, phosphatidylcholine, phosphatidylserine, and lysophosphatidylcholine. DEHP exposure significantly reduced the amounts of monounsaturated and polyunsaturated fatty acids ($P < 0.05$; and ω-3 and 7 series) when organizing by lipid class. DEHP administration also significantly decreased the levels of docosahexaenoic acid in cholesterol esters (43% reduction), diacylglycerol (60%), phosphatidylserine (33%), lysophosphatidylcholine (35%), and sphingomyelin (40%; $P < 0.05$) and the levels of arachidonic acid in cholesterol esters and lysophosphatidylcholine (~33% reduction; $P < 0.05$).

The author commented that normal fetal development was dependent on a sufficient supply of fatty acids and lipids. Various adverse effects such as deficits in learning, and altered behavior and vision have been correlated to low fatty acid levels (esp. docosahexaenoic acid). Exposure to DEHP during pregnancy changes the disposition of fatty acids and lipids in fetal brain tissue, suggesting that it can alter fetal neural development. In particular, changes in free cholesterol and sphingomyelin during an active period of neuronogenesis (i.e., Gd 15 to birth in rat) may affect CNS myelination. Changes in polyunsaturated fatty acid levels (i.e., docosahexaenoic acid and arachidonic acid) may also affect neuronal membranes. Changes in fatty acid composition can occur through interaction with the dam, since many fatty acids derive from maternal circulation and are transported by carrier proteins across the placenta into fetal circulation. Alternatively, adverse effects may result from direct interaction of DEHP with the fetus, since DEHP has been shown to cross the placenta and accumulate in the fetus. DEHP-

induced effects on peroxisomes may also affect neural development, since many of the lipid/essential fatty acid transporters and metabolizing enzymes that determine homeostasis are regulated by the peroxisome proliferator-activated nuclear hormone receptor family.

Xu *et al.* (2008) determined the effects of DEHP on placental and fetal essential fatty acid (EFA) homeostasis. Pregnant female Sprague-Dawley rats were gavage dosed with DEHP (0, 750, 1500 mg/kg) during Gd 0 to 19. The maternal, fetal, and junctional and labyrinthine areas of the placenta were isolated on Gd 20. Placental transfer and fetal distribution of fatty acids was determined in placental and fetal tissues (brain, liver, heart, intestine, blood) by using ^{14}C and ^{3}H labeled fatty acids. Placental production of total prostaglandins was also determined by homogenizing placental tissue and analyzing via a prostaglandin screening ELISA. Expression of PPAR isoforms (α, β, γ), EFA transporters (membrane-associated FAT/CD36, FATP1, FABPpm, and cytoplasmic HFABP), EFA metabolic enzymes (CYP4A1, COX-1, COX-2) were also determined in placental tissue by using semi-quantitative RT-PCR and Western Blot.

RT-PCR-determined expression of PPARα and PPARγ was significantly increased in a dose-dependent fashion in the junctional tissue ($P < 0.05$ to 0.01) and labyrinthine tissue ($P <$ 0.01 to 0.001) of the placenta following exposure to 750 and 1500 mg/kg doses. The expression of PPARβ was largely unchanged in either area of the placenta following dosing with DEHP. A similar trend was seen when measuring protein content via Western Blot. The magnitude of the change was consistently greater in the labyrinthine tissue when compared to the junctional tissue. Significant dose-dependent increases ($P < 0.05$ to 0.01) were also seen in the EFA transporters FAT/CD36 (labyrinthine), FATP1 (junctional and labyrinthine), and HFABP (junctional and labyrinthine) following RT-PCR analysis. Western Blot protein analysis mirrored these data and also demonstrated a non-significant, but dose-dependent, increase in the expression of the FABPpm transporter in both placental tissue areas. For both analytical methods, significant DEHP-induced increases in transporter expression were observed to a greater extent in the labyrinthine than in the junctional area of the placenta. A significant dose-dependent increase in the expression of the EFA metabolizing enzyme CYP4A1 was demonstrated in junctional and labyrinthine areas of the placenta ($P < 0.05$ to 0.001) via RT-PCR. Dose-dependent, but non-significant increases in COX-1 were also observed in both tissue areas following dosing with DEHP. A significant dose-dependent decrease in COX-2 was reported in the junctional area of the placenta. This change was not observed in the labyrinthine area of the placenta. Western blot analysis also demonstrated that protein production of CYP4A1 was increased in a dose-dependent fashion and that COX-2 production was decreased in a dose dependent fashion in junctional, but not labyrinthine tissue following exposure to DEHP. Exposure to 1500 mg/kg DEHP also significantly reduced the amount of arachidonic acid (an example of ω-6 EFA) in the maternal plasma and fetal plasma ($P < 0.05$ to 0.01), but increased the amount in the whole placenta ($P < 0.001$). Exposure to 1500 mg/kg of DEHP also increased the amount of docosahexaenoic acid (a representative of ω-3 EFA) in maternal plasma ($P < 0.01$), but decreased it in fetal plasma ($P < 0.05$). Similar exposures also significantly decreased the amount

of arachidonic acid in organs (70 to 50% reductions; fetal heart > intestine > liver > brain; $P < 0.01$ to 0.001), and docosahexaenoic acid in the fetal brain (50% reduction; $P < 0.001$). A significant dose-dependent decrease ($P < 0.05$ to 0.01) in the production of total prostaglandins was also reported following exposure to DEHP and DEHP and the selective COX-2 inhibitor NS398.

This study demonstrated that DEHP exposure alters the essential fatty acid homestasis in maternal, placental, and fetal tissues. The author states that administration of 750 and 1500 mg/kg-day results in peak plasma DEHP concentrations of about 2 µg/mL and 3 to 4 µg/mL, and MEHP concentrations of 65 µg/mL and 136 µg/mL, respectively, in rat dams. Both concentrations are higher than that in human maternal plasma (1.15 and 2.05 µg/mL, respectively), but not that from people exposed long-term to DEHP containing devices (70 to 80 µg/mL). The author also reports that PPARα and PPARγ, but not PPARβ, are increased in the placenta following exposure to DEHP. This is of interest because PPARβ has a primary role in giant cell differentiation and placenta development. EFA transporters (FAT/CD36, FATP1, and HFABP) were also increased in the labyrinthine placental tissue following DEHP exposure. The authors postulate that this is more than likely related to the activation of PPARs. Enzymes that are involved in the metabolism of long-chain fatty acids to eicosanoids (i.e., CYP4A1, COX-2) were also affected by DEHP exposures. As with the EFA transporters, changes in CYP4A1 may be related to changes in PPARα because it has been shown that these metabolic enzymes are transcriptionally regulated by PPARs. Inhibition of the COX-2 enzyme is important because it catalyzes the biosynthesis of prostaglandins that are important in parturition. The authors also demonstrated that the normal transfer of arachidonic acid and docosahexaenoic acid was altered, and mentioned that these fatty acids were important in developing visual and brain systems in the fetus. Tha authors concluded that these results were consistent with what has been observed in *in vitro* studies.

Appendix 4. Phthalate Chemical Product List

CAS Number	Phthalate Chemical Name	CAS Number	Phthalate Chemical Name
21395-09-5	(+/-)-mono-2-octylphthalate	27554-26-3	diisooctyl phthalate
68296-97-9	(±)-2-octyl hydrogen phthalate		diisophentyl phthalate
23276-77-9	(1-ethylhexyl) hydrogen phthalate	27253-26-5	diisotridecyl phthalate
67939-28-0	(butylstannylidyne)tris(thioethylene) triisooctyl triphthalate	96507-86-7	diisoundecyl phthalate
68928-78-9	(dibutylstannylene)bis(thioethylene) diisooctyl diphthalate	85507-79-5	diisoundecyl phthalate
1322-94-7	(dimethylcyclohexyl) hydrogen phthalate	17840-25-4	dilithium isophthalate
84473-57-4	[2-[bis(2-hydroxyethyl)amino]ethyl] hydrogen phthalate	15968-00-0	dilithium phthalate
55334-51-5	[4-(methoxycarbonyl)phenyl]methyl methyl terephthalate	14309-54-7	dimethyl 1,4-Cyclohexadiene-1,2-dicarboxylate; dimethyl 3,6-Dihydrophthalate
26761-40-0	1,2-benzenedicarboxylic acid diisodecyl ester; diisodecyl phthalate	18014-00-1	dimethyl 2,5-dibromoterephthalate
2055-00-7	1,2-ethanediyl dimethyl phthalate	3293-89-8	dimethyl 2,5-dichloroterephthalate
118-99-0	1,3-diphenylguanidinium phthalate	5292-51-3	dimethyl 2,5-difluoroterephthalate
40139-96-6	1-[2-(methacryloyloxy)-1-methylethyl] hydrogen sulphophthalate	35636-63-6	dimethyl 2-[[1-[[(2,3-dihydro-2-oxo-1H-benzimidazol-5-yl)amino]carbonyl]-2-oxopropyl]azo]terephthalate
60728-41-8	1-methyl 2-aminoterephthalate	67906-31-4	dimethyl 2-[[2-[(2-methoxyphenyl)amino]-2-oxo-1-(1,4,5,6-tetrahydro-4,6-dioxo-1,3,5-triazin-2-yl)ethyl]azo]terephthalate
65859-45-2	1-methyl-2-[(2-methyl-1-oxoallyl)oxy]ethyl hydrogen phthalate	5292-47-7	dimethyl 2-fluoroterephthalate
20566-35-2	2-(2-hydroxyethoxy)ethyl 2-hydroxypropyl 3,4,5,6-tetabromophthalate	14186-60-8	dimethyl 2-methylterephthalate
2202-98-4	2-(2-hydroxyethoxy)ethyl hydrogen phthalate	55447-98-8	dimethyl 2-sulphoterephthalate
51986-91-5	2,2'-diethyl dihydrogen 4,4'-carbonylbisphthalate	52656-24-3	dimethyl 4-[(3-aminophenyl)hydroxymethyl]phthalate
43135-99-5	2,2-dimethylpropane-1,3-diyl dihexahydrophthalate	51832-31-6	dimethyl 4-aminophthalate
35512-59-5	2,2-dimethylpropane-1,3-diyl phthalate	39617-05-5	dimethyl 4-dimethylaminophthalate
85851-76-9	2,5-dimethylheptyl 4-methyloctyl phthalate	5985-24-0	dimethyl 4-hydroxyisophthalate
85851-77-0	2,5-dimethylheptyl 6-methyloctyl phthalate	59340-47-5	dimethyl 4-iodophthalate
85391-52-2	2,5-dimethylheptyl nonyl phthalate	22955-73-3	dimethyl 4-methoxyisophthalate
54380-33-5	2-[(2-methyl-1-oxoallyl)oxy]ethyl hydrogen 3-chloro-2-hydroxypropylphthalate	23038-61-1	dimethyl 4-methylisophthalate
41284-31-5	2-[[4-(2,2-dicyanovinyl)-3-methylphenyl]ethylamino]ethyl methyl terephthalate	20116-65-8	dimethyl 4-methylphthalate
38056-88-1	2-acryloyloxyethyl 2-hydroxyethyl phthalate	610-22-0	dimethyl 4-nitrophthalate
30697-40-6	2-acryloyloxyethyl hydrogen phthalate	3748-70-7	dimethyl 5-(1-hydroxy-N-octadecyl-2-naphthamido)isophthalate
61827-64-3	2-ethylhexyl 2-methylpropyl phthalate	70364-24-8	dimethyl 5-(N-tosylsulphamoyl)isophthalate; potassium salt
85661-32-1	2-ethylhexyl 3-methoxypropyl phthalate	29920-31-8	dimethyl 5-[[1-[[(2,3-dihydro-2-oxo-1H-benzimidazol-5-yl)amino]carbonyl]-2-oxopropyl]azoterephthalate
89-13-4	2-ethylhexyl 8-methylnonyl phthalate	51760-21-5	dimethyl 5-bromoisophthalate
53272-22-3	2-ethylhexyl isodecyl phthalate	20330-90-9	dimethyl 5-chloroisophthalate
85851-92-9	2-ethylhexyl isononyl phthalate	13036-02-7	dimethyl 5-hydroxyisophthalate; dimethyl 5-hydroxybenzene-1,3-dicarboxylate~5-hydroxyisophthalic acid dimethyl ester
98072-28-7	2-ethylhexyl isotridecyl phthalate	51839-15-7	dimethyl 5-iodoisophthalate

