National Toxicology Program
U.S. Department of Health and Human Services

Center For The Evaluation Of Risks To Human Reproduction

NTP-CERHR EXPERT PANEL REPORT

on

DI(2-ETHYLHEXYL)PHTHALATE

OCTOBER, 2000 **NTP-CERHR-DEHP-00**

PREFACE

The National Toxicology Program (NTP) and the National Institute of Environmental Health Sciences established the NTP Center for the Evaluation of Risks to Human Reproduction (CERHR) in June, 1998. The purpose of the Center is to provide timely, unbiased, scientifically sound evaluations of human and experimental evidence for adverse effects on reproduction, including development, caused by agents to which humans may be exposed.

The following seven phthalate esters were selected for the initial evaluation by the Center: butyl benzyl phthalate, di(2-ethylhexyl) phthalate, di-isodecyl phthalate, di-isononyl phthalate, di-n-butyl phthalate, di-n-hexyl phthalate, and di-n-octyl phthalate. Phthalate esters are used as plasticizers in a wide range of polyvinyl chloride-based consumer products. These chemicals were selected for the initial evaluation by the CERHR based on their high production volume, extent of human exposures, use in children's products, published evidence of reproductive or developmental toxicity, and public concern.

This evaluation is the result of three public Expert Panel meetings and 15 months of deliberations by a 16-member panel of experts made up of government and non-government scientists. This report has been reviewed by the CERHR Core Committee made up of representatives of NTP-participating agencies, by CERHR staff scientists, and by members of the Phthalates Expert Panel. This report is a product of the Expert Panel and is intended to (1) interpret the strength of scientific evidence that a given exposure or exposure circumstance may pose a hazard to reproduction and the health and welfare of children; (2) provide objective and scientifically thorough assessments of the scientific evidence that adverse reproductive/development health effects are associated with exposure to specific chemicals or classes of chemicals, including descriptions of any uncertainties that would diminish confidence in assessment of risks; and (3) identify knowledge gaps to help establish research and testing priorities.

The Expert Panel Reports on phthalates will be a central part of the subsequent NTP report that will also include public comments on the Panel Reports and any relevant information that has become available since completion of the Expert Panel Reports. The NTP report will be transmitted to the appropriate Federal and State Agencies, the public, and the scientific community.

The NTP-CERHR is headquartered at NIEHS, Research Triangle Park, NC and is staffed and administered by scientists and support personnel at NIEHS and at Sciences International, Inc., Alexandria, Virginia.

Reports can be obtained from the website (http://cerhr.niehs.nih.gov**) or from:**

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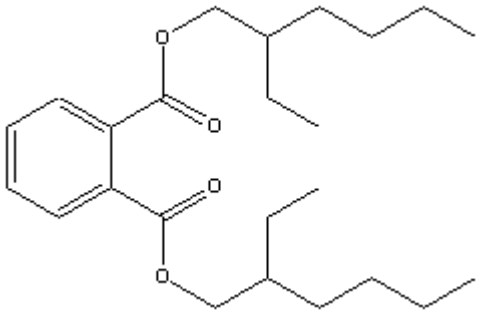
Di (2-Ethylhexyl) Phthalate

1.0 CHEMISTRY, USAGE, AND EXPOSURE

1.1 Chemistry

Figure 1: Chemical Structure of Di-(2-Ethylhexyl) Phthalate



Di(2-ethylhexyl) phthalate (DEHP) (CAS RN 117-81-7) is produced by reacting 2-ethylhexanol (2-EH) with phthalic anhydride (*1*). The reaction is either conducted in the presence of an acid or metal catalyst or at a high temperature.

Table 1: Physicochemical Properties of DEHP

Property	**Value**
Chemical Formula	$C_{24}H_{38}O_4$
Molecular Weight	390.62
Vapor Pressure	1.0 x 10^{-7} mmHg at 25°C
Melting Point	-47°C
Boiling Point	386°C
Specific Gravity	0.986
Solubility in Water	essentially insoluble – 3 μg/L
Log K_{ow}	7.50

(*1*)

1.2 Exposure and Usage

1.2.1 Overview

DEHP is used as a plasticizer of polyvinyl chloride (PVC) in the manufacture of a wide variety of consumer products. According to Aristech Chemical Company, the production volume of DEHP is approaching 2 million tons (*2*). Important examples of DEHP use include: building products (flooring and pavements, roof coverings, wallpaper, polymeric coatings, tubes and containers, wire and cable insulation), car products (vinyl upholstery, car seats, underbody coating, trim), clothing (footwear, raincoats), food packaging, children's products (toys, crib bumpers), and medical devices. It is currently the only phthalate plasticizer used in PVC medical devices. Exposure to DEHP in children's products has declined in recent years. In the United States and Canada, DEHP is no longer used in toys intended for mouthing (nipples, teethers, pacifiers, rattles), but is still found in larger toys used by older children.

Release into the environment

DEHP enters the environment in several different ways. These include: during production, distribution, and incorporation into PVC resin; disposal in industrial and municipal landfills; waste incineration; and leaching from consumer products during use or after disposal. The release of DEHP directly into the atmosphere is believed to be the most important mode of entry into the environment.

Table 2: Toxic Release Inventory Data

Total Release (pounds/year)					
Year	Air	Water	Underground Injection	Land	Total Environmental
1987	1,682,435	3,595	500	34,794	1,721,324
1992	873,783	947	35	101,712	976,477
1997	213,621	303	0	71,004	284,928

(*3*, *4*)

DEHP is a common laboratory contaminant from analytical equipment, a fact that was not completely appreciated until the mid-1980s. Special protocols have since been developed and must be employed to avoid contamination of samples. Data gathered prior to the development of these protocols may be misleading and should be interpreted with caution.

Because of its low vapor pressure and poor water solubility, concentrations of DEHP in outdoor air and water are low; they range from 10^0 to 10^2 nanograms per cubic meter (ng/m^3) and 10^{-3} to 10^1 parts per billion (ppb), respectively (*1*). DEHP concentrations are often either at or below detection limits. DEHP adsorbs strongly to sediments and aerosol particulates; it also bioaccumulates in invertebrates, fish, and plants. Biomagnification is not observed; biodispersal occurs at higher trophic levels in the food chain because of metabolism. Biodegradation proceeds well in aerobic, nutrient-rich environments over weeks, but may be extremely slow (many years) under anaerobic conditions (*5*). The aerobic biodegradation of dialkyl phthalates begins with hydrolysis to the monoester (mono (2-ethylhexyl) phthalate [MEHP]) and alcohol (2–ethyl hexanol [2-EH]) (*6*). The monoester is converted to phthalic acid and ultimately to pyruvate and oxaloacetate or acetyl CoA and succinate. Studies reporting levels of MEHP or 2-EH in environmental media were not found.

General population exposure

The general population is exposed to DEHP in food, water, and air, via inhalation and ingestion. The largest source of general population exposure to DEHP is dietary, followed by indoor air. Dietary exposures result both from DEHP accumulating in certain foods and from leaching of DEHP during processing, packaging, and storing. A few food surveys show a range of DEHP content, but fatty foods, including dairy, fish, meat, and oils, contain the most (*7*, *8*). Dietary exposures may vary from country to

country because DEHP is introduced through various food processing and packaging techniques which differ internationally. There are no known studies that report levels of DEHP metabolites (MEHP, 2-EH, or 2-ethylhexanoic acid [2-EHA]) in foods.

Few studies on indoor air exist. Concentrations of DEHP have been documented from 8 ng/m^3 (*9*) to 3 micrograms per cubic meter (μg/m^3) (*7*), but most measurements are in the 10–100 ng/m^3 range.

Assumptions about the general population's diet do not apply to infants and children who consume significantly different types and amounts of food than do adults (*10*). DEHP leaches from food processing equipment and from food wrappings into fatty foods, including dairy products. Infants and children take in much more diary product per kilogram of body weight than do adults. Single-serving convenience or snack foods may also contain DEHP. Because children eat more of these foods, exposure to DEHP could be disproportionately high for children (*11*). Meek estimated environmental exposures using age categories which clearly show the differences in exposure to DEHP from all compartments, but most specifically from food (Table 3).

Table 3. Estimated Daily Intake of DEHP by the Population of Canada
Expressed in micrograms per kilogram body weight per day (μg/kg bw/day)

	Age				
Substrate/ Medium	**0.0–0.5 yr**	**0.5–4 yr**	**5–11 yr**	**12–19 yr**	**20–70 yr**
Ambient Air: Great Lakes Region	0.00003–0.0003	0.00003–0.0003	0.00004–0.0004	0.00003–0.0003	0.00003–0.0003
Indoor Air	0.86	0.99	1.2	0.95	0.85
Drinking Water	0.13–0.38	0.06–0.18	0.03–0.10	0.02–0.07	0.02–0.06
Food	7.9	18	13	7.2	4.9
Soil	0.000064	0.000042	0.00014	0.000004	0.00003
Total Estimated Intake	8.9–9.1	19	14	8.2	5.8

(*7*)

DEHP has been found in infant formula (*12-15*). Levels of DEHP found in infant formula in the US are lower than those reported from other countries (*12-15*). DEHP can also be present in products like toys and mosquito repellents, and in cosmetics, to which children will be exposed at different levels than adults, (*16, 17*).

DEHP is used in some children's toys (*18-20*). Health Canada analyzed 41 children's products made in the US, China, and Thailand for the presence of diisononyl phthalate (DINP) and DEHP. DEHP was detected in 23 of the products at concentrations of 0.002–0.19% dry weight. DEHP was found in 1 of 5 US-manufactured, 22 of 35 Chinese, and 1 of 2 Thai products. Criteria for the selection of products were not discussed in any of these surveys. No information on market share, length of availability on the market, or estimates of the numbers of products in circulation was noted in any study. Only Health Canada listed product number, country of origin, manufacturer/distributor, and brand. All studies listed a product description. Marin (*18*) analyzed 15 samples of materials used in toys in Spain. The authors noted that the PVC contained a mixture of plasticizers including DINP, DEHP, and diisodecyl phthalate (DIDP), but reported only the DEHP content. A range of <0.1–34% DEHP dry weight was reported and

6 of 15 samples contained >10% DEHP dry weight. Rastogi (*20*) found DEHP in 3 of 4 teethers (0.01–0.07 % dry weight) and 2 of 3 dolls (0.12 and 22.4 % dry weight). Lay (*21*) found 4 commercially available pacifiers that contained 31.4–41.6% dry weight DEHP. Since this paper was published in 1987, it is likely that the pacifiers studied are no longer available in the US.

Steiner et al. (*22*) measured migration of DEHP into a saliva simulant under static and dynamic conditions (simple shaking with glass balls and glass plates, and simulated chewing with glass dentures) using a standardized PVC film. They also performed three 3-hour and two 6-hour sucking tests using the same adult volunteers, collecting all saliva. The range of extraction varied by a factor of 40 among the various experimental scenarios. Adult sucking was comparable to static methods, which were the lowest migrations, at 64 ± 14 µg DEHP/dm^2 film and 41 ± 9 µg DEHP/g film, respectively. The highest dynamic extraction resulted in 1,006 ± 484 µg DEHP/g film.

A recent Austrian risk assessment suggests that children's exposures to DEHP from sucking or chewing on PVC articles could reach 85 µg/kg bw/day or higher (*23*).

The range of exposure in the general population from all sources–excluding non-dietary ingestion, medical, and occupational was estimated by Doull et al. to be 3–30 µg/kg bw/day (*24*).

1.2.2 Occupational exposure

Occupational exposure to DEHP occurs during the manufacture and processing of this compound. Workers may be exposed to relatively high concentrations during the compounding of DEHP with PVC resins (*5*). The major route of exposure is inhalation (*24*). Maximum occupational exposures should not exceed 700 µg/kg bw/day if the workplace air concentrations meet the OSHA standard.

Studies based upon workplace air measurements in Europe and the former USSR estimate occupational exposures from <2–6,600 µg/kg bw/day (*8*). The American Chemistry Council (ACC, formerly CMA) (*1*) cites six studies that indicate that exposures in the US are generally below 1 mg/m^3 during production of phthalates and below 2 mg/m^3 during production of PVC. They estimated an exposure of less than 143 µg/kg bw per workday for phthalate production workers. The corresponding exposure for PVC production workers was 286 µg/kg bw per workday.

Occupational exposures to users of products containing DEHP have not been studied.

1.2.3 Medical exposure

1.2.3.1 DEHP

Overview

A variety of medical procedures require the use of specialized equipment. Such equipment is frequently made of PVC or contains PVC components. The PVC preferred contains DEHP as its plasticizer because DEHP provides the PVC with desired mechanical properties. These mechanical properties include flexibility, strength, suitability for use at a wide range of temperatures, suitability for various sterilization processes, resistance to kinking, optical clarity, weldability, barrier capability, centrifugability, and bondability. PVC can be softened and shaped into many designs without cracking or leaking, an important performance characteristic for medical devices. Medical procedures that use PVC-containing devices include: hemodialysis; transfusion of whole blood, platelets, or plasma; extracorporeal

oxygenation; cardiopulmonary bypass; administration of intravenous (IV) fluids; and enteral and parenteral feedings. Respiratory therapy, such as artificial ventilation, also uses PVC products that contain DEHP.

PVC-based medical devices generally contain 20–40% DEHP by weight. A typical formulation of a plasticized PVC container used for storing blood, blood components, and other aqueous solutions is given below.

Table 4: Formula for a Typical PVC Container (Medical)

PVC polymer	~ 55%
DEHP plasticizer	~ 40%
Zinc octanoate	~ 1%
Calcium or zinc stearate	~ 1%
N,N' di-acyl ethylene diamine	~ 1%
Epoxy soya or linseed oil	~ 10%

In medical devices, DEHP exists in the PVC matrix loosely attached to the resin in a semi-solid or gel-like structure. Since the DEHP plasticizer is not chemically bound to PVC, it can leach out when the medical device contacts fluids such as blood, plasma, and drug solutions, or it can be released and migrate when the device is heated. The rate at which DEHP migrates from the medical device into the stored material depends on the storage conditions (temperature of the fluid contacting the device, the amount of fluid, the contact time, and the extent of shaking or flow rate of the fluid) and the lipophilicity of the fluid. Higher temperatures and shaking during procedures such as autoclaving and irradiation increase the migration rates of plasticizers. Since DEHP is lipophilic, it will readily dissolve in whole blood, plasma, platelet concentrate, lipid-containing fluids (such as IV lipid emulsion), total parenteral nutrition solution, and solutions containing Polysorbate 80 and other formulation aids used to solubilize some IV medications.

DEHP in Blood and Blood Products

There are extensive data on the levels of DEHP that leach into blood, blood components, and blood derivatives. The mean amount of DEHP in blood or blood components reportedly ranges from 10 to 650 μg/mL depending on storage conditions and product. Table 5 lists selected data on DEHP levels in various blood products.

Except for red blood cells resuspended in additive solutions, the amount of DEHP in units of blood or blood components (1 unit=450 mL) nearing their recommended storage shelf-life ranges from 66.7–122 μg/mL. Red blood cells resuspended in additive solutions can be stored at 4°C for up to 42 days. Assuming a constant migration rate of DEHP of 1–4 mg/14 days (which appears to be an overestimation, since leaching rates usually decrease with time), a unit of red blood cells would contain approximately 6.7–26.7 μg/mL of DEHP after 42 days. Freezing blood products, such as red blood cells or plasma components during storage, prevents the migration of DEHP (*25-27*). When blood products are frozen shortly after collection, such as for fresh frozen plasma (less than 8 hours after collection) or RP15 (recovered plasma frozen within 15 hours of collection), minimal amounts of DEHP leach into the collected material.

TABLE 5: DEHP in Selected Blood Components under Similar Conditions

Exposure Source*	DEHP concentration: mean +/- SD (range) µg/mL	Comments; (Source)
Whole Blood	83.1±9.1 72.5 ±9.0	2 bag types, stored 21 day, curvilinear extraction; (*28*)
Whole Blood	46.4 (34.8–52.6)	21 day, 5°C, 83–100% extraction; (*29*)
Whole Blood	39.8±2.8 44.2±10.3	21–-24 day, 4°C, CPD 21–24 day, 4°C, ACD; (*30*)
Packed RBC	14.3±1.2 13.4±2.6	21–24 day, 4°C, CPD 21–24 day, 4°C, ACD; (*30*)
Packed RBC	38.6 (32.6–46.1)	13 day, 5°C, 83–100% extraction; (*29*)
Platelets	190	assumes 2 day storage, 5–10 units/day and 60 mL/unit; (*31*)
Platelets	270	Stored 72 hours; (*32*)
Platelets	288 ± 33.5	Stored 72 hours @ 20°C; (*33*)
Platelets	180-382 (26.4–82.4)	Pooled platelets; (*34*)
Platelets	4.68±0.53 to 649.5±4.6	5 day storage, 6 different bag types; (*32*)
Plasma	17–24 71–109	Stored 1 day, not corrected for incomplete extraction stored 30 days, not corrected, (*35*)
Plasma	261±16.4 475.4±54.6	2 day, 22°C 5 day, 22°C, (*36*)
Plasma	145±20	stored 24 days, (*37*)
Plasma	5.5 + 11	stored 72 hours, (*33*)

*****vehicle in PVC infusion sets and storage bags
ACD = Acid Citrate Dextrose
CPD = Citrate Phosphate Dextrose
SD = Standard deviation reported by authors.

Other IV Infusates

IV administration of solutions and drugs may also result in exposure to DEHP. As expected, very little PVC leaches into normal saline solutions from PVC storage bags even after long periods of storage (*38*). However, several studies have found that if normal saline and glucose solutions in PVC bags are agitated, DEHP may from an emulsion, increasing the amount of DEHP extracted into the solutions (*39-43*). Values of 285 µg/L (*41*), 340 µg/L (*42*), and 360 µg/L (*43*) have been reported for water and 160 µg/L for saline (*42*), suggesting that DEHP has lower solubility or emulsion formation capacity in saline than in water. A 1 liter saline IV bag could contain up to 45 µg of DEHP. However, DEHP may form an emulsion which would increase the amount of DEHP to as much as 360 µg/L in IV solution and ~160 µg/L in saline.

A variety of drugs are administered intravenously by adding them to PVC IV bags. The rate at which DEHP is extracted from the bags into the drug solution depends on the hydrophobicity of the drug formulation.

Pharmaceutical solvents such as ethanol and polyethylene glycol do not affect the extraction of DEHP from PVC storage bags (*44*). In contrast, a variety of formulation aids, including Polysorbate 80 (Tween 80) and castor oil (Cremophor EL), dramatically increase the rate of DEHP extraction (*44, 45*). For example, according to one report (*44*), 3.1–237 mg of DEHP leached into 1 liter of Polysorbate 80 solution stored in a PVC bag for 1 day at 24ºC.

Several studies (*44-48*) have identified a variety of drug formulations that significantly increase the extraction of DEHP from the PVC container into the solution. These drugs include: cefoperazone (Cefobid Bulk), chlordiazepoxide HCL (Librium), ciprofloxacin (Cipro IV), cimetidene (Tagamet), cyclosporine (Sandimmune), etoposide (VePesid), fluconazole (Diflucan), metronidazole HCl (Flagyl IV), micronazole (Monistat IV), paclitaxel (Taxol), tacrolimus (Prograf), taxotere (Docetaxel), teniposide (Vumon), total parenteral nutrition formulations, and vitamin A.

One study (*46*) reported that the concentration of DEHP in a cyclosporine solution in 5% dextrose stored in a PVC bag at room temperature reached 72+/-13 µg/mL after 8 hours and 668+/-96 µg/mL after 24 hours.

Total parenteral nutrition (TPN) formulations contain amino acids, dextrose, electrolytes, and lipids. The presence of lipids have been shown to increase extraction of DEHP from PVC bags (*47*). In TPN formulations without added lipids, there was no measurable amount of DEHP. In TPN formulations with added lipids, the concentration of DEHP in the TPN solution increased with time and storage temperature. TPN solutions are administered within 24–36 hours of mixing. Mazur et al. (*47*) found that the highest concentrations of DEHP reached 1.0+/-0.4 µg/mL within 24 hours and 3.1+/-0.7 µg/mL within 48 hours.

The highest DEHP concentrations are reached when the drugs are pre-mixed in IV bags and the pre-mixed solution is agitated for 24 hours. However, it should be noted that clinicians and nurses are familiar with the increased leaching of DEHP from PVC containers into lipophilic drug formulations. Pearson and Trissel (*44*) have recommended that these drug formulations be prepared in non-PVC containers and administered through non-PVC tubing. The labeling of such formulations includes a warning to that effect. For example, the directions for use for Taxol include the following warning: "Data collected for the presence of the extractable plasticizer DEHP [di-(2-ethylhexyl) phthalate] show that levels increase with time and concentration when dilutions are prepared in PVC containers. Consequently, the use of plasticized PVC containers and administration sets is not recommended."

Enteral Infusions

Exposure from enteral infusions has not been studied; however, it represents another possible source of DEHP to ill individuals. Both the bags holding enteral nutrition fluids and the tubing may contain DEHP.

DEHP Extraction from Tubing

PVC tubing is used in numerous medical applications such as hemodialysis, extracorporeal membrane oxygenation (ECMO), mechanical ventilation of infants and adults, and feeding tubes. *In vitro* experiments with tubing (*8*) have shown that DEHP is extracted in amounts ranging from 1.0 to 6.5 mg/hour or 7.7 µg/mL /hour from dialysis tubing (*49-52*). Levels as high as 3.5–4 mg/L have been reported to be extracted per hour during extracorporeal oxygenation (*53*). However, a study by Karle et al. (*54*) suggests much lower extraction rates. This study also suggested that ECMO using heparin-coated tubing would expose the patient to little or no DEHP. PVC tubing plasticized with DEHP is also used in breathing circuits both at home and in the hospital. There are no reliable data on the rate of extraction of DEHP from respiratory tubing. The rate of leaching from enteral feeding tubes has not been measured,

but should not exceed the rate of extraction of DEHP from tubing by platelet-rich plasma, which is 0.011 mg/cm^2. Some breast pumps may also utilize PVC tubing.

Medical Procedures

Exposure to DEHP from medical procedures is highly variable and determined by the handling of the devices during storage and use, and by the fluids that contact the medical device. Long storage or use time, increased temperature, and agitation all increase leaching of DEHP from medical devices. Leaching is also enhanced by increased lipid content or by the lipophilic nature of liquids that contact DEHP in medical devices. Unanticipated exposure may result from infusing parenteral drugs or infusates like lipid solutions through DEHP-containing infusion sets against manufacturer specifications. Additional DEHP exposure may result from contamination during sterilization (autoclaving) and irradiation. Variability in exposure estimates can result from the analytical techniques used to measure DEHP and the care with which contamination from analytic equipment is prevented.

In general, medical procedures that require hours or days, like hemodialysis or ECMO, result in higher exposures than brief medical procedures like infusion of packed red blood cells or administration of IV medications. Chronic or recurrent treatments like hemodialysis in chronic renal failure patients or multiple long-term transfusions in cancer victims can result in cumulatively high exposures. Intensive procedures like exchange transfusions in neonates can result in acutely high exposures. Table 6 includes published data on DEHP exposures from various medical procedures in adults.

Total exposure is often calculated based upon timed, point measurements of DEHP, either from the medical apparatus itself, or from the recipient patient, using estimates of treatment time and frequency. Table 7 shows published data on DEHP and metabolites before and after some medical procedures. Several authors have published rates of DEHP leaching from various medical devices which are used to calculate total anticipated exposures. All exposure calculations not based upon *in vivo* measurements are subject to error based upon scenario assumptions. For example, using data from Rubin (*34*) on DEHP in pooled platelets, twice a week platelet transfusions in a pancytopenic patient could result in total exposure over 6 months from a low estimate of 2.7 grams to a high estimate of 8.6 grams. Exposure estimates will be increased by assuming long storage time, room or body temperature storage or use, and high-range concentrations or large volume procedures, and will correspondingly be lowered by assuming the opposite. The variability in measured DEHP concentrations, number of parameters that can affect leaching, and numerous variables in clinical use make precise determination of exposures impossible. A comparison of orders of magnitude for various procedures might be more useful.

TABLE 6: DEHP Exposure by Medical Procedure (Adults)

Medical Procedure	DEHP concentration: mean +/- SD (range) µg/mL	Procedure Time	Exposure/Time	Comments; (Source)
CABG (n=10)	1.8–2.3 (blood)	83.9±15.9 minutes	15.4–72.9 mg/op	calculated from post-measures; (*55*)
Heart Transplant (n=3)	Not Stated	184±54 minutes	2.3–21 mg/ transfusion	Calculated from post-measures. Cyclosporin may contribute significantly; (*55*)
Jarvik Bridging (n=2)	Not Stated	Several days	3.8–167.9 mg/day	(*55*)

Medical Procedure	DEHP concentration: mean +/- SD (range) µg/mL	Procedure Time	Exposure/Time	Comments; (Source)
Hemodialysis (n=27)	0.75±0.4 (0.25–1.95) (serum)	5 hours	≥250 mg/year	60–70% extraction, not corrected, concentration postdialysis; (*50*)
Hemodialysis (n=3)	Not Stated	4 hours	60 mg/session	calculated from post- (n=3) dialysis concentration and flow rate; (*56*)
Hemodialysis (n=17)	1.67 (0.4-4.2) (serum)	3 hours	1,000 mg/year	Calculated year dose based on linear extraction coefficient; r (*52*)
Hemodialysis (n=9)	not given	5 hours	80 (9–150) mg/session	calculated AUC from (n=9) hourly measures; (*57*)
Hemodialysis (n=11)	1.91±2.1 (blood)	4 hours	105 (23.8–360) mg/session 16 g (3.7–56)/year	AUC calculated; (*58*)
Hemodialysis (n=21)	not given	4 hours	16.6 mg/session (3.6–59.6)	AUC calculated; (*59*)
CAPD (n=7)	0.079 (0.032–0.21) (serum)	Continuous	500mg/year	yearly dose for DEHP and PA; (*60*)

AUC = Area Under Curve
CABG = Coronary Artery Bypass Graft
CAPD = Continuous Ambulatory Peritoneal Dialysis
PA = Phthalic Acid
SD=Standard deviation reported by authors.

Table 7: Measured Blood Levels DEHP/MEHP Before and After Selected Medical Procedures

Procedure	Age I=infant A=adult	Timing	DEHP concentration: mean +/- SD (range) µg/mL		MEHP concentration: mean +/- SD (range) µg/mL		Comments	Reference
			Pre DEHP concentration	Post DEHP concentration	Pre MEHP concentration	Post MEHP concentration		
Cardiac Bypass	I	Acute	0.76 (0.27–1.66)	3.38 (1.10–5.06)	1.03 (0–0.16)	(0.06–2.66)	Decline to pre-op levels within 24 hours n=7,	(*55*)
Exchange Transfusion	I	Acute	<1.0–1.9	15.3 (6.1–21.6)	NR	NR	n=16, Serum	(*61*)
Exchange Transfusion	I	Acute	NR	5.8–19.6	Maximum	5 µg/mL	n=4, $T_{½}$ = ~ 10 hrs (11 exchanges),	(*62*)

Procedure	Age I=infant A=adult	Timing	DEHP concentration: mean +/- SD (range) µg/mL		MEHP concentration: mean +/- SD (range) µg/mL		Comments	Reference
			Pre DEHP concentration	Post DEHP concentration	Pre MEHP concentration	Post MEHP concentration		
							Plasma	
Exchange Transfusion	I	Acute	0.24 (<.1–.55)	7.84 (3.4–11.1)	(.02–1.17)	6.41 (2.36–15.05)	n=6 (but 9 transfusions)	(*63*)
ECMO	I	Sub-Acute	0 8.3±5.7 (maximum)	SD 1.3±2.9 µg/mL (decanulation)	NR	NR	Control Pts all neg. Mean 1 pt with 5.9 µg/mL DEHP after tx. n=5, Plasma	(*54*)
Cardiac Bypass	A	Acute	NR	1.8–2.3	NR	(0.15–1.7)	Decline to pre-op levels by 24 hours n=10, Serum	(*55*)
Surgery	A	Acute	NR	8.4 (3–14)	NR	NR	3 µg/mL in control (n=1), Serum	(*64*)
Platelet Transfusion	A	Acute	NR	6.7 (3.4–8.3)	NR	NR	n = 6, $T_{½}$ 28 ±4.3 min Plasma	(*34*)
Hemodialysis	A	Chronic	0.43 ±0.19 µg/mL (SD)	NR	NR	NR	DEHP level in Control: 0.62 ± 0.12 µg/mL (n=5) n=21 hemodialysis patients, Plasma	(*59*)
Hemodialysis	A	Chronic	0.156±0.074 0.170± 0.112	0.827±.45 0.541±0.171	0.025± 0.015 0.035± 0.041	0.121±0.049 0.17±0.072	n=14 patients with pruritis Serum. n=7 patients without pruritis, Serum	(*65*)

SD=Standard deviation reported by study authors.
NR=Not reported.