98088-96-1	2-ethylhexyl isoundecyl phthalate	57052-99-0	dimethyl 5-nitroisophthalate
63468-13-3	2-ethylhexyl methyl terephthalate	138-25-0	dimethyl 5-sulphoisophthalate
85851-86-1	2-ethylhexyl nonyl phthalate	18643-86-2	dimethyl bromoterephthalate
85391-47-5	2-ethylhexyl undecyl phthalate	1687-29-2	dimethyl cis-1,2-cyclohexanedicarboxylate; dimethyl cis-hexahydrophthalate
17689-42-8	2-hydroxyethyl hydrogen phthalate	36928-64-0	dimethyl dihydrogen 4,4'-carbonylbisphthalate
46828-05-1	3,5-bis(methoxycarbonyl)phenylisocyanate; dimethyl-5-(isocyanato)isophthalate	5292-45-5	dimethyl nitroterephthalate; nitroterephthalic acid dimethyl ester
97692-55-2	3-ethylheptyl 2,5-dimethylheptyl phthalate	131-11-3	dimethyl phthalate
85851-79-2	3-ethylheptyl 4-methyloctyl phthalate	120-61-6	dimethyl terephthalate
85851-80-5	3-ethylheptyl 6-methyloctyl phthalate	3965-55-7	dimethyl-5-sulfoisophthalate sodium salt; DMSIP
13365-26-9	3-nitrophthalic acid dimethyl ester; dimethyl-3-nitrophthalate	27987-25-3	dimethylcyclohexyl-phthalate; DMCHP
25333-72-6	4,5alpha-epoxy-14-hydroxy-3-methoxy-17-methyl-6-oxomorphinan hydrogen terephthalate	84-74-2	di-n-butyl phthalate = DBP; phthalic acid di-n-butyl ester
22479-95-4	4-hydroxy phthalic acid dimethyl ester; dimethyl-4-hydroxy phthalate	4654-26-6	di-n-octyl terephthalate; dioctyl terephthalate
85851-78-1	4-methyloctyl 6-methyloctyl phthalate	131-18-0	di-n-pentyl phthalate; dipentylphthalate
85391-53-3	4-methyloctyl nonyl phthalate	14117-96-5	dioctadecyl phthalate
152699-63-3	5-aminoisophthalic acid dibenzyl ester; dibenzyl 5-amino isophthalate	137-89-3	dioctyl isophthalate
119-05-1	6-methylheptyl 8-methylnonyl phthalate	10578-33-3	dioleoyl phthalate
85391-54-4	6-methyloctyl nonyl phthalate	1539-04-4	diphenyl terephthalate
85409-84-3	8-methylnonyl phenylmethyl phthalate	17573-13-6	diphenylguanidine phthalate
3179-56-4	acetyl cyclohexane sulfonyl peroxide; 29% in phthalate plasticizer	18824-74-3	dipotassium 3,4,5,6-tetrabromophthalate
3814-58-2	allyl 2,3-epoxypropyl phthalate	15968-02-2	dipotassium 5-tert-butyliso phthalate
13654-74-5	aluminium tetrabromophthalate (3:2)	4409-98-7	dipotassium phthalate
67952-97-0	aluminium tridecyl phthalate (1:3:3)	4409-98-7	dipotassium phthalate
85959-15-5	ammonium dihydrogen 3-sulphonatophthalate		dipropargyl isophthalate
65229-11-0	ammonium dihydrogen 4-sulphonatophthalate		dipropargyl phthalate
83968-68-7	ammonium sodium hydrogen 5-sulphonatoisophthalate; compound with hexane-1,6-diamine (2:1)		dipropargyl terephthalate
50930-79-5	aniline hydrogen phthalate; 99%	68003-45-2	disodium 2-dodecyl 4-sulphonatophthalate
15656-86-7	barium phthalate	53566-35-1	disodium 4-hydroxyisophthalate
16883-83-3	benzyl 3-isobutyryloxy-1-isopropyl-2,2-dimethylpropyl phthalate	68189-35-5	disodium dodecyl 4-sulphonatophthalate
26386-42-5	benzyl butyl terephthalate	51821-29-5	disodium hydrogen sulphonatophthalate
27215-22-1	benzyl isooctyl phthalate	10028-70-3	disodium terephthalate
1248-43-7	benzyl octyl phthalate	25357-79-3	disodium tetrabromophthalate
21578-94-9	bis(1,1-dimethylethyl) dioxyterephthalate	2155-71-7	di-tert-butyl diperoxyphthalate
117-85-1	bis(2-(2-ethoxyethoxy)ethyl) phthalate	43039-86-7	di-tert-butyl peroxyhexahydroterephthalate
62240-27-1	bis(2,2,2-trifluoroethyl) phthalate	30448-43-2	di-tert-butyl phthalate
7415-86-3	bis(2,3-dibromopropyl) phthalate	119-06-2	ditridecyl phthalate; bis(tridecyl) Phthalate
97890-18-1	bis(2,3-epoxypropyl) 3,4,5,6-tetrabromophthalate	3648-20-2	diundecyl phthalate

97890-17-0	bis(2,3-epoxypropyl) 3,4,5,6-tetrachlorophthalate	21577-80-0	dodecyl hydrogen phthalate
7195-43-9	bis(2,3-epoxypropyl) isophthalate	85-71-2	ethoxycarbonylmethyl methyl phthalate
7195-45-1	bis(2,3-epoxypropyl) phthalate	29092-13-5	ethyl hydrogen tetrabromoterephthalate
7195-44-0	bis(2,3-epoxypropyl) terephthalate	4196-98-9	ethylene phthalate
57376-95-1	bis(2,4,6-tribromophenyl) terephthalate	51834-16-3	hexadecyl hydrogen phthalate
85391-48-6	bis(2,5-dimethylheptyl) phthalate	64084-40-8	hexadecyl octadecyl phthalate
605-54-9	bis(2-ethoxyethyl)phthalate	75673-16-4	hexyl 2-ethylhexyl phthalate
7299-89-0	bis(2-ethylbutyl) phthalate	61702-81-6	hexyl isodecyl phthalate
70152-36-2	bis(2-ethylheptyl) phthalate	71850-12-9	hexyl isooctyl phthalate
85409-66-1	bis(2-ethylhexyl) 4-(isopropyl)-5-methylphthalate	85851-89-4	hexyl isotridecyl phthalate
26040-51-7	bis(2-ethylhexyl) tetrabromophthalate	61827-62-1	hexyl octyl phthalate
6422-86-2	bis(2-ethylhexyl)terephthalate; 98.5%; DOTP	9050-31-1	hydroxypropyl methyl cellulose phthalate; methyl hydroxypropyl cellulose phthalate
85851-82-7	bis(2-ethylnonyl) phthalate	9050-31-1	hydroxypropyl methyl cellulose phthalate; hydroxypropyl methyl cellulose phthalat
85851-81-6	bis(2-ethyloctyl) phthalate	9050-31-1	hypromellose phthalate
7259-89-4	bis(2-hydroxyethyl) 5-nitroisophthalate	52118-12-4	iron phthalate (2:3)
84-73-1	bis(2-hydroxyethyl) phthalate	30833-53-5	isobutyl hydrogen phthalate
959-26-2	bis(2-hydroxyethyl)terephthalate; terephthalic acid bis(2-hydroxyethyl)ester	85168-75-8	isodecyl isononyl phthalate
117-82-8	bis(2-methoxyethyl)phthalate; di(2-methoxyethyl) phthalate	42343-35-1	isodecyl isooctyl phthalate
85851-83-8	bis(2-methyldecyl) phthalate	85168-77-0	isodecyl isotridecyl phthalate
70857-56-6	bis(2-methyloctyl) phthalate	94979-22-3	isodecyl isoundecyl phthalate
84787-86-0	bis(2-methylpropyl) 4-(dimethylamino)phthalate	85851-91-8	isodecyl nonyl phthalate
117-83-9	bis(2-n-butoxyethyl)phthalate; phthalic acid bis(2-butoxyethyl) ester	1330-96-7	isodecyl octyl phthalate
101012-82-2	bis(2-oxo-2-phenylethyl) phthalate	96507-81-2	isodecyl undecyl phthalate
53306-54-0	bis(2-propylheptyl) phthalate	96532-79-5	isononyl isooctyl phthalate
85851-84-9	bis(2-propylhexyl) phthalate	85168-76-9	isononyl isotridecyl phthalate
85851-85-0	bis(2-propyloctyl) phthalate	85168-79-2	isononyl isoundecyl phthalate
37832-65-8	bis(3,3,5-trimethylcyclohexyl) phthalate	98088-97-2	isononyl nonyl phthalate
14103-61-8	bis(3,5,5-trimethylhexyl) phthalate	85851-88-3	isononyl octyl phthalate
85409-67-2	bis(3-cyclohexylpropyl) phthalate	96507-82-3	isononyl undecyl phthalate
85391-51-1	bis(3-ethylheptyl) phthalate	67907-16-8	isooctyl 2-mercaptoethyl phthalate
85661-30-9	bis(3-methoxypropyl) phthalate	72512-75-5	isooctyl 2-phenoxyethyl terephthalate
20198-64-5	bis(3-phenylpropyl) phthalate	30849-48-0	isooctyl hydrogen phthalate
146-50-9	bis(4-methyl-2-pentyl)phthalate	94979-21-2	isooctyl isotridecyl phthalate
85391-50-0	bis(4-methyloctyl) phthalate	96532-80-8	isooctyl isoundecyl phthalate
85391-49-7	bis(6-methyloctyl) phthalate	96507-85-6	isooctyl nonyl phthalate
89-16-7	bis(8-methylnonyl) phthalate	96507-84-5	isooctyl undecyl phthalate