Adults may be exposed acutely or chronically to DEHP. Hemodialysis is the procedure which results in the highest doses because of the nature of the procedure and the chronic repetitive nature of the treatment. The most reliable numbers come from studies which include calculations of the differences from AUC of pre- and post-infusion measurements of DEHP from the machine ports serially over the 3–5 hour hemodialysis session. Yearly doses using this technique, assuming twice-weekly dialysis 52 weeks per year, yield estimate ranges of 0.9–56 grams. Using the average exposure from the three studies using direct measurements and AUC calculations, the range of yearly exposure from twice-weekly dialysis decreases to 8.3–10.9 grams. The amount of 250 mg/year is from Lewis et al. (*50*), but this is an older study which neither corrected for incomplete extraction, nor accounted for DEHP absorbed during dialysis. The wide range of estimates may be due to patient variation (e.g., hematocrit, triglycerides, and serum cholesterol), differences in dialysis protocols, and differences in the circuits themselves. All of these studies, though carefully conducted, are based upon small patient numbers which further complicates the reliability of exposure estimates.

Pediatric exposure represents a special case. Exchange transfusions, ECMO, and cardiopulmonary by-pass for correction of congenital anomalies all represent high exposure scenarios. Table 8 includes some of these neonatal exposures. Replacement blood transfusions and hyperalimentation with protein and lipids are routine procedures in neonatal intensive care units, yet no direct measures of DEHP exposures were located. Exposures for replacement blood transfusions, usually in volumes of 10 cc/kg/transfusion can be calculated using published data on DEHP levels in whole blood, but require assumptions about the age of the blood, storage temperatures, and specifics of blood products selected (e.g., whole blood vs reconstituted packed red blood cells). For example, using the data from both Sjoberg et al. studies (*62, 63*) on DEHP in whole blood used in exchange transfusions, a standard transfusion of 10 cc/kg of whole blood would expose a neonate to an average of 490 μg/kg/transfusion with a range of 140–850 μg/kg bw/transfusion. Using data from Peck (*28*), the average could be 700–800 μg/kg bw/transfusion with a range based on two standard deviations of 540–10,000 μg/kg bw/transfusion. Packed red cell concentrates which have a lower DEHP content are often reconstituted with fresh frozen plasma and used as replacement in neonates, so these whole blood estimates, while variable, may also be high for some centers or scenarios. Whether these differences are meaningful depends on the toxicokinetics of DEHP and its metabolites.

Table 8 : DEHP Exposure (Neonates)

Medical Procedure	DEHP concentration: mean +/- SD (range) μg/mL	Procedure Time	Exposure: mean (range)	Comments	Source
Congenital Heart Repair	1.8 (0.73–4.66) DEHP in blood from patient	1–4 hours	1.8 μg/mL/hr (0.3–4.7 μg/mL/hr)	Dose in whole blood, difference before and after procedure	(*55*)
Exchange Transfusion	47.0 (4.3–123.1) DEHP in whole blood	1.25–3 hours	Dose not calculated.	Pre- and post-Treatment DEHP concentration measured	(*61*)
Exchange Transfusion	58.5 (36.82–84.9) DEHP in whole blood	Not Stated	1,700-4,200 μg/kg bw	Calculated from bags	(*62*)
Exchange Transfusion	38 (13.82–71.87) DEHP in plasma or whole blood	Not Stated	1,800 μg/kg bw(840-3,300 μg/kg bw)	Area under curve calculation	(*63*)

SD=Standard deviation reported by author.

ECMO, used for refractory respiratory failure in both premature infants and term infants, gives one of the highest single-course exposures to DEHP. Two studies estimate exposure based upon a study of small numbers of infants using different ECMO circuits. Schneider (*53*) estimated that a 4 kg infant would be exposed to 42,000 μg/kg bw DEHP after 3 days and 140,000 μg/kg bw after 10 days of ECMO therapy. More recently, Karle (*54*) studied three different circuits and estimated much lower exposures. For two uncoated circuits at 3 days, she estimated an exposure of 4,700 μg/kg bw and 10,000 μg/kg bw which rose at 10 days to 15,500 μg/kg bw and 34,900 μg/kg bw. A third circuit coated with heparin was found not to leach DEHP, resulting in no DEHP exposure from the ECMO circuit. Karle (*54*) made these calculations from periodic blood sampling and *in vitro* leaching rates on the three circuits, using as controls babies with similarly severe medical conditions which were treated with mechanical ventilation rather than ECMO. She found no accumulation of DEHP in the mechanically ventilated babies. AUC

calculations were not performed.

Some components of breathing circuits, notably oxygen and humidifier tubing, suction catheters, and endotracheal tubes, are commonly made from PVC. However, there are no reliable published data on the release of DEHP from these devices. Roth et al. (*66*) studied five pre-term infants who were ventilated using heated respiratory tubing and humidified air flow. They reported that concentrations of DEHP in the condensate collected from the water traps of the respirator tubing ranged from <0.001 to 4.1 mg/L. Based on these values, Roth et al. estimated that the infants could have received inhalation doses of DEHP ranging from 0.001 to 4.2 mg/hour. The reported DEHP concentrations in the condensate appear to have been seriously overestimated due to a sampling error. Furthermore, DEHP levels in the condensate cannot provide a reliable estimate of inhalation exposure since the infants were not exposed to the condensate.

The worst possible case of DEHP exposure during ventilation would occur if infants were exposed to air saturated with DEHP throughout the procedure. Respiratory tubing used with ventilators is currently made from polyethylene and contains no DEHP. Health Canada recently confirmed this by testing the 13 leading North American brands.

Latini et al. (*67*) recently measured the DEHP content of neonatal endotracheal tubes before and after use. They reported a loss of 0.06–0.12 mg DEHP per mg sample after use. This would represent a loss of up to 60–120 mg DEHP for a typical 1 g endotracheal tube or 11–22 mg DEHP per day, since the average length of the procedure was 129.5 hrs. (Additional details obtained from the authors.)

This study cannot be used to estimate exposure of a neonate to DEHP from the endotracheal tubes. The DEHP measurements involved overnight extraction in chloroform:methanol; these conditions are much harsher than those present *in vivo*. Levels of DEHP extracted by air and mucus may be considerably lower. Furthermore, if DEHP is extracted by mucus, some of the mucus would be removed by suctioning during the procedure. There are no published data on the rate of extraction of DEHP from PVC by mucus. This potentially important source of exposure needs to be further quantified.

All studies of DEHP exposure from medical devices consider only single exposure sources. Even within these single source exposure estimates, there is a wide range. In part, this may be due to patient variation (e.g., hematocrit, triglyceride, and cholesterol), differences in medical procedure protocols (e.g., ECMO or hemodialysis flow rates), and differences in the medical devices themselves (e.g., coated vs non-coated ECMO circuits). All of these estimates are based upon very few patients which further complicates the precision of exposure estimates.

For many patients, particularly critically ill neonates, examining single sources of exposure (e.g., ECMO or ventilation) may substantially underestimate exposure. Babies who require ECMO, for example, also require multiple replacement blood transfusions, parenteral feeding, medications, and IV fluids. Many of these other inputs could substantially increase DEHP exposure. DEHP also passes into breast milk which, when available, is used in some critically ill, hospitalized babies as part of enteral nutrition.

Prenatal exposure through medical procedures also occurs and has not been studied. Pregnancy is possible for women on chronic hemodialysis, and DEHP is known to cross the placenta. Maternal transfusions or intrauterine fetal blood transfusions and *in utero* fetal surgery are additional, though uncommon, sources of exposure.

1.2.3.2 MEHP

Few data have been generated on MEHP and medical exposure. MEHP has been measured in some infusates. These data are displayed in Table 9.

MEHP is the monoester metabolite of DEHP. Exposure results from *in vitro* conversion of leached DEHP to MEHP during storage of blood products, and from *in vivo* conversion of circulating DEHP to MEHP. *In vitro* conversion of DEHP to MEHP is enhanced by increased storage time and temperature (*68*). Storage at 4°C significantly inhibits conversion to MEHP and storing at -30°C prevents it entirely (*33*, *68*). *In vivo* production of MEHP is dependent upon the route of exposure. Oral administration results in the greatest conversion of DEHP to MEHP. Most medical exposures, however, result from IV administration of DEHP and MEHP in procedures which involve transfusion or processing of blood. Tables 10 and 11 display published levels of MEHP in adults and neonates. Conversion by plasma enzymes during IV administration is much slower than that observed when equivalent doses are administered orally (*8*). Exposure via the peritoneal cavity during continuous ambulatory peritoneal dialysis (CAPD,) despite potentially high concentrations in the dialysate, does not seem to result in systemic absorption (*52*).

Conversion of DEHP to MEHP is heat sensitive and has been shown to increase when plasma protein concentrates are transported during summer months (*68*). Processes which require physiologic temperatures are more likely to increase exposure to MEHP. (Data are sparse and firm conclusions cannot be drawn. Additional studies on *in vitro* conversion rates as well as analysis of intra-vascular conversion rates during hemodialysis, ECMO, and cardiac bypass would be very useful.) While the dose of MEHP received during various medical procedures from conversion of DEHP in storage or during use can be estimated even with these few data, it is difficult to interpret without adding the *in vivo* conversion of circulating DEHP which may be at least as great if not greater. MEHP is never an isolated contaminant, but always accompanies DEHP which is almost always present in much larger concentrations.

Table 9: MEHP Exposure by Infusate

Exposure Source	**MEHP concentration: mean +/- SD (range) µg/mL**	**Comments (Source)**
Peritoneal Dialysate	0.177 (0.137–0.239) 0.051(0.032–0.211) 0.022 (0.005–0.058)	Before dialysis 2 hours 4 hours (*60*)
Peritoneal Dialysate	239–396 µg/L	Stored 0–12 months (*52*)
Whole Blood	2.3±0.3 8.8±3.0	Bag A. 21 day storage, 4°C Bag B 21 day storage, 4°C (*28*)
Whole Blood	2.1 (0.6–5.1)	Unspecified for exchanges (*63*)
Whole Blood	7.05 (3.03–15.64)	Unspecified for exchanges (*62*)
Plasma	2.1±0.1	Stored 72 hours, 22°C (*33*)
Platelets	26.4±1.9	Stored 72 hours, 22°C (*33*)
Platelets	0 2.24±0.09 2.5±1.27 4.45±1.2 22.5±.9	0 day storage, 6 bag types (*32*) 5 day storage, 6 bag types
25% NS* Albumin CO.A** CO.B summer transport CO.C winter transport CO.C	10±1 13±10 330±48 176±63	Other protein fractions also evaluated, CO.C used outdated plasma to make protein fractions (*68*)

- NS = Normal Serum; **CO.A, CO.B, and CO.C = Company A, B, and C, respectively.
- SD=Standard deviation reported by authors.

Table 10: MEHP Exposure by Medical Procedure (Adults)

Medical Procedure	MEHP Level: Mean (range) µg/mL	Procedure Time	Exposure/Time	Comments and Source
CABG	0.15–1.7 from Swan catheter	83.9±15.9 minutes	2,200–80,000 µg/operation	Calculated from post-measures (*55*)
Heart Transplant	not given	1.3–5.6 hours	450–2,500 µg/operation	Calculated from post measures, cyclosporin may contribute (*55*)
Bridging period Jarvik 7–70 TAAH	not given	several days	250–18,800 µg/day	(*55*)
CAPD	0.009 (0.001–0.022) controls 0.0145 (0.005–0.023)	Not Stated	Not Stated	No change in median serum concentration over 4 hours measurement (*60*)
CAPD	0	Not Stated	None absorbed	(*52*)
Hemodialysis	1.33±0.58	4 hours	Not Stated	AUC, circulating MEHP (*58*)

CABG = Coronary Artery Bypass Graft
CAPD = Continuous Ambulatory Peritoneal Dialysis

Table 11: MEHP Exposure by Medical Procedures (Neonates)

Medical Procedure	MEHP Level: Mean (range) µg/mL	Exposure: Mean (range)	Comments and Source
Congenital Heart Repair	0.66–2.66 from Swan catheter	0.6 (0.03–2.7) µg/mL/hr	retained dose in whole blood, difference before and after procedure (*55*)
Exchange Transfusion	Max measured in infants, 5 µg/mL	100 (5–200) µg/kg	AUC calculation (*63*)
Exchange Transfusion	7.06 in infants (3.03–15.6)	360 (160–680) µg/kg	Linear extraction assumed (*62*)

Conclusion

Medical exposures to DEHP and MEHP are highly variable and only partially studied. They range from tenths of milligrams per kilogram of body weight as with a single blood transfusion, to tens of grams per person for chronic hemodialysis patients. Most single-source exposure scenarios can be reasonably estimated within one or two orders of magnitude, but additional variability is possible depending on assumptions made. The best studies of single-source exposure measure delivered dose using AUC calculations for dialysis (*58, 59*) and exchange transfusion (*62, 63*). Table 12 displays the average and range of single-source exposures from these three investigators in juxtaposition to the assumed general population exposure, excluding non-dietary sources in infants and toddlers, and the maximum allowable occupational exposures by OSHA standards. These exposures were calculated from the data tables in the individual published reports and rounded to the nearest 10 for easy comparison. Hemodialysis daily dose was calculated by assuming 156 sessions per year in a 70 kg adult. Replacement transfusion was calculated for the average, highest, and lowest DEHP concentrations reported in the blood bags used for the exchange transfusions in the Sjoberg (*62, 63*) study. Most other published exposure estimates are modeled from spot measurements of DEHP in blood in a few individuals, or calculated from leaching rates from DEHP tubing, assuming linear extraction.

Table 12. Comparison of DEHP Exposures – General & Medical Sources

Procedure Resulting in Exposure	DEHP Exposure (µg/kg bw/day)
General population — Primarily Ingestion	3–30 (*24*)
Maximum Allowable Occupational Exposure to DEHP—Inhalation	700 (*24*)
Selected Medical Exposure to DEHP-IV [1]	Average (range)
• Neonatal Replacement Transfusion-short-term, acute (10 cc/kg bw/tx)	300 (140–720) (*63*)[2]
• Double Volume Exchange Transfusion-short-term, acute (neonate)	1,800 (840–3,300) (*63*)[3]
• Adult Hemodialysis-long-term, averaged over 1 year (70 kg, 156 sessions/yr)	640 (150–2,200) (*58*)[4]
• Adult Hemodialysis—long-term, averaged over 1 year (70 kg, 156 sessions/yr)	100 (20–360) (*59*)[4]

[1]These numbers will not match those reported by the authors due to rounding and utilization of tabular data.
[2]Calculated from reported DEHP concentration in blood bags averaged (range) and rounded to nearest 10.
[3] Calculated from reported area under curve (AUC) dose to patients, averaged and (range) rounded to nearest 10.
[4] Calculated from reported AUC dose at average (range), 3 sessions per week, 52 weeks, 70 kg, for 1 year.

The summary for Section 1 is located in Section 5.1.1.

2.0 GENERAL TOXICOLOGICAL AND BIOLOGICAL PARAMETERS

2.1 General Toxicity

Subchronic and chronic toxicity studies were reviewed with consideration for the following: study design and thoroughness of the evaluation; whether the study was conducted under current guidelines; whether dose-response relationships were established; number of doses tested; whether the concentration of the substance in the feed was verified analytically; method of fixation of the testes; relevance of route and, whether peroxisome proliferation was evaluated. These studies were not designed to fully evaluate reproductive toxicity; effects on testes are noted, but not further discussed. The tremendous amount of literature on peroxisome proliferation and its relevance to humans is not reviewed here.

2.1.1 DEHP

Humans: Oral

There were no human oral data located for Expert Panel review.

Rodents: Oral

The most extensive short-term rodent studies were conducted by British Industrial Biological Research Association (BIBRA) (*69*) and Hazelton (*70*). Supporting studies include Mangham et al. (*71*) and Mann et al. (*72*).

BIBRA (*69*) administered DEHP in the diet at concentrations of 0, 0.01, 0.1, 0.6, 1.2, and 2.5% to groups of 5 male and 5 female Fischer 344 rats (6 weeks old) for 21 days. Average dose levels for males were reported to be 0, 11, 105, 667, 1,224, and 2,101 mg/kg bw/day; the doses were 0, 12, 109, 643, 1,197, and 1,892 mg/kg bw/day for females. The feed was analyzed for DEHP content. At necropsy, the liver, kidneys, and testes were weighed and preserved in formalin; part of the liver was preserved in osmium for electron microscopy (EM) and part was saved for biochemical analysis. Liver samples were analyzed for protein, cyanide-insensitive palmitoyl-CoA oxidase, and microsomal lauric acid hydroxylation. Statistically significant differences ($p < 0.05$) between rats exposed to 2.5% and controls included decreased body weight (44% in males and 38% in females on day 21), increased absolute and relative liver weight, decreased absolute testes and kidney weights, decreased serum triglycerides (males only), increased serum triglycerides (females only), and increased hepatic activities of palmitoyl-coenzyme A oxidase. Increased absolute and relative liver weights, decreased serum triglycerides (males only), increased serum triglycerides (females only), and increased palmitoyl-coenzyme A oxidation were also observed at the 0.6 and 1.2% levels. At dosage levels of 0.1%, serum total cholesterol levels were decreased. Increased hepatic activities of lauric acid 11- and 12-hydroxylases were measured in male rats exposed to 0.1% DEHP and in female rats exposed to 1.2% DEHP. Light microscopy revealed decreased hepatic cytoplasmic basophilia (1.2 and 2.5% groups, males at 0.6%), increased hepatic cytoplasmic eosinophilia (2.5% males), and severe-to-moderate testicular atrophy (2.5% males). EM revealed increases in the number and ultrastructural appearance of hepatic peroxisomes in all males exposed to 0.1% and in females exposed to doses 0.6%.

This study provides evidence that the liver and testes are target organs of DEHP in F344 rats following 3 weeks of exposure. The principal effect on the testes was severe atrophy. The principal effects on the liver were increased weight and induction of peroxisome proliferation as determined by EM and activity of cyanide-insensitive palmitoyl CoA oxidase. Metabolic processes associated with the liver were also affected. Kidney weights were increased, but there were no histologic changes. This study provides a LOAEL of 0.6% (643–667 mg/kg bw/day) and a NOAEL of 0.1% (105–109 mg/kg bw/day).

Hazelton (*70*) (WEB Table 1) exposed groups of 10 male and 10 female $B6C3F_1$ mice (6 weeks old at study initiation) to DEHP in the diet at concentrations of 0, 1,000, 5,000, 10,000, or 25,000 ppm for 4 weeks. These dietary concentrations corresponded to doses of 0, 245, 1,209, 2,579, and 6,992 mg/kg bw/day for males and 0, 270, 1,427, 2,897, and 7,899 mg/kg bw/day for females (calculated as the average of the reported weekly mean mg/kg bw/day intakes). The diets were analyzed by high pressure liquid chromatography (HPLC) to confirm the concentrations of DEHP. Food consumption and body weights were recorded weekly. Clinical observations, hematology, serum clinical chemistry, organ weights (liver with gallbladder, kidneys, and testes with epididymides), and gross pathology and histopathology (major tissues and organs) were used to assess toxicity. All tissues were preserved in 10% buffered formalin. Increased mortality was observed in the 25,000 ppm male and female mice; 4/10 males and 3/10 females died. Clinical signs noted at 25,000 ppm included hunched posture, thin appearance, hypoactivity, tremors, urine stains, dyspnea, and rough haircoat. Male mice in the 5,000 and 25,000 ppm groups weighed significantly less than controls (5 and 28%, respectively, lower than controls at termination); decreased body weight was also observed in the 10,000 ppm group (8% lower than controls at termination), but the difference was not statistically significant. Significantly lower body weights were also observed in the 25,000 ppm females (29% lower than controls at termination). Decreases in food consumption were initially observed in the 25,000 ppm group, but by the fourth week of the study, food consumption was similar to the control group. A number of statistically significant

hematological alterations were observed, including decreased erythrocyte levels in 25,000 ppm males and decreased hemoglobin and hematocrit levels in 5,000 ppm males and 10,000 ppm females. A small number of blood samples were analyzed for serum clinical chemistry parameters, thus precluding a statistical analysis of these data. Significant alterations in organ weights included increases in absolute and relative liver weights at 5,000 ppm and higher in males and females, decreases in absolute kidney weights in the 5,000 ppm males, decreases in relative kidney weights in 5,000 ppm males, increases in relative kidney weight in 25,000 ppm females, and decreases in absolute and relative testes weights in the 10,000 and 25,000 ppm males. Histological alterations were observed in the liver, kidney, testes, ovary, and thymus. In the liver, increases in the incidence of hepatocellular hypertrophy and focal necrosis were observed in males and females at doses of 5,000 ppm and higher. Acute inflammation was observed in the kidneys of male mice exposed to 5,000 ppm and higher and female mice exposed to 10,000 or 25,000 ppm. In the thymus, atrophy was observed in the 25,000 ppm males and females. Testicular atrophy was observed in the 25,000 ppm group and the absence of corpora lutea in the ovary was observed in the 25,000 ppm group. No adverse effects were observed at 1,000 ppm (245 and 270 mg/kg bw/day for male and female mice, respectively).

This study provides evidence that the liver, kidney, thymus, ovary, and testes are target organs of DEHP in $B6C3F_1$ mice following 4 weeks of exposure. The principal effect on the testes and thymus was atrophy, and loss of corpora lutea was observed in the ovary. Decreased weight and acute inflammation were noted in the kidney. Increased weight and hepatocellular hypertrophy were noted in the liver. In addition, there were some blood effects. The study provides a LOAEL of 5,000 ppm (1,209–1,427 mg/kg bw/day) and a NOAEL of 1,000 ppm (245–270 mg/kg bw/day).

The studies of Mangham et al. (*71*) and Mann et al. (*72*) also provide evidence for effects on the liver and testes. Both are single high-dose studies and are therefore limited. The studies are summarized below.

Mangham et al. (*71*) administered 0 or 2,500 mg/kg bw/day DEHP by gavage to Wistar rats, 6/sex/group, (45–50 g body weight at study commencement) for 7 or 21 days. The liver and testes were weighed and examined histologically and ultrastructurally. Treatment with DEHP resulted in significant reductions in mean body weight and food consumption in the male rats. Relative liver weights were significantly increased by 95% in males and 75% in females at 7 days, and by 148% in males and 122% in females at 21 days. Liver enlargement was accompanied by alterations in several marker enzyme systems. Mitochondrial succinate dehydrogenase levels were significantly reduced in male but not female animals. There was no evidence of histological lesions of the liver in either sex, but ultrastructural studies showed proliferation of the endoplasmic reticulum, an increase in the number of peroxisomes, and mitochondrial changes. Relative testes weights were significantly decreased in males at 7 (36%) and 21 (44%) days, and histological examination showed atrophy of the seminiferous tubules.

Mann et al. (*72*) administered male Wistar rats (4 weeks old at study initiation) dietary concentrations of 0 (n=18) or 2% (n=12) DEHP for up to 21 days. Using actual food intake levels and rat body weights on the day of sacrifice, a dose of 1,824 mg/kg bw/day was calculated. Food intake was monitored biweekly but the frequency of body weight measurements was not clear. It was not stated whether samples of the diet were analyzed for actual DEHP content. Six control animals and 4 treated animals were sacrificed at 3, 10, or 21 days. Testes and liver weights were recorded, and testes and liver histopathology (preserved in formalin), liver enzyme activity, and liver peroxisome proliferation were examined. Levels of thyroid hormones in serum and thyroid histopathology were also examined and reported by Hinton (*73*). Mean body weights were significantly reduced following 10 (7%) and 21 (7.6%) days of treatment, but food consumption was comparable to control. Absolute liver weights were significantly increased following 3 (47%), 10 (81%), and 21 (140%) days of treatment. Examination of the livers showed a progression of effects following 3, 10, and 21 days of treatment that included midzonal and periportal accumulation of

small droplets of lipid. Hepatomegaly accompanied by an initial burst of mitosis was observed only at day 3. Proliferation of hepatic peroxisomes was evident by increases in cyanide-insensitive palmitoyl CoA oxidase, glycerophosphate dehydrogenase, and catalase activity, by smooth endoplasmic reticulum proliferation accompanied by induction of peroxisomal fatty acid oxidation, damage to the peroxisomal membranes as demonstrated by increased leakage of catalase to the cytosol, centrilobular loss of glycogen and decreases in glucose-6-phosphatase activity, and in low molecular weight-reducing agents. Serum thyroxine and triiodothyronine levels were not significantly affected by treatment with DEHP (*73*). However, microscopic changes, indicating possible thyroid hyperactivity, were observed following treatment with DEHP. The changes included increased lysosomal numbers and size, enlarged Golgi apparatus, and mitochondrial damage. In addition, absolute testes weight was significantly reduced by 9% following 21 days of treatment and was accompanied by histological evidence of atrophy.

Dalgaard et al. (*74*) gavage dosed 10 male Wistar rats/group (~160 g) with DEHP in soya oil at 0, 125, 250, 500, or 1,000 mg/kg bw/day for 9 weeks. In a second study, they gavage dosed 10 male Wistar rats/group (~160 g) with DEHP in soya oil at 0, 1,000, 5,000, and 10,000 mg/kg bw/day for 4 weeks. Some rats were tested for behavior and sensory motor function at an unspecified time period. On the day before sacrifice, blood was collected for clinical biochemistry analysis. At sacrifice, rats were necropsied and organs were weighed and collected (testes fixed in Bouin's fixative) for a histological analysis. Histopathology was only examined in control and high-dose rats.

In the first study, treated animals experienced no effects on body weight gain, clinical signs, mortality, or behavior. There were no histopathological effects in any organs of the high-dose rats, including testes and liver. The only effects observed were reduced serum cholesterol levels in rats exposed to 125, 500, and 1,000 mg/kg bw/day and increased relative liver weight in groups exposed to 500 mg/kg bw/day and higher. In the second study, reduction in body weight and food intake was noted in rats exposed to 5,000 mg/kg bw/day and higher. Two animals in the 10,000 mg/kg bw/day group died of emaciation. A significant dose-related reduction in forelimb grip strength was observed in DEHP-treated rats. Relative weight effects in non-reproductive organs included increased liver weight in all treated animals, increased kidney and brain weight in mid- and high-dose animals, and increased adrenal gland weight in the high-dose group. Authors did not discuss histological effects in these organs and it is not clear if the analyses were conducted. There were no consistent effects on serum biochemistry. Dalgaard et al. (*74*) had a male fertility component in the second experiment with higher doses. These results are discussed in Section 4.

Three 90-day studies of DEHP have been conducted in rodents. Poon et al. (*75*) (WEB Table 2) administered groups of 10 male and 10 female Sprague-Dawley rats (~4-6 weeks old based on body weights) diets containing 0, 5, 50, 500, or 5,000 ppm DEHP for 13 weeks. The authors estimated that these dietary concentrations corresponded to average DEHP doses of 0, 0.4, 3.7, 38, and 375 mg/kg bw/day, respectively, for the male rats and 0, 0.4, 4.2, 42, and 419 mg/kg bw/day for the female rats. Food consumption and body weights were recorded weekly and cage-side observations were recorded daily. At the termination of the study, the following parameters were assessed: hematology, clinical chemistry, organ weights, and histopathology. The testes and epididymides were preserved in Zenkers fluid and the other tissues were preserved in formalin. There were no clinical signs of toxicity in the treated groups, and body weights and food consumption were comparable among treated and control groups. Relative kidney weights were significantly increased by 15% in males and 12% in females fed the 5,000 ppm diet. Females in the 5,000 ppm group exhibited a significant increase in hepatic aminopyrine-N-demethylase activity and a significant reduction in the concentration of serum cholesterol. Males in the 5,000 ppm group exhibited significant decreases in red blood cell counts and serum hemoglobin levels, and significant increases in serum albumin and potassium levels. The albumin:globulin ratio was significantly higher in males and females in the 5,000 ppm group. Slightly lower, but statistically significant activities of alanine aminotransferase and aspartate aminotransferase

were noted in females in the 500 ppm group, while alanine aminotransferase activity was decreased in males at all dose levels (not statistically significant). Histological examination showed minimal-to-mild hepatocellular hypertrophy in all rats of both sexes in the 5,000 ppm group. One male and two females in this group also had minimal focal necrosis. In the testes, minimal Sertoli cell vacuolation was noted in 7/10 males in the 500 ppm group, and 9/10 males in the 5,000 ppm group exhibited bilateral, multifocal, or complete atrophy of the seminiferous tubules with complete loss of spermatogenesis and cytoplasmic vacuolation of the Sertoli cells lining the tubules. Exposure to 5,000 ppm DEHP resulted in mild histological changes in the thyroid, consisting of reduced follicle size and colloid density.

This study provides evidence that the liver and testes are target organs of DEHP in Sprague-Dawley rats. The principal effect in the liver was increased weight with associated histologic changes. Metabolic processes associated with the liver were also affected. The principle effects in the testes were atrophy of the seminiferous tubules and loss of spermatogenesis. There was some indication of a possible effect on kidneys (weight increase only), blood, and thyroid (mild histologic changes) at high doses. The study provides a LOAEL of 500 ppm (38–42 mg/kg bw/day) and a NOAEL of 50 ppm (3.7–4.2 mg/kg bw/day).