159852-53-6	bis(hexafluoroisopropyl)terephthalate	1459-93-4	isophthalic acid dimethyl ester; dimethyl isophthalate
82001-21-6	bis(pentabromobenzyl) tetrabromophthalate	744-45-6	isophthalic acid diphenyl ester; diphenyl isophthalate
94441-98-2	bis(pentabromobenzyl) tetrabromoterephthalate	1877-71-0	isophthalic acid monomethyl ester; monomethyl isophthalate
57212-63-2	bis(pentabromophenyl) terephthalate	93843-14-2	isotridecyl hydrogen phthalate
93951-36-1	bis[(1-methyl-1-phenylethyl)phenyl] isophthalate	85168-78-1	isotridecyl isoundecyl phthalate
3388-01-0	bis[(tetrahydrofuran-2-yl)methyl] phthalate	85851-90-7	isotridecyl nonyl phthalate
36388-36-0	bis[[1,4a-dimethyl-7-(1-methylethyl)tetradecahydrophenanthryl]methyl] phthalate	98072-29-8	isotridecyl undecyl phthalate
57569-40-1	bis[2-(1,1-dimethylethyl)-6-[[3-(1,1-dimethylethyl)-2-hydroxy-5-methylphenyl]methyl]-4-methylphenyl] terephthalate	96507-78-7	isoundecyl nonyl phthalate
32741-83-6	bis[2-(azidoformyloxy)ethyl] isophthalate	96507-79-8	isoundecyl undecyl phthalate
94088-05-8	bis[2-[(2-methyl-1-oxoallyl)oxy]ethyl] 2,5-bis(chloroformyl)terephthalate	93839-98-6	lead 3-(acetamido)phthalate
94088-04-7	bis[2-[(2-methyl-1-oxoallyl)oxy]ethyl] 4,6-bis(chloroformyl)isophthalate	60580-60-1	lead 5-nitroterephthalate
33374-28-6	butoxyethyl butyl phthalate	38787-87-0	lead isophthalate
85-69-8	butyl 2-ethylhexyl phthalate	6838-85-3	lead phthalate
85-68-7	butyl benzyl phthalate	16183-12-3	lead phthalate
84-64-0	butyl cyclohexyl phthalate	17976-43-1	lead phthalate (dibasic)
42597-49-9	butyl hydrogen tetrabromophthalate	42596-02-1	lithium terephthalate
24261-19-6	butyl hydrogen tetrachlorophthalate	68123-44-4	magnesium 4,4'-carbonylbisphthalate (2:1)
17851-53-5	butyl isobutyl phthalate	78948-87-5	magnesium bis(monoperoxyphthalate) hexahydrate; monoperoxyphthalic acid magnesium salt hexahydrate
42343-36-2	butyl isodecyl phthalate	84665-66-7	magnesium monoperoxyphthalate hexahydrate; monoperoxyphthalic acid magnesium salt 6H2O
3461-31-2	butyl nonyl phthalate	549-14-4	magnesium phthalate
84-78-6	butyl octyl phthalate	67801-55-2	methyl (4-methylphenyl)methyl terephthalate
89-19-0	butyl-n-decyl phthalate	39973-15-4	methyl hydrogen 4-(m-aminobenzoyl)phthalate
94275-93-1	cadmium (1-ethylhexyl) phthalate (1:2:2)	23843-86-9	methyl hydrogen 4-[(3-aminophenyl)hydroxymethyl]phthalate
94247-16-2	cadmium isooctyl phthalate (1:2:2)	6725-72-0	methyl phenyl terephthalate
94275-94-2	cadmium octyl phthalate (1:2:2)	4376-18-5	monomethyl phthalate; methyl hydrogen phthalate
5064-27-7	cadmium phthalate	131-70-4	n-butyl hydrogen phthalate; butyl hydrogen Phthalate
23239-68-1	calcium dibenzyl diphthalate	39020-35-4	nitroterephthalate dimethyl ester
94248-52-9	calcium dichlorophthalate	119-07-3	n-octyl-n-decyl phthalate
94275-90-8	calcium hydrogen 3,4,5,6-tetrachlorophthalate	24539-59-1	nonyl hydrogen phthalate
84681-97-0	calcium octadecyl phthalate (1:2:2)	65185-89-9	nonyl undecyl phthalate
5793-85-1	calcium phthalate	17181-26-9	octadecyl hydrogen phthalate
16130-76-0	calcium terephthalate	5393-19-1	octyl hydrogen phthalate
9004-38-0	cellulose acetophthalate; cellulose acetate phthalate	523-31-9	phthalic acid dibenzyl ester; dibenzyl phthalate
6732-01-0	cholesterol hydrogen phthalate	84-66-2	phthalic acid diethyl ester; diethyl phthalate ; DEP
51084-32-3	cobalt methyl terephthalate (1:2:2)	84-69-5	phthalic acid diisobutyl ester; di-isobutyl phthalate

34262-88-9	cobalt terephthalate	84-76-4	phthalic acid di-n-nonyl ester; di-n-nonyl phthalate
68123-45-5	copper 4,4'-carbonylbisphthalate (2:1)	117-84-0	phthalic acid di-n-octyl ester; di-n-octylphthalate
5423-38-1	copper dibutyl diphthalate	25053-15-0	poly(diallyl phthalate); average MW 65000 (gpc)
10027-30-2	copper phthalate	25038-59-9	poly(ethylene terephthalate); polyethylene terephthalate = PET
6190-36-9	cotarnine phthalate	29382-68-1	polyvinyl hydrogen phthalate
1169-98-8	cyclohexyl 2-ethylhexyl phthalate	10433-41-7	potassium dimethyl 5-sulphonatoisophthalate
5334-09-8	cyclohexyl isobutyl phthalate	877-24-7	potassium hydrogen phthalate; phthalic acid monopotassium salt~potassium biphthalate
71486-48-1	cyclohexyl isooctyl phthalate		potassium monomethyl terephthalate
85391-46-4	decyl 2-ethylhexyl phthalate	29801-94-3	potassium phthalate (2:1)
25724-58-7	decyl hexyl phthalate	97552-48-2	propane-1,3-diyl isophthalate
24539-60-4	decyl hydrogen phthalate	32657-12-8	S,S-bis[[4-(1,1-dimethylethyl)-3-hydroxy-2,6-dimethylphenyl]methyl] terephthalate
96507-83-4	decyl isononyl phthalate	24066-77-1	sodium (2,3-dihydroxypropyl) phthalate
53363-96-5	decyl isooctyl phthalate	83249-61-0	sodium 2-[(1-oxooctadec-9-enyl)amino]ethyl phthalate
98072-27-6	decyl isotridecyl phthalate	25425-73-4	sodium 2-ethylhexyl phthalate
96507-80-1	decyl isoundecyl phthalate	20259-91-0	sodium decyl phthalate
96507-76-5	decyl nonyl phthalate	73309-51-0	sodium diethyl 2-[(2-amino-8-hydroxy-6-sulphonatonaphthyl)azo]terephthalate
19295-82-0	decyl undecyl phthalate	33562-89-9	sodium dihydrogen 4-sulphonatophthalate
94023-12-8	D-glucitol phthalate; cyclic	66687-30-7	sodium dihydrogen 5-(3-sulphonatopropoxy)isophthalate
117-81-7	di-(2-Ethylhexyl)phthalate; dioctylphthalate	31352-31-5	sodium dimethyl 5-(3-sulphonatopropoxy)phthalate
62736-00-9	di(D-glucitol) phthalate	83781-01-5	sodium hydrogen 3(or 4)-sulphophthalate
110-22-5	diacetyl peroxide; 25% solution in dimethyl phthalate	68966-32-5	sodium hydrogen 3-chlorophthalate
13846-31-6	diallyl hexahydrophthalate	93762-14-2	sodium isobutyl phthalate
1087-21-4	diallyl isophthalate	94248-71-2	sodium isooctyl phthalate
131-17-9	diallyl phthalate	94108-00-6	sodium nonyl phthalate
131-71-9	diallyl phthalate practical	827-27-0	sodium phthalate
1026-92-2	diallyl terephthalate	15596-76-6	sodium terephthalate
7495-85-4	diallyl tetrahydrophthalate	94108-01-7	sodium tridecyl phthalate
523-24-0	diammonium phthalate	94248-20-1	strontium dichlorophthalate
523-31-9	dibenzyl phthalate; phthalic acid dibenzyl ester	94275-91-9	strontium hydrogen 3,4,5,6-tetrachlorophthalate (1:2)
19851-61-7	dibenzyl terephthalate	636-09-9	terephthalic acid diethyl ester; diethyl terephthalate
3126-90-7	dibutyl isophthalate	173550-97-5	terephthalic acid mono(2-bromoethyl) ester; 2-bromoethyl hydrogen terephthalate
1962-75-0	dibutyl terephthalate	33693-84-4	tert-butyl hydrogen phthalate
3015-66-5	dibutyl tetrachlorophthalate	15042-77-0	tert-butyl monoperphthalate
68515-51-5	di-C6-10-Phthalate	49693-09-6	tetrabromo phthalic acid diallyl ester; diallyl tetrabromo phthalate
68515-41-3	di-C7-9-Phthalate	49693-09-	tetrabromophthalic acid diallyl ester; diallyl tetrabromo phthalate

68515-43-5	di-C9-11-Phthalate; 1,2-benzoldicarbonsäure di-9-11-verzweigte und lineare Alkylester	68123-46-6	tetracesium 4,4'-carbonylbisphthalate
131-15-7	dicapryl phthalate	67846-42-8	tetraethyl 4,4'-[2,2,2-trifluoro-1-(trifluoromethyl)ethylidene]bis(phthalate)
84-61-7	dicyclohexyl phthalate; DCHP	35395-64-3	tetrahydrofurfuryl hydrogen phthalate
68443-43-6	didecyl 2-ethyl-2-[[(2-methyl-1-oxoallyl)oxy]methyl]propane-1,3-diylphthalate	68226-87-9	tetralithium 4,4'-carbonylbisphthalate
84-77-5	didecyl phthalate; di-n-decyl phthalate	68516-73-4	tetramethyl 2,2'-[1,4-phenylenebis[imino(1-acetyl-2-oxoethane-1,2-diyl)azo]]bisterephthalate
2432-90-8	didoceyl phthalate; 98%	85050-00-6	tetramethyl 5,5'-[(1,4-dioxo-1,4-butanediyl)diimino]bisisophthalate
65701-07-7	diethyl 4,4'-carbonylbis(hydrogen phthalate); compound with benzene-m-diamine	79723-02-7	tetramethylammonium hydrogen phthalate
65701-06-6	diethyl 4,4'-carbonylbis(hydrogen phthalate); compound with p,p'-methylenedianiline (1:1)	56585-48-9	tetrapotassium 4,4'-carbonylbisphthalate
64139-21-5	diethyl 4-hydroxyphthalate	68123-47-7	tetrarubidium 4,4'-carbonylbisphthalate
636-53-3	diethyl isophthalate; DEIP	67892-57-3	tetrasodium 4,4'-[(1-methylethylidene)bis(1,4-phenyleneoxy)]bisphthalate
3648-21-3	diheptyl phthalate; di-n-heptyl phthalate	68123-48-8	tetrasodium 4,4'-carbonylbisphthalate
13372-18-4	dihexadecyl phthalate	68226-88-0	tricesium hydrogen 4,4'-carbonylbisphthalate
84-75-3	dihexyl phthalate	61886-60-0	tridecyl isodecyl phthalate
605-50-5	diisoamyl phthalate; DIAP	67907-14-6	triisooctyl (methylstannylidyne)tris(thioethylene) triphthalate
70969-58-3	diisobutyl hexahydrophthalate	71686-04-9	trilithium 5-sulphonatoisophthalate
18699-48-4	diisobutyl terephthalate	68226-92-6	trilithium hydrogen 4,4'carbonylbisphthalate
90937-19-2	diisoheptyl phthalate	68226-90-4	tripotassium hydrogen 4,4'-carbonylbisphthalate
71850-09-4	diisohexyl phthalate	68226-89-1	trirubidium hydrogen 4,4'-carbonylbisphthalate
68515-50-4	diisohexyl phthalate	68226-91-5	trisodium hydrogen 4,4'-carbonylbisphthalate
68515-48-0	diisononyl phthalate; DINP	52642-40-7	trisodium sulphonatophthalate
28553-12-0	di-''isononyl'' phthalate; diisononyl phthalate	51622-03-8	undecyl hydrogen phthalate
67907-15-7	diisooctyl (dimethylstannylene)bis(thioethylene)phthalate	60580-61-2	zinc 5-nitroisophthalate
71850-11-8	diisooctyl isophthalate	2880-85-5	zinc phthalate

*Source: Chemos GmbH Chemical Product list: http://www.chemos-group.com

Appendix 5.1 Summary Table of DEHP-induced *in vitro* Genotoxic Effects

(retrieved from ECB, 2008; ATSDR, 2002; and IARC, 2000)

DEHP-induced *in vitro* Genotoxic Effects					
Species/Test System (Strain)	End Point	Doses (ECB, 2008) – **LED/HID Doses** (IARC, 2000)	Conclusion - WITH activation[1]	Conclusion - WITHOUT activation[1]	Citation
Bacterial Systems					
Salmonella typhimurium	Gene mutation	N/A	Negative	Negative	Astill *et al.*,1986 ; ATSDR, 2002
S. typhimurium	Gene mutation	N/A	Negative	Negative	Barber *et al.*,1987; ATSDR, 2002
S. typhimurium (TA100, TA1535, TA1537, TA1538, TA98)	Reverse gene mutation	0.1, 0.5, 2.5, 5.0, 10.0 µl/plate (98-9800 µg/plate; GLP) **9860**	Negative (arochlor-induced rat liver S9)	Negative	Kirby *et al.*,1983; Nuodex, 1980; IARC, 2000; ATSDR, 2002; ECB, 2008
S. typhimurium (TA100, TA1535, TA1537, TA1538, TA98)	Reverse gene mutation	0.2 µl/plate **(MEHP)**	Negative	Negative	Kirby *et al.*,1983; IARC, 2000
S. typhimurium (TA100, TA1535, TA1537, TA1538, TA98)	Reverse gene mutation	1 µl/plate **(2-ethylhexanol)**	Negative	Negative	Kirby *et al.*,1983; IARC, 2000
S. typhimurium (TA98, TA100)	Reverse gene mutation	Up to 1000 µg/plate	Negative (rat S9 mix)	Negative – (ECB) {Positive, ATSDR}	Kozumbo *et al.*,1982; ATSDR, 2002; ECB, 2008
S. typhimurium (TA98)	Gene mutation	N/A	Negative	Negative	Sato *et al.*,1994; ATSDR, 2002
S. typhimurium (TA102)	Reverse gene mutation	0, 1.0, 2.5, 5.0, 10.0, 20.0 µmol/plate (391-7812 µg/plate)	Negative (various enzymes)	Negative	Schmezer *et al.*,1988; ATSDR, 2002; ECB, 2008
S. typhimurium (TA102)	Reverse gene mutation	Up to 5000 µg/plate; GL	Negative (rat S9 mix)	Negative	Jung *et al.*,1992; ECB, 2008
S. typhimurium (TA100)	Gene mutation	N/A	Negative	Negative	Seed 1982; ATSDR, 2002
S. typhimurium (TA98, TA100)	Reverse gene mutation	0, 30, 39, 3900 µg/mL	Negative (rat S9 mix)	Negative	Warren *et al.*,1982; ECB, 2008
S. typhimurium (TA100, TA1535, TA1537, TA1538, TA98)	Reverse gene mutation	0.5, 5.0, 50, 500, 5000 µg/plate; GLP	Negative (rat S9 mix)	Negative	Eastman Kodak, 1984; DiVincenzo *et al.*,1985; ECB, 2008
S. typhimurium (TA100, TA1535, TA1537, TA1538, TA98)	Reverse gene mutation	0.02, 0.06, 0.20, 0.66, 2.00 ml urine from rats treated 15 days with 2000 mg/kg-day	Negative (rat S9 mix)	Negative	Eastman Kodak, 1984; DiVincenzo *et al.*,1985; ECB, 2008
S. typhimurium (TA100, TA1535, TA1537, TA1538, TA98)	Reverse gene mutation	0.15, 0.47, 1.50, 4.74, 15.0, 47.43, 150.0 µl/plate (147-14,700 µg/plate; GLP)	Negative (arochlor-induced rat liver S9)	Negative	CMA, 1982d; ECB, 2008

S. typhimurium (TA100, TA1535, TA1537, TA98)	Reverse gene mutation	100-2000 µg/plate	Negative (rat S9 mix)	Negative	Agarwal *et al.*,1985; ECB, 2008
S. typhimurium (TM677)	Forward gene mutation	50, 200, 500 µg/mL **500**	Negative (rat S9 mix)	Negative	Liber, 1985; IARC, 2000; ECB, 2008
S. typhimurium (TA100, TA98)	Reverse gene mutation	4000 µg/mL	Negative	Negative	Robertson *et al.*,1983; IARC, 2000
S. typhimurium (TA 1537, TA98, TA7001, TA7002, TA7003, TA7004, TA7005, TA7006)	Reverse gene mutation	1000 µg/mL	Negative	Negative	Gee *et al.*,1998; IARC, 2000
S. typhimurium	Gene mutation	N/A	Negative	Negative	Tennant *et al.*,1987; ATSDR, 2002
S. typhimurium (TA100)	Reverse gene mutation	5 mg/plate	Marginally Positive (rat S9 mix, ECB) {positive, ATSDR}	N/A	Tomita *et al.*,1982b; ATSDR, 2002; ECB, 2008
S. typhimurium (TA100)	Reverse gene mutation	1250µg/plate **(MEHP)**	Negative	Negative	Tomita *et al.*,1982b; IARC, 2000
S. typhimurium (TA98, TA100)	Reverse gene mutation	50, 100, 200, 500, 1000, 2000 µg/plate **2000**	Negative (rat S9 mix)	Negative	Yoshikawa *et al.*,1983; IARC, 2000; ATSDR, 2002; ECB, 2008
S. typhimurium (TA100, TA102, TA97, TA98)	Reverse gene mutation	0, 320, 1000, 3200, 10,000 µg/plate **10,000**	Negative (rat S9 mix)	Negative	Baker and Bonin, 1985; IARC, 2000; ECB, 2008
S. typhimurium (TA100, TA102, TA97, TA98)	Reverse gene mutation	0, 100, 200, 500, 1000, 2000, 5000 µg/plate **5000**	Negative (rat S9 mix)	Negative	Matsushima *et al.*,1985; IARC, 2000; ECB, 2008
S. typhimurium (TA100, TA1535, TA1537, TA1538, TA98)	Reverse gene mutation	50, 100, 500, 1000, 2000 µg/plate **5000**	Negative (rat S9 mix)	Negative	Rexroat and Probst, 1985; IARC, 2000; ECB, 2008
S. typhimurium (TA100, TA1535, TA97, TA98)	Reverse gene mutation	10,000 µg/mL	Negative	Negative	Zeiger and Haworth, 1985; IARC, 2000
S. typhimurium (TA100, TA1535, TA1537, TA98)	Reverse gene mutation	100, 333, 1000, 3333, 10,000 µg/plate; GL **10,000**	Negative (rat hamster S9 mix)	Negative	Zeiger *et al.*,1985a,b {1982}; IARC, 2000; ECB, 2008
S. typhimurium (TA100, TA102, TA97, TA98)	Reverse gene mutation	10,000 µg/mL	Negative	Negative	Nohmi *et al.*,1985; IARC, 2000
S. typhimurium (TA100, TA102, TA97, TA98)	Reverse gene mutation	1000 µg/plate **(MEHP)**	Negative	Negative	Dirven *et al.*,1991; IARC, 2000
S. typhimurium (TA100, TA102, TA97, TA98)	Reverse gene mutation	1000µg/plate **(5OH-MEHP; IX)**	Negative	Negative	Dirven *et al.*,1991; IARC, 2000
S. typhimurium (TA100, TA102, TA97, TA98)	Reverse gene mutation	1000µg/plate **(5oxo-MEHP; VI)**	Negative	Negative	Dirven *et al.*,1991; IARC, 2000