Hazelton (*76*) (WEB Table 3) administered groups of 10 male and 10 female Fischer 344 rats (8 weeks old at study initiation) diets containing 0, 1,000, 4,000, 12,500, or 25,000 ppm DEHP for 13 weeks. The diets were analyzed for DEHP content. The authors estimated that these dietary concentrations corresponded to average DEHP doses of 0, 63, 261, 850, and 1,724 mg/kg bw/day, respectively, for the male rats and 0, 73, 302, 918 and 1,858 mg/kg bw/day, respectively, for the female rats. Food consumption and body weights were recorded weekly. Prior to study termination, ophthalmoscopic examinations were conducted and at study termination the following parameters were assessed: hematology, serum clinical chemistry, urinalysis, organ weights (brain, lung, kidney, liver, spleen, testes with epididymides, and uterus), and gross necropsy and histopathology. No deaths were observed. An increased incidence of urine stains was observed in the 12,500 and 25,000 ppm groups; the incidence of other clinical observations did not appear to be related to the DEHP exposure. Significant decreases in mean body weight were observed in the female rats exposed to 12,500 ppm (weeks 3–14) and 25,000 ppm (weeks 2–14) and in males exposed to 25,000 ppm (weeks 4–14). At study termination, mean body weights were reduced by 7 and 18% in females at 12,500 and 25,000 ppm, respectively, and by 17% in males at 25,000 ppm. Food consumption was significantly reduced in these groups during some weeks of the study, but there was no consistent pattern. Mild anemia, as indicated by effects on red cell counts, hematocrit, and/or hemoglobin levels were observed in males exposed to 4,000 ppm or greater and in females exposed to 25,000 ppm. A number of serum clinical chemistry alterations were observed, including increased glucose levels in the rats exposed to 12,500 ppm DEHP and higher, and increases in serum, blood urea nitrogen (BUN), total protein, and albumin levels, and decreases in serum globulin levels at 4,000 ppm and higher. Alterations in urinalysis results were observed in the 25,000 ppm group, including a significant increase in occult blood and decrease in urine protein in males and a slight decrease in urine pH in the females. A number of statistically significant alterations in organ weights were observed; these included decreased absolute lung weight (25,000 ppm males and females), increased absolute and relative liver weights (1,000 ppm males and 4,000 ppm males and females), increased absolute kidney weights (12,500 ppm males and 1,000, 4,000, and 12,5000 ppm females), increased relative kidney weights (4,000 ppm males and 12,500 ppm males and females), decreased absolute spleen weight (25,000 ppm males and females), decreased absolute brain stem weight (25,000 ppm females), increased relative brain stem weight (25,000 ppm males and females), decreased absolute and relative uterine weight (25,000 ppm females), and decreased absolute and relative testicular (with epididymides) weight (25,000 ppm males). Histological alterations were observed in the pituitary, liver, kidney, stomach, adrenal cortex, testes, and epididymides; the pituitary, stomach, and adrenals were only examined in the control and 25,000 ppm groups. In the pituitary, an increase in castration cells was noted in males at 25,000 ppm. In the adrenals, there was an increased vacuolization/width of the zona glomerulosa in males and females at 25,000 ppm. In the stomach, erosion was noted in males at 25,000

ppm. In the kidney, pigment in the proximal tubules was observed in both sexes exposed to 12,500 and 25,000 ppm. In the liver, hepatocellular hypertrophy was noted in both sexes at 12,500 and 25,000 ppm. Bilateral testicular atrophy with aspermia in the epididymides was noted in males at 25,000 ppm.

This study provides evidence that a variety of organs are affected by DEHP in F344 rats following 13 weeks of exposure. The principal effect on the testes was atrophy with aspermia. In the pituitary, there was an increase in castration cells in males. Increased weight and hepatocellular hypertrophy were noted in the liver. Increased weight and pigment in the proximal tubules were noted in the kidney. Mild anemia was noted as well as weight changes in the uterus, spleen, and brain stem. In addition, there were some blood effects. The study provides a LOAEL of 1,000 ppm (63–73 mg/kg bw/day).

NTP (*77*) conducted subchronic studies with mice and rats exposed to DEHP in the diet. Groups of 10 male and 10 female F344 rats (5–6 weeks old at the study inception) were fed diets containing 0, 1,600, 3,100, 6,300, 12,500, or 25,000 ppm DEHP (>99% pure) for 13 weeks. Groups of 10 male and 10 female $B6C3F_1$ mice of a similar age were similarly exposed to diets containing 0, 800, 1,600, 3,100, 6,300, or 12,500 ppm DEHP. Using reference body weights and the allometric equation for food consumption recommended by U.S. EPA (*78*), dosage levels are calculated to be 0, 160, 320, 641, 1,282, and 2,563 mg/kg bw/day for male rats and 0, 182, 364, 727, 1,454, and 2,908 mg/kg bw/day for female rats. Estimated dosage levels are 0, 144, 289, 578, 1,156, and 2,311 mg/kg bw/day for male mice and 0, 157, 314, 629, 1,258, and 2,516 mg/kg bw/day for female mice. At the end of 13 weeks, necropsies were performed on all animals and tissues were prepared for histopathological examination. No significant increases in treatment-related deaths were observed in either species. Seven of the 10 male mice exposed to 12,500 ppm died, but 6 of these deaths were attributed to accidents. Depressions of body weight gain greater than 10% were observed for male (53% depression) and female (29%) rats exposed to 25,000 ppm. Testicular atrophy was noted in male rats exposed to 12,500 or 25,000 ppm. In mice, body weight gain depressions of 10% or more were observed in males fed concentrations of 3,100 ppm and in all female mice except those fed 1,600 ppm. Histopathological alterations of tissues were not apparent in the treated mice.

This study provides evidence that the testes is a target organ of DEHP in F344 rats. As in other studies, DEHP exposure resulted in testicular atrophy. It is not clear (given the dose range) why effects were not noted in the liver in this study. In mice, the only effect noted was reduced body weight. Confidence in this study is limited. In rats, a NOAEL of 6,300 ppm (641 mg/kg bw/day) and LOAEL of 12,500 ppm (1,282 mg/kg bw/day) for testicular effects were identified. In mice, the LOAEL is 3,100 ppm (578 mg/kg bw/day) for decreased body weight, and the NOAEL is 1,600 ppm (289 mg/kg bw/day).

Although limited by the small sample size, Mitchell et al. (*79*) conducted a study with multiple sacrifices to provide a time course of events. Mitchell et al. provided groups of 4 male and 4 female Wistar rats with diets containing DEHP at concentrations sufficient to maintain nominal dosage levels of 50, 200, or 1,000 mg/kg bw/day for 3, 7, 14, or 28 days, or for 9 months. Control groups of six males and six females were provided a normal diet. For each sacrifice time, abdominal organs were removed and examined histologically; in addition, EM and biochemical examinations were conducted on livers. More extensive necropsies were performed only at the 9-month sacrifice. Decreased body weights were observed at 9 months in male rats treated with 200 or 1,000 mg/kg bw/day and in female rats exposed to 1,000 mg/kg bw/day. Increased absolute liver weights were observed after 9 months of exposure in all treated male groups and in females exposed to doses of 200 mg/kg bw/day. Hypertrophy of the hepatocytes, centrilobular loss of glycogen, and a decrease in glucose-6-phosphatase activity were observed in livers of rats exposed for 28 days or 9 months to 200 or 1,000 mg/kg bw/day; only marginal changes in these 3 parameters were observed at 50 mg/kg bw/day. Proliferation of peroxisomes and smooth endoplasmic reticulum proliferation were observed at 14 and 28 days and at 9 months in liver

cells of treated males from all exposure groups; a comparable response in treated females was observed only after 9 months of exposure. Slight kidney alterations (enlargement of droplets in the proximal tubules) were observed in rats treated with doses of 200 mg/kg bw/day at 9 months. Thyroid lesions (basophilic deposits in the colloid and enlargement of lysosomes) were also observed in rats treated with the highest dose level. No alterations in testes weight were observed after 9 months of exposure.

Several chronic carcinogenicity studies of DEHP have been conducted. The most useful are the studies by NTP (*77*) and the recent studies by David et al. (*80*). Other studies have been criticized for a variety of reasons and are not summarized here.

In the NTP study, Kluwe et al. (*77*) administered groups of 50 male and 50 female F344 rats diets containing 0, 6,000, or 12,000 ppm DEHP and $B6C3F_1$ mice were fed diets containing 0, 3,000, or 6,000 ppm DEHP for 103 weeks. Mean daily ingestions of DEHP were calculated to be 322 and 674 mg/kg bw/day for male rats, 394 and 774 mg/kg bw/day for female rats, 672 and 1,325 mg/kg bw/day for male mice, and 799 and 1,821 mg/kg bw/day for female mice. Treatment did not affect survival rates for either species. Food consumption was comparable among control and treated groups in both species. Mean body weight gains were significantly reduced in male rats at 6,000 and 12,000 ppm, female rats at 12,000 ppm, and female mice at 3,000 and 6,000 ppm. Rats in the 12,000 ppm group and mice at 6,000 ppm exhibited severe seminiferous tubular degeneration and testicular atrophy and the tubules were devoid of spermatocytes and germinal epithelium. The cells in the anterior pituitary were hypertrophied in 45% of the male rats at 12,000 ppm. The incidence of animals with foci of clear changes in the liver was increased in male and female rats at 6,000 and 12,000 ppm. Male mice at 6,000 ppm exhibited a significant increase in the incidence of chronic inflammation of the kidney. Hepatocellular carcinomas were significantly increased in female rats at 12,000 ppm, male mice at 6,000 ppm, and female mice at 3,000 and 6,000 ppm. The combined incidence of animals with hepatocellular carcinomas or neoplastic nodules was significantly increased in male rats at 12,000 ppm and female rats at 6,000 ppm, while the combined incidence of animals with hepatocellular carcinomas or adenomas was significantly increased in male mice at 3,000 ppm.

This study provides further evidence that the liver, kidney, testes, and pituitary are target organs of DEHP following 2 years of exposure. Testicular atrophy and loss of spermatocytes were seen in rats and mice. Hepatocellular adenomas and carcinomas were observed in both sexes of both species. Hypertrophy of the anterior pituitary was noted in male rats and chronic inflammation of the kidney was noted in male mice. The LOAEL for testicular effects was 12,000 ppm in rats (674 mg/kg bw/day) and 6,000 ppm for mice (1,325 mg/kg bw/day), and the NOAEL was 6,000 ppm in rats (322 mg/kg bw/day) and 3,000 ppm for mice (672 mg/kg bw/day).

David et al. (*80, 81*) recently conducted 2-year bioassays in rats and mice. In the rat study, F344 rats were administered dietary concentrations of 0 (n = 80 males, 80 females), 100 (n = 50 males, 50 females), 500 (n = 55 males, 55 females), 2,500 (n = 65 males, 65 females), or 12,500 ppm (n = 80 males, 80 females) DEHP for 104 weeks. This corresponded to average daily doses of 0, 5.8, 29, 147, and 789 mg/kg bw/day for males, and 0, 7.3, 36, 182, and 939 mg/kg bw/day for females. Ten rats/sex from the control and two highest dose groups were sacrificed at week 78 for a complete necropsy and histopathological examination of tissues from major organs. During week 105, the remaining rats were sacrificed, necropsied, and tissues examined histopathologically. In addition, a sixth group was administered a diet containing 12,500 ppm DEHP (n = 55 males, 55 females) for 78 weeks, followed by a 26-week recovery period during which a basal diet was provided. All relevant parameters for a chronic/carcinogenicity study were evaluated. In addition, hepatic cell and peroxisome proliferation were evaluated at weeks 1, 2, 13, and 79, and at study termination.

Survival was decreased in both sexes at 2,500 and 12,500 ppm. Mean body weight gain was slightly reduced in males at 2,500 ppm and significantly reduced in both sexes at 12,500 ppm. At 2,500 ppm, there was a significant increase in serum albumin and absolute and/or relative liver weight in both sexes. Induction of peroxisome proliferation was evident in both sexes at 104 weeks. At 12,500 ppm there was a significant decrease in erythrocyte count, hemoglobin, and hematocrit, as well as a significant increase in serum urea nitrogen and serum albumin, in both sexes. Absolute and relative liver weights were significantly increased in both sexes accompanied by a significant increase in peroxisome proliferation. Histologic changes in the liver included diffuse hepatocellular enlargement, increased cytoplasmic eosinophilia, and increased pigment Kupffer cells/hepatocytes. Absolute and relative kidney weights were significantly increased in both sexes at 12,500 ppm accompanied by an increase in the incidence and severity of mineralization of the renal papilla in males, and an increased incidence and/or severity of tubule cell pigment seen in both sexes. In addition, male kidneys had an increased severity of chronic progressive nephropathy. At 12,500 ppm absolute and relative testes weights were significantly decreased. An increased incidence of bilateral aspermatogenesis was observed in the high dose group at week 78 and in rats treated with 500 ppm DEHP and higher at week 105. The incidence of interstitial cell neoplasms was decreased in treated rats. Increased castration cells were observed in the pituitary of males at 12,500 ppm at both time periods. There was a significant increase in hepatocellular neoplasms in males at 2,500 and 12,500 ppm and females at 12,500 ppm. In addition, males at 12,500 ppm exhibited a significant increase in the incidence of monocellular leukemia; however, it is not clear whether this was treatment-related since the incidence was within that observed for historical controls and this effect has not been observed in other chronic studies.

Following the 26-week recovery period (after 78 weeks of treatment), treatment-related liver enlargement appeared to be reversible; hepatic peroxisome proliferation activity, diffuse hepatocellular enlargement, and increased cytoplasmic eosinophilia were comparable among control and recovery animals in both sexes. At study termination, hepatocellular neoplasms (adenomas and carcinomas) were present, but the incidence for both sexes was decreased compared to the animals receiving 12,500 ppm DEHP for 104 weeks; hepatocellular neoplasms were observed in 32.7 and 42.5% of the males in the recovery and 12,500 ppm groups, respectively, and in 18.2 and 27.5% of the females in the recovery and 12,500 ppm groups, respectively. There was no evidence of reversibility of DEHP-related effects in the testes, pituitary, or kidney.

In the mouse study, David et al. (*80*) administered B6C3F_1 mice dietary concentrations of 0 (n = 70 males, 70 females), 100 (n = 60 males, 60 females), 500 (n = 65 males, 65 females), 1,500 (n = 65 males, 65 females), or 6,000 ppm (n = 70 males, 70 females) DEHP for 104 weeks. These dose levels corresponded to an average daily dose of 0, 19, 99, 292, and 1,266 mg/kg bw/day for males and 0, 24, 117, 354, and 1,458 mg/kg bw/day for females. In addition, a sixth group (n = 55 males, 55 females) was administered 6,000 ppm DEHP for 78 weeks followed by a 26-week recovery period; this corresponded to an average dose of 1,157 mg/kg bw/day for males and 1,254 mg/kg bw/day for females. In addition to the usual analyses, hepatic cell and peroxisome proliferation were analyzed.

Exposure to 500 ppm resulted in a significant increase in liver weights in males and a significant increase in peroxisome proliferation in both sexes. There were no histologic changes noted in the liver. Exposure to 1,500 ppm resulted in a significant increase in liver weights and peroxisome proliferation in both sexes. Kidney weights were significantly reduced in males, and the incidence and/or severity of chronic progressive nephropathy was increased in both sexes. In males, testes weights were significantly reduced, accompanied by an increased incidence and severity of bilateral hypospermia and immature/abnormal sperm in the epididymis. The incidence of hepatocellular neoplasms (adenomas/carcinomas) was significantly increased in both sexes at 1,500 ppm. Exposure to 6,000 ppm resulted in increased mortality, with deaths most frequently associated with hepatocellular neoplasia. Total body weight gain was decreased in both sexes, associated with poor health and/or antemortem condition; there was

increased ventral-abdominal swelling and distended abdomens reflecting enlarged livers and/or liver masses. There was a significant increase in liver weights and hepatic peroxisome proliferation in both sexes. Hepatocellular adenomas and carcinomas were significantly increased in both sexes. Non-neoplastic histologic changes included diffuse hepatocellular enlargement, increased cytoplasmic eosinophilia, and presence of pigment. Kidney weights were significantly reduced in both sexes accompanied with an increased incidence and/or severity of chronic progressive nephropathy in both sexes. In males, testes weights were significantly reduced accompanied by an increased incidence and severity of bilateral hypospermia and immature/abnormal sperm in the epididymis.

Following the 26-week recovery period (after 78 weeks of treatment), some of these effects appeared reversible or did not progress after cessation of treatment. At the end of the recovery period, hepatic peroxisome activity was comparable to control. Absolute and relative liver weights were still significantly increased but not as much as observed in the group receiving treatment for 104 weeks. Diffuse hepatocellular enlargement and increased cytoplasmic eosinophilia were not observed in the recovery group, and the incidence of pigment was reduced. In addition, the incidence of hepatocellular adenomas and carcinomas was reduced in the male recovery group. However, in the female recovery group, the incidence of adenomas was reduced but the incidence of carcinomas was increased. Absolute and relative kidney weights were still significantly reduced in the recovery group, but the severity of the chronic nephropathy was less in both sexes compared to that observed following treatment for 104 weeks. The decrease in testes weight and associated hypospermia was also less severe.

The rat study provides further evidence that the liver, kidney, testes, and pituitary are target organs of DEHP following 2 years of exposure. In addition, anemia was noted at the high dose. Testicular atrophy and loss of spermatocytes was observed, and there was an increase in castration cells in the pituitary of males. Kidney weights were increased accompanied by tubule cell pigment and chronic progressive nephropathy (males). Liver weights were increased accompanied by peroxisome proliferation. Hepatocellular adenomas and carcinomas were observed in both sexes. Treatment-related hepatic enlargement appeared to be reversible after 78 weeks of exposure, but effects on the testes, pituitary, and kidney were not. The LOAEL for effects on the pituitary, and kidney was 12,500 ppm (789–939 mg/kg bw/day), and the NOAEL was 2,500 ppm (147–182 mg/kg bw/day). The LOAEL for effects on the liver was 2,500 ppm (147–182 mg/kg bw/day), and the NOAEL was 500 ppm (29–36 mg/kg bw/day). NOAELs and LOAELs for testicular effects are discussed in Section 4.

The mouse study shows similar effects on the liver, kidneys, and testes. Testicular atrophy and loss of spermatocytes was observed. Kidney weights were reduced accompanied by tubule cell pigment and chronic progressive nephropathy. Liver weights were increased accompanied by peroxisome proliferation. Hepatocellular adenomas and carcinomas were observed in both sexes. Treatment-related effects on the liver, testes, and kidney appeared to be partially reversible after 78 weeks of exposure. The LOAEL for effects on the testes and kidney was 1,500 ppm (292–354 mg/kg bw/day), and the NOAEL was 500 ppm (99–117 mg/kg bw/day). The LOAEL for effects on the liver was 500 ppm (99–117 mg/kg bw/day), and the NOAEL was 100 ppm (19–24 mg/kg bw/day).

Other Species: Oral

A number of studies have been conducted to ascertain the species specificity of the liver and testicular effects. These have included studies in hamsters, guinea pigs, dogs, ferrets, marmosets, and monkeys. All of the studies are limited for a variety of reasons (e.g., small sample sizes, single doses tested). However, they provide useful information and are summarized below.

The most valuable study was conducted in marmosets. Kurata et al. (*82*) (WEB Table 4) administered groups of 4 male and 4 female marmosets doses of 0, 100, 500, or 2,500 mg/kg bw/day DEHP in corn oil by gavage for 13 weeks. Body weights were recorded more than once weekly and clinical observations were recorded daily. Blood samples were collected for clinical chemistry prior to treatment, and at 4 and 13 weeks after commencement of treatment. Blood levels of cholecystokinin, testosterone, and estradiol were measured prior to treatment and at study termination. At study termination, the animals were necropsied, organ weights were recorded, and organs were preserved for histopathology. Portions of the liver and testes were prepared for electron microscopy. In addition, hepatic peroxisomal enzyme activities and P450 content were analyzed, as well as testicular zinc levels. Males given 2,500 mg/kg bw/day exhibited a significant reduction in mean body weight. There were no treatment-related effects on blood chemistry, testosterone, estradiol or cholecystokinin levels, testicular zinc levels, organ weights, or organ histopathology. The number of peroxisomes, peroxisome morphology, and peroxisomal enzyme activities were comparable among treated and control groups. The males treated with 500 and 2,500 mg/kg bw/day showed a significant increase in mean volume of peroxisomes (1.3- and 1.4-fold increase in the 500 and 2,500 mg/kg bw/day groups, respectively). Cytochrome P450 were elevated in males in all treated groups and in females in the 500 and 2,500 mg/kg bw/day groups, but the differences were not statistically significant.

Given the thorough analysis in this study, the lack of testis effects is consistent with the concept that marmosets are less or at most, equally sensitive than rodents to the effects of DEHP.

Pugh et al. (*83*) gavage dosed 2-year-old (prepubertal) male cynomolgus monkeys (4/group) with 0 or 500 mg/kg bw/day DEHP in methylcellulose for 14 days (Web Table 50). According to Short et al. (*84*), 500 mg/kg bw/day is the maximum dose that can be absorbed by the monkeys. On day 15, the animals were sacrificed and the tissues were removed, weighed and fixed in formalin for histopathological evaluation. Hematology, serum chemistry, and urine analysis were conducted. Peroxisomal proliferation was examined by measuring peroxisomal beta oxidation activity and replicative DNA synthesis. Gap junctional intercellular communication was determined in liver. Reproductive organ effects from this study are discussed in Section 4. There were no clinical signs or changes in body weight gain. Diffuse hepatocellular vacuolation was observed in one animal. There were no effects on any of the other systemic parameters examined.

Rhodes et al. (*85*) reported that a 14-day exposure to 2,000 mg/kg of orally administered DEHP produced less marked effects, such as peroxisomal proliferation in the marmoset, than those produced in rats (Web Table 51). This difference in sensitivity of response corresponded with quantitative differences in metabolic disposition. The marmoset excreted DEHP metabolites primarily in the conjugated form, whereas unconjugated metabolites were predominantly excreted in the urine of the rat. In addition, the marmoset excreted more products of the omega-1 oxidation pathway than did the rat.

Lake et al. (*86*) reported that the magnitude of DEHP-induced hepatic peroxisomal proliferation and increased liver weight was much less in hamsters than in rats treated with gavage doses of 0, 25, 100, 250, or 1,000 mg/kg bw/day for 14 days. In this study, the NOAELs and LOAELs for hepatic responses were 25 and 100 mg/kg bw/day for rats, respectively, compared with 250 and 1,000 mg/kg bw/day in hamsters.

Lake et al. (*87*) administered 18-month-old male albino ferrets diets containing 0 (n=6) or 1% DEHP (n=7) for 14 months. The authors calculated that the mean daily intake of DEHP was 1,200 mg/kg bw/day, but owing to the seasonal fluctuation in the body weight of the ferret, the daily intake ranged from 650 to 2,000 mg/kg bw/day. At the end of the treatment period, livers were removed for biochemical and histochemical analyses. Histological examinations were conducted on the brain, heart, adrenals, thyroid, trachea, esophagus, bladder, lung, and kidney. There was a significant reduction in

mean body weight in the ferrets receiving the 1% DEHP diet. Absolute and relative liver weights were significantly increased in the treated group. In addition, liver cell enlargement, lysosomal changes, and dilation of the endoplasmic reticulum were noted, as well as the depression of a number of marker enzyme activities. In the testes, a few seminiferous tubules showed a complete or almost complete absence of germinal epithelium in 3/7 treated animals. No lesions were noted in other organs that were examined.

Short et al. (*84*) administered gavage doses of 0, 100, or 500 mg/kg bw/day DEHP to 2 male cynomolgus monkeys per group for 25 days and observed no treatment-related changes in liver weights, hepatic activities of peroxisomal enzymes, or liver structure revealed by light microscopy or EM.

Carpenter et al. (*88*) assessed the oral toxicity of DEHP in guinea pigs, dogs, and rats. Groups of hybrid guinea pigs were fed diets containing 0 (24 males and 22 females), 0.04 (23 males and 23 females), or 0.13% (24 males and 23 females) commercial DEHP (Flexol Plasticizer DOP, registered trademark of Union Carbide and Carbon Corp.) for 1 year, starting when the animals were 23 days old. From food intake and body weight data, the authors estimated the daily intakes at 0, 19, or 64 mg/kg bw/day. No exposure-related effects on body weight, kidney weight, or histopathology of the kidney, liver, lung, spleen, or testes were observed. A significant ($p < 0.05$) increase in relative liver weight was observed in the female guinea pigs exposed to 19 or 64 mg/kg bw/day DEHP.

Gelatin capsules containing DEHP were orally administered to 4 dogs (2 cocker spaniels and 2 wire-haired terriers, sex not specified) for 5 days/week for 1 year (*88*). A similar group of two cocker spaniels and two wire-haired terriers served as a vehicle control group. The daily dosages were 0.03 mL/kg (29.5 mg/kg bw/day, using a density of 0.9843 g/mL) for the first 4 weeks, followed by dosages of 0.06 mL/kg bw/day (59.1 mg/kg bw/day) for the remainder of the treatment period. The time-weighted average dose, adjusted for intermittent administration, is approximately 41 mg/kg bw/day. Body weight gain, liver weight, or kidney weight were not significantly different in the treated dogs compared with values for the control group. Treated dogs displayed no gross or microscopic pathological alterations in the lungs, heart, liver, stomach, upper and lower intestine, cecum, spleen, adrenal, ovary, testes, bladder, or thyroid.

In the rat study, groups of 32 male and 32 female Sherman rats (60 days of age) were maintained for 2 years on diets containing 0, 0.04, 0.13, or 0.4% DEHP (*88*). From food consumption and body weight data, the authors calculated the approximate dosage levels to be 0, 20, 60, or 195 mg/kg bw/day DEHP. The animals were allowed to breed, and an F_1 group of approximately 80 rats was maintained for 1 year on a 0.4% diet. Mortality incidences in the treated and control F_1 groups were high, but not compound-related; 46.2 and 42.7% of the F_1 rats in the respective groups lived to 1 year. There were no compound-related effects in the F_0 or F_1 groups on mortality, life expectancy, or hematology or histopathology of organs. The only statistically significant ($p < 0.05$) adverse effects noted were decreased body weight gain and increased kidney and liver weights in both F_0 and F_1 rats exposed to 0.4% DEHP (195 mg/kg bw/day).

Humans: Inhalation

There is limited information about DEHP toxicity in humans exposed by inhalation. No controlled epidemiological studies are available. Increased incidence of neuropathy was reported in groups occupationally exposed to several phthalates, including DEHP. However, the association is considered questionable due to the lack of a control group and presence of other exposure components (phthalic anhydride and respective alcohols) that could have caused this effect (*89*). Exposure to DEHP-containing

fumes in the thermo-plastic industry has been associated with decreased erythrocyte and platelet counts and increased isotransferrin ratio (*90*).

A series of case studies describing lung disorders resembling hyaline membrane disease in three pre-term infants ventilated with PVC respiratory tubes for 4 weeks has been published (*66*).

Laboratory Animals: Inhalation

Klimisch et al. (*91*) (WEB Table 5) evaluated systemic and reproductive effects of DEHP in Wistar rats. Male and female rats were exposed head only to DEHP mists (mass median aerodynamic diameter < 1.2 µm) for 6 hours/day, 5 days/week, for 28 days at concentrations of 0, 0.01, 0.05, and 1.0 mg/L. Assuming 100% depositions and absorption, the authors estimated doses of 0, 2.3, 11, and 230 mg/kg bw/day for males and 0, 3.6, 18, and 360 mg/kg bw/day for females. Clinical signs were recorded daily and body weights were recorded twice weekly. Ten rats/sex/group were sacrificed, necropsied, and evaluated for histological, hematological, and blood chemistry effects and two rats/sex/group were evaluated for peroxisome proliferation immediately after exposure. Fifteen males per group were mated to untreated females for 10 days at 2 and 6 weeks after exposure. Five rats/sex/group were sacrificed 8 weeks after exposure and were evaluated for histological and blood chemistry effects to determine if DEHP effects were reversible. Dams were sacrificed either 14 days after mating or following the end of the mating period. Corpora lutea were counted and implantation sites were examined. Immediately after exposure, there was a significant increase in relative lung weight in males at 1 mg/L and this was accompanied by thickening of alveolar septums and foam cell proliferation. Relative liver weights were significantly increased in males and females at 1 mg/L, but there was no evidence of peroxisome proliferation or histological damage. Plasma albumin was significantly increased in both sexes and plasma inorganic phosphate was increased in males at 1 mg/L. None of these effects were observed after the 8-week recovery period. DEHP did not affect fertility in male rats as determined by mating success, fertility index, and pre- and post-implantation loss. Histopathological evaluations revealed no adverse testicular effects.

Humans: Parenteral

A single report of 29 infants undergoing ECMO found a statistically significant positive association between both free hemoglobin and DEHP serum levels and the degree of cholestasis. A causal link between DEHP and either increased hemolysis or cholestasis is speculative (*92*).

Laboratory Animals: Parenteral

Rhodes et al. (*85*) report a rat oral DEHP study (n=10 M/F Wistar-derived rats, 2 g/kg bw/day for 14 days in corn oil) and two marmoset studies: an oral study (n=5 M/F, 2 g/kg bw/day for 14 days in corn oil) and an intraperitoneal (IP; injection into the abdominal cavity) study (n=5 marmosets, 1 g/kg bw/day for 14 days in corn oil). At necropsy, blood was taken for measures of plasma triglycerides and cholesterol, a gross examination was made, and "selected tissues were taken for microscopic examination . . ." which apparently included testes. A variety of enzyme activities were measured from hepatic subcellular fractions. Toxicokinetic studies were also performed, and are reviewed in Section 2.2.

Only rats (receiving 2 g/kg bw/day by the oral route) showed an increase in liver weight and a decrease in testis weight. No histology results were presented. The marmoset data are confusing and poorly reported: a single set of bargraphs is presented, while two studies were performed. In the published report, the data are much less clear: the authors state that organ weights were not changed in marmosets either at 2 g/kg bw/day orally or 1 g/kg bw/day IP but provide no data. However, in an EPA docket which contains these data, there are tables that list the organ weights and histology findings, and DEHP

clearly caused no change in histopathology or in testis weights: mean absolute weights for control and treated marmoset testes are 0.803 and 0.876 g, with a variance of 0.315. Based on histology and biochemical measures, peroxisomes were strongly induced in male rats, but not female rats and not in marmosets.

The alternate route, IP, was effective in showing differences between species for a number of hepatic measures. However, this study used only a single dose level for each route. In addition, the reader does not know the length of time between the last dose and death. Another methodologic shortcoming was that testes were fixed and stained inappropriately. Finally, the study protocol does not require gestational or perinatal dosing or evaluation of endpoints of reproductive development.

Because of the limited confidence that can be placed in the testis evaluation and the use of a single dose level, no meaningful LOAEL/NOAEL can be determined from these data.

Sjoberg et al. (*93*) looked at the effects of IV administration of DEHP on hepatic histology and serum enzymes and testicular histology in 40-day-old male Sprague Dawley rats. The DEHP was emulsified with egg yolk phosphatides and administered in a glycerol solution. The control group was administered vehicle that was exactly the same, but without the DEHP. Doses of DEHP were 5, 50, or 500 mg/kg. Rats (n=5–6/group) were 40 days old. DEHP was administered every other day for 6 administrations. Therefore the time weighted average doses were 2.5, 25, and 250 mg/kg bw/day. Two to three hours. after the last infusion, 1 mL of blood was taken for clinical chemistry measures, and bromsulphophthalein (BSP) was administered to measure renal clearance. Approximately 20 minutes after BSP infusion, the animals were killed and various organs removed. Liver blocks were fixed in glutaraldehyde.

There was a dose-related decrease in body weight gain (~40% reduced at the high dose). Relative liver weight was increased at the middle and top doses by 4% and 35%, respectively. BSP clearance was unchanged; clinical chemistry measures were unchanged, reflecting no increased leakage of liver enzymes into the blood. Hepatic peroxisomes were increased by 32, 26, and 41% in the 5, 50, and 500 mg/kg dose groups, respectively. The authors concluded that the high dose change was an effect. Liver and kidney histology appeared unchanged except for the hepatic peroxisomal increase. Testicular effects are discussed in Section 4.

Since the study used appropriate controls, excellent histology, sensitive measures, multiple dose levels, and an appropriate route, the Panel is confident in the findings.

However, the study protocol does not require measures of the developing reproductive system; neither does it require functional measures, but with these subtle effects, no changes in reproductive system function would be expected (i.e., fertility would be unchanged). In addition, the animals were older than the likely most sensitive developmental age. A LOAEL of 500 mg/kg bw (250 mg/kg bw/day) was identified based on hepatic changes. The NOAEL was 50 mg/kg bw (25 mg/kg bw/day).

Confidence in this study is high, because of the care taken in the methods and measures, and the fact that the changes seen in the liver mimic those found by other investigators. The Panel is confident that the authors found any effects that were present in these animals.

Jacobson et al. (*94*) studied liver effects in 6-month-old rhesus monkeys transfused with plasma from DEHP-plasticized bags. Groups of 2–3 monkeys were transfused weekly for 1 year with platelet-rich plasma stored at 22°C (Group 1) or 4°C (Group 2). A third group was transfused biweekly for 6 months with platelet poor plasma stored at 22°C (Group 3). Average <u>total</u> exposures to DEHP for groups 1–3 respectively were 27, 8, and 32 mg/kg. One group of three control monkeys was not transfused and a group of two control monkeys was transfused weekly for 6 months with platelet-rich plasma stored in

polyethylene bags. Impairment of hepatic perfusion and/or infiltration was indicated in 2 of 3 monkeys in Group 1 and in one of 2 monkeys in Group 2 by a consistent reduction in baseline ^{99m}Tc scan activity from 3 months of treatment to 14 months post-treatment. In those same monkeys, altered liver function was indicated by altered BSP clearance that persisted until 14 months post-treatment.

Abnormal histological findings consisting of vacuolized cells, necrosis, increased numbers of Kupffer cells or inflammatory cell infiltration were observed at 1 year in six of the seven monkeys perfused with plasma from PVC-plasticized bags. The histological findings persisted for up to 14 months post-treatment. There was no evidence of abnormal liver function or histology in the monkeys who were not transfused or were transfused with plasma from polyethylene bags. Serum chemistry measurements were not affected in either treated or control animals. There are several factors that confound the interpretation of the study: 1) inconsistent responses in the two groups that received the largest (and similar) DEHP exposure; 2) pooled plasma was re-transfused into the monkeys, which provided the possibility of the development of allergic reaction to foreign proteins; and 3) the authors reported that there was a tuberculosis outbreak in the monkey colony, which might have contributed to the hepatic effects.

Greener et al. (*95*) IV dosed 2–4 day-old rats (12/group; strain not specified) with DEHP at 0, 31, 92, or 165 mg/kg bw/day for 18 days. The vehicle was 4% bovine serum albumin (BSA) in 0.9% sodium chloride. One group of 12 control rats was not treated and 2 other groups of 12 control rats were IV dosed with 0.9% sodium chloride or 4% BSA. Twenty-four hours after the last treatment, blood was drawn for hematological and biochemical analysis. The rats were necropsied and select organs were examined histologically (brain, heart, lungs, liver, spleen, kidneys, eyes, and digestive tract). Reproductive organs were not weighed or examined histologically.

Significant effects in the high dose rats (165 mg/kg bw/day) included reduced body weight gain, increased liver weight and liver to body weight ratio, and increased serum glutamic oxaloacetic tansaminase (SGOT) activity. Histological lesions were not observed in the liver or other organs examined. The Food and Drug Administration reviewed this study and selected a NOAEL of 92 mg/kg bw/day (*96*).

2.1.2 2-EHA/2-EH

Oral

There were no human data located for Expert Panel review.

Juberg et al. (*97*) (WEB Tables 6 and 7) administered groups of 10 male and female F344 rats and B6C3F$_1$ mice dietary concentrations of 0, 0.1, 0.5, or 1.5% 2-EHA for 13 weeks (6 weeks of age at study initiation). Additional groups of rats and mice (10/sex/group) were fed either 0 or 1.5% 2-EHA for 13 weeks followed by a 4-week recovery period. The concentrations of 2-EHA in the diets were verified analytically. Based on food consumption and body weight, the authors calculated doses of 61, 303, and 917 mg/kg bw/day for male rats and 71, 360, and 1,068 mg/kg bw/day for female rats; the doses for male mice were 180, 885, and 2,728 mg/kg bw/day, and 205, 1,038, and 3,139 mg/kg bw/day for female mice. Clinical signs of toxicity were recorded twice each workday, food consumption was recorded twice weekly, and body weights were recorded weekly. During the last week of the 90-day study, the eyes of 5 male and female animals from each group were examined; since no effects were noted, the remainder of the animals were not examined. At necropsy, hematological analyses, urinalysis, and clinical chemistry were conducted. The liver, kidneys, adrenal glands, testes, ovaries, and brain were weighed. All tissues were preserved in 10% buffered formalin and examined histologically.

There were no deaths or clinical signs of toxicity during the study. Beginning on day 4, the mean food consumption was reduced by 3–5% in male rats and 8–10% in female rats; mean food consumption by mice on the 1.5% diet was equal to or slightly lower than controls throughout the study. Mean body weights were significantly reduced beginning at week 2 in rats and mice fed the 1.5% diet. At the end of the treatment period, mean body weights were significantly reduced by 8% in male rats, 10% in female rats, 5% in male mice, and 14% in female mice compared to controls. During the recovery phase, body weight gain for rats that had received 1.5% 2-EHA increased so that at the end of the recovery period mean body weights were 5% lower in males and 3% lower in females compared to the controls. The body weight decrements in the mice persisted throughout the recovery phase. After 13 weeks, lower triglyceride levels occurred in male mice fed 1.5% 2-EHA and female mice fed 0.5 or 1.5% 2-EHA, but not in other groups. Cholesterol levels were significantly higher in all male rat test groups and in female rats and male and female mice fed 0.5 or 1.5% 2-EHA; this effect was reversible following the 28-day recovery period. Mice and rats fed the 0.5 and 1.5% diets exhibited significant increases in absolute and relative liver weight. Following the 28-day recovery period, absolute liver weights were comparable among the control and 1.5% 2-EHA groups. Hepatocyte hypertrophy was observed in males and females of both species fed 1.5% 2-EHA, and in male rats and mice fed 0.5% 2-EHA, but with decreased incidence and severity. Proximal renal tubule cytoplasmic basophilia was noted in male and female mice fed 1.5% 2-EHA, and hyperkeratosis of the mucosa of the forestomach was noted in male mice fed 1.5% 2-EHA. Following the recovery period, only 1/10 male and 1/10 female mice showed hepatocyte hypertrophy, suggesting that the liver changes are reversible. Renal changes were not observed following the recovery period. No testicular effects were noted. Testes and kidney to body weight ratios were also affected as noted in WEB Tables 6 and 7.

This study provides evidence that the liver is a target organ of 2-EHA in F344 rats and $B6C3F_1$ mice. The principal effects were increased liver weights associated with hepatocyte hypertrophy and reduced cytoplasmic vacuolization. Metabolic processes associated with the liver were also affected. Observed histopathological and clinical pathological changes were reversible following a 4-week recovery period. Proximal renal tubule cytoplasmic basophilia was noted in male and female mice fed 1.5% 2-EHA, and hyperkeratosis of the mucosa of the forestomach was noted in male mice fed 1.5% 2-EHA. These effects were reversible. No testicular effects were noted. This study provided a NOAEL of 0.1% 2-EHA for rats and mice (61 and 71 mg/kg bw/day for male and female rats, and 180 and 205 mg/kg bw/day for male and female mice).

Astill et al. (*98*) (WEB Table 8 and WEB Table 9) administered groups of 10 male and female F344 rats and $B6C3F_1$ mice doses of 0, 25, 125, 250, and 500 mg/kg bw/day EH in Cremophor EL by gavage for 13 weeks. Clinical observations were recorded daily, and body weights and food consumption were recorded weekly. At necropsy, hematological analyses, urinalysis, and clinical chemistry were conducted. The liver, kidneys, adrenal glands, stomach, ovaries, and brain were weighed. All tissues were preserved in 4% buffered formalin and examined histologically. In addition, three animals/sex/dose were treated for 13 weeks and their livers were used for determination of cyanide-insensitive palmitoyl CoA activities and protein concentration.

One female mouse at 250 mg/kg bw/day died. There were no other mortalities or clinical signs of toxicity noted during the study. There was a significant reduction in mean body weight gain in rats at 500 mg/kg bw/day, beginning at week 4 in males and week 11 in females; at week 13, mean body weights were reduced by 7% in males and 6% in females compared to controls. Food consumption was comparable among treated and control groups for both species. Female rats at 250 and 500 mg/kg bw/day had 30 and 36% decreases in serum ALT activities, respectively. Female rats at 500 mg/kg bw/day had a 16% decrease in serum cholesterol concentration and male rats at 500 mg/kg bw/day had 13% decreases in total protein and albumin concentrations. There was a 25% increase in reticulocyte numbers in male and

female rats at 500 mg/kg bw/day. Clinical pathology parameters were comparable among treated and control mice. At 500 mg/kg bw/day in the rat there were increased relative liver (29% male, 15% female), kidney (16% male, 6% female), stomach (11% male, 16% female), and testes (6%) weights compared to controls. In rats treated with 250 mg/kg bw/day, there were increases in relative weights of kidney, liver, stomach (females only), and ovaries. Histopathology revealed acanthosis of the forestomach mucosa in 2/10 males and 5/10 females, and a moderate decrease in hepatic peripheral lobular fatty infiltration in 4/10 males and 2/10 females and adrenal-cell hyperplasia in 3/10 female rats at 500 mg/kg bw/day. In male mice, there was a significant increase in relative stomach and liver weights at 250 and 500 mg/kg bw/day (stomach, 14 and 13%; liver, 6.9 and 5.9%). Moderate focal or multifocal acanthosis of the forestomach was noted in 2/10 male and 1/10 female mice at 500 mg/kg bw/day. The activities of hepatic cyanide-insensitive palmitoyl CoA were significantly increased in male (6.5-fold) and female (3.4-fold) rats at 500 mg/kg bw/day; no effects were noted in mice.

This study provides evidence that the liver, kidney, stomach, and perhaps testes are target organs of 2-EH in F344 rats. The principal effects were increased liver and forestomach weights with associated histologic changes. Metabolic processes associated with the liver were also affected. In $B6C3F_1$ mice, the target organs were the stomach and liver (females only). The only effects were increased stomach weights in males and histologic changes in the forestomach (male and female) and liver (female). EH induced peroxisome proliferation was observed in rats, but not mice, as determined by increased activity of hepatic cyanide-insensitive palmitoyl CoA. This study provided a LOAEL of 250 mg/kg bw/day and a NOAEL of 125 mg/kg bw/day for both species.

Astill et al. (*99*) administered groups of 50 male and 50 female F344 rats and $B6C3F_1$ mice EH in Cremophor EL by gavage. For rats, doses of 0 (water and vehicle controls), 50, 150, and 500 mg/kg bw/day were administered for 24 months, and doses of 0 (water and vehicle controls), 50, 200, and 750 mg/kg bw/day were administered to mice for 18 months. Clinical observations were recorded daily, and body weights and food consumption were recorded weekly. At necropsy, hematological analyses, urinalysis, and clinical chemistry were conducted. The liver, kidneys, adrenal glands, stomach, ovaries, and brain were weighed. All tissues were preserved in 4% buffered formalin and examined histologically. In addition, 3 animals/sex/dose were treated for 13 weeks and their livers were used for determination of cyanide-insensitive palmitoyl CoA activities and protein concentration.

Statistical comparisons of data were made between vehicle controls and treatment groups. There were no differences of biological significance between data from vehicle and water control groups. In rats, there were no treatment-related changes at 50 mg/kg bw/day. There was reduced body weight gain at 150 mg/kg bw/day (M, 16%; F, 12%) and 500 mg/kg bw/day (M, 33%; F, 31%) and an increased incidence of lethargy and unkemptness. There were dose-related increases in relative liver, stomach, brain, kidney, and testes weights at sacrifice. Female rat mortality was markedly increased at 500 mg/kg bw/day. There was marked aspiration-induced bronchopneumonia in rats at 500 mg/kg bw/day; hematological, gross, and microscopic changes, including tumors, were otherwise comparable among all rat groups. In mice at 50 and 200 mg/kg bw/day there were no dose-related changes and essentially no time-dependent or time-independent adverse trends in liver tumor incidence at the 5% significance level. At 750 mg/kg bw/day mouse body weight gain was reduced (M, 26%; F, 24%), and mortality increased (M and F, 30%) versus vehicle controls. At 750 mg/kg bw/day there was a slight increase in nonneoplastic focal hyperplasia in the forestomach of mice (M 5/50, F 4/50) compared to vehicle controls (M 1/50, F 1/50). There were increases in mouse relative liver (F, 21%) and stomach (M, 13%; F, 19%) weights at 750 mg/kg bw/day. There was a 12% incidence of hepatic basophilic foci and an 18% incidence of hepatocellular carcinomas in male mice at 750 mg/kg bw/day, not statistically significant compared with either control by Fisher's exact test. There was a 12% incidence of hepatic basophilic foci and a 10% incidence of hepatocellular carcinomas in female mice at 750 mg/kg, statistically significant (p

< 0.05) compared with vehicle but not with water controls by Fisher's exact test. There were no metastases. Time-dependent and independent statistical analyses showed an adverse trend in the incidence of hepatocellular carcinomas in male and female mice, correlated with toxicity (expressed as mortality) at 750 mg/kg bw/day. The time-adjusted incidence of hepatocellular carcinomas in male mice (18.8%) was within the historical normal range at the testing facility (0–22%), but that in females (13.1%) lay outside the normal range (0–2%). Under the conditions of these studies 2-EH was not oncogenic in rats, but there were weak trends in hepatocellular carcinoma incidence in mice at high-dose levels which may have been associated with toxicity. Direct comparison of any tumorogenic effects of 2-EH given alone to female mice with those due to 2-EH formed *in vivo* from DEHP is limited by the high mortality in female mice at equivalent doses of 2-EH. While 2-EH may be a contributing factor in the hepatocellular carcinogenesis in female mice associated with the chronic administration of DEHP, it is unlikely to be the entire proximate carcinogen.

This study provides evidence that the liver, stomach, kidney, brain, and testes are target organs of EH in F344 rats. The principle effect was increased organ weights. There were no increases in tumors in the rat. In $B6C3F_1$ mice, increased weights were noted in the stomach, liver (females), kidneys, brain, and testes. There were weak trends in hepatocellular carcinoma incidence in mice at high dose levels which may have been associated with toxicity.

Inhalation

Klimisch et al. (*100*) administered groups of 10 male and 10 female Wistar rats (7 weeks of age at study initiation) EH vapors at concentrations of 0, 15, 40, or 120 ppm 6 hours/day, 5 days/week, for 90 days. Samples of the inhalation atmospheres were analyzed at 15-minute intervals by gas chromatography. Body weights were recorded weekly. As specified in the protocol, ophthalmological examinations were conducted prior to and at study termination. At necropsy, hematological analyses and clinical chemistry were conducted. Protein concentration and the activity of cyanide-insensitive palmitoyl CoA were measured. The liver, lungs, kidneys, adrenal glands, and testes were weighed. All tissues were preserved in 4% formaldehyde solution for histologic evaluation. No effects were noted in any of the groups treated with concentrations as high as 120 ppm (vapor saturation at 20^0C).

Mode of Action for DEHP

Liver. It is well understood that DEHP produces a range of hepatic effects (induction of peroxisomes; increased Cyp4A1; PCoA) and hepatocellular adenoma and carcinoma in rat and mouse studies. The induction of these effects is believed due to activation of PPAR-alpha. In PPAR-alpha knockout mice, administration of DEHP does not result in the induction of hepatic effects or tumors, unlike the tumor response in wild type control animals. Peroxisomal proliferation was not observed in marmosets exposed with up to 2,500 mg/kg bw/day or in cynomolgus monkeys dosed with up to 500 mg/kg bw/day (*82, 84*). However an increase in peroxisomal volume, a presumed biological response, was noted in the marmosets. In humans, PPAR-alpha, is activated upstream of, and regulates different enzymes from, those noted in the rat. Subsequently, peroxisomal proliferation does not occur and induction of these enzymes does not appear to produce toxic responses in humans. Taking note of the large body of research data in this area, IARC recently re-evaluated DEHP and changed its classification to “not classifiable as to carcinogenicity in humans” from the previous classification of “possibly carcinogenic to humans.” In contrast to hepatic effects, renal toxicities are noted in PPAR-alpha knockout mice. The panel is aware that a CPSC review of DEHP is in progress.

The modes of action for developmental and reproductive effects are addressed in Sections 3 and 4 respectively.

2.2 Toxicokinetics

DEHP

Phthalate Moiety

Absorption

Rodents: Dermal
Dermal absorption was slow in two studies conducted in rats (*101, 102*). In these studies, 95 or 86% of the applied dose remained at the site of application after 5 or 7 days, respectively. In a study in guinea pigs, dermal absorption was also slow, with only 3 and 21% of the applied dose absorbed and excreted after 1 and 7 days, respectively (*103*). Deisenger et al. (*104*) demonstrated that dermal absorption of DEHP from a plastic film is slower than absorption of neat DEHP in rats. A plastic film containing 40% radiolabeled DEHP was applied to rat skin for 24 hours then removed. At the end of 7 days, it was determined that 0.01064% of the DEHP was absorbed by the animals.

Rodents: Oral
DEHP is rapidly absorbed from the gut of rodents, mostly in the form of the monoester because of the rapid hydrolysis of the DEHP by gut lipases (*105-108*). In a study to determine the dose-dependence of the form of DEHP (DEHP or MEHP) absorbed from the gut (*109*), rats showed a sharp increase in the amount of intact DEHP reaching the liver when the dose exceeded 0.43% of the diet, but mice showed no such threshold, presumably because mice have higher levels of DEHP-hydrolase in the intestines than do rats.

Rodents: Inhalation
Radiolabeled DEHP was rapidly absorbed in rats exposed by inhalation to 100 mg/m^3 DEHP for 6 hours either singly or repeatedly (*110*).

Rodents: Other Routes
Studies in rats indicate that DEHP can cross the placenta (*111*) and is present in breast milk of lactating dams (*112, 113*). Rats dosed orally, or by intraaterial (IA) or IP injection of DEHP indicated a marked route-dependency in formation of MEHP. Eighty percent of the oral dose was converted to MEHP while only 1% of the IA or IP dose was converted (*107*). IP injection of radiolabeled DEHP into pregnant rats resulted in radioactivity being widely distributed in maternal blood and fetal tissues.

Non-human Primates: Oral
In a series of studies comparing the absorption of orally administered DEHP in rats and marmosets (*85, 114-116*), marmosets absorbed less than rats. Tissue levels of DEHP-derived ^{14}C in the monkeys were about 20% or less than those in rats. Fecal excretion was about 70% in monkeys, compared to 30% in rats.

Non-human Primates: Intraperitoneal
^{14}C-DEHP was injected IP into marmosets (*115*). About 85% of the dose remained in the peritoneal cavity with only 0.6% of the administered dose recovered in tissues after 7 days.

Humans: Dermal
No human *in vivo* dermal studies were found. However, Barber et al. (*117*) and Scott et al. (*118*) compared *in vitro* absorption of DEHP through rat and human skin and found that DEHP was more rapidly absorbed through rat skin.

Humans: Oral
Two male volunteers were dosed with 30 mg DEHP as a single dose or with 10 mg/kg bw/day for 4 days (*119*). Approximately 13% of the administered dose was excreted in urine, suggesting less absorption than is observed in rat studies, but similar absorption to that observed in monkeys.

Humans: Inhalation
The absorption of inhaled DEHP has not been studied formally in humans, but indirect evidence for pulmonary absorption has been observed in infants ventilated with PVC respiratory tubes (*66*) and in workers occupationally exposed to DEHP (*120, 121*). The evidence in infants was based on demonstrated urinary levels of DEHP, but not MEHP. For workers, pre- and post-shift urine that were analyzed indicated the presence of DEHP and its metabolites.

Humans: Other Routes
Humans have been exposed to DEHP via plastic materials used in medical treatment devices or storage bags. In patients receiving blood stored in plastic bags or undergoing hemodialysis, the ratio of DEHP to its monoester in their blood is much higher than if the DEHP is received orally (*34*).

In a study of 11 patients undergoing maintenance hemodialysis for treatment of renal failure, peak concentrations of DEHP in blood were between 5 and 50 μg/mL. Concentrations of the metabolite, MEHP, ranged from ~1/3 to 6 times the DEHP concentrations (*58*). Half-life for clearance of DEHP from human blood has been reported as 28 minutes for the initial rapid phase that reflects distribution of DEHP within the body (*34*), followed by a slower clearance phase of 10–12 hours (*63*). This bi-phasic clearance of DEHP is also reflected in data from hemodialysis patients (*50*).

DEHP plasma concentrations in infants during ECMO therapy, measured daily until 3 days after decanulation in a prospective comparative clinical study versus non-ECMO neonatal patients, were shown to be greater in the early course of ECMO therapy (the average highest concentration at any time of the bypass being 8.3 μg/mL, or 2 mg/kg versus non-detectable in the control), but most patients cleared the compound from the plasma before decanulation (*54*). The authors speculated that DEHP might have been either efficiently metabolized by the newborn, or redistributed into various tissues. DEHP levels in necropsy tissues (heart and gastrointestinal) from premature neonates who received varying quantities of blood products were found to be significantly higher in comparison to those of infants who had not received blood transfusions (*122*). However, in a clinical study of hospitalized adult postmortem patients with or without a history of exchange transfusions of blood stored in PVC containers during their last hospitalization, no correlation was found between DEHP tissue levels and transfusion history (*123*).

Rodents: Biotransformation

Studies by Albro et al. (*124, 125*) have described the metabolites of DEHP found in the urine of exposed rats (Figure 2, Table 13). The qualitative nature of the metabolism is similar in mammalian species studied. DEHP is converted to a large number of metabolites. There is no evidence for transformation of the aromatic part of the molecule nor for oxidation of the side chains on the intact DEHP. Thus, the metabolites are side-chain modifications of MEHP or oxidation products of the 2-EH cleaved from DEHP to form MEHP. The formation of the monoester from the parent diester is achieved by lipases located

mainly in the gut, liver, lung, and pancreas. The 2-EH is oxidized (ω- and ω-1-oxidation with subsequent β-oxidation) to acetate and CO_2. The oxidative metabolism of MEHP begins with hydroxylation of the ethylhexyl side chain at five different positions resulting in the formation of primary and secondary alcohols. The alcohols are then oxidized to diacids or diketoacids. The diacids are subject to alpha or beta oxidation in mitochondria and peroxisomes to yield shorter diacids, but never beyond the branch point in the ethylhexyl chain.

Primates, Including Humans: Biotransformation

The major metabolic difference between metabolism of DEHP in rats and primates is that oxidative metabolism of MEHP appears to play a dominant role in rats with approximately 75% of the urinary metabolites consisting of dicarboxylic acids (metabolites V and I), whereas the major metabolites in primates are MEHP and metabolite IX (a secondary alcohol). Another major difference is the degree of conjugation of the metabolites with glucuronic acid. Urinary glucuronides are absent in rats, low (15%) in hamsters, moderate (60–65%) in mice and guinea pigs, and high (65–80%) in primates, including humans. (8)

Distribution

Most distribution studies have been based on radiolabeled DEHP, without identification of the form of the radioactivity. The studies indicate that ^{14}C from absorbed ^{14}C-DEHP is widely distributed in tissues (none in the brain) without evidence of accumulation. In rats exposed by inhalation (*110*), the highest concentrations of ^{14}C were in the lungs, liver, and kidney. In a comparative oral study in rats and marmosets (*85*), tissue distribution patterns of the ^{14}C were similar in the two species, but the amount was lower in marmosets because of the poorer uptake from the gut in monkeys.

Rodents: Excretion

Rats exposed by inhalation to 100 mg ^{14}C-DEHP/m^3 for 6 hours, singly or repeatedly, excreted 90% of the ^{14}C within 72 hours with 52% in urine and 39% in feces. Excretion in the feces was linear with a half-life of 22 hours; urinary excretion was biphasic with half-lives of 10 and 22 hours. Oral studies in rats (*126*) indicated that 80% of the administered ^{14}C-DEHP was excreted in urine and feces within 5–7 days. Total ^{14}C in urine was slightly higher than in feces. About 5% of the dose was excreted via the bile.

In rats administered ^{14}C-DEHP (100 mg/kg body weight) orally, excreta were collected for 6 days. Most of the ^{14}C (62%) was excreted in the feces, 30% was in urine, and 4% was in exhaled air (*127, 128*). Less than 2% of the dose remained in the carcass and tissues.

In rats dosed orally with much more ^{14}C-DEHP (2,000 mg/kg) (*129*), half of the dose was excreted in the feces and half in the urine over a 4-day period and less than 0.1% was retained in tissues. Similar results were obtained if the rats were pretreated for 6 or 13 days with the same amount of unlabeled DEHP.

Male and female rats exposed by gavage daily for 14 days to 2,000 mg/kg bw/day excreted 97% (male) and 96% (female) given on the last day within 24 hours. Of this, 54% was in the urine and 42% in feces (*114*).

In a study in rats designed to determine the effect of dose on excretion of orally administered DEHP, it was found that urinary excretion increased with dose and fecal excretion decreased (*130*). Urinary excretion represented 53, 64, and 67% of the dose after administration of 85, 550, and 1,000 mg/kg, respectively. The fecal excretion for the same doses was 36, 28, and 26%, respectively. Prior exposure to DEHP did not affect this pattern.

Dosing of rats by IV perfusion resulted in similar excretion rates into urine and feces as after oral dosing (*126*). Cannulation of the bile duct indicated that 24% of the dose could be recovered in bile within 24 hours, whereas only 5% was in bile following oral administration.

Non-human Primates: Excretion

In a study in male Cynomolgus monkeys, 2 animals received an oral dose of 100 mg ^{14}C-DEHP/kg and 2 received a dose of 500 mg/kg (*84, 131*). Most of the radioactivity was excreted within 24 hours and after 96 hours only 0.2% of the dose remained in the tissues. The urinary excretion for each animal was 20 and 55% at the low dose and 4 and 13% at the high dose. Fecal excretion was 49 and 39% at the low dose and 69 and 56% at the high dose. Urinary metabolites were identified and indicated a major species difference from the rats. Two of the major metabolites identified in the urine of rats (metabolite I, the end-product of $-oxidation of V, and metabolite VI, which is believed to be the proximate peroxisome stimulator in rodents) were minor metabolites in monkey urine.