S. typhimurium (TA100, TA102, TA97, TA98)	Reverse gene mutation	1000µg/plate **(5cx-MEPP; V)**	Negative	Negative	Dirven *et al.*,1991; IARC, 2000
S. typhimurium (TA100, TA1535, TA1537, TA1538, TA2637, TA98)	Spot test	500 µg/plate	N/A	Negative	Agarwal *et al.*,1985; ECB, 2008
Escherichia coli (PQ37)	Gene mutation	N/A	Negative	Negative	Sato *et al.*,1994; ATSDR, 2002
E. coli (WP2uvrA)$^{+}$	Reverse gene mutation	50, 100, 200, 500, 1000, 2000 µg/plate	Negative (rat S9 mix)	Negative	Yoshikawa *et al.*,1983; IARC, 2000; ATSDR, 2002; ECB, 2008
E. coli (WP2uvrA)	Reverse gene mutation	50, 100, 200, 500, 1000, 2000 µg/plate **2000**	Negative (rat S9 mix)	Negative	Yoshikawa *et al.*,1983; ATSDR, 2002; ECB, 2008
S. typhimurium	Azaguanine resistance	N/A	Negative	Negative	Seed, 1982; ATSDR, 2002
Bacillus subtilis (rec assay)	DNA damage – differential toxicity	500 µg/disc (plate)	NT {negative, ATSDR}	Negative	Tomita *et al.*,1982b; IARC, 2000; ATSDR, 2002; ECB, 2008
Bacillus subtilis (rec assay)	DNA damage – differential toxicity	50-300 µg/disc (plate) 400-500 µg/disc (plate) **(MEHP)**	NT	Positive (400-500); Negative (50-300)	Tomita *et al.*,1982b; IARC, 2000; ECB, 2008
Bacillus subtilis (rec assay)	DNA damage – differential toxicity	500 µg/disc (plate) **(2-ethylhexanol)**	NT	Negative {slightly positive, ECB}	Tomita *et al.*,1982b; IARC, 2000; ECB, 2008
Bacillus subtilis (rec assay)	DNA damage – differential toxicity	500 µg/disc (plate) **(phthalic acid)**	NT	Negative	Tomita *et al.*,1982b; IARC, 2000
Eukaryotic Systems					
Saccharomyces cerevisae (JD1, D7-144, D7)	Gene conversion	200, 500, 1000, 2000, 3000, 5000 µg/mL **5000**	Negative (ATSDR) {eqiovocal for mitotic segregation, ECB, rat S9 mix}	Negative	Parry and Eckardt, 1985; IARC, 2000; ATSDR, 2002; ECB, 2008
S. cerevisae (D7)	Gene conversion	0, 40, 200, 1000, 5000 µg/mL **5000**	Weak Positive (positive; S9 mix at 5000, ECB)	Weak Positive (positive at 5000, ECB)	Arni, 1985; IARC, 2000; ECB, 2008
S. cerevisae	Gene conversion	2000 µg/mL	Negative	Negative	Brooks *et al.*,1985; IARC, 2000
S. cerevisae (PV-2, PV-3, PV-4a,b)	Gene conversion	1-1000 µg/mL **1000**	Negative	Negative	Inge-Vechtomov *et al.*,1985; IARC, 2000; ECB, 2008
S. cerevisae (D7-144)	Gene conversion	1541, 3081, 6163, 12,325 nL/mL (1510-12,080 µg/mL) **1500**	Positive (negative/positive, ECB; S9 mix)	Positive (negative/positive, ECB)	Mehta and von Borstel, 1985; IARC, 2000; ECB, 2008

S. cerevisae (D61M, D6)	Mitotic aneuploidy	5000 µg/mL	Positive	Positive	Parry and Eckardt, 1985; IARC, 2000; ATSDR, 2002
S. cerevisae (D7)	Mitotic aneuploidy	Up to 50 µL/mL (49 µL/mL)	N/A	Positive – increase in hyperploidy (chromosome no > 22)	Parry, 1985; ECB, 2008
S. cerevisae (D61.M)	Mitotic aneuploidy	Saturated solution	N/A	Negative	Zimmerman *et al.*,1985; ECB, 2008
S. cerevisae (D7)	Homozygosis	0, 40, 200, 1000, 5000 µg/mL **5000**	Negative(S9 mix)	Negative	Arni, 1985; IARC, 2000; ECB, 2008
S. cerevisae (PV-2, PV-3, PV-4a,b)	Homozygosis	1-1000 µg/mL **1000**	Negative	Negative	Inge-Vechtomov *et al.*,1985; IARC, 2000; ECB, 2008
S. cerevisae (D61M, D6){D7}	Mitotic segregation	Up to 50 µL/mL (49µg/mL)	N/A {negative – ATSDR}	Negative	Parry, 1985; ATSDR, 2002; ECB, 2008
S. cerevisae (D7)	Gene mutation– point mutation	Up to 50 µL/mL (49µg/mL) {mg/mL, ECB)	N/A	Negative	Parry, 1985; ECB, 2008
S. cerevisae (PV-1, PV-2, PV-3)	Forward gene mutation	1-1000 µg/mL **1000**	Negative (rat S9 mix)	Negative	Inge-Vechtomov *et al.*,1985; IARC, 2000; ECB, 2008
S. cerevisae (XV185-14C, D7, RM52, D6, D5, D6-1)	Reverse gene mutation	200, 500, 1000, 2000, 3000, 5000 µg/mL **5000**	Negative (rat S9 mix)	Negative	Parry and Eckardt, 1985; IARC, 2000; ATSDR, 2002; ECB, 2008
S. cerevisae (D7)	Reverse gene mutation – point mutation	0, 40, 200, 1000, 5000 µg/mL **5000**	Negative (S9 mix)	Negative	Arni, 1985; IARC, 2000; ECB, 2008
S. cerevisae (PV-1, PV-2, PV-3)	Reverse gene mutation	1-1000 µg/mL **1000**	Negative (rat S9 mix)	Negative	Inge-Vechtomov *et al.*,1985; IARC, 2000
S. cerevisae (XV185-14C, RM52)	Reverse gene mutation	1541, 3081, 6163, 12325 nl/mL (1510-12080 µg/mL) **1500**	Positive (equivocal, ECB; rat S9 mix)	Positive (equivocal, ECB)	Mehta and von Borstel, 1985; IARC, 2000; ECB, 2008
Schizosaccharomyces pombe (P1)	Gene mutation	N/A	Negative	Negative	Parry *et al.*,1985; ATSDR, 2002
S. pombe (P1)	Forward gene mutation	369, 738, 1467, 2935, 5870 µg/mL **5900**	Equivocal (ECB; rat S9 mix) {negative, IARC}	Negative (equivocal, ECB)	Loprieno *et al.*,1985; IARC, 2000; ECB, 2008
S. cerevisae (RS112)	Deletion assay, Interchromosomal recombination assay	0, 3000, 10,000, 30,000, 100,000, 200,000 µg/mL **200,000**	Negative (rat S9 mix)	Negative	Carls and Schiestl, 1994; IARC, 2000; ECB, 2008
Aspergillus niger (P1)	Mitotic segregation	N/A	Negative	N/A	Parry *et al.*,1985; ATSDR, 2002

Aspergillus nidulans (strain P1)	Haploid, mutation	0, 2465, 4930, 9860 µg/mL **9900**	NT	Negative	Carere *et al.*,1985; IARC, 2000
Aspergillus nidulans (strain P1)	Non-disjunction	0, 2465, 4930, 9860 µg/mL **9900**	NT	Negative	Carere *et al.*,1985; IARC, 2000
Aspergillus nidulans (strain P1)	Mitotic crossing-over	0, 2465, 4930, 9860 µg/mL **9900**	NT	Negative	Carere *et al.*,1985; IARC, 2000; ECB, 2008
Drosphila melanogaster	Crossing-over/recombination	39,000 µg/g food	NT	Negative	Würgler *et al.*,1985; IARC, 2000
D. melanogaster	Somatic mutation (unstable eye mosaic test)	96 hours, addition to culture medium of 10, 20, 40, 80, 160, 320 mM (3.9-125 mg/mL) (6930 µg/cm^2 food surface)	NT	Weak Positive (positive at 20 mM, negative at other concentrations, ECB)	Fujikawa *et al.*,1985; IARC, 2000; ECB, 2008
D. melanogaster	Somatic mutation	4 days, feed 2 mM (0.78 mg/mL) **780 µg/g food**	NT	Weak Positive (questionable results)	Vogel, 1985; IARC, 2000; ECB, 2008
D. melanogaster	Somatic mutation (wing spot tests)	48, 72, 96 hours, feed 200 mM (78 mg/mL) **39,000 µg/g food**	NT	Negative (negative except for induction of twin spots after 48 hours, ECB)	Würgler *et al.*,1985; IARC, 2000; ECB, 2008
D. melanogaster	Somatic mutation (wing spot tests)	48 hours, feed 200 mM (78 mg/mL)	N/A	Positive for large single spots; ambiguous for twin spots; negative for small single spots	Graf *et al.*,1989; ECB, 2008
Mammalian Systems					
Mouse lymphoma L5178Y cells, *Tk* locus	Mutagenicity – gene mutation	250 µL/mL	Negative	Negative	Astill *et al.*,1986; IARC, 2000; ATSDR, 2002
Mouse lymphoma L5178Y cells, *Tk* locus	Mutagenicity – gene mutation	0.016-1.0 µL/mL (15-206 µg/mL; -S9); 0.067-5.0 µL/mL (66-4900 µg/mL; +S9) {980, IARC}	Negative (S9 mix)	Weak Positive (Negative, ATSDR, ECB)	Kirby *et al.*,1983; Nuodex, 1981d; IARC, 2000; ATSDR, 2002; ECB, 2008
Mouse lymphoma L5178Y cells, *Tk* locus	Mutagenicity – gene mutation	0.3 µL/mL **(MEHP)**	Negative	Negative	Kirby *et al.*,1983; IARC, 2000
Mouse lymphoma L5178Y cells, *Tk* locus	Mutagenicity – gene mutation	0.3 µL/mL **(2-ethylhexanol)**	Negative	Negative	Kirby *et al.*,1983; IARC, 2000

Mouse lymphoma L5178Y cells, *Tk* locus	Mutagenicity – gene mutation	184-2468 µg/mL(+S9); 22-301 µg/mL (-S9) {2500, IARC}	Negative for trifluorothymidine resistance (S9 mix)	Equivocal	Amacher and Turner, 1985; IARC, 2000; ECB, 2008
Mouse lymphoma L5178Y cells, *Tk* locus	Mutagenicity – gene mutation	250, 500, 1000, 2000, 3000, 5000 nL/mL (245-4900 µg/mL) **4900**	Negative (rat S9 mix)	Negative	Myhr *et al.*,1985; IARC, 2000; ECB, 2008
Mouse lymphoma L5178Y cells, *Tk* locus	Mutagenicity – gene mutation	10, 20, 30, 40, 50, 100, 200, 400, 620 µg/mL (-S9), 1.0, 2.5, 5.0, 7.5, 10, 20, 40, 80 µg/mL (+S9) **7.5**	Weak Positive (Positive, ECB; S9 mix)	Weak Positive (Positive, ECB)	Oberly *et al.*,1985; IARC, 2000; ECB, 2008
Mouse lymphoma L5178Y cells, *Tk* locus, clone 372 +/+	Mutagenicity – gene mutation	0, 78, 392, 1962, 9810 µg/mL **9800**	Negative (rat S9 mix)	Negative	Styles *et al.*,1985; IARC, 2000; ECB, 2008
Mouse lymphoma cells	Mutagenicity	N/A	Negative	Negative	Tennant *et al.*,1987; ATSDR, 2002
CHO-K1-BH4 Chinese hamster ovary (CHO) cells	Forward mutation – mutant frequency	5, 10, 20, 40, 80 nl/ml (4.9-78 µg/mL; GLP)	Negative (rat S9 mix)	Negative	CMA, 1985; ECB, 2008
Mouse lymphoma L5178Y cells, ouabain and 6-thioguanine resistance	Mutagenicity – gene mutation – fluctuation assay	12.5-200 µg/mL **200**	Negative (rat S9 mix)	Negative	Garner and Campbell, 1985; IARC, 2000; ECB, 2008
Mouse lymphoma L5178Y cells, ouabain resistance	Mutagenicity – gene mutation	9800 µg/mL	Negative	Negative	Styles *et al.*,1985; IARC, 2000
BALB/c-3T3 mouse embryo cells, ouabain resistance	Mutagenicity – gene mutation	0, 79, 250, 791, 2000, 7910 nl/mL (77-7752 µg/mL) {1960, IARC}	Negative (rat S9 mix)	NT	Matthews *et al.*,1985; IARC, 2000; ECB, 2008
Human lymphoblasts (TK6, AHH-1)	Gene mutation	0, 200, 250, 400, 600, 750, 800, 1000 µg/mL (TK-6 +S9); (AHH-1 – S9) **1000**	Negative (rat S9 mix)	Negative	Crespi *et al.*,1985; IARC, 2000; ECB, 2008
Rat hepatocytes	DNA damage – DNA single strand breaks	1.25, 2.5, 3.125, 5.0, 12.5, 25.0 µmol/tube (488-9765 µg/tube) **9750**	N/A {negative, ATSDR}	Negative {N/A, ATSDR}	Schmezer *et al.*,1988; ATSDR, 2002; ECB, 2008
Syrian hamster hepatocytes	DNA damage – DNA single strand breaks	1.25, 2.5, 3.125, 5.0, 12.5, 25.0 µmol/tube (488-9765 µg/tube) **9750**	NT{negative, ATSDR}	Negative {N/A, ATSDR}	Schmezer *et al.*,1988; IARC, 2000; ATSDR, 2002; ECB, 2008
Rat hepatocytes	DNA repair	N/A	Negative	N/A	Astill *et al.*,1986; ATSDR, 2002
Rat hepatocytes	DNA repair	N/A	Negative	N/A	Hodgson *et al.*,1982; ATSDR, 2002
Rat hepatocytes	DNA damage and repair - single strand breaks	391, 1172, 3907 µg/mL	NT	Negative	Bradley, 1985; IARC, 2000; ECB, 2008