Male and female marmosets dosed with 2,000 mg/kg bw/day for 14 days (*85, 114-116*) excreted 62% (male) or 76% (female) of a ^{14}C-labeled DEHP dose in 24 hours. Almost all of the radioactivity was in feces, with only 1% in urine. Ninety-eight percent of the fecal radioactivity was in the form of DEHP, suggesting that DEHP is not hydrolyzed and absorbed well from the marmoset gut.

IV administration of DEHP in monkeys resulted in excretion of 40% in urine and 20% in feces over a 7-day period. Around 28% of the dose remained in the lungs with minimal dose in other tissues (*115*). After IP administration, only 10% of the dose was excreted in urine and 4% in feces. Most of the IP dose remained in the peritoneal cavity with minimal amounts in tissues.

Humans: Excretion

In workers likely to be exposed to DEHP, urinary metabolites increased approximately 2-fold during the workshift (*121*). Two male volunteers received 30 mg DEHP as a single dose or 10 mg/day for 4 days. Approximately 13% of the dose was excreted in urine within 24 hours with a urinary elimination half-life of about 12 hours. About 35% of the metabolites were unconjugated (*119*). In another study with a human volunteer, a man was given a single oral dose of 213 mg DEHP (*132*). More than 99% of the urinary metabolites were conjugated to $-glucuronic acid. Six patients receiving platelet concentrates containing 18–38 mg/dL of DEHP showed plasma DEHP levels of 0.34–0.83 mg/dL at termination of the transfusion (*34*). Urinary excretion accounted for 60–90% of the total dose within 24 hours. Two cancer patients received larger doses of DEHP (95 mg and 174 mg) via platelet concentrate infusions (*133*). Approximately 80% of the urinary metabolites were conjugated to glucuronide.

The glucuronidation pathways of children do not mature glucuronidation until they are 3 months old. Thus, this important clearance mechanism is not fully available to neonates and young infants (*134*).

Side Chain-associated Toxicokinetics

MEHP given directly to rats by IV, oral, or IP administrations was cleared with a half-life of approximately 3 hours (*107*).

The metabolism of 2-EH, the secondary alcohol metabolite of DEHP, was studied in male rats (*135*). Radiolabeled 2-EH was rapidly absorbed from the gut and excreted as $^{14}CO_2$ in exhaled air (6–7%), urine (80–82%), and feces (8–9%). Metabolites in urine indicated the 2-EH was metabolized mainly via oxidation to acetate and CO_2.

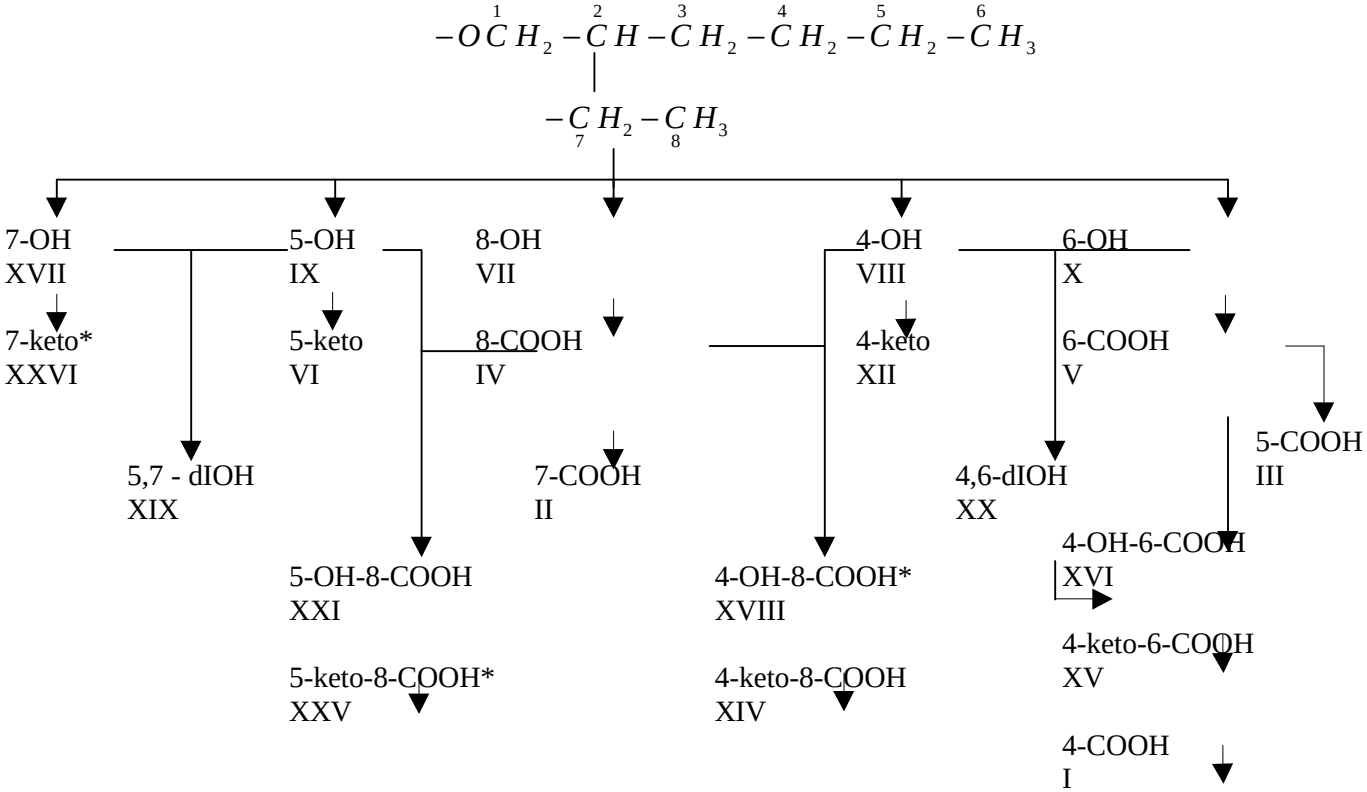
Models

A physiologically-based pharmacokinetic (PBPK) model of the distribution of DEHP and its metabolite MEHP in rats administered DEHP by various routes has been developed by Keys et al. (*136*). The model provides a valuable tool for extrapolation of tissue doses among various routes of exposure and for various exposure regimens. With modifications, such a model should allow extrapolation among species, among individuals of different ages, between genders, and for the pregnant versus the non-pregnant state. The model allows estimation of the internal dose to specific target tissues as the basis for evaluations of risk, rather than use estimates of total exposure or total internal dose.

The initial model developed by Keys et al. (*136*) was a flow-limited model that failed to adequately simulate the experimental data. Addition of factors to account for diffusion-limited membrane transport, enterohepatic circulation and ion trapping (pH-trapping) of the MEHP all improved the model fit. The pH-trapping model gave the best prediction.

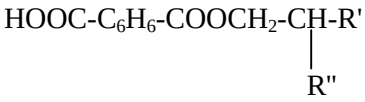
The model of Keys et al. (*136*) can be used to reduce uncertainty in extrapolating from animal data to predictions for human dosimetry and risk

Figure 2: Metabolites from DEHP Excreted into the Urine of Rats. (Only the ethylhexyl chain and its metabolic alterations are shown.)



(*124*, *125*)

Table 13: Structure of DEHP Metabolites



Metabolite		R'	R"
I	2-ethyl-3-carboxypropyl phthalate	$-CH_2COOH$	$-CH_2CH_3$
II	2-carboxyhexyl phthalate	$-[CH_2]_3CH_3$	-COOH
III	2-ethyl-4-carboxybutyl phthalate	$-[CH_2]_2COOH$	$-CH_2CH_3$
IV	2-carboxymethylhexyl phthalate	$-[CH_2]_3CH_3$	$-CH_2COOH$
V	2-ethyl-5-carboxypentyl phthalate	$-[CH_2]_3COOH$	$-CH_2CH_3$
VI	2-ethyl-5-oxyhexyl phthalate	$-[CH_2]_2-CO-CH_3$	$-CH_2CH_3$
VII	2-(2-hydroxyethyl)hexyl phthalate	$-[CH_2]_3CH_3$	$-CH_2CH_2OH$
VIII	2-ethyl-4-hydroxyhexyl phthalate	$-CH_2-CHOH-CH_2CH_3$	$-CH_2CH_3$
IX	2-ethyl-5-hydroxyhexyl phthalate	$-[CH_2]_2]-CHOH-CH_3$	$-CH_2CH_3$
X	2-ethyl-6-hydroxyhexyl phthalate	$-[CH_2]_2CH_2OH$	$-CH_2CH_3$
XI	2-ethyl-pentyl phthalate	$-[CH_2]_3$	$-CH_2CH_3$
XII	2-ethyl-4-oxyhexyl phthalate	$-CH_2-CO-CH_2CH_3$	$-CH_2CH_3$
XIV	2-carboxymethyl-4-oxyhexyl phthalate	$-CH_2-CO-CH_2CH_3$	$-CH_2COOH$
XV	2-ethyl-4-oxy-6-carboxyhexyl phthalate	$-CH_2CO-CH_2COOH$	$-CH_2CH_3$
XVI	2-ethyl-4-hydroxy-6-carboxyhexyl phthalate	$-CH_2-CHOH-CH_2COOH$	$-CH_2CH_3$
XVII	2-(1-hydroxyethyl)hexyl phthalate	$-[CH_2]_3CH_3$	$-CHOH-CH_3$
XVIII	2-carboxymethyl-4-hydroxyhexyl phthalate	$-CH_2-CHOH-CH_2CH_3$	$-CH_2COOH$
XIX	2-(1-hydroxyethyl)-5-hydroxyhexyl phthalate	$-[CH_2]_2-CHOH-CH_3$	$-CHOH-CH_3$
XX	2-ethyl-4,6-dihydroxyhexyl phthalate	$-CH_2CHOH-CH_2CH_2OH$	$-CH_2CH_3$
XXI	2-carboxymethyl-5-hydroxyhexyl phthalate	$-[CH_2]_2-CHOH-CH_3$	$-CH_2COOH$
XXV	2-carboxymethyl-5-oxyhexyl phthalate	$-[CH_2]_2-CO-CH_3$	$-CH_2COOH$
XXVI	2-(1-oxyethyl)hexyl phthalate	$-[CH_2]_3CH_3$	$-CO-CH_3$

(*124*)

2.3 Genetic Toxicity

The genotoxic effects of DEHP and MEHP have been thoroughly investigated in a number of short term tests that include gene mutation studies in bacteria (Ames assay, E. coli WP2 uvrA+/uvrA-), yeast (S. cervisiae D6 to test for aneuploidy and spindle effects), mammalian cells, and rodents (point mutations in HGPRT- locus using Chinese hamster V79 cells) in the absence and presence of microsomal activation (*137*). DEHP is considered non-mutagenic by Kemikalieinspektionen (KEMI) (*137*). Chromosome effects, sister chromatid exchange, micronucleus effects, unscheduled DNA synthesis, Rec assay, and cell transformation were also examined (*138*). DEHP failed to accelerate the accumulation of spontaneous mutations via indirect mutagenic mechanisms in the lacI transgenic mouse mutation assay following a 4-month feeding period at dose levels positive in the 2-year bioassays (*139, 140*).

The frequency of chromosomal aberrations in blood lymphocytes were investigated in occupationally exposed workers in a DEHP production plant in Germany. Exposure levels ranged from 0.0006 to

0.01 ppm (0.01–0.16 mg/m^3). In comparison to a control group of 20 workers, no evidence of an increased frequency of chromosome aberrations could be found. However, the study is considered to be inadequate for the evaluation of genotoxicity to DEHP in humans because of the small number of workers examined and the low exposure levels (*137*). In general, negative results have been noted in all of these systems. However, one group has reported positive tests for point mutations in bacteria and mammalian cells and in tests for chromosome abnormalities *in vivo*, in the Rec assay, and in an unconventional cell transformation test (*141*). Negative results have also been reported with MEHP in the majority of these assays. However, the same group as before reported positive results in tests for point mutations in bacteria, in *in vitro* tests for chromosome anomalies, and in the Rec assay (*138*). Taken together with all the results both positive and negative, DEHP and MEHP are considered to be non-mutagenic substances (*137*).

2-EH was examined in two mutagenicity assays with *Salmonella* and tested negative in one (*142*) and weakly positive in the second (*143*).

2-EHA tested negative in a mutagenicity assay with *Salmonella* (*144*), but produced dose-related increases in sister chromatid exchanges in cultured human lymphocytes (*145*).

The summary for Section 2, including general toxicity, toxicokinetics, and genetic toxicity, is located in Section 5.1.2.

3.0 DEVELOPMENTAL TOXICITY DATA

3.1 Human Data

There were no human data located for Expert Panel review.

3.2 Experimental Animal Toxicity

Forty-one studies were evaluated for the effects of DEHP or its metabolites, MEHP, 2-EH, and 2-EHA, on development in mammals. Three DEHP studies from two laboratories explored the effects of dietary exposure in mice (*146-149*); other laboratories provided studies in mice on the effects of short-duration gavage or IP injections (*150-155*). In rats, there were two dietary studies from a single laboratory (*149, 156, 157*); gavage studies (*111, 158-160*); a drinking water study (*161*); an inhalation study (*162*); a study of DEHP-plasma extracts administered intravenously (*163, 164*); and studies using short-duration oral or IP exposures (*165-167*). Two reproductive studies also addressed some developmental endpoints (*168, 169*).

For DEHP metabolites, 18 studies were reviewed. For MEHP, four short duration dietary, gavage, or IP exposure studies in the mouse and rat were reviewed (*153, 155, 170, 171*), as was a single IV route study in the rabbit (*172*). Five studies of 2-EH toxicity in rats and mice were reviewed (*166, 173-176*). Eight developmental toxicity studies on 2-EHA were reviewed (*166, 177-182*), including one study in rabbits (*183*). One study on the developmental effects of phthalic acid has also been reviewed (*184*). The primary goal of some of the DEHP metabolite studies was to explore their role in the developmental toxicity of DEHP. 2-EHA has been studied as a teratogen in its own right. One study has addressed mechanism of action (*177*).

Studies are reviewed in the following order: administration in diet or drinking water, by repeated or single gavage, by injection, and by inhalation. One embryo culture study and one investigation of mechanism were also reviewed.

3.2.1 DEHP

The best quantitative information on the effects of DEHP on development is provided by the NTP-sponsored dietary exposure studies in the mouse and rat (*146, 149, 156, 157*). The studies use similar dose ranges and identify similar NOAELs and LOAELs. The results of the Tyl et al. mouse study (*149*) were essentially replicated in a recent independent study (*150*). The Panel has high confidence in the quality of these studies and their ability to provide accurate NOAELs and LOAELs for oral exposures. The remaining studies provide support to the findings of those studies under a variety of experimental conditions. The limited scope and design of parenteral and inhalation studies precludes accurate identification of NOAELs and LOAELs by these routes.

Administration in diet or drinking water

Tyl et al. (*149, 185*) (WEB Table 10) evaluated the developmental effects of DEHP administered in feed of CD-1 mice. From gd 0 to 17 animals were fed a diet containing 0, 0.025, 0.05, 0.10, or 0.15% DEHP (0, 44, 91, 191, or 293 mg/kg bw/day; estimated by author). Group sizes ranged from 30 to 31 pregnant animals at sacrifice. Maternal body weights and food and water consumption were recorded throughout the treatment period. At sacrifice on gd 17, information on liver and uterine weights, number of corpora lutea, and fetal viability, growth, and morphology was obtained.

Maternal body weight was reduced on gd 12, 16, and 17 at 191 and 293 mg/kg bw/day in diet. Gestation weight gain, but not corrected weight gain, was reduced (~33%) at 191 and 293 mg/kg bw/day. Food consumption adjusted for body weight was elevated at 293 mg/kg bw/day. Clinical signs limited to rough coat and lethargy were reported to have occurred in some females in the 91, 191, and 293 mg/kg bw/day groups. No significant effects were reported for females in the 44 mg/kg bw/day dose group. Thus the maternal NOAEL was 44 mg/kg bw/day.

The number and percentage of resorptions per litter was increased in a dose-dependent manner with values significantly higher than controls at 191 and 293 mg/kg bw/day. The litters of 6 dams at 191 mg/kg bw/day and 14 at 293 mg/kg bw/day were fully resorbed, resulting in a greater percentage of litters containing nonlive implants in all but the 44 and 91 mg/kg bw/day groups. Live litter size was reduced in a dose-related manner with values significantly lower than controls at 191 and 293 mg/kg bw/day. Female fetal body weight was reduced at 191 and 293 mg/kg bw/day, and male weight was reduced at 293 mg/kg bw/day. Major malformations observed included cardiovascular malformation (major vessels and heart), axial and appendicular skeleton defects, open eye, and exencephaly. The NOAEL for developmental toxicity was 44 mg/kg bw/day.

Tyl et al. (*149, 157*) (WEB Table 11) fed Fischer 344 rats a diet containing 0, 0.5, 1.0, 1.5, and 2.0% DEHP (0, 357, 666, 856, and 1,055 mg/kg bw/day; estimated by authors). Dosing began on day 0 of gestation and continued to gd 20. Group sizes ranged from 24 to 25. Maternal body weights and food and water consumption were recorded throughout the treatment period. At sacrifice on gd 21, information on liver and uterine weights, number of corpora lutea, and fetal viability, growth, and morphology was obtained.

Maternal weight gain and corrected maternal weight gain were reduced during gestation at 666, 856, and 1,055 mg/kg bw/day. Piloerection and rough fur coat were also seen at these three doses. Food consumption (g/kg bw/day) was lower in all treated groups. In contrast, water consumption was greater in the 3 lowest dose groups (357, 666, and 856 mg/kg bw/day). Liver weights were higher in all treatment groups.

The percentages of resorptions, nonlive, and affected (nonlive plus malformed) conceptions were increased in a dose-related manner and were significantly different from controls at 1,055 mg/kg bw/day. The ability of DEHP to induce embryolethality increased abruptly from the control level of about 4 to 57% for doses between 856 and 1,055 mg/kg bw/day. Fetal body weight was significantly reduced at the three highest dose levels in both sexes, but was significantly higher than control at the low dose. A similar enhancement of fetal weight was seen at the lowest dose in a mouse study (*149, 185*) using a similar protocol. There was a significant dose related linear trend for the percentage of malformed fetuses per litter (1.27, 0, 1.92, 3.13, and 2.87%), but no pairwise comparison was significant. Treatment-related variations or other signs of developmental delay were not observed.

Price et al. (*156*) (WEB Table 13) administered 0, 0.25, 0.5, or 1.0% (0, 164, 313, or 573 mg/kg bw/day; estimated by author) in the diet to Fischer 344 rats from gd 0 to 20. The design is similar to Tyl et al. (*149, 157*), except that postnatal growth and development of the offspring were evaluated after normal delivery. Food consumption during the treatment period was reduced in the two highest dose groups, and maternal weight gain was reduced at the highest dose. Post-implantation mortality was significantly increased at 313 mg/kg bw/day; a similar increase was noted at the high dose, but was not significantly different from control. On postnatal day (pnd) 1, average live litter size and average pup body weight were decreased in a dose-related manner. With increasing dose, litter sizes at birth were 9.47, 9.30, 8.18, and 8.0, while average pup weights were 4.91, 4.86, 4.75, and 4.52 g. Both measures were significantly reduced at the high dose. Pup body weights in the high-dose group were not different from controls by pnd 4. There were no other effects on postnatal growth, viability, age at acquisition of developmental landmarks, and levels of spontaneous locomotor activity on pnd 21, 35 and/or 38, or 55. The NOAEL[1] for developmental toxicity was 164 mg/kg bw/day. The F_1 rats were mated and results of the study are discussed in Section 4.

Price et al. (*146*) (WEB Table 14) conducted a study in CD-1 mice of the postnatal effects of prenatal exposure to DEHP. The design is similar to Tyl et al. (*149, 185*) except that developmental toxicity was evaluated postnatally rather than at the end of pregnancy. Pregnant mice, 28–29 per group, were fed a diet containing 0, 0.01, 0.025, or 0.05% DEHP, resulting in mean doses of 0, 19, 48, or 95 mg/kg bw/day (estimated by authors), respectively, from gd 0 to 17. Dams were evaluated for maternal toxicity and their offspring were evaluated for viability, growth, and development. The F_1 pups were then mated within dose groups and their F_2 offspring were evaluated for viability and growth through pnd 4.

There were no observed adverse effects on the (F_0) females during pregnancy. The only indication of a maternal DEHP effect was a trend for decreasing body weight gain on pnd 4 and 7 that was not significant in a pairwise comparison with controls. For F_1 litters the percent prenatal mortality was significantly increased at the 95 mg/kg bw/day dose with a concomitant decrease in live litter size at pnd 1. On pnd 4, the percentage of viable pups was also decreased in litters exposed to 95 mg/kg bw/day. No other effects of DEHP upon growth, viability, age at acquisition of landmarks, or spontaneous locomotor activity were seen in any dose groups on pnd 14, 21, or 50. The NOAEL for developmental toxicity was 48 mg/kg bw/day and the maternal NOAEL was 95 mg/kg bw/day. No adverse effects were consistently observed upon the reproductive performance of the F_1 generation or the F_2 offspring through pnd 4.

Some developmental effects (litter size, reproduction, maturation) have been observed in reproductive studies (*168, 169, 186*). The studies and developmental effects will be briefly discussed in this section. Specific details of the studies can be found in the reproductive toxicity section.

In a continuous breeding study, 18–40 mating pairs of CD-1 mice were exposed to DEHP through diet at concentrations of 0, 14, 141, and 425 mg/kg bw/day for 98 days (*168, 186*) (WEB Table 15). Litters born during the breeding period were evaluated and sacrificed so the pairs could continue mating. Evaluation of developmental effects was complicated by complete infertility in the 425 mg/kg bw/day group and infertility in 15/19 breeding pairs in the 141 mg/kg bw/day group. However, it was noted that the number of live pups/litter was significantly reduced in the 141 mg/kg bw/day group relative to controls (n=5 vs 11). No effects were observed in the 14 mg/kg bw/day group.

In a dose-setting assay for a two generation reproductive toxicity assay, 9–10 male and female Wistar rats were exposed to DEHP in feed at concentrations of 0, 100, 339, and 1,060 mg/kg bw/day for 70 days prior to mating until the end of the lactation period (*169*) (WEB Table 16). Postnatal pup survival was reduced in all treatment groups. A loss of spermatocytes was observed in 2/10 and 7/9 pups examined in the 339 and 1,060 mg/kg bw/day groups, respectively. Additional effects observed in the high-dose group included a decrease in live litter size (n=9.9 vs 15.1), decreased weight gain in pups, a transiently increased number of male pups with areolas and nipples (84 vs 0%), and increased time for vaginal opening (3 days) in female pups and preputial separation (4 days) in male pups. Authors indicated that the delay in vaginal opening was most likely due to decreased body weights in female pups. Decreased weight gain in dams of the 1,060 mg/kg bw/day group was accompanied by decreased food intake.

Schilling et al. (*169*) continued to expose the F_1 pups to the same DEHP concentrations as their parents. When sexually mature, 7–10 pairs of F_1 rats/group were mated within dose groups. A decrease in live litter size was observed and the anogenital distance was reduced by 13% in the male F_2 offspring. On pnd 2, the F_1 males were sacrificed and organs were weighed and examined histologically. Liver to body weight ratio was increased while testes to body weight ratio and absolute epididymis weight were decreased. There was some microscopic evidence of testes damage only at the high dose (1,060 mg/kg bw/day).

Arcadi et al. (*161*) (WEB Table 17) studied the effects of DEHP on pre- and postnatal development in Long-Evans rats. Twelve rats/group were exposed to DEHP through drinking water at 0, 32.5, or 325 µL/L throughout the gestation and lactation period with exposure ending on pnd 21. Although drinking water intake was not precisely measured, the authors estimated exposure concentrations of 0, 3.0–3.5, and 30–35 mg/kg/day during gestation, with an approximate 30% increase during lactation as a result of increased water intake. Plasma DEHP levels were measured in 7–8 dams/group on pnd 21. Liver, kidney, and testes were weighed and examined histologically in 8 male pups/group (1 pup/8 litters) on pnd 21, 28, 35, 42, and 56. On pnd 30, neurobehavioral function was assessed in female pups by observing their ability to walk on a beam in order to avoid negative stimuli.

Treated dams experienced no changes in body weight gain or appearance. On pnd 21, plasma levels of 1.417±0.21 and 0.197±0.031 µg/mL DEHP were measured in dams exposed to 30–35 and 3.0–3.5 mg/kg bw/day, respectively. A plasma level of 0.496±0.063 µg/mL DEHP was measured in pups from the high-dose group on pnd 21, but DEHP was not detected in pups at any other time point. On each sacrifice day, a reduction in testes to body weight ratio in both dose groups was accompanied by histopathological effects in all high-dose rats and 6–8 low-dose rats. Histological effects consisted of disorganized tubular epithelium with detachment of the spermatogonial cells from the basal membrane. Spermatogenesis in the high-dose group was disrupted throughout the treatment period. Liver to body weight ratio was significantly increased in both treatment groups and was accompanied by subendothelial edema of the

centrilobular vein and portal spaces with mild cellular infiltration in approximately half the rats in each treatment group at ages of 4-weeks and younger. The incidence of hepatic histopathology continued to drop in pups killed at later time periods and was no longer observed in pups killed at 8 weeks of age. Kidney to body weight ratio was significantly reduced in pups from the high-dose group only between the ages of 3–5 weeks. Renal effects in both treated groups included glomerulonephritis and dilation of renal tubuli in all rats of the high-dose group and about half the rats in the low-dose group. Light fibrosis of the kidneys was observed in all treated rats. By 6 weeks of age, there were no signs of glomerulonephritis in the low-dose group and the incidence of glomerulonephritis in the high-dose group and dilated renal tubules in both groups dropped to 1–2/8 pups per treatment group. Renal fibrosis was observed in the majority of treated rats up to 8 weeks of age. In the neurobehavioral testing, female pups from the high-dose group walked across a beam at a slower rate than control animals. The study by Arcadi et al. (*161*) is limited due to uncertainty of dose levels resulting from a lack of measurement of DEHP levels in drinking water and drinking water intake. The authors were contacted, but this issue was not resolved.

Administration by gavage

Huntingdon Life Sciences (*150*) conducted a prenatal developmental toxicity study of DEHP in CD-1 mice (WEB Table 12). On gd 6–15, mice were gavaged with DEHP at 0, 40, 200, or 1,000 mg/kg bw/day. A control group of 30 mice was gavaged with vehicle (carboxymethylcellulose with 0.1% Tween 80). Clinical signs were evaluated daily and maternal body weight was measured on gd 0, 2, 6, 9,12, 15, and 17. At sacrifice on gd 17, each treatment group contained 13–14 animals. Maternal reproductive organs were evaluated for corpora lutea, implantation sites, and resorption sites. Uteri from non-pregnant animals were stained to check for implantation sites. Fetuses were weighed, sexed, and evaluated for external malformations. One half of the fetuses were examined for visceral malformations and the other half were examined for skeletal malformations.

Evidence of maternal toxicity in the 1,000 mg/kg bw/day group included a significant decrease in weight gain. Authors reported reduced food intake on gd 15–16, but the results were not statistically significant. A significant increase in maternal liver to body weight ratio was also observed in the 1,000 mg/kg bw/day group. Fetal toxicity included significant decreases in live fetuses which were apparently caused by significant increases in early and late resorptions and post-implantation losses. The incidence of malformations and variations was not statistically analyzed, but the authors did note some dose-related increases in the incidence of certain defects in the 200 and 1,000 mg/kg bw/day groups. Vascular defects, dilated sinuses, and intramuscular nasal hemorrhage were noted in the 200 mg/kg bw/day treatment group. In the 1,000 mg/kg bw/day group, external malformations included cleft palate and ablepharon (missing eyelids). Visceral malformations or variations included defects in hearts, blood vessels, lungs, kidneys, ureters, and retinas. Skeletal variations or malformations included incomplete ossification, abnormalities in cranial sutures, extra or fused ribs, and missing, fused, or malformed vertebrae. A maternal NOAEL of 200 mg/kg bw/day and developmental NOAEL of 40 mg/kg bw/day were selected by the study authors.

Hellwig et al. (*158*) (WEB Table 18) evaluated the prenatal development toxicity of DEHP in Wistar rats. On gd 6–15, rats were gavaged with DEHP at 0, 40, 200, or 1,000 mg/kg bw/day. Control rats were administered the olive oil vehicle. Maternal body weight was measured on gd 0, 6, 10, 15, and 20. At sacrifice on gd 20, each group contained 9–10 animals. Maternal uteri were weighed and corpora lutea and implantation sites were counted. Fetuses were weighed and evaluated for external malformations. Half of the fetuses were examined for visceral malformations and the other half were examined for skeletal malformations.

Maternal effects were evidenced by vaginal hemorrhage in 2/9 dams in the 1,000 mg/kg bw/day group. Significant increases in the liver and kidney to body weight ratios, decrease in uterine weight, and reduction in body weight gain from gd 15 to 20 were also observed in the 1,000 mg/kg bw/day group.

Early resorptions were significantly increased in the 1,000 mg/kg bw/day group as a result of increased postimplantation loss. Fetal body weights in the 1,000 mg/kg bw/day group were significantly lower. The number of fetuses per litter with external, soft tissue, and skeletal malformations was significantly increased in the 1,000 mg/kg bw/day group and 70% of fetuses per litter were malformed. The types of malformations that appeared to be dose-related included defects of the tail, brain, urinary tract, sex organs, vertebrae, and sternum. Malformations in sex organs included hypoplasia of uterine horns and abnormal position of ovaries or testes. The incidence of soft tissue variations and skeletal retardations was also significantly increased in the 1,000 mg/kg bw/day group.

In an abstract by Parks et al. (*187*), Sprague-Dawley rats were dosed by gavage with 750 mg/kg bw/day of BBP, DEHP, or corn oil (vehicle) from gd 14 through pnd 3. On pnd 2, anogenital distance (AGD), and testis weight were measured. Testes weights and AGD were significantly decreased and the incidence of retained areolae on pnd 13 were increased for both DEHP- and BBP-exposed male pups. Testicular testosterone production was evaluated *in vitro* from male fetuses/pups on gd 17, 18, and 19, and pnd 2; it was significantly reduced on gd 18 and 19 (maximal) and on pnd 2 for DEHP, but not for BBP. The antiandrogenic effects observed in male rats from perinatal exposure to DEHP (and other phthalates) may be due to reduced androgen production in fetal Leydig cells; the authors suggest that testes is the target organ for perinatal phthalate exposure. It is not yet known whether these effects are mediated via direct action of phthalates on the fetal Leydig cell or through alterations of Sertoli cell paracrine secretions.

Narotsky and Kavlock (*160*) (WEB Table 19) conducted a prenatal toxicity screen of DEHP in Fischer 344 rats. On gd 6–19, 9–13 rats were gavaged with DEHP in corn oil at concentrations of 0, 1,125, or 1,500 mg/kg bw/day. Control rats were administered the corn oil vehicle. Maternal body weight was measured on gd 6, 8, 10, 13, 16, and 20. Dams were allowed to litter and pups were counted, weighed, and examined on pnd 1 and 6. Dams and pups were sacrificed on pnd 6. Pups were examined for visceral abnormalities and dams were examined for implantation sites. The uteri of females that did not produce a litter were stained with 10% ammonium sulfide and examined for resorptions.

Parturition was delayed in both treated groups and the delay was significant at the lower dose. Both groups of treated dams experienced a weight loss (~5 g) and severe weight loss occurred in one dam of the high-dose group. Piloerection was observed primarily in the late stages of pregnancy. Most treated dams experienced vaginal bleeding or there were no indications that delivery occurred. During necropsy it was noted that deliveries had occurred in most dams although pups were never observed. Prenatal loss occurred at a rate of approximately 100 and 98% in the low- and high-dose group, respectively. By pnd 6, there were no surviving pups. A few dead pups were available for examination, but the number available was not specified. Observed pup defects included anophthomalia or microphthalmia in two low-dose pups from the same litter, missing or short innominate arteries in four low-dose pups from two litters, and cleft palate and renal agenesis in one high-dose pup.

The developmental toxicity screen was repeated at doses of 0, 333, 500, 750, or 1,125 mg/kg/day that were administered on gd 6–15 (*159*) (WEB Table 19). All other aspects of the experiment were the same as those described in Narotsky and Kavlock (*160*). There were no signs of maternal toxicity, but dams in the 750 and 1,125 mg/kg bw/day groups experienced unspecified delays in parturition. In pups, an increased incidence of anophthomalia and microphthalmia was observed at the 750 and 1,125 mg/kg bw/day groups (2.8 and 4.3% of pups affected, respectively). The total number of pups and litters examined was not stated.

Yagi et al. (*155*), Nakamura et al. (*151*), and Tomita et al. (*154*) (WEB Table 20) evaluated the developmental effects of DEHP in ddY-Slc mice. DEHP was administered by gavage in doses correlating to 1/30, 1/12, 1/6, 1/4, 1/3, or 1/1 of the acute oral LD_{50} (30mL/kg bw) (986, 2,465, 4,930, 7,395, 9,860,

or 29,580 mg/kg bw/day) on gd 6, 7, 8, 9, or 10 to groups of 3–8 pregnant dams. Olive oil was administered to a control group of four pregnant mice on gd 7 only. In a separate experiment, a single dose of 0, 0.05, 0.1, or 1.0 mL/kg DEHP (0, 49.3, 98.6, or 986 mg/kg) was administered orally to groups of 10–31 pregnant dams on gd 7, but treatment of control animals was not described. Dam body weight was measured daily. All animals were sacrificed on gd 18. Maternal corpora lutea were counted. Fetuses were examined for gross external and skeletal malformations.

Decreased maternal weight gain was observed in mice receiving doses of 4,930–29,580 mg/kg bw/day on gd 7–10. A significant decrease in fetal body weight was observed in all treatment groups. Some fetal mortality and malformations were observed in groups treated with 986 mg/kg bw/day and higher. The highest incidence was observed in those groups treated on gd 7 and 8. No skeletal malformations were observed in the dose groups treated on gd 10, and a few (16.7%) were observed at the highest dose only (29,580 mg/kg bw/day) on gd 9.

Shiota et al. (*147, 148*) (WEB Table 21) examined the developmental effects of DEHP in ICR-JCL mice. DEHP was administered in the diet at levels of 0, 0.05, 0.1, 0.2, 0.4, and 1.0%. Reported intakes for these diets were 0, 70, 190, 400, 830, and 2,200 mg/kg bw/day. There were 7–24 females per dose group, treated from day 0 to day 18 of pregnancy. Body weights and food and water consumption were recorded daily. On gd 18, females were sacrificed by cervical dislocation and the uterus was removed and examined. Live fetuses were weighed. Subsequently, half of the live fetuses were examined for skeletal morphology and half for internal soft tissue morphology.

The weight gain of pregnant females receiving 400, 830, and 2,200 mg/kg bw/day in the diet was significantly reduced during gestation. No other maternal information was reported. All fetuses from females given 830 and 2,200 mg/kg bw/day DEHP died. At 0, 70, and 190 mg/kg bw/day, the mortality rates were 5.0, 7.5, and 31% respectively. At the 400 mg/kg bw/day dose level, prenatal mortality rates of 83 and 68% were observed in the 1980 and 1982 studies, respectively. Due to the high incidence of mortality, there were few live fetuses at term in the 400 mg/kg bw/day DEHP group. Fetal body weights were significantly reduced at 400 mg/kg bw/day DEHP. The incidence of malformed fetuses was 0, 0, and 5.3% at 0, 70, and 190 mg/kg bw/day, respectively. In the 400 mg/kg bw/day dose group, 41 and 26% of the pups had malformations in the 1980 and 1982 studies, respectively. Malformations included exencephaly, spina bifida, malformed tail, gastroschisis, club foot, and open eyelids. The LOAEL for developmental toxicity was 70 mg/kg bw/day. The interpretation of this study is limited by the small numbers of litters examined in each dose group.

Shiota and Mima (*153*) (WEB Table 22) evaluated the developmental toxicity of DEHP and MEHP administered orally or intraperitoneally to ICR mice. The agents were dissolved in olive oil and given by stomach tube or IP injections on gd 7, 8, and 9 in a volume of 0.5 mL/100 g bw. Oral dosages of DEHP were 0, 250, 500, 1,000, and 2,000 mg/kg bw/day; IP doses were 0, 500, 1,000, 2,000, 4,000, and 8,000 mg/kg bw/day. Group sizes for orally-administered and IP doses ranged from 9 to 11 and 3 to 9 pregnant females, respectively.

Oral doses of 2,000 mg/kg bw/day induced a low incidence of lethality and full litter loss in almost all female ICR mice. The NOAEL for maternal toxicity was reported to be 1,000 mg/kg bw/day. Oral doses of DEHP greater than 500 mg/kg bw/day produced clear indications of developmental toxicity (embryonic death, fetal growth retardation, and multiple major malformations). There were indications of increased malformations at the 250 and 500 mg/kg bw/day dose levels as well, suggesting the lack of a clear NOAEL for developmental toxicity in this study. Following IP administration of DEHP, full litter loss was seen at 8,000 mg/kg bw/day in 2/3 dams. There was no significant increase in malformations following ip exposure.

Ritter et al. (*166*) (WEB Table 23) compared the developmental toxicity caused by treatment of rats with either DEHP, 2-EH , or 2-EHA. Pregnant Wistar rats (7–10 per dose group) received a single gavage dose on gd 12 of either 12.5 or 25 mmol/kg bw (4,882 or 9,764 mg/kg bw) DEHP, 6.25 or 12.5 mmol/kg 2-EH (814 or 1,628 mg 2-EH/kg bw), or 6.25 or 12.5 mmol/kg of 2-EHA (902 or 1,803 mg 2-EHA/kg bw). Dams were sacrificed on gd 20. Fetuses were weighed, counted, and examined for gross external, visceral, and skeletal malformations. Multiple manifestations of developmental toxicity were seen on gd 20, including apparent growth inhibition, decreased viability (as described in WEB Table 23), and increased incidence of malformations involving several organ systems. No information on maternal response was provided. Defects caused by all three agents were similar. Malformations frequently observed included hydronephrosis, levocardia, septal defects, short and kinky tails, ectrodactyly, misplaced digits, and bowed radius. On an equimolar basis, DEHP was the least potent, 2-EH was intermediate, and 2-EHA was the most potent. The results are compatible with the hypothesis that 2-EHA is involved in the teratogenic actions of DEHP.

Srivastava et al. (*111*) (WEB Table 24) investigated the effects of exposure to DEHP during organogenesis on the biochemistry of the fetal liver, including cytochrome c-oxidase (CCO), malate dehydrogenase (MD), succinate dehydrogenase (SD), and adenosine triphosphatase (ATPase). DEHP was dissolved in ground nut oil and administered by gavage to rats on gd 6–15 at a dose level of 1,000 mg/kg bw/day. Retarded fetal growth and an increase in relative fetal liver weight were observed on gd 20. The activities of SD, MD, CCO, and ATPase were decreased in the fetal liver. No mortality or gross behavioral abnormalities were observed in the treated females, nor was there a significant difference in food intake among the treated and control groups. The rate of pregnancy weight gain was decreased in DEHP-exposed animals. There were no gross pathologic changes in major maternal organs. This study provides evidence that prenatal exposure to DEHP can affect the biochemical development of the liver, but the effects are confounded by the marked reductions in maternal body weight gain (15 vs 68 grams in controls) during gestation in the DEHP-treated females, and by the large decrement in fetal body weight in the DEHP group (down about 25% from the control value). Only a single dose level was employed, and therefore little can be said about the dose-response relationship. Overall, the study provides only supplemental information.

Administration by Injection (IV and IP)

Lewandowski et al. (*163*) (*164*) (WEB Table 25) evaluated the teratogenicity of the DEHP plasma extracts of PL-146 and PL-130 (plastics used in the manufacture of blood storage bags) in pregnant rats by administering IV doses during gd 6–15. Test materials were obtained by extraction of PL-146 and PL-130 (cut into 1.5 x 3 cm strips) into rat plasma for 21 days at 4°C. DEHP levels were approximately 185 µg/mL. A lower concentration (50 µg/mL) was prepared by dilution. The DEHP-plasma was injected into rats at a volume of 20 mL/kg daily into the tail vein at a rate of 3 mL/min from gd 6 to 15. Reported doses of PL-130 extracts were 1.3 and 4.7 mg/kg bw/day, while those of PL-146 were 1.4 and 5.3 mg/kg bw/day. The control group received plasma only. There were 25 pregnant females per dose group.

Maternal DEHP-plasma concentrations were measured at 19.9 µg/mL at 3–10 minutes following injection and at 5.4 µg/mL at 24-hours post treatment. DEHP concentrations in urine were below the detection limit. No effects were seen on maternal body weight, embryo/fetal viability, fetal weights, crown-rump length, or morphology. Under these conditions of exposure, plasma-extractable DEHP from two types of plastics did not appear to demonstrate developmental toxicity in rats. No treatment-related anomalies were present. However, the exposure levels (reported < 5.3 mg/kg bw/day) were low relative to doses administered in feeding studies and a comparison of developmental toxicity from the oral versus IV route is therefore not possible. Therefore, the study should be considered only supplemental.

Singh et al. (*167*) (WEB Table 25) administered 5 or 10 mL/kg bw (4,930 and 9,860 mg/kg bw) to groups of 5 Sprague-Dawley rats by IP injections on gd 5, 10, and 15. On gd 20, the females were sacrificed. The following observations were recorded: corpora lutea, viability of implants, fetal weight, and external and skeletal morphology. Maternal toxicity was not evaluated in this study. The percentage implant resorptions were 0, 8.2, and 26.8 at doses of 0, 4,930, and 9,860 mg/kg bw, respectively. A decrease in fetal weights was observed at both treatment levels. Gross anomalies were observed only at the 9,860 mg/kg bw dose level (22% versus 0 in both the control and low-dose groups). The lack of information on maternal toxicity and route of exposure limit the utility of this study.

Peters and Cook (*165*) (WEB Table 26) conducted a series of experiments to determine the effects of IP DEHP injection on implantation and pregnancy outcome in Sprague-Dawley rats. These studies used high doses and small group size. In the first experiment, 5 rats/group were injected with saline or 2 or 4 mL/kg DEHP (0, 1,972, or 3,944 mg/kg bw/day) on gd 3, 6, and 9. Dams were allowed to litter and wean pups. Implantation sites were examined in dams that died or delivered dead pups. At the 3,944 mg/kg bw/day dose, implantation occurred in only one dam that experienced vaginal bleeding and died during delivery. Implantation occurred in only 2 dams of the 1,972 mg/kg bw/day group. One dam experienced vaginal bleeding and died during delivery. The number of pups weaned by the surviving dam was much lower than the average control number (n=4 vs 10).

In the second experiment, Peters and Cook (*165*) injected dams intraperitoneally with saline or 2 mL/kg DEHP (0 or 1,972 mg/kg bw/day) on gd 1, 3, 6, 9, or 3 and 6, or 6 and 9, or 3, 6, and 9. All other details were the same as the first experiment. The number of dams that died or were sacrificed during delivery were 2, 5, 1, and 3 in the groups injected on days 6; 9; 6 and 9; and 3, 6 and 9, respectively. Fetal retention was noted in all dams that died or were sacrificed during delivery. The numbers of dams with implantations were reduced in all groups injected prior to day 6 with the exception of the day 1 group. Average number of live pups and pups weaned was reduced in surviving dams of all treated groups except for the day 6 and 9 group. However, the description in the text for the day 6 and 9 group appears to conflict with the information outlined in a table.

Lastly, Peters and Cook (*165*) mated an unspecified number of female offspring from the second experiment and allowed them to deliver their litters. Females from treated dams experienced no significant differences in average litter size. No other reproductive parameters were evaluated. The utility of these studies for risk assessment purposes is limited due to small group size, relevancy of exposure route, few dose levels, and insufficient information provided.

Administration by Inhalation

Merkle et al. (*162*) (WEB Table 27) studied the effects of DEHP administered via inhalation to Wistar rats. Ultrafine particles (mass median aerodynamic diameter less than 1.2 µm) were separated by glass cyclone and fed to a head/nose inhalation system. Rats had a 5-day acclimatization period in the chamber prior to addition of DEHP. Females were exposed for 6 hours/day on gd 6–15. There were 25 females per dose group. Dose levels were 0, 0.01, 0.05, and 0.3 mg DEHP/L (0, 2.8, 14, 84 mg/kg bw/day**)**. The authors stated that the high dose was chosen on the basis of a pilot experiment (two rats per dose) which revealed moderate-to-marked hepatic peroxisome proliferation at 0.5 and 1.0 mg DEHP/L (140 and 280 mg/kg bw/day, estimated by CERHR). On gd 20, twenty females per group were sacrificed and evaluated for gross pathology, uterine weights, implantation status, as well as fetal and placental weights, and fetal visceral and skeletal morphology. The remaining 5 females per group were allowed to give birth and their offspring were followed for viability and reflex development for 21 days.

In the rats sacrificed on gd 20, no differences were noted in body weight, weight gain, clinical signs, number of corpora lutea, or uterine weight. There was a significant reduction in live litter size in the 14 mg/kg bw/day dose group, but not in the 84 mg/kg bw/day group. There was no effect on fetal weight. A slight, but significant, increase in the incidence of retarded development (mostly renal pelves dilations) was seen at the high dose.

In the postnatal component of the study, no effect on offspring viability or physical and reflex development (auricle opening on day 4, eye opening on day 16, righting test on day 6, gripping reflex on day 13, and hearing test on day 21) was noted. A significant decrease in dam body weight was recorded on postpartum day 21; offspring body weights were not recorded.

This study has several limitations. No maternal toxicity was indicated by the indices in the study. However, neither liver weight nor histology were evaluated in the main study. There was evidence for mild developmental retardation (hydroureter, dilated renal pelves) at the high (84 mg/kg bw/day) concentration. However, the authors did not consider this effect to be treatment related, citing evidence of similar and greater incidence in historical control groups of Wistar rats. Further developmental retardation was not reflected in body weight or skeletal ossification data. Unfortunately, the reproductive tract was not examined in the pups in the postnatal portion of the Merkle et al. (*162*) study. The potential toxicity by inhalation should be studied further, since if the developmental retardation observed is reproducible, the NOAEL would be lower than that reported for any other rat developmental toxicity study despite the lack of DEHP hydrolysis in the GI tract.

Mechanism

Peters et al. (*152*) (WEB Table 28) evaluated mechanisms of DEHP-induced developmental toxicity in F_4 C57BL/6N X Sv/129 wild-type mice, and peroxisome proliferator-activated receptor-α (PPAR-alpha)-null type mice. PPAR-alpha mediates responses associated with peroxisomal proliferators and those responses are lacking in null-type mice. Groups of 20–28 mice/genotype were gavaged with either corn oil or 1,000 mg/kg bw DEHP in corn oil on gd 8–9. Maternal body weights were measured on gd 0, 8, 9,10, and 18. Half the dams were sacrificed on gd 10 and the other half on gd 18. Maternal livers were weighed, and liver mRNA and zinc analysis were conducted on gd 10. Implantation sites were examined on gd 10 and 18. Fetuses were examined for visible heartbeat, neural tube defects, and zinc levels on gd 10, and weighed and observed for gross external malformations on gd 18.

Transcription of CYP4A1 (P-450 4A1) mRNA, which is known to increase in response to peroxisome proliferation, was significantly increased in treated wild-type mice, but unaffected in treated PPAR-alpha-null mice. Body weight gain was significantly reduced in treated wild- and null-type dams sacrificed on gd 18, but effects on food intake were not discussed. Liver to body weight ratios were significantly increased in treated wild- and null-type dams. Hepatic levels of zinc and metallothionein, a compound which sequesters zinc in the liver, were significantly increased in treated dams of both genotypes. However, there was no effect on zinc levels in plasma. Significantly decreased levels of zinc were measured in fetuses of treated dams of both genotypes. Additional effects observed in fetuses of both groups of treated dams included significant increases in resorptions (gd 18), reductions in live fetuses (gd 10 and 18), and in fetal crown-rump length (gd 10) and weight. Teratogenic effects included significant increases in neural tube defects and external malformations that consisted primarily of exencephaly in fetuses of both genotypes of treated rats. Soft tissue and skeletal malformations were not evaluated. The authors concluded that DEHP-induced developmental toxicity and alterations in zinc metabolism are not mediated through PPAR-alpha. Limitations of the study included the short duration of exposure (gd 8–9) and the lack of soft tissue and skeletal defect evaluation.

Although Peters et al. (*152*) found DEHP-induced developmental toxicity in the PPAR-alpha knock out mouse, recent studies indicate that rat embryos express three PPAR isotypes (alpha, beta or delta, and gamma), (*188, 189*). MEHP, but not 2-EHA, has recently been found to activate PPAR -gamma in an *in vitro* assay (*190*). Further, valproic acid, a structural isomer of 2-EHA, was found to activate PPAR-delta *in vitro* (*191*). Involvement of PPAR with retinoid X receptor (RXR) heterodimers suggests that interesting, development-specific roles for embryonic PPAR may be discovered (*192, 193*). It may be premature to rule out a role of PPAR activation in DEHP-induced toxicity to the rodent embryo

Embryo Culture

In vitro embryo culture studies provide limited information relevant to DEHP developmental toxicity. Hansen and Grafton (*194*) used DEHP at concentrations of 0–2% culture medium and found effects on embryonic growth and development at and above 0.5%. This study used rat embryos and the scoring methods of Brown and Fabro (*195*).

3.2.2 MEHP

Studies are ordered by route of administration: administration in diet is reviewed first followed by repeated or single gavage studies. Several of the studies compared DEHP and MEHP. The Price et al. (*171*) diet administration study was coordinated with the Tyl et al. (*149, 185*) study of DEHP. There was one study which used IV administration (*172*).

Price et al. (*171*) (WEB Table 29) evaluated the developmental toxicity of MEHP in CD-1 mice fed a diet containing the chemical on gd 0–17. Groups of 25–27 mice received doses of 0, 0.017, 0.035, 0.07, or 0.14% MEHP in feed. Average doses were reported as 0, 35, 73, 134, or 269 mg/kg bw/day MEHP. Doses were selected to be approximate molar equivalents to the DEHP doses studied by Tyl et al. (*149, 185*) in the same mouse strain using a similar protocol. Maternal body weights and food and water consumption were recorded throughout the treatment period. At scheduled sacrifice on gd 17, the numbers of resorptions and dead or live fetuses were recorded. All fetuses were weighed, and live fetuses were sexed and examined for external, visceral, and skeletal malformations.

MEHP-exposed females exhibited no clinical signs of maternal toxicity, and food and water consumption were similar to those of controls. There was a decrease in the adjusted body weight gain of mice in the 269 mg/kg bw/day dose group. The relative liver weights of mice fed a diet containing 134 and 269 mg/kg bw/day increased. The maternal NOAEL was stated to be 134 mg/kg bw/day.

The percent litters with resorptions increased at all dose levels. The numerical values increased in a dose-related manner, reaching 77% in the high-dose group. A significant linear decrease in average number of live fetuses per litter was observed with increasing dose level; values for the 73, 134, and 269 mg/kg bw/day dose groups were significantly different from controls by pairwise comparison. Fetal malformations were observed in a significantly higher percentage of litters at dose levels of 73 mg/kg bw/day and greater, and in a significantly higher percentage of fetuses at doses of 134 mg/kg bw/day and higher. MEHP exposure was associated with an increase in skeletal and visceral malformations, with the latter increase primarily due to cardiovascular malformations. A NOAEL for developmental toxicity was not observed in this study. The LOAEL (based on incidence of litters with resorptions) was 35 mg/kg bw/day MEHP. The Panel has high confidence in the quality of this study and its ability to identify the developmental LOAEL for oral exposure.

The authors compared these data with the results of Tyl et al. (*149, 185*) and concluded that maternal and developmental effects of MEHP and DEHP exposure were qualitatively similar at approximately

equimolar doses administered under comparable experimental conditions. For example, well-defined developmental toxicity was observed at 0.23 and 0.26 mmol/kg bw/day for DEHP and MEHP, respectively. Both chemicals produced an increased incidence of prenatal mortality and malformations. However, apparent differences in the quantitative responses to the two chemicals were observed for specific endpoints. The data suggest that MEHP is more effective in producing prenatal mortality whereas DEHP produces greater fetal growth retardation and malformations.

The potential teratogenicity of MEHP was investigated in the Wistar rat as part of a larger experiment (*170*) (WEB Table 30). Two experiments were conducted, the first with 4 groups of 15 animals, receiving doses of 0, 225, 450, and 900 mg/kg bw/day on gd 6–15 by gavage. Due to excessive maternal lethality, the experiment was repeated with 4 groups, 15 rats per group, receiving doses of 0, 50, 100, or 200 mg/kg bw/day. On gd 22, females were sacrificed. Maternal deciduomas were counted and adjusted gestational weight gain was measured. Fetuses were counted and litter weight determined. Litters were divided and fetuses were examined for skeletal (2/3) or visceral (1/3) malformations.

Maternal lethality occurred at doses greater than or equal to 225 mg/kg bw/day. Reduced maternal body weight gain at doses of 100 mg/kg bw/day or greater occurred, although the dose-response pattern was relatively flat for this endpoint and statistical significance was not reached for most doses. The only indications of developmental toxicity were full litter resorptions and reduced fetal weight at 450 mg/kg bw/day. Due to sparse presentation of data, including no presentation of fetal morphology data, this paper was considered to provide only supplemental information. A maternal NOAEL of 50 mg/kg bw/day and a development NOAEL of 225 mg/kg bw/day were identified.

Shiota and Mima (*153*) (WEB Table 31) evaluated the developmental toxicity of DEHP and MEHP administered orally or intraperitoneally to ICR mice. The agents were dissolved in olive oil and given by stomach tube or intraperitoneal injections on gd 7, 8, and 9 in a volume of 0.5 mL/100 g bw. MEHP was administered orally at doses of 0, 50, 100, 200, and 400 mg/kg bw/day and intraperitoneally at 0, 50, 100, and 200 mg/kg bw/day. Group sizes for MEHP doses ranged from 6 to 13 for oral doses and 9 to 14 for intraperitoneally administered doses.

Oral administration of 200 or 400 mg/kg bw/day MEHP on gd 7–9 induced maternal lethality. Full litter resorptions were seen at doses as low as 100 mg/kg bw/day in 3/12 dams, making 50 mg/kg bw/day the NOAEL for maternal toxicity as reported in this study. No effects on embryo/fetal viability or fetal weight were observed. Collectively, three fetuses in MEHP litters had cleft palate, suggestive of an adverse effect, but the presentation was pooled over all treated groups (the authors stated there was no evidence of a dose-response relationship). Following IP administration of MEHP on gd 7–9, maternal lethality was seen at 100 and 200 mg/kg bw/day with complete maternal mortality at the highest dose (NOAEL = 50 mg/kg bw/day). Abortion was observed in 4/12 females in the 100 mg/kg bw/day. There were no indications of developmental toxicity in surviving litters.

This paper has several severe limitations for use in risk assessment including its small sample size, limited maternal and fetal evaluation, and lack of detail in reporting. For DEHP, IP exposure was less toxic to the females than oral exposure. The reverse was true for MEHP exposure. This may be because a significant degree of hydrolysis to MEHP occurred in the gastrointestinal tract following oral dosing. It is also apparent that MEHP was more toxic than DEHP when administered orally to the female by approximately an order of magnitude.

The embryotoxicity and fetotoxicity of DEHP and MEHP were evaluated in ddY-Slc(SPF) x CBA(SPF) hybrid mice (*155*) (WEB Table 32). DEHP was given by oral intubation (10 mL/kg) at doses between 0.05 and 30 mL/kg bw (493 and 29,580 mg/kg bw); these were fractions of the LD_{50} ranging from 1/60 to 1/1. This was not a full factorial dose experiment. There were 3–8 females per dose group, except in the

"Low Dose DEHP," where group sizes ranged from 10 to31 females. For MEHP, the dose groups were 0.1, 0.5, and 1.0 mL/kg bw (104, 520, and 1,040 mg/kg bw/day) (1/15, 5/15, or 10/15 of the estimated LD_{50}) on days 7, 8, or 9. Again, there was not a complete block design (2 dose levels were used on day 7, 3 on day 8, and 1 on day 9). Olive oil was used as the vehicle for MEHP and was given to control females only on day 7. Group sizes for MEHP were 2–8 females. Maternal body weights were recorded daily during gestation, but the data were only partially summarized. Embryo/fetal viability, growth, and gross and skeletal abnormalities were recorded at term.

The authors reported the same toxic effects on the fetus with a smaller dose of MEHP than DEHP when given on gd 8, suggesting a role for MEHP in the developmental toxicity of DEHP. Adverse developmental effects included increased fetal mortality, decreased fetal weight, and increased abnormalities. Those effects are outlined in greater detail in Web Table 32.

These studies involved very small sample sizes, used quite wide-spread dose separations, and did not report the data in great detail. The results support other studies in terms of the types of effects noted, but are considered supplemental studies for the purposes of risk assessment.

Thomas et al. (*172*) (WEB Table 33) studied groups of 11 New Zealand white rabbits which received bolus IV doses of MEHP dissolved in 0.9% saline in volumes ranging from 0.5 to 0.9 mL on gd 6–18. Controls consisted of 11 sham and 11 saline-injected dams. Dose levels were reported as 0, 1.14, 5.69, or 11.38 mg/kg bw/day. [Doses were based on the amount that a patient might receive in 1, 5, or 11 units of blood that had been stored in plastic bags.] Fetuses were examined on gd 30 for viability, and gross, visceral, and skeletal morphology. One of eleven saline injected females died during gestation. In the treated groups, 2 of 11, and 4 of 11 females in the mid- and high-dose groups died, respectively. In most cases convulsion occurred before death. Paralysis was seen in 2 dams in the 11.4 mg/kg bw/day group. There were no significant effects on maternal lung, liver, kidney, heart, or adrenal weights in treated females. There were no effects on fetal viability, body weights, or morphology. The apparent acute neurotoxicity of the injection protocol limits the utility of this study.