Chinese Hamster Ovary cells	DNA damage and repair - single strand breaks	391, 1170, 1950, 2730, 3910 µg/mL {39,000, IARC}	N/A {negative, IARC, ECB}	Negative	Douglas *et al.*,1985, 1986; IARC, 2000; ECB, 2008
Syrian hamster embryo (SHE) cells	DNA damage and repair - DNA adenovirus (SA7) transformation	0, 0.2, 0.3, 0.6, 1.3, 2.6 mM (78-1016 µg/mL)	N/A	Equivocal	Hatch and Anderson, 1985; ECB, 2008
Human primary hepatocytes	DNA repair - UDS	0.1, 1, 10 mM (39-3900 µg/mL); GL	NT {negative, ATSDR}	Negative {N/A, ATSDR}	Butterworth *et al.*,1984; IARC, 2000; ATSDR, 2002; ECB, 2008
Human primary hepatocytes	DNA repair - UDS	139 µg/mL **(MEHP)**	NT	Negative	Butterworth *et al.*,1984; IARC, 2000
Rat primary hepatocytes	DNA repair-UDS	0.1, 1, 10 mM (39-3900 µg/mL; GL) **3900**	NT {negative, ATSDR}	Negative {N/A, ATSDR}	Butterworth *et al.*,1984; IARC, 2000; ATSDR, 2002; ECB, 2008
Rat primary hepatocytes	DNA repair-UDS	0.01, 0.1, 1, 10 mM (3.9-3900 µg/mL) **3900**	NT {negative, ATSDR}	Negative {N/A, ATSDR}	Kornbrust *et al.*,1984; IARC, 2000; ATSDR, 2002; ECB, 2008
Rat primary hepatocytes	DNA repair-UDS	0.19, 0.39, 1.95, 3.9, 19.5, 39, 195, 390, 1950, 3900 µg/mL **3900**	NT {negative, ATSDR}	Negative {N/A, ATSDR}	Probst and Hill, 1985; IARC, 2000; ATSDR, 2002; ECB, 2008
Rat primary hepatocytes	DNA repair-URP, UDS	0.1, 1, 10, 100, 1000, 10,000 µg/mL **10,000**	NT	Negative	Williams *et al.*,1985; IARC, 2000; ECB, 2008
Rat primary hepatocytes	DNA repair-URP, UDS	1000 µg/mL	NT	Negative	Astill *et al.*,1986; IARC, 2000
Rat primary hepatocytes	DNA repair-URP, UDS	0.078, 0.156, 0.313, 0.625, 1.25, 2.5, 5.0, 10.0 µL/mL (76-9800 µg/mL; GLP)	N/A	Negative	Nuodex, 1981e; ECB, 2008
B6C3F$_1$ Mouse primary hepatocytes	DNA repair-UIA, UDS	0.01, 0.1, 1 mM (3.9-390 µg/mL) **390**	NT {negative, ATSDR}	Negative {N/A, ATSDR}	Smith-Oliver and Butterworth 1987; IARC, 2000; ATSDR, 2002; ECB, 2008
B6C3F$_1$ Mouse primary hepatocytes	DNA repair-UIA, UDS	139 µg/mL **(MEHP)**	NT	Negative	Smith-Oliver and Butterworth 1987; IARC, 2000
V79 cells	DNA repair	N/A	Negative	N/A	Kornbrust *et al.*,1984; ATSDR, 2002
Chinese Hamster Don cells	Sister chromatid exchange	3900 µg/mL	NT {negative, ATSDR}	Negative {N/A, ATSDR}	Abe and Sasaki, 1977; IARC, 2000; ATSDR, 2002
Chinese Hamster Ovary cells	Sister chromatid exchange	N/A	Negative	N/A	Phillips *et al.*,1982; ATSDR, 2002

Chinese Hamster Ovary cells	Sister chromatid exchange	N/A	Positive	N/A	Tennant *et al.*,1987; ATSDR, 2002
Chinese Hamster Ovary cells	Sister chromatid exchange	3.9, 19.5, 39, 195, 390, 1170, 2340, 3900 µg/mL	Negative (rat S9 mix)	Negative	Douglas *et al.*,1985, 1986; IARC, 2000; ECB, 2008
Chinese Hamster Ovary cells	Sister chromatid exchange	5, 16, 50, 160, 500, 1600, 3000, 4000, 5000 µg/mL **5000**	Negative (rat S9 mix)	Weak Positive (Negative, ECB)	Gulati *et al.*,1985; Gulati *et al.*,1989; IARC, 2000; ECB, 2008
Chinese Hamster Ovary cells	Sister chromatid exchange	N/A	Negative	N/A	Tennant *et al.*,1987; ATSDR, 2002
Rat liver (RL-4)	Sister chromatid exchange	0, 125, 250, 500, 1000 µg/mL	NT {negative, ATSDR}	Negative {N/A, ATSDR}	Priston and Dean, 1985; IARC, 2000; ATSDR, 2002; ECB, 2008
Human peripheral lymphocytes	Sister chromatid exchange	10, 100, 1000 µg/mL **1000**	Negative (rat S9 mix)	Negative	Obe at al., 1985; IARC, 2000; ECB, 2008
Human lymphocytes (co-culture with rat liver cells)	Sister chromatid exchange	39 µg/mL	Weak Positive	Negative	Lindahl-Kiessling *et al.*,1989; IARC, 2000
Chinese hamster V79 cells	Sister chromatid exchange	25 µg/mL **(MEHP)**	NT	Positive	Tomita *et al.*,1982; IARC, 2000
Human lymphocytes {human hepatocytes, ATSDR}	Cytogenetic assay; Chromosomal aberrations	3.2, 15.7, 30.6, 45.0, 61.3, 75.4 µg/mL **75**	NT {negative, ATSDR}	Negative {N/A, ATSDR}	Turner *et al.*,1974; IARC, 2000; ATSDR, 2002; ECB, 2008
Human leucocytes {lymphocytes}	Cytogenetic assay; Chromosomal aberrations	0.06, 0.6, 6, 60 µg/mL **60**	NT {negative, ATSDR}	Negative for breaks, gaps, abnormal forms {N/A, ATSDR}	Stenchever *et al.*,1976; IARC, 2000; ATSDR, 2002; ECB, 2008
Human fetal lung cells	Cytogenetic assay; Chromosomal aberrations	6 µg/mL	N/A	Negative for breaks, gaps, abnormal forms, aneuploidy	Stenchever *et al.*,1976; ECB, 2008
Chinese Hamster Ovary cells	Cytogenetic assay; Chromosomal aberrations	0.5, 1.0, 2.0 mM (195-780 µg/mL) **781**	NT {negative, ATSDR}	Negative for CA {N/A, ATSDR}	Phillips *et al.*,1982; IARC, 2000; ATSDR, 2002; ECB, 2008
Rat liver cells (RL4)	Cytogenetic assay; Chromosomal aberrations	0, 125, 250, 500, 1000 µg/mL **1000**	NT {negative, ATSDR}	Negative for CA {N/A, ATSDR}	Priston and Dean, 1985; Shell, 1983; IARC, 2000; ATSDR, 2002; ECB, 2008
Chinese Hamster Don cells	Chromosomal aberrations	3900 µg/mL	NT	Negative	Abe and Sasaki, 1977; IARC, 2000
Chinese Hamster lung cells	Chromosomal aberrations	160 µg/mL	NT	Negative	Ishidate and Odashima, 1977; IARC, 2000
Chinese Hamster liver cells	Chromosomal aberrations	50 µg/mL	NT	Negative	Danford, 1985; IARC, 2000

Chinese Hamster Ovary cells	Cytogenetic assays; Chromosomal aberrations	50, 160, 500, 1600, 2000, 3000, 4000, 5000 µg/mL **5000**	Negative (rat S9 mix)	Negative	Gulati *et al.*,1985; Gulati *et al.*,1989; IARC, 2000; ECB, 2008
Chinese hamster lung fibroblasts (CHL)	Cytogenetic assays; Chromosomal aberrations	1375, 2750, 4130 µg/mL **4130**	Negative for CA	Negative for CA	Ishidate and Sofuni, 1985; IARC, 2000; ECB, 2008
Syrian hamster embryo (SHE) cells	Cytogenetic assays; Chromosomal aberrations	0, 1, 3, 10, 30, 100 µM (0.39-39 µg/mL) **39**	Positive (rat S9 mix)	Negative (Positive, ECB)	Tsutsui *et al.*,1993; IARC, 2000; ECB, 2008
Syrian hamster embryo (SHE) cells	Chromosomal aberrations	2.8 µg/mL **(MEHP)**	Positive	Negative	Tsutsui *et al.*,1993; IARC, 2000
Human lymphocytes	Chromosomal aberrations	160 µg/mL	NT	Negative	Tsuchiya and Hattori, 1976; IARC, 2000
Chinese Hamster Ovary cells	Micronucleus formation	0.1, 1, 10, 100 mM (3.9-3900 µg/mL) **3900**	Negative (rat S9 mix)	Negative	Douglas *et al.*,1985; IARC, 2000; ECB, 2008
Rat hepatocytes	Micronucleus formation	3900 µg/mL	NT	Negative	Müller-Tegethoff *et al.*,1995; IARC, 2000
Syrian hamster embryo (SHE) cells	Micronucleus formation	N/A	NT	Positive	Fritzenschaf *et al.*,1993; IARC, 2000
Chinese Hamster SV40-transformed liver cells	Selective DNA amplification	N/A	Negative	N/A	Schmezer *et al.*,1988; ATSDR, 2002
Chinese Hamster Ovary cells	Cell transformation	N/A	Positive	N/A	Sanner and Rivedal, 1985; ATSDR, 2002
Mouse JB6 epidermal cells	Cell transformation	N/A	Positive	N/A	Diwan *et al.*,1985; ATSDR, 2002
Syrian hamster embryo (SHE) cells	Cell transformation	N/A	Negative	N/A	Astill *et al.*,1986; ATSDR, 2002
Syrian hamster embryo (SHE) cells	Cell transformation	0.01, 0.1, 1.0, 10, 100 µg/mL **1**	NT	Positive – 0.2-0.9% transf. colonies in > 0.1 µg/mL	Barret and Lamb, 1985; IARC, 2000; ECB, 2008
Syrian hamster embryo (SHE) cells	Cell transformation	0.8-300 µg/mL **4**	NT	Positive – transformation freq. up to 6%	Sanner and Rivedal, 1985; IARC, 2000; ECB, 2008
Syrian hamster embryo (SHE) cells	Cell transformation	10 µg/mL	NT {positive, ATSDR)	Positive {N/A, ATSDR)	Mikalsen *et al.*,1990; IARC, 2000; ATSDR, 2002
Syrian hamster embryo (SHE) cells	Cell transformation	23 µg/mL **(MEHP)**	NT	Positive	Mikalsen *et al.*,1990; IARC, 2000
Syrian hamster embryo (SHE) cells	Cell transformation	77 µM (30 µg/mL) **30**	NT	Positive – transf. in 12/1, 197 colonies	Mikalsen and Sanner, 1993; IARC, 2000; ECB, 2008