3.2.3 2-EH

Studies with oral administration are discussed first. EH is volatile and there is one study via inhalation. A study by cutaneous administration is also reviewed. None of the studies were considered adequate for identification of LOAELs and NOAELs.

Oral Administration

Developmental effects following oral intake of 2-EH have been studied in CD-1 Swiss mice (*175*) (WEB Table 34**)**. On gd 0–17, groups of 27 mice were fed diets containing 0, 0.009, 0.03, or 0.09% 2-EH (0, 17, 59, or 191 mg/kg bw/day; estimated author). Because of its volatility, the 2-EH was contained in microcapsules. Dams were weighed approximately every 3 days and were sacrificed on gd 17. Maternal livers and uteri were weighed. Corpora lutea were counted and resorption sites were examined by staining with ammonium sulfide. Fetuses were sexed, weighed, and examined for external, skeletal, and visceral malformations.

There were no signs of maternal toxicity following 2-EH treatment. The only effect in dams was a significant increase in food intake at the highest dose level. 2-EH treatment did not affect implantations, resorptions, fetal growth, or malformations. Because earlier experiments demonstrated maternal and developmental toxicity at approximately equimolar concentrations of DEHP (*149, 185*), the authors

concluded that 2-EH is not essentially involved in toxicity associated with DEHP administration. However, other investigators have demonstrated developmental toxicity following gavage dosing with higher concentrations of 2-EH.

Ritter et al. (*166*) (WEB Table 35) studied the effects of 2-EH on prenatal development in Wistar rats and compared the effects to those produced by DEHP and 2-EHA. Groups of ≥ 7 rats were gavaged with undiluted 2-EH at 0, 6.25, or 12.5 mmoles/kg (0, 814, or 1,629 mg/kg bw) on gd 12. Dams were sacrificed on gd 20 and implantation sites were examined. Fetuses were weighed and observed for viability and external, visceral, and skeletal malformations.

Toxicity in dams was not discussed. Fetal effects observed at both doses included decreased weight and increased malformations. Resorptions were also increased at the lower dose level. The types of malformations observed most frequently included hydronephrosis and defects of the heart, tail, and limbs. Levels of statistical significance were unclear for most fetal observations, but dose-response relationships were evident for effects excluding resorptions. Fetal toxicity was similar to that induced by DEHP and 2-EH. 2-EHA was the most potent compound, followed by 2-EH and DEHP. Use of this study for risk assessment is limited due to lack of statistical analyses.

Hellwig et al. (*196*) (WEB Table 36) evaluated the prenatal development toxicity of 2-EH in Wistar rats. On gd 6–15, 10 rats/group were gavaged with 2-EH in water with 0.005% Cremophor EL at concentrations of 0, 130, 650, or 1,300 mg/kg bw/day. Two groups of ten control rats each were administered water or vehicle. Maternal body weight and food intake were measured daily. At sacrifice on gd 20, maternal uteri were weighed and corpora lutea and implantation sites were counted. Fetuses were weighed and evaluated for external malformations. Half of the fetuses were examined for visceral malformations and the other half were examined for skeletal malformations. The numbers of litters evaluated from control to high-dose group were 19, 10, 9, and 2.

Six of ten dams in the high-dose group (1,300 mg/kg bw/day) died between gd 9 and 13. Clinical signs of toxicity included nasal discharge, salivation, and central nervous system depression. Body weight gain and food intake were reduced. At necropsy, hepatic discoloration and pulmonary edema were observed. For the mid-dose group (650 mg/kg bw/day group), the authors reported that "slight maternal toxicity was visible." Fetal effects included a significant increase in post-implantation loss (54.7 vs. 8.2% in controls) and resorptions (550% increase) in the high-dose group and decreased fetal body weight in the mid- (9.5% decrease) and high-dose (25% decrease) group. Dose-related increases were noted for fetal malformations (0.8–1.4, 2.3, 5.5, and 17.9% in 3/19, 3/10, 4/9, and 2/2 litters at 0, 130, 650, and 1,300 mg/kg bw/day, respectively), variations (31.5–37.1, 31.5, 38.6, and 71.4% in 18/19, 9/10, 8/9, and 2/2 litters at 0, 130, 650, and 1,300 mg/kg bw/day, respectively), and retardations (22.6–26, 23.8, 40.2, and 53.6% in 18/19, 8/10, 9/9, and 2/2 litters at 0, 130, 650, and 1,300 mg/kg bw/day, respectively). The effects were only significant in the high-dose group and, with the exception of increased incidence of dilated renal pelves and anal atresia/acaudia in one rat, only the skeleton was affected. Skeletal defects included malformed, misaligned, or missing vertebrae. Variations such as accessory and rudimentary lumbar and cervical ribs and delayed ossification were also observed. Although not statistically significant in the mid-dose group, the authors reported an increased incidence of fetuses with misformed vertebrae, rudimentary cervical ribs, and delayed ossification.

Administration by Inhalation

Nelson et al. (*173*) (WEB Table 37) evaluated the prenatal development toxicity of 2-EH in Sprague Dawley rats. On gd 1–19, 15 rats/group breathed 0, or 850 mg/m^3 2-EH vapors for 7 hours/day. Using average measured body weights during the course of the experiment (312.5 g) and EPA (*78*) assumptions

for inhalation rates (0.330 m^3/day), a dosage of 262 mg/kg bw/day was calculated. That dosage was the highest that could be obtained while maintaining 2-EH in the vapor phase. Maternal body weight and food intake were measured weekly. At sacrifice on gd 20, maternal uteri were examined for corpora lutea and implantation sites. Fetuses were weighed, sexed, and examined for gross external malformations. Half the fetuses were examined for visceral malformations and the other half for skeletal malformations. Signs of maternal toxicity were not observed and the only effect in dams was an approximately 10–15% reduction in food intake. The only fetal effect described was a small, non-significant delay in ossification.

Dermal Administration

In a prenatal developmental toxicity study, pregnant Fischer 344 rats were exposed to 2-EH by occluded cutaneous application, 6 hours per day on gd 6–15, at doses of 0, 0.3, 1.0, or 3.0 mL/kg bw/day (0, 252, 840, 2,520 mg/kg bw/day; estimated by author) (*174, 197*) (WEB Table 38). There were 25 rats in each dose group with control rats exposed to deionized water. Maternal body weights were recorded on gd 0, 6, 12, 15, 18, and 21. At scheduled sacrifice on gd 21, data were recorded for maternal liver, spleen, adrenals, thymus, and kidney weights, number of corpora lutea, uterine weight (full), number of implantation sites, sex and number of live and dead fetuses, number of early and late resorptions, individual fetal weights, and external abnormalities. Approximately half of the live fetuses were examined for visceral malformations. The other half were evaluated for skeletal abnormalities.

At the 1.0 and 3.0 mL/kg bw/day doses there was a dose-related cellular exfoliation and erythema at the site of application of the chemical. There was also a reduction in weight gain for the period of gd 6–9 in the 3.0 mL/kg bw/day rats. No developmental toxicity was observed at any dose level. Confidence in the study is reduced due to lack of a clearly maternally toxic dose.

3.2.4 2-EHA

Most studies of 2-EHA were conducted by gavage, and a number of these compared 2-EHA to other short chain carboxylic acids, including valproic acid. Drinking water studies with prenatal and postnatal endpoints are available. One mechanism study and two embryo culture studies were also reviewed.

Administration by Gavage

A study was performed to assess the prenatal developmental toxicity potential of 2-EHA in Fischer 344 rats (*182*) (WEB Table 39). 2-EHA, diluted in corn oil, was administered by gavage on gd 6–15 to groups of 25 pregnant rats at doses of 0, 100, 250, or 500 mg/kg bw/day. Maternal body weight and food consumption were recorded at predetermined intervals throughout gestation. Animals were sacrificed on gd 21 and the following were recorded: maternal liver weights, number of corpora lutea, uterine weight (full), number of implantation sites, sex and number of live and dead fetuses, number of early and late resorptions, individual fetal weights, and external abnormalities. Fetuses were examined for visceral or skeletal abnormalities. There were no statistically significant differences between groups for gestational maternal body weight gain or for food consumption.

Treatment-related clinical signs including hypoactivity and ataxia were observed in rats receiving the 500 mg/kg bw/day dose. Fetal body weights per litter were also significantly reduced at this dose. There was consistent evidence of fetotoxicity in the 500 mg/kg bw/day dose group, consisting of skeletal variations in 26/94 fetuses. Fetal skeletal variations exhibited significantly different incidences (19 increases in incidence; 7 reductions in incidence) relative to those in controls. Slight evidence of

fetotoxicity was observed at 250 mg/kg bw/day, consisting of reduced ossification in skeletal districts without other signs of fetotoxicity. No embryotoxicity or teratogenicity was observed at any dose level. The NOAEL for maternal toxicity was given as 250 mg/kg bw/day, the NOAEL for developmental toxicity as 100 mg/kg bw/day.

Timed pregnant New Zealand White rabbits (11/treated group, 22 in control group) were administered 2-EHA to assess its maternal and developmental toxicity (*183*) (WEB Table 40). 2-EHA was given by gavage at doses of 0, 25, 125 and 250 mg/kg bw/day in corn oil at a volume of 2 mL/kg on gd 6–18. Maternal food consumption was measured and clinical observations were taken daily throughout the study. Maternal body weights were recorded at gd 0, 6, 12, 15, 18, and 29. At sacrifice on gd 29, body weight, liver weight, and gravid uterine weight were measured. The number of corpora lutea, implantation sites, resorptions, dead fetuses, and live fetuses were recorded. Live fetuses were examined for external, visceral, and skeletal malformations.

During the study period, one doe died at each dose of 125 and 250 mg/kg bw/day. Maternal toxicity in the form of reduced weight gain was observed at the 250 mg/kg bw/day dose for gd 18–29. There were no effects of treatment on maternal body weight (absolute or corrected), gravid uterine weight, or absolute or relative liver weight. No effects were seen on implantations/litter, sex ratio, or fetal body weights/litter. There was no significant increase in incidence of malformation in any treated group. Administration of 2-EHA resulted in maternal toxicity at 125 and 250 mg/kg bw/day as evidenced by death of 1 doe in each dose group and abortion in 1 doe in the 125 mg/kg bw/day group. There was no embryotoxicity, fetotoxicity, or teratogenicity observed. The NOAEL reported by the author for maternal toxicity was 25 mg/kg bw/day. The NOAEL for developmental toxicity was 250 mg/kg bw/day. Confidence is limited due to the absence of a clearly maternally toxic dose.

Ritter et al. (*166*) (WEB Table 41) compared the developmental effects of 2-EHA with DEHP and 2-EH in rats. Pregnant Wistar rats (7–10 per dose group) received a single gavage dose on gd 12 of 6.25 or 12.5 mmol/kg 2-EHA (902 or 1,803 mg/kg bw). All animals were sacrificed on gd 20. Fetuses were weighed, counted, sexed, and examined for gross external, visceral, and skeletal malformations. No information on maternal response was provided, which limits the ability to interpret the findings of this study. Defects caused by all three agents were similar. Malformations frequently observed included hydronephrosis, levocardia, IV septal defects, short and kinky tails, ectrodactyly, misplaced digits, and bowed radius. On an equimolar basis, 2-EHA was the most potent, resulting in 67.8% of the surviving fetuses being malformed. When DEHP and 2-EHA were administered, 4.5 and 22.2% of the surviving fetuses were malformed, respectively. The results are compatible with the hypothesis that 2-EHA is the proximate teratogen.

Scott et al. (*181*) (WEB Table 42) studied the effects of 2-EHA on prenatal development in Sprague-Dawley rats. Groups of 7–10 rats were gavaged with undiluted 2-EHA at 0, 12.5, or 15.625 mmoles/kg (0, 1,803, or 2,253 mg 2- EHA/kg bw) on gd 12. Dams were sacrificed on gd 20 and implantation sites were evaluated. Fetuses were observed, weighed, and sexed. Two-thirds of the fetuses were examined for visceral malformations and one third for skeletal malformations.

Toxicity in dams was not discussed and thus limited interpretation. Fetal effects observed at both doses included decreased weight and increased resorptions and malformations. The types of malformations observed most frequently included defects of the heart and appendicular skeleton. Statistical significance for fetal effects was not discussed, but there appeared to be a dose-response relationship for effects on resorptions and fetal weight.

The studies of Ritter et al. (*166*) and Scott et al. (*181*) also compared the toxicity of 2-EHA to that of its structural isomer, valproic acid (VPA). Doses of VPA and 2-EHA (VPA 6.5 mmol/kg, 2-EHA 12.5 mmol/kg) that produced roughly similar overall malformation rates (VPA 68%, 2-EHA 48%) were used to compare the pattern of developmental toxicity. The authors report a greater incidence of cardiovascular malformation with VPA than 2-EHA, while the relative incidence of skeletal malformations depended on the test species (Wistar in Ritter et al. (*166*), Sprague-Dawley in Scott et al. (*181*)). 2-EHA produced skeletal defects in the Wistar rats, but VPA produced somewhat more limb defects in Sprague Dawley rats. Thus, both similarities and differences in the actions of VPA and 2-EHA were noted. The study did not include a third, structurally unrelated teratogen which would be expected to differ from the two monocarboxylic acids, which would have been a valuable comparison.

The greater overall potency of VPA (two-fold higher than 2-EHA) was attributed to its greater bioavailability relative to 2-EHA. Both maternal plasma concentrations and embryo concentrations of the two acids were roughly equivalent at the doses considered equivalent for teratogenic action. The pharmacokinetic data indicated a very prolonged peak exposure resulting from the single gavage administration.

In the third study of VPA and 2-EHA, a number of short chain carboxylic acids were tested in the Chernoff-Kavlock assay for rats (*198*). In this test paradigm, compounds are administered by gavage on gd 6–15, pregnancies terminate in spontaneous delivery, and litters are followed to pnd 6 when they are sacrificed for skeletal examination. The developmental toxicity endpoints were pup weight and viability, external malformations, and skeletal malformations as detected postnatally. VPA and 2-EHA both produced reduced maternal weight gain, prolonged gestation, rales, reduced activity in dams, and perinatal pup loss. The 2-EHA toxicity was more severe at the doses used; 900 mg/kg bw/day led to death of 4/15 dams and 1,200 mg/kg bw/day killed 6/15 dams. Both 2-EHA and VPA led to reduced pup weights and postnatal viability, extra presacral vertebrae, extra cervical ribs, fused ribs, and lumbar ribs in the day-6 pups. External malformations mentioned were oligodactyly in the VPA pups and syndactyly and vestigial tail in the 2-EHA pups. Thus, this study also supports the similarity of the developmental toxicity syndrome produced by VPA and 2-EHA.

These studies were conducted to test the hypothesis that short chain organic (carboxylic) acids as a group are teratogenic. The strict structural requirement for this action was considered to be a chain longer than 1 carbon (methyl) and branching at the C2 position. Both VPA and 2-EHA fit this requirement; a variety of compounds that do or do not meet the criteria have been tested with results consistent with the theory. Exposure of the embryo to carboxylic acids is suggested to be enhanced because of ion trapping in the slightly alkaline environment of late embryonic development (weak acids enter the alkaline environment, become neutralized, and are not able to migrate out as ions). No mechanism of action for the developmental toxicity of the short chain organic acids has been demonstrated. Embryo culture experiments related to this issue are reviewed below.

Administration by Drinking Water

The effect of sodium salt of 2-EHA on prenatal development was studied in Wistar rats (*179*) (WEB Table 43). Groups of 20–21 pregnant females received 2-EHA in drinking water at doses of either 0, 100, 300, or 600 mg/kg bw/day on gd 6–19. The treated rats were killed on gd 20, and standard maternal and fetal parameters were observed and recorded. Every second fetus was selected for subsequent evaluation for skeletal anomalies; all other fetuses were examined for visceral anomalies.

No clinical or behavioral signs of toxicity were observed in any of the pregnant rats. Decreased body weight gain and water consumption were observed in females that received 600 mg/kg bw/day 2-EHA. The authors reported that there were no effects on food consumption. There were no chemical-related

effects on the number of implantations, resorptions, or live fetuses. Mean fetal body weight/litter was reduced in the 600 mg/kg bw/day and in female fetuses in the 300 mg/kg bw/day dose groups. Mean placental weight was also reduced at the 300 and 600 mg/kg bw/day dose groups. There was an increase in the incidence of clubfoot at the 300 and 600 mg/kg bw/day dose groups; no other gross malformations were increased to a statistical level of significance. An increased level of skeletal variations was manifested as wavy ribs in all treatment groups; the incidence did not increase with dose. Reduced levels of ossification were also observed in fetuses from the 600 mg/kg bw/day group. The apparent developmental LOAEL was thus 300 mg/kg bw/day with a NOAEL of 100 mg/kg bw/day.

The effect of 2-EHA on reproduction and postnatal development was reported (*180*) (WEB Table 44). Male and female Wistar rats received the sodium salt of 2-EHA in drinking water at doses of 0, 100, 300, or 600 mg/kg bw/day. There were 21–24 rats in each dose group. Male rats were exposed to the chemical for 10 weeks and females for 2 weeks prior to mating, both sexes were exposed during the mating trial and females throughout the gestation and lactation period. No effects were observed on body weight, feed consumption, or water consumption except for the period of pregnancy for females that received the 600 mg/kg bw/day dose, where water consumption and weight gain were decreased.

Mean litter size at pnd 0 was reduced at the 600 mg/kg bw/day dose. The pup body weights at lactation days 7 and 14 were also reduced in the 600 mg/kg bw/day dose group; the body weight of these pups had recovered at time of weaning (21 days). There were no differences in the lactation index values of the control and treated groups. There was a dose-related increase in the incidence of kinky tail with significant effects observed at the 300 and 600 mg/kg bw/day dose groups. Delays in landmarks of physical development and several reflexes were clearly present in pups from the 600 mg/kg bw/day dose group. A delay in auricle opening was also observed in pups in the 300 mg/kg bw/day dose group. The NOAEL and LOAEL are thus the same as for the previous study, and the Panel has confidence in these values.

Mechanism

A study has been directed at determining whether 2-EHA-induced developmental toxicity is secondary to elicitation of an acute phase response in rats (*177*). In response to stress or trauma, an acute phase response can occur in which hepatic production of proteins, including the zinc-binding protein metallothionein (MT), is increased. Consequently, zinc is withdrawn from the circulation through binding to liver MT and zinc supply to the embryo is reduced. Since zinc deficiency is teratogenic, it could be suggested that induction of an acute phase response by high doses of toxicants can produce teratogenesis through this mechanism. Also, some data on elicitation of acute phase response have also been produced for DEHP (*152*). The hypothesis for the studies was stated by Bui et al. (*177*), "agents which result in the induction of maternal toxicity can be predicted to result in compromised fetal development if the insult is sufficient to result in a marked stimulation of MT synthesis, unless supplemental maternal dietary Zinc is provided."

Bui et al. (*177*) studied pregnant Sprague-Dawley rats. On gd 11.5, six rats/group were gavaged with 0, 3.13, 6.25, 9.38 or 12.5 mmol 2-EHA/kg (0, 451, 902, 1,355, or 1,804 mg 2-EHA/kg bw) in corn oil and a control group of 8 rats was gavaged with corn oil. Eight hours later, dams were gavaged with radioactive zinc (^{65}Zn). Rats were also gavaged with equimolar concentrations of 2-EH. Dams were sacrificed on gd 12.5 and analyzed for zinc metabolism and concentrations in maternal liver and plasma. Zinc levels in fetuses were also measured. Hepatic levels of zinc and metallothionein, a compound that sequesters zinc, were significantly increased in a dose-related manner in dams exposed to 901 mg 2-EHA/kg bw and higher. There were no changes in maternal plasma zinc concentrations, but the zinc content of fetuses was significantly lower in dams exposed to 1,353 mg 2-EHA/kg bw and higher. Similar effects were noted with 2-EH but the responses were not dose-related.

Because of the strong dose-related effect observed with 2-EHA in the first experiment, Bui et al. (*177*) decided to study 2-EHA in additional experiments on the influence of dietary zinc on developmental toxicity in Sprague-Dawley rats (WEB Table 45). Groups of 7–10 rats were fed diets with low, adequate, and supplemental zinc levels (1.15, 25.44, and 97.46 μg zinc/g diet, respectively). On gd 8–15, the rats on each zinc diet were gavaged with either corn oil or 3.5 mmol 2-EHA/kg/day (483 mg/kg bw/day). At sacrifice on day 16, body weights, implantation sites, and zinc metabolism were evaluated in dams. Fetuses were measured, weighed, and examined for external malformations. Fetal effects were also evaluated in groups of 7–9 dams fed low or adequate zinc diets, gavaged with corn oil or 483 mg/kg bw/day on gd 8–15, and sacrificed on gd 19.

The primary focus of this results summary is the comparison of effects in 2-EHA-treated vs untreated dams that were fed adequate zinc diets. Dams exposed to 2-EHA experienced a significant reduction in body weight gain (gravid uterus weight excluded) on gd 16 accompanied by a reduction in food intake. A further decrease in body weight gain was observed in 2-EHA-treated dams that were fed low zinc diets. Similar effects on maternal body weight gain were observed in dams sacrificed on gd 19. Maternal levels of zinc in liver and plasma and metallothionein levels in liver were related to dietary zinc intake. Significant effects in fetuses treated with 2-EHA included increased resorptions (23 vs 5%), and decreased fetal weight (9%) and crown-rump length (9%) in the groups sacrificed on gd 19, but not gd 16. The percentage of fetuses with malformations was increased in the 2-EHA-treated groups sacrificed on gd 16 and 19, but statistical significance was obtained only in the gd 16 sacrifice group. The mean percentage of fetuses per litter with encephaloceles (14 vs 0%) and tail abnormalities (26 vs 2%) was significantly increased in the gd 16 sacrifice group. A lower and non-significant increase in tail defects (7.9 vs 0%) was observed in fetuses from the gd 19 sacrifice group. A low zinc diet in addition to 2-EHA exposure resulted in an enhancement of adverse fetal effects including resorptions, decreased fetal weight and crown-rump length, and increased fetuses with encephaloceles and tail abnormalities. Zinc supplementation appeared to have a protective effect in rats sacrificed on gd 16 because significantly fewer malformations were observed in fetuses of 2-EHA-treated dams receiving supplemental zinc compared to fetuses of treated dams fed low and adequate zinc diets.

Several types of data were produced that support the hypothesis that 2-EHA acts through zinc deficiency: 1) DEHP (1,000 mg/kg, one administration) produced higher maternal liver MT and zinc, and lowered embryonic zinc (nmol/embryo) the day after dosing. 2) 2-EHA (6.25, 9.38, 12.5 mmol/kg) produced higher maternal liver MT and zinc, greater hepatic uptake of ^{65}Zinc, and lower embryo uptake of ^{65}Zinc 18 hours after a single dose. Neither DEHP or 2-EHA influenced maternal plasma zinc. After repeated 2-EHA gavage (3.5 mmol/kg), a much smaller increase in maternal MT concentration was seen, with no increase in maternal liver zinc. 3) Supplemental zinc increased maternal liver MT but not plasma or liver zinc (embryo zinc not reported). 4) Supplemental zinc decreased the percentage of fetuses per litter with encephalocele and tail anomalies the day after treatment was completed (gd 16) and also percent fetuses/litter with >1 anomaly.

The Peters study (*152*) used a single gavage dose, while the Bui et al. study (*177*) used gavage dosing throughout embryogenesis. However, it seems unlikely that the dispersed dosing that occurs throughout the day with food and drinking water administration would elicit an acute phase response. Peak dose levels are lower with this type of administration than with gavage. Further, we do not know the zinc content of the rodent diets used when DEHP or 2-EHA were administered in diet or drinking water to understand whether they correspond to the adequate or supplemental (1, 25, or 100 μg zinc/g diet) levels of the Bui et al. study (*177*). Finally, the data on single versus multiple administrations is sparse, but in the case of 2-EHA it strongly suggests that stressful/traumatic aspects of gavage dosing are mitigated with

repetition. Indeed, the data suggest that a single corn oil gavage also induces an acute phase response (compare study 1 and 2 of Bui et al. (*177*)).

Hauck et al. (*178*) assessed the stereoselectivity of 2-EHA teratogenicity. The R and S enantiomers and the racemic mixture of 2-EHA were injected intraperitoneally 4 times on gd 7–8 to NMRI mice at a dose of 500 mg 2-EHA/kg bw (3mmol/kg). This model has been demonstrated to be sensitive to induction of exencephaly. After sacrifice on gd 18, the following were recorded: number of implantations, embryolethality, weight of living fetuses, and whether fetuses had exencephaly. The maternal effects were not reported. Administration of the S enantiomer resulted in 1% exencephaly, while the R enantiomer resulted in 59% exencephaly. Administration of the racemic mixture resulted in a 32% rate of exencephaly. This report further supports the structural selectivity of teratogenic effects of short chain carboxylic acids.

In considering extrapolation of animal findings on developmental toxicity to humans, the similarity in developmental toxicity actions between the DEHP metabolite 2-EHA and that of valproic acid (VPA, 2-pentylpropanoic acid) may be relevant. VPA is used clinically as an anticonvulsant and is a known human and animal teratogen. 2-EHA and VPA are structural isomers; they are both carboxylic acids with eight-carbon alkyl chains. Thus, the comparable actions of 2-EHA and VPA in animal models as reviewed earlier (*166, 181, 198*) strengthen a generalization of the relevance of animal findings on 2-EHA to humans.

Embryo Culture

In vitro embryo culture studies help provide a context for whole animal experiments. Two studies of 2-EHA are available, both using rat embryos and the scoring methods of Brown and Fabro. Brown et al. (*199*) compared a series of short chain carboxylic acids (SCCA) at 1 mM concentration, including 2-EHA. 2-EHA led to reduced fetal protein content and a small increase in abnormal embryos, but was considered to be in the least effective group of SCCA. Bui et al. (*177*) used serum from 2-EHA-treated rats, but the 2-EHA content was not provided, so that results cannot definitely be attributed to 2-EHA.

3.2.5 Phthalic Acid

Ema et al., who have published extensively on the developmental toxicity of phthalates, evaluated phthalic acid, (PA) which is a common metabolite of phthalate-based plasticizers (*184*) (WEB Table 46). Pregnant Wistar rats, 11 per dose group, were given a dose of 0, 1.25, 2.5, or 5.0% in the diet on gd 7–16 of pregnancy. These concentrations in diet were calculated by the authors to result in an average intake of 0, 1,021, 1,763, and 2,981 mg/kg bw/day. Maternal body weight and food consumption were recorded daily. On gd 20 (presence of sperm in vaginal smear equaled day 0), the dams were killed, weighed intact and with the gravid uterus removed; the number of live and dead fetuses were also counted. Live fetuses were sexed, weighed, and examined for external malformations; approximately 2/3 of fetuses were prepared and examined for skeletal malformations and 1/3 selected for Bouin's fixation and visceral examination using the free hand razor technique of Wilson.

Neither death nor clinical signs of toxicity were observed in the pregnant rats, although reduced weight gain and food consumption were observed throughout the treatment period in the rat groups fed 2.5 and 5.0% PA in the diet. No significant differences between treated groups and the control group were detected in corpora lutea/litter, implantation, dead fetuses/litter, live fetuses/litter, or sex ratios/litter. Male fetuses in the 5.0% group were significantly lighter in weight. No fetuses with external, internal, or

skeletal malformations were observed. The degree of ossification, as indicated by the number of ossification centers in caudal vertebrae, was significantly lower in pups from the 5.0% group. The authors concluded that maternal toxicity is suggested by a roughly proportional decrease in maternal food consumption and weight gain at the 2.5 and 5.0% dose of PA in the feed. PA was considered to have an effect on prenatal development of rat offspring as evidenced by reduced fetal weight and degree of ossification in pups from the 5.0 % group. It seems likely that PA possesses adverse effects on prenatal development of rat offspring at a dietary dose of 5.0% and that PA has no adverse effects on fetal development at dietary doses of 2.5% and lower, thus providing a LOAEL of 2,981 mg/kg bw/day and a NOAEL of 1,763 mg/kg bw/day. The authors consider it unlikely that PA is a major contributing factor in the developmental toxicity of phthalic acid esters.

The summary for Section 3 is located in Section 5.1.3.

4.0 REPRODUCTIVE TOXICITY

4.1 Human Data

There were no human data located for Expert Panel review.

4.2 Experimental Animal Toxicity

4.2.1 DEHP

Sixty-eight studies were reviewed in the evaluation of the reproductive toxicity of DEHP. Collectively, these studies were undertaken predominantly in rodents and build on the original observation that DEHP produced testicular atrophy in a subchronic toxicity study (*200*). The literature contains many redundant studies, usually at high doses (e.g., 2 g/kg, usually in rats), all of which show similar effects on the testes. A number of more specific studies in the rat have attempted to investigate the mode of action of DEHP using *in vivo* and *in vitro* protocols. It should be noted that a multigeneration reproduction study does not yet exist for DEHP, and this review must, perforce, utilize numerous studies that are suboptimal for one or more reasons, which will be clarified at each point. Several studies are of peripheral importance, and will be discussed later. The papers summarized here illustrate important facets of DEHP-induced reproductive effects.