Syrian hamster embryo (SHE) cells	Cell transformation	0, 3, 10, 30, 100 µM (1.2-39 µg/mL) **1.2**	Positive (S9 mix)	Weak Positive {positive, ECB}	Tsutsui *et al.*,1993; IARC, 2000; ECB, 2008
Syrian hamster embryo (SHE) cells	Cell transformation	56 µg/mL **(MEHP)**	Weak Positive	Negative	Tsutsui *et al.*,1993; IARC, 2000
Mouse C3H/10T½ fibroblasts	Cell transformation	0-100µM (39µg/mL) **3.9**	NT {negative, ATSDR}	Negative {N/A, ATSDR}	Sanchez *et al.*,1987; IARC, 2000; ATSDR, 2002; ECB, 2008
Mouse C3H/10T½ fibroblasts	Cell transformation	417 µg/mL **(MEHP)**	NT	Negative	Sanchez *et al.*,1987; IARC, 2000
Primary rat tracheal epithelial cells	Cell transformation	37.5 µg/mL	N/A	Positive	Steele *et al.*,1989; ECB, 2008
Mouse C3H/10T½ fibroblasts	Cell transformation	10, 20, 40 µg/mL (-S9); 250, 500, 1000 µg/mL (+S9) **40**	Weak Positive {negative, ECB}	Weak Positive {negative, ECB}	Lawrence and McGregor, 1985; IARC, 2000; ECB, 2008
BALB/3T3 mouse embryo cells	Cell transformation	0, 0.9, 3.5, 7.0, 12.0, 21.0 µg/mL (-RLC); 0, 10,000, 25,000, 50,000, nl/mL (9800-49,000 µg/mL; +RLC) **25,000**	Negative	Negative	Matthews *et al.*,1985; IARC, 2000; ECB, 2008
BALB/3T3 mouse cells	Cell transformation	20 µg/mL	Negative	Negative	Astill *et al.*,1986; IARC, 2000
RLV/Fischer rat	Cell transformation	1000 µg/mL	NT	Positive	Suk and Humphreys, 1985; IARC, 2000
SA7/Syrian hamster embryo cells	Cell transformation	500 µg/mL	NT	Positive	Hatch and Anderson, 1985; IARC, 2000
Syrian hamster embryo (SHE) cells	Cell transformation	39 µg/mL	NT	Positive	Dhalluin *et al.*,1998; IARC, 2000
Syrian hamster embryo (SHE) cells	Cell transformation	13-4000 µg/mL	N/A	Positive	Jones *et al.*,1988; ECB, 2008
Syrian hamster embryo (SHE) cells	Cell transformation	0-75 µM (0-29 µg/mL)	N/A	Positive	Sanner *et al.*,1991; ECB, 2008
Mouse Balb/c-3T3 clone I13 C14 cells	Cell transformation	0.498, 4.98, 12.5, 24.9, 49.8 µg/mL	N/A	Negative	Nuodex, 1981c; ECB, 2008
Mouse Balb/c-3T3 clone I13 C14 cells	Cell transformation	1.64-32.8 µg/mL	N/A	Negative	Nuodex, 1981d; ECB, 2008
Mouse Balb/c-3T3 clone A31 cells	Cell transformation	0.1, 0.3, 1.0 µL/mL (98-980 µg/mL; +S9); 0.01, 0.1, 1.0 µL/mL (9.8-980 µg/mL; -S9)	Negative (rat S9 mix)	Positive	Nuodex, 1981f; ECB, 2008
Chinese hamster V79 fibroblasts	Inhibition of Gap junction intercellular communication	3 µg/mL	NT {positive, ATSDR}	Positive {N/A, ATSDR}	Malcolm and Mills, 1989; ; IARC, 2000; ATSDR, 2002

Chinese hamster V79 fibroblasts	Inhibition of Gap junction intercellular communication – metabolic cooperation assay	Up to 300 nM (0.12 µg/mL) **0.1**	NT {negative, ATSDR}	Negative {N/A, ATSDR)	Kornburst *et al.*,1984; IARC, 2000; ATSDR, 2002; ECB, 2008
Chinese hamster V79 fibroblasts	Inhibition of Gap junction intercellular communication – metabolic cooperation assay	0.5-200 µg/mL **5**	NT	Positive	Elmoore *et al.*,1985; IARC, 2000; ECB, 2008
Chinese hamster V79 fibroblasts	Inhibition of Gap junction intercellular communication – metabolic cooperation assay	0, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5 mM (3.9-195 µg/mL)	N/A	Negative	Umeda *et al.*,1985; ECB, 2008
Chinese hamster V79 fibroblasts	Inhibition of Gap junction intercellular communication	10 µg/mL	NT	Positive	Malcolm and Mills, 1989; IARC, 2000
Chinese hamster V79 fibroblasts	Inhibition of Gap junction intercellular communication	78 µg/mL	NT	Positive	Vang *et al.*,1993; IARC, 2000
Syrian hamster embryo cells	Inhibition of Gap junction intercellular communication	30 µg/mL	NT	Positive	Mikalsen and Sanner, 1993; IARC, 2000
Chinese hamster V79 fibroblasts and Syrian hamster embryo cells	Inhibition of Gap junction intercellular communication	10 µg/mL	NT	Positive	Cruciani *et al.*,1997; IARC, 2000
Chinese hamster V79 fibroblasts and Syrian hamster embryo cells	Inhibition of Gap junction intercellular communication	28 µg/mL **(MEHP)**	NT	Positive	Cruciani *et al.*,1997; IARC, 2000
Rat hepatocytes	DNA binding	N/A	Negative	N/A	Gupta *et al.*,1985; ATSDR, 2002
Human fetal lung cells	Aneupoidy	6 µg/mL	NT {negative, ATSDR}	Negative {N/A, ATSDR}	Stenchever *et al.*,1976; IARC, 2000; ATSDR, 2002
Chinese Hamster liver cells	Aneupoidy	50 µg/mL	NT	Weak Positive	Danford, 1985; IARC, 2000
Rat liver cells (RL-4)	Polyploidy, aneuploidy	1000 µg/mL	NT {negative, ATSDR}	Negative {N/A, ATSDR}	Priston and Dean, 1985; IARC, 2000; ATSDR, 2002

Chinese hamster liver cells, Chinese Hamster 1-L primary liver cells	Cytogenetic assay - Mitotic aberrations	5, 12.5, 25, 50 mg/mL **50**	NT	Weak Positive {positive} – spindle effects	Parry, 1985; IARC, 2000; ECB, 2008
Chinese hamster liver cells, Chinese Hamster 1-L primary liver cells	Cytogenetic assay - Mitotic aberrations	Up to 50 µL/mL (49 mg/mL)	N/A	Weak Positive {positive} – increase in hyperploidy (chromosome no > 22)	Parry *et al.*,1984; ECB, 2008
Syrian hamster embryo (SHE) cells	Ornithine decarboxylase superinduction	39 µL/mL	NT	Negative	Dhalluin *et al.*,1998; IARC, 2000
Human blood - leucocytes	Comet assay; DNA damage	156 µL/mL	Negative	Positive	Anderson *et al.*,1999; IARC, 2000; ATSDR, 2002
Sprague-Dawley rat, body fluids	Urine, microbial mutagenicity	2000 x 15 po	Negative	Negative	DiVencenzo *et al.*,1985; IARC, 2000

CA – Chromosome aberrations
CT – Cell transformation
GL – Guideline study
GLP – Good Laboratory Practice
IP – Intraperitoneal
N/A – not applicable/ specified
NT – not tested
PO – er os, oral administration
UDS – Unscheduled DNA synthesis

[1]Yellow highlight denotes positive tests. Tan highlights denote marginally positive or equivocal tests.
[2]Test results in {parentheses} denote occasions where source materials differed in interpretation.

Appendix 5.2 Summary Table of DEHP-induced *in vivo* Genotoxic Effects

(retrieved from ECB, 2008; IARC, 2000; and ATSDR, 2002)

DEHP-induced *in vivo* Genotoxic Effects				
Species/Test System (Strain)	End Point	Doses (IARC, 2000; ECB, 2008; NICNAS 2008)	Conclusion [1]	Citation
Mammalian Systems				
Human leucocytes	Chromosomal aberrations	0.01-0.16 mg/m^3	Negative	Thiess and Fleig, 1978; ATSDR, 2002
Syrian Hamster (SH) embryo cells (F)	Chromosomal aberrations	Single gavage dose (0, 3750, 7500, 15,000 mg/kg) at Gd 11{7500 x 1 po, IARC)	Positive	Tomita *et al.*,1982b; IARC, 2000; ATSDR, 2002; ECB, 2008
Fischer 344 rat bone marrow (5 M per group)	Chromosomal aberrations	0.5, 1.7, 5.0 ml/kg-day (500, 1700, 5000 mg/kg-day) gavage daily for 5 days {4900 x 5 po, IARC}	Negative	Putman *et al.*,1983; Nuodex, 1981g; IARC, 2000; ECB, 2008
Syrian Hamster (SH) embryo cells (F)	Cell transformation	Single gavage dose (0, 3750, 7500, 15,000 mg/kg-day at Gd 11) {7500 po}	Positive	Tomita *et al.*,1982b; IARC, 2000; ATSDR, 2002; ECB, 2008
Syrian Hamster (SH) embryo cells	8AG/6TG-resistant mutation	N/A	Positive (?)	Tomita *et al.*,1982b; ATSDR, 2002
Rat bone marrow	Micronucleus formation	N/A	Negative	Putman *et al.*,1983; ATSDR, 2002
Rat bone marrow	Mitotic Index	N/A	Negative	Putman *et al.*,1983; ATSDR, 2002
Mouse	Dominant lethal test	N/A	Negative	Rushbrook *et al.*,1982; ATSDR, 2002
Mouse	Dominant lethal test	980x3sc	Positive	Autian, 1982; IARC, 2000; ATSDR, 2002
ICR Swiss mouse (10 M per group)	Dominant lethal test	12,530, 18,790, 25,060 mg/kg single intraperitoneal dose {12,780 x 1 ip}	Positive	Singh *et al.*,1974; IARC, 2000; ATSDR, 2002; ECB, 2008
ICR Swiss mouse	Dominant lethal test	980 x 3sc	Positive	Agarwal *et al.*,1985; IARC, 2000
CD-1 mice (10 M per group)	Dominant lethal test	12,500, 25,000 mg/kg single gavage	Negative	Hamano *et al.*,1979; ECB, 2008
ICR/SIM mice (25 M per group)	Dominant lethal test	2465, 4930, 9860 mg/kg gavage dose for 5 days; GLP	Negative	Nuodex, 1981b; ECB, 2008
Mouse bone marrow	Micronucleus formation	5000 x 1 po	Negative	Astill *et al.*,1986; IARC, 2000; ATSDR, 2002

$B6C3F_1$ Mouse erythrocytes	Micronucleus formation	6000 x 5 ip	Negative	Douglas *et al.*,1986; IARC, 2000; ATSDR, 2002
Mouse bone marrow	Micronucleus formation	N/A	Negative	Putman *et al.*,1983; ATSDR, 2002
Fischer 344 rat hepatocyte DNA	DNA binding - covalent	390 mg/kg-day	Negative	Gupta *et al.*,1985; IARC, 2000
Fischer 344 rats (F)	DNA binding	Radiolabeled 500 mg/kg Radiolabeled carbonyl-^{14}C or alcohol-^{14}C, ± 4 week prefeeding period with 1% in diet	Carbonyl-^{14}C – negative Alcohol-^{14}C - positive	BASF, 1982; ECB, 2008
Fischer 344 rat liver DNA	DNA binding - covalent	10,000 mg/kg diet 11 days	Positive(ATSDR)/Negative (IARC)	Albro *et al.*,1982a; IARC, 2000; ATSDR, 2002
Fischer 344 rat liver DNA (3 M per group)	DNA binding – covalent – DNA adduct formation	2000 mg/kg-day - Once via gavage daily for 3 days	Negative for adduct formation	Gupta *et al.*,1985; IARC, 2000; ATSDR, 2002; ECB, 2008
Fischer 344 rat liver DNA	DNA binding - covalent	500 x 1 po	Negative	Lutz, 1986; IARC, 2000; ATSDR, 2002
Fischer 344 rat liver DNA	DNA binding - covalent	10,000 mg/kg diet 4 wk	Negative	Von Däniken *et al.*,1984; IARC, 2000; ATSDR, 2002
Fischer 344 rat hepatocytes (M)	DNA repair- UDS – alkaline elution assay	150 mg/kg-day, One gavage daily for 14 days; 500 mg/kg, one gavage dose at 2, 12, 24, 48 hours prior to sacrifice, 12,000 mg/kg diet (600 mg/kg-day) for 30 days followed by 500 mg/kg gavage	Negative	Butterworth *et al.*,1984; IARC, 2000; ATSDR, 2002; ECB, 2008
Fischer 344 rat hepatocytes (liver)	DNA repair - UDS	12,000 mg/kg diet 28d	Negative	Cattley *et al.*,1988; IARC, 2000; ATSDR, 2002
Sprague-Dawley rat hepatocytes (M; liver)	DNA repair - UDS	5000 mg/kg gavage for 4-8 weeks; 2% feed (1000 mg/kg-day) for 4-8 weeks followed by 5000 mg/kg gavage	Negative	Kornbrust *et al.*,1984; IARC, 2000; ATSDR, 2002; ECB, 2008
Rat liver	DNA repair	N/A	Positive	Hayashi *et al.*,1998; ATSDR, 2002
$B6C3F_1$ mouse hepatocytes (M; liver)	DNA repair - UDS	10, 100, 500 mg/kg for 7, 14, 28 days; 6000 mg/kg feed (1200 mg/kg-day) for 7, 14, 28 days	Negative	Smith Oliver and Butterworth, 1987; IARC, 2000; ATSDR, 2002; ECB, 2008
Primary rat hepatocytes	Stimulation of DNA synthesis	200µM (78µg/mL)	Positive	Reddy *et al.*,1992; ECB, 2008
Fischer 344 rats (4 M per group)	Stimulation of DNA synthesis	1.73 mmol/kg (676 mg/kg) single gavage dose	Increased ratio of thymidine incorporation	Büsser and Lutz, 1987; ECB, 2008

Fischer 344 rats (5-10 M per group)	Replicative DNA synthesis	1.2% in diet (500 mg/kg-day) for 1, 2, 4, 8, 18, 77, 151, 365 days	Pulse labeling index of hepatocyte nuclei increased at only 2 days; Pump infusion tech. increase in hepatic nuclear labeling at 8 days	Marsman *et al.*,1988; ECB, 2008
Fischer 344 rats (6 M per group)	Replicative DNA synthesis	1.2% (600 mg/kg-day) in diet for 8 weeks	Urinary bladder epithelium labeling index unaffected	Hagiwara *et al.*,1990; ECB, 2008
Fischer 344 rats (M)	Replicative DNA synthesis	1000, 2000 mg/kg once via gavage or subcutaneous injection	Synthesis increased both doses at 24 hours but not 39 or 48 hours after administration	Uno *et al.*,1994; ECB, 2008
Alderly Park rats (10 M and 10F)	Replicative DNA synthesis	2000 mg/kg-day via gavage for 14 days; GLP	Significant decrease in ^{3}H-thymidine uptake in hepatocytes (M) and proximal tubule cells (M&F)	ICI, 1982b; ECB, 2008
Marmosets (5M & 5F)	Replicative DNA synthesis	2ml/kg-day (1960 mg/kg-day) via gavage for 14 days; GLP	No difference in ^{3}H-thymidine uptake	ICI, 1982b; ECB, 2008
B6C3F$_1$ mice (48 M per group)	Replicative DNA synthesis	0, 6000, 12000 mg/kg (1000, 2000 mg/kg-day) in diet for 2, 8, 24, 40 weeks	Hepatic labeling index increased at 2000 mg/kg-day (24, 40 wk), thymidine kinase activity increased at 2 wk and decreased at 8 wk (2000 mg/kg-day), increased at 2 and 40 wk (1000 mg/kg-day)	Ward *et al.*,1988; ECB, 2008
Rat liver	Strand breaks	N/A	Negative	Butterworth *et al.*,1984; ATSDR, 2002
Wistar rat liver	DNA strand breaks	2000 x 28 po	Negative	Elliott and Elcombe, 1985; IARC, 2000; ATSDR, 2002
Wistar rat liver	DNA strand breaks	500 x 14 po **(MEHP)**	Negative	Elliott and Elcombe, 1985; IARC, 2000
Fischer 344 rat liver (4 M per group)	DNA single-strand breaks	2, 40, 78 weeks, diet 2% (900 mg/kg-day) 20,000 mg/kg diet 78 wk	Negative (positive – 5-fold increase in single strand breaks in tumor bearing rats, ECB)	Tamura *et al.*,1991; IARC, 2000; ATSDR, 2002; ECB, 2008
Fischer 344 rat liver	DNA base modification – DNA oxidative damage	12,000 mg/kg diet 22 wk	Negative	Cattley and Glover, 1993; IARC, 2000; ATSDR, 2002
Fischer 344 rat liver (3 M per group)	DNA base modification – DNA oxidative damage	1, 2, 3, 6, 9, 12 months – diet 1.2% (600 mg/kg-day) **12,000 mg/kg diet 1 yr 12,000 mg/kg diet over 1-2 wk**	Positive (oxidative DNA damage 2-fold increased after 1 month, but no oxidative damage in kidney DNA)	Takagi *et al.*,1990a, 1990b; IARC, 2000; ATSDR, 2002; ECB, 2008
Rat liver	Tetraploid nuclei	N/A	Positive	Ahmed *et al.*,1989; ATSDR, 2002

Fischer rat hepatocytes	Aneupoidy	12,000 mg/kg diet 7 days	Negative	Hasmall and Roberts, 1997; IARC, 2000
Rat kidney	Tumor promotion	N/A	Positive	Kurokawa *et al.*,1988; ATSDR, 2002
S. typhimurium (TA100): Rat host-mediated assay	Gene mutation	N/A	Negative	Kozumbo *et al.*,1982; ATSDR, 2002
C57BL/6f lacl transgenic mouse liver	Transgenic mouse mutation assay	3000, 6000 mg/kg (600, 1200 mg/kg-day) **6000 mg/kg diet 120 days**	Negative, no inc. in mutant frequency in the lacl gene of liver DNA	Gunz *et al.*,1993; IARC, 2000; ECB, 2008
Cynomolgous monkey liver cells	Inhibition of Gap junction intercellular communication	500 x 14 po	Negative	IARC, 2000
$B6C3F_1$ mice	Sperm morphology	6000 x 5 ip	Negative	Douglas *et al.*,1986; IARC, 2000
Sprague-Dawley rats	Sperm morphology	5200 x 5 ip	Negative	Douglas *et al.*,1986; IARC, 2000
Insect Systems				
D. melanogaster (Canton-S)	Sex-linked recessive lethal mutation	20 mg/kg single dose injection	Negative – percentage of lethal was 0.03 compared to 0.05 in controls	Yoon *et al.*,1985; IARC, 2000; ATSDR, 2002; ECB, 2008
D. melanogaster (Canton-S)	Sex-linked recessive lethal mutation	18,600 µg/g in feed, no exposure period	Negative – percentage of lethal was 0.07 compared to 0.11 in controls	Zimmering *et al.*,1989; IARC, 2000; ECB, 2008
D. melanogaster	DNA double strand breakage	7540 µg/g food	Negative	Kawai, 1998; IARC, 2000
D. melanogaster	DNA repair test	7540 µg/g food	Negative	Kawai, 1998; IARC, 2000
D. melanogaster	Wing spot test, mutation	7540µg/g food	Negative	Kawai, 1998; IARC, 2000

GL – Guideline study
GLP – Good Laboratory Practice
IP – Intraperitoneal
N/A – not applicable/ specified
NT – not tested
PO – er os, oral administration
UDS – Unscheduled DNA synthesis
[1]Yellow highlight denotes positive tests. Tan highlights denote marginally positive or equivocal tests.
[2]Test results in {parentheses} denote occasions where source materials differed in result interpretation.