It is appropriate to review how these studies are rated. The Panel was most certain about those studies which were thorough evaluations of numerous reproductive processes (studies that examined both the structure and function of the reproductive system). While recent reviews point to not-unexpected relationships between gamete measures taken at necropsy and fertility (*201*), this link is not completely predictable, and many events occur during the process of reproduction that are not captured by measuring sperm count or motility or estrous cycle length and regularity. Thus, measuring reproductive function is important. In addition, recent mechanistic studies have documented that phthalates are more potent reproductive toxicants at lower doses when exposure occurs during gestation (*202-205*). The most sensitive endpoints are those that monitor the development and formation of the reproductive system: testes descent, prepuce separation (also known as balanopreputial separation) in males, and vaginal opening and onset of estrous cycling in females. Based on all these considerations, a study was given more weight if it 1) assessed reproductive function in both F_0 and F_1 generations (at least); 2) dosed the female parent during gestation and evaluated the offspring after birth; 3) monitored the development of the offspring's reproductive system; 4) looked for malformations in the reproductive system of the

offspring; and 5) evaluated estrous cycle and sperm number, motility, and shape at necropsy. In addition, to ensure that the animals received the amount of phthalate intended by the investigators, a study was rated more highly if the dosing medium (corn oil or feed or water) was chemically analyzed for phthalate content. Finally, confidence in the veracity of a response was increased if the changes show a relationship to dose.

The Panel also addressed the sufficiency of the data for drawing conclusions about reproductive toxicity. If two or more studies find the same or similar effects, the Panel concluded that the data were sufficient to conclude that this compound produces that effect. If only one study was available, there was greater uncertainty about both the replicability of the effect and the dose level at which it occurred. In that case, the Panel stated that the data are sufficient to show a *likely* effect to reflect the 'thinness' of the database.

Oral Administration

The key study for the quantitative assessment of the reproductive toxicity of DEHP is reported by Reel et al. (*186*) and Lamb et al. (*168*) (WEB Table 15). CD-1 Swiss mice, 11 weeks old at the start of exposure, were used for continuous breeding phase and cross-over mating studies. There were 20 breeding pairs in each treated dose group, and 40 pairs in the control group. DEHP was mixed with feed to levels of 0, 0.01, 0.1, and 0.3% (w/w); this yielded calculated doses of 0, 14, 141, and 425 mg/kg bw/day[2]. Following a 7-day premating period, the mice were housed as breeding pairs for 98 days. Litters were removed immediately after birth. Endpoints in-life were clinical signs, parental body weight and food consumption, fertility (numbers of pairs producing a litter/total number of breeding pairs), number of litters/pair, number of live pups/litter, proportion of pups born alive, sex ratio, pup body weights within 24 hours of birth, and water consumption.

There was clear indication that DEHP affected fertility when administered in the diet. At 425 mg/kg bw/day no breeding pairs delivered a litter. At 141 mg/kg bw/day, fertility was significantly reduced as evidenced by fewer litters, fewer pups/litter, and fewer pups born alive.

A cross-over mating study was conducted between the 425 mg/kg bw/day treatment group and the controls. Fertility was significantly reduced in the groups of treated males and control females and the groups of treated females and control males. The treated females produced no litters and only 4/20 treated males sired a litter. Only the control and high-dose groups were necropsied. High-dose males had reduced testicular and epididymal weights and histologic evidence of seminiferous tubular destruction accompanied by major changes in epididymal sperm number, morphology, and motility. In addition, the males had decreased prostate weight, reduced serum testosterone, and elevated LH and FSH. There were no histopathological effects in the reproductive tracts of high dose females, but the weight of the reproductive tract was lower than controls (probably because the animals were not pregnant).

In Lamb et al. (*168*), the high-dose group was infertile, the mid-dose group was affected, and the low-dose group was unaffected. Thus, the NOAEL was a calculated dose of ~14 mg/kg bw/day. The LOAEL was ~141 mg/kg bw/day, based on reductions in litter size and in proportions of pairs having litters.

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[2] Doses were converted to mg/kg bw/day assuming mice consume 5.1 g/day and weigh 36 g, regardless of treatment group. Both assumptions are based on average food consumption and weights reported in Lamb et al. (*168*) and results in an average consumption rate of 5.1 mg/day. Doses were converted from percentages to mg/kg (0.01% = 100 mg/kg; 0.1% = 1000 mg/kg; 0.3% = 3000 mg/kg). Dose concentration was verified by HPLC. The following formula was used to calculate the concentration:

$$\text{Dose (mg/kg bw/day)} = \frac{\text{Food consumption (kg/day)} \times \text{concentration in diet (mg/kg)}}{\text{weight of mice (kg)}}$$

Example:

$$\text{For 0.01\% in diet} = \frac{0.0051 \text{ kg/day} \times 100 \text{ mg/kg}}{0.036 \text{ kg}} = 14 \text{ mg/kg bw/day}$$

These dose groups were not evaluated at necropsy, and the lack of assessment of reproductive development or the performance of the second generations leads The Panel to state that while our confidence in the quality of the study is high, our confidence is moderate-to-low that these doses correctly represent the true LOAEL and NOAEL.

In a recent report (WEB Table 16) by Schilling et al. (*169*), data were presented for the dose-setting phase of a full two-generation study of DEHP in rats. In this dose-range finding study performed according to OECD Guideline 416, groups of 10 male and female Wistar rats were exposed to DEHP in feed at 1,000, 3,000, or 9,000 ppm, which resulted in consumptions of ~110, 339, and 1,060 mg/kg bw/day. Exposure was continuous to the end of the study. The F_0 rats were mated within dose groups after 10 weeks of treatment; 6–10 F_1 pups were reared and mated as adults. Adult food consumption and body weights were measured weekly; neonate weights were taken throughout lactation. Anogenital distance was measured in the F_2 neonates only. Parental organ weights were taken; histopathology of testes and epididymis was performed. Feed was analyzed for DEHP concentration.

DEHP exposure did not alter weight gain in F_0 males or females or estrous cyclicity in females, though female body weight and food intake during gestation and lactation were reduced at the high dose. Mating indices were unchanged, although the high-dose females had a higher rate of postimplantation loss and delivered fewer pups (9.9 vs 15.1 in controls). There were significant reductions in survival to pnd 4 in the 1,000 and 9,000 ppm groups, and survival to pnd 21 was reduced at 3,000 ppm. Weight gain to pnd 21 was lower at 9,000 ppm. The sex ratio of the F_1 pups was not affected by DEHP. The proportion of male pups with detectable nipples or areolae (from control to high-dose groups, respectively) was 0, 0, 1.4, and 84%; statistical significance was achieved at the highest dose. There was a significant delay (by ~3 days) in vaginal opening at the high dose, which was attributed by the authors to a significantly lower weight (~21%) in these animals. Males at 9,000 ppm evidenced a significant 4-day delay in prepuce separation. The number of F_1 adult females mated were 10, 10, 8, and 7, from control to high dose, respectively. Two females at the high dose did not become pregnant, leaving five litters for evaluation. Mean litter sizes of F_2 pups were 13.7, 13.6, 12.5, and 9.0; the reduction in litter size at the high-dose group was statistically significant. The sex ratio was unchanged, as was absolute pup weight. Male pup anogenital distance (AGD) was reduced by 3, 6, and 13%. The effect in the high-dose group was statistically significant. Anogenital distance was unchanged in female F_2 pups.

Terminal body weights and testes weights of the F_0 adult males were unchanged, while relative liver weight was significantly increased by 15 and 39% in the mid- and high-dose groups, respectively. For females, terminal body weight was significantly reduced by ~17% at 9,000 ppm, while relative liver weights were significantly increased by ~6, 23, and 38% (low to high doses, respectively), and absolute ovary weights were significantly reduced at the high dose by ~25%. For the F_1 animals, male body weight was non-significantly reduced at 9,000 ppm, while relative liver weight was significantly increased by ~33%, and the relative weight of the testes and absolute weight of the epididymis was reduced by ~22 and 20%, respectively. These weights were unchanged at 3,000 ppm. There was microscopic evidence of damage in testes at 9,000 ppm, but not 3,000 or 1,000 ppm. Spermatocyte loss was observed in 2/10 F_1 pups exposed to 3,000 ppm and 7/9 F_1 pups exposed to 9,000 ppm. F_1 adult female terminal body and organ weights were not taken or reported, but gestational weight gain was reduced in F_1 dams.

The reduced F_0 fertility, increased liver weight, testicular toxicity, and reduced ovary weights are consistent with previous reports, which lends credence to both. Additionally, the retained male nipples and delay in preputial separation at 9,000 ppm would be expected from an anti-androgen, which several of the phthalates appear to mimic (*204*). Thus, these data are consistent with other reports. The reduced pup survival at 1,000 ppm and higher is not reflected in other reports, and will be viewed with caution until it is observed again in the upcoming main study.

Except for the irregular reduced pup survival, all the reproductive effects were noted only at 9,000 ppm: reduced gonad weights, reduced fertility, and the male reproductive developmental abnormalities (retained nipples, reduced AGD, delayed prepuce separation). Assuming the pup survival effect will not be repeated in the main study and that these reproductive effects (which are consistent with many other reports) will be repeated, then the reproductive LOAEL is ~1,060 mg/kg bw/day and the reproductive NOAEL is ~339 mg/kg bw/day, based on the fertility and gonadal and reproductive development effects. For liver weight changes (observed at the lowest dose in the F_1 adults), the LOAEL is 110 mg/kg bw/day, and there is no NOAEL.

Confidence in the quality of the study is high, based on the reputation of this group (i.e., their documented history of conducting acceptable studies) and on the fact that the study was done to the new OECD guideline. Confidence that this study found the true NOAEL is moderate-to-high, and is 1) reduced because the numbers of animals are relatively low, and female reproductive function was not fully assessed; and 2) raised because endpoints of reproductive development were examined, and second generation effects were examined with accepted methods.

A study by Arcadi et al. (*161*) (WEB Table 17) was notable for its exposure during pre- and postnatal development. Neat phthalate was dissolved in the drinking water by 30 minutes of sonication. Drinking water intake was not directly measured, and phthalate content of the water was not determined. Phthalate content of maternal plasma was determined on the last day of dosing. Tissues were fixed in Bouin's. In this report, Long-Evans adult rats (n=12/group) were exposed to DEHP in the drinking water with 0, 32.5 or 325 μL/L, starting the first day of gestation (the day after a sperm-positive vaginal smear). Exposure continued until pnd 21; there was no exposure of the pups after weaning. On pnd 2, litters were culled to 7 pups/dam. At selected ages (3, 4, 5, 6, and 8 weeks of age), eight pups from each group were sacrificed and evaluated for organ weight and histological appearance of testes, liver, and kidney. Behavioral testing (ability to walk a beam) was performed in female pups on pnd 30. Estimated consumptions were 3–3.5 mg/kg bw/day, and 30–35 mg/kg bw/day in the 2 groups, respectively, though water consumption was not measured. Maternal body weight was not affected and clinical and gross appearance was normal. There were no differences among the groups in pregnancy or lactational indices, or in litter sizes or viability. Plasma-DEHP concentration was 1.417 ± 0.21 μg/mL in 8 high-dose dams on pnd 21. Low-dose dams had a blood-DEHP concentration of 0.197 ± 0.031 μg/mL. Body weights of pups were not statistically reduced by DEHP exposure, though absolute kidney weight was reduced at all times in both exposed groups with associated reversible histopathology. There was increased relative liver weight in both groups. And there were reductions in absolute and relative testes weight: low dose was ~ 12% less than controls, while the high dose weighed ~30% less than controls. At the earlier ages evaluated, both exposed groups showed significant seminiferous epithelial disorganization and delayed development. By 56 days of age, the low-dose group evidenced some continued seminiferous tubular disorganization, but the effect appears to be more characterized as a delay in development, while spermatogenesis in the high-dose animals was clearly disrupted. Beam-walking took longer in the high-dose females at pnd 30.

In the Arcadi et al. (*161*) study, both exposed groups showed adverse effects on testicular weight and structure. Based on this, the LOAEL in this study is 32.5 μL DEHP/L, producing an estimated intake of 3 mg/kg bw/day. There was no NOAEL. Based on the relationship between testes weight/histopathology and sperm production, and the relationships between sperm numbers and fertility (*206*), it would be expected that at least the high-dose animals would be sub-fertile, and the low-dose animals might have reduced fertility if evaluated sufficiently. The Panel's confidence in the quality of the study is low because the authors had no chemical verification of the dosing solution, and they did not put their blood-

DEHP concentration data into context with other studies. The authors were contacted, but these issues were not resolved.

Although this study design exposed rats during the appropriate window of development and analyzed the tissues appropriately and seemingly well, measures of reproductive development were not monitored, and there are few studies linking these external measures of development (hypospadias, testes descent, prepuce separation, nipple development) with testes structure.

Another rat study which incorporated a breeding phase were identified (*207*) (WEB Table 47). DEHP was given in the diet for 60 days to adult male Fischer-344 rats (24/group) at 0, 320, 1,250, 5,000, or 20,000 ppm (reported as 0, 17.5, 69.2, 284.1, and 1,156.4 mg/kg bw/day). Body weight and food consumption were measured weekly. After 60 days of treatment, males were returned to control diet and housed with virgin females for a 5-day mating period. Approximately 1/3 of the males were sacrificed and necropsied at the end of the mating period; the rest were sacrificed after a further 60 days without DEHP exposure, to evaluate recovery. Different animal group sizes were used for different endpoints (i.e., 8/group for pathology, 16/group for recovery, all 24 males mated 1:2 with females in the fertility trial). Testes were fixed in Bouin's; epididymal sperm measures were taken. Litters were evaluated for number and viability of offspring.

There was a reduction in body weight gain in the 1,156 mg/kg bw/day group and a transient decrease in body weight in the 284 mg/kg bw/day group. Absolute and relative testes and epididymal weights and absolute prostate weights were decreased at 1,156 mg/kg bw/day. At 1,156 mg/kg bw/day, severe testicular degeneration, reductions in epididymal sperm density and motility, and an increase in abnormal forms were observed. Marked histopathological effects were found only at the top dose; 5,000 ppm was without testicular structural effects. Mean litter size was reduced by ~15% at 1,156 mg/kg bw/day, but other measures of function were not affected at any dose. Partial recovery of the testicular lesion was noted in the recovery group. Serum levels of FSH were increased and the testicular levels of zinc were reduced in the 1,156 mg/kg bw group.

The data are indicative of a toxic effect although the functional reproductive effect is surprisingly weak in view of the testicular/epididymal changes. The NOAEL was 284 mg/kg bw/day and the LOAEL 1,156 mg/kg bw/day for reproductive effects.

Although the Panel's confidence in the quality of the study is moderate-to-high because of the fact that both structure and function were evaluated conjointly, confidence that this study actually identified the correct LOAEL/NOAEL is low because the wrong ages were exposed and the most sensitive endpoints were not examined.

Dalgaard et al. (*74*) gavaged 10 male Wistar rats/group (~160 g) with DEHP in soya oil at 0, 1,000, 5,000, and 10,000 mg/kg bw/day for 4 weeks. During the last week of the study, fertility in high dose rats was assessed by mating treated males with untreated females for 7 days and examining implantation sites and fetuses in dams at 15 days post mating. Following sacrifice, rats were necropsied and organs were weighed. Testes from all dose groups were fixed in Bouin's and examined histologically.

A reduction in body weight and food intake was noted in rats exposed to 5,000 mg/kg bw/day and higher. Two animals in the 10,000 mg/kg bw/day group died of emaciation. In the mating experiment, there were dose-related reductions in male fertility. There was no evidence of mating in 1/10, 3/10, 4/10, and 5/8 males from the low- to high-dose group, but the results were not statistically significant. The number of pregnant females from the low- to high-dose group was 9/10, 8/10, 6/10, and 1/8 with statistical significance achieved in the high-dose group. The pregnant female in the high dose group had only one implantation. Effects observed in males at necropsy included reductions in relative testis weight in the

high- and mid-dose groups and relative epididymis and seminal vesicles weight in the high-dose group. Severe testicular lesions were observed in 2/10 rats in the mid-dose group and 7/8 rats in the high-dose groups. The testicular examination revealed disorganized architecture, atrophied tubules with massive spermatid and spermatocyte loss with the majority of the germinal epithelium lined with spermatogonia and Sertoli cells or only Sertoli cells, and slight diffuse Leydig cell hyperplasia. Expression of vimentin (a possible marker of Sertoli cell damage) was increased in areas of greater testicular damage.

A study that started out with pubertal animals is reported by Poon et al. (*75*) (WEB Table 2). They exposed young (~100 gram) Sprague-Dawley rats (n=10/sex/group) to well-characterized DEHP levels in feed at 0, 5, 50, 500, or 5,000 ppm for 13 weeks. Food consumption and body weights were measured weekly. At the end of the 13-week exposure period, animals were killed and necropsied. Testes were fixed in Zenker's. Other tissues were weighed and fixed in neutral buffered formalin (NBF). Sperm measures were not taken. A subsequent DNOP study used 5,000 ppm DEHP as a positive control.

Calculated intakes of DEHP for males were 0.4, 3.7, 38, and 375 mg/kg bw/day, respectively. Female intakes were 0.4, 4.2, 42 and 419 mg/kg bw/day. Non-reproductive systemic effects are discussed in Section 2. Minimal Sertoli cell vacuolation was observed in 7/10 rats at 500 ppm; testicular histopathology was more severe at 5,000 ppm. While the text does not mention changes at other doses, a table in the report (Table 3) lists no Sertoli cell vacuolation in controls, minimal focal vacuolation in 4/10 treated rats in the lowest 2 dose groups, minimal multifocal effects in 7/10 rats at 500 ppm, and more severe effects at 5,000 ppm. The severity score shows a monotonic dose-response: from control to high dose, the severity is 0, 0.2, 0.5, 1.0, and 2.4 out of a maximum possible score of 4. That same table lists the number of rats showing seminiferous tubular atrophy from the control to the top dose as 1, 3, 1, 0, and 9, respectively. A strict interpretation of this table would have vacuolation showing up at 5 ppm, but atrophy not showing up until 5,000 ppm. The DEHP effects at 5,000 ppm were repeated in the DNOP study that used DEHP as a positive control.

The LOAEL as listed by the authors is 38 mg/kg bw/day, which would be for Sertoli cell vacuolation. This would make the NOAEL 3.7 mg/kg bw/day.

Confidence in the study is moderate-to-high, increased by the methods of testicular evaluation, and lowered by the fact that the study is not designed to measure reproductive performance. Confidence that the authors have found the true NOAEL is moderate-to-low, because while pubertal animals were examined, there was no gestational exposure and no evaluation of the likely most sensitive endpoints.

David et al. (*81*) reported testicular effects in Fischer-344 rats in a chronic study. The 6-week-old rats (50–80 males/group) were fed diets containing 0, 100, 500, 2,500, or 12,500 ppm DEHP (0, 5.8, 29, 147, and 789 mg/kg bw/day for males) for 104 weeks. Additional study details and non-reproductive systemic effects are discussed under Section 2.0. Testes weight (absolute and relative) was reduced in rats of the high-dose group. Aspermatogenesis was observed in 10/10 rats of the 789 mg/kg bw/day group at study week 78. The effect was not observed in rats treated with 147 mg/kg bw/day or in the control group. At study week 105, the incidence of aspermatogenesis was significantly increased in rats exposed to 29 mg/kg bw/day and higher. The percentage of rats affected from the control to high-dose group was 58, 64, 78, 74, and 97%, respectively. Pituitary castration cells were observed in high-dose male rats at both time periods. Interestingly, the incidence of interstitial cell tumors was lower in high dose rats.

The Panel has confidence in the quality of this study because the doses were sufficient, the route was appropriate (oral), there was verification of the dosing medium (feed), the numbers of animals examined were sufficient, and the interpretation of changes in Leydig cell tumor occurrence was insightful. Additionally, at the high dose organ weight effect was consistent with a significant microscopic lesion

reported by the authors. However, the study utilized suboptimal testis fixation, which might obscure an early vacuolar lesion, which DEHP produces.

The authors state that the NOAEL for testicular toxicity was 146 mg/kg bw/day. However, because of the clear dose-response increase in the proportion of each group showing aspermatogenesis, the Panel concludes that the NOAEL for testis effects is 5.8 mg/kg bw/day, which the Panel rounded to 6 mg/kg bw/day.

Ishihara et al. (*208*) fed 5-week-old male Sprague-Dawley rats (n=15/group) diets containing 0, 1, or 2% DEHP for 2, 4, or 6 weeks. The authors stated that the high-dose group was exposed to DEHP at approximately 1,000 mg/kg bw/day. The exposure in the low-dose group can therefore be approximated at 500 mg/kg bw/day. At each sacrifice time, the testes, kidney, and liver from five rats/group were weighed. Testes were preserved in Bouin's solution, and examined histologically. Spermatogenic disturbance was measured by the Johnsen's scoring system that gives a score of 1 for a lack of cells in seminiferous tubules and a score of 10 for complete spermatogenesis.

Bodyweights were reduced at week 6 for the low-dose group and at weeks 2, 4, and 6 for the high-dose group. Liver enlargement (based on statistically significant changes in relative organ weight) was observed in the low-dose group at 2 and 4 weeks and in the high-dose group at each time period. Absolute and relative kidney weights were unaffected in the low-dose group, but absolute kidney weights were reduced in the high-dose group at weeks 2 and 4. Liver and kidneys were not examined histologically. Absolute and relative testes weights were unaffected in the low-dose group, but were significantly lower in the high-dose group at each time period. Spermatogenic disturbance was observed in some seminiferous tubules in the low-dose groups but according to study authors, the lesions were not significant compared to controls. Testicular lesions were observed at all time points in the high dose group and included marked atrophy of the seminiferous tubules and disappearance of spermatids and spermatocytes with the presence of only Sertoli cells and no or few spermatogonia. In a second experiment, it was noted that supplementation with vitamins A and C reduced the severity of testicular lesions.

Kurata et al. (*82*) (WEB Table 4) dosed male and female marmosets, 12–15 months-old at the start of the study, 4/sex/dose, to DEHP by gavage in corn oil for 13 weeks to levels of 100, 500, and 2,500 mg/kg bw/day. This age of marmoset corresponds to puberty. Purity of the compound was assessed at the start and end of the study; dosing solutions were apparently not analyzed for DEHP. After 13 weeks, animals were killed and necropsied. The liver was evaluated for peroxisomal activity and structure by EM. Testis zinc concentration was determined. Testes were fixed in NBF and examined by light and EM.

The high dose (2,500) reduced weight gain in males early on, but terminal weights were not different from controls. Testes weight and structure were unchanged by any dose of DEHP at either the light or EM level. Testicular zinc concentration, which is significantly decreased after toxic phthalate exposure in rats, concomitant with major germ cell loss, was unchanged in these marmosets. See comments regarding the study by Rhodes et al. (*85*), below on absorption of DEHP in primates.

There were no reproductive effects, so a LOAEL cannot be assigned. The reproductive NOAEL is 2,500 mg/kg bw/day. The systemic NOAEL is discussed in Section 2.

The Panel's confidence in the quality of the study is moderate-to-high, because animals were exposed to sufficient amounts for adequate duration and authors closely examined the tissue. However, confidence that the authors found the real NOAEL or LOAEL is moderate-to-low, because the most susceptible age of animal was not exposed and the most sensitive endpoints were not monitored.

Pugh et al (*83*) gavage dosed 2 year old (prepubertal) cynomolgus monkeys (n=4 per group) with 500 mg/kg bw/day DEHP suspended in methylcellulose for 14 days (Web Table 50). This dose was calculated to be the maximal dose that would be absorbed by these primates by Short et al. (*84*). Other animals in the study were dosed with DINP or clofibrate, and were examined histologically for tissue changes as seen in rats. After 14 days of exposure, the animals were killed, tissues removed and weighed, then fixed in formalin and embedded in paraffin for tissue sectioning and staining with hematoxylin and eosin. No microscopic lesions were noted in the testes of these animals. Systemic effects are discussed in Section 2.0

Although this study examined a relatively small number of animals exposed to DEHP for a relatively brief duration (14 days), the species and route are relevant, the maximal likely dose that would be absorbed was administered, and appropriate target organs were studied.

If the cynomolgus monkey were as sensitive as a juvenile rat to the effects of DEHP, testicular histopathology would have been observed. If the monkeys were only as sensitive as an adult rat, this dose would have been ineffective in producing testicular toxicity. Thus, while this study is useful in confirming that monkeys are not as sensitive as the most vulnerable of other model species, it is not useful in confidently placing the monkey along the spectrum of susceptibility to DEHP-induced testicular damage. As such, it is of limited use to the Panel in determining the likely risk of DEHP to human reproduction.

Rhodes et al. (*85*) examined testicular effects in DEHP-treated marmosets. The study is discussed under the parenteral exposure section since the effects of oral exposures were compared to IP exposure.

Gray et al. (*202*) administered DEHP by gavage in corn oil at 750 mg/kg bw/day to pregnant Sprague Dawley rats from gd 14 to lactation day 3 (WEB Table 48). In another study, the exposure started on pnd 21, and continued through maturation, mating, gestation, and lactation. Only one generation of rats was directly dosed. Measures of reproductive development were taken.

Males from dosed dams showed reduced anogenital distance at birth, significant increases in percent males with areolas (88.9% vs 0 in controls), and numbers of areolas in males (8 vs 0 in controls), and in percent males with reproductive malformations and whole-organ agenesis or atrophy (91% vs 0 in controls), as reduced pup body weights at birth. In addition, when these males matured, their reproductive organs weighed less than controls. Collectively, this pattern of results is consistent with at least a degree of antiandrogenic activity. However, this is significantly different from the pattern produced by the "standard" antiandrogen flutamide (*202*). This suggests that there are likely other mechanisms of action. The data from Li et al. (*209*) suggest an antiproliferative effect of DEHP, which that author suspects is related to changes in the expression of cell-cycle control genes (*210*). The prostate and seminal vesicle and testis agenesis produced by DEHP, but not seen after flutamide exposure, would be consistent with such an effect.

In two studies (*146, 156*) in F344 rats and CD-1 mice, dams received DEHP in the diet at levels up to 573 or 96 mg/kg bw/day, respectively, throughout the major portion of pregnancy. Following gestation, dams were permitted to give birth and rear their litters through pnd 4. No impairment of reproductive function was observed in the F_1 generation, even though there was decreased viability in the litters (during the early postnatal period) from which these F_1 pups were selected. The F_1 rats were mated and there was no effect on F_2 growth, viability, or development through pnd 4.

Administration by Inhalation

Klimisch et al. (*91*) (WEB Table 5) exposed 27 male and 17 female rats per dose level to DEHP aerosols at 0.01, 0.05, or 1.0 mg/L. Assuming 100% deposition and absorption, this gave approximate dosages of 2.3, 11, and 230 mg/kg bw/day for males and 3.6, 18, and 360 mg/kg bw/day for females. Exposure was 6 hours/day, 5 days/week, for 4 weeks. At the end of exposure, some males were sacrificed and necropsied, and others were mated twice to untreated females. The mating animals were sacrificed 8 weeks after the end of exposure. The test atmospheres were analyzed for DEHP.

Systemic effects are discussed Section 2. In the fertility trials, there was no decrease in mating performance or pre- or post-implantation losses, although no data are given for litter size. The authors state that "A few sporadic changes that were not attributable to treatment were seen for . . . male reproductive organs. . .," although no additional data were given. Specifics on testes fixation were not provided; no sperm parameters were measured.

The Panel's confidence in the conduct of this study is moderate-to-high, given the other toxicology experience of this group and the chemistry expertise. Confidence that this study found a real NOAEL for reproduction is low, because the most sensitive measures of reproduction were not measured, and the most likely vulnerable period was not assessed. In addition, peroxisome proliferation was not detectable in the liver, which suggests insufficient internal dosing. Conversely, this suggests poor absorption and distribution after aerosol inhalation.

Parenteral Exposure

Rhodes et al. (*85*) report a rat oral DEHP study (n=10 M/F Wistar-derived rats, 2,000 mg/kg bw/day for 14 days in corn oil) and two marmoset studies: an oral study with 5 males and 5 females (12–18 months old) exposed to 2,000 mg/kg bw/day in corn oil for 14 days (Web Table 51) and an IP study (five 24-month-old males exposed to 1,000 mg/kg bw/day in corn oil for 14 days). At necropsy, blood was taken for measures of plasma triglycerides and cholesterol, a gross examination was made, and "selected tissues were preserved in buffered formol saline for microscopic examination. . .," which apparently included testes. A variety of enzyme activities were measured from hepatic subcellular fractions. Toxicokinetic studies were also performed, and are reviewed in Section 2.3.

Only rats receiving 2,000 mg/kg bw/day by the oral route showed an increase in liver weight and a decrease in testis weight. No histology results were presented. The marmoset data are confusing and poorly reported: a single set of bar graphs are presented, while two studies were performed. In the published report, data are much less clear; the authors state that organ weights were not changed in marmosets either at 2,000 mg/kg bw/day orally or 1,000 mg/kg bw/day IP, but provide no data. However, in an EPA docket which contains these data, there are tables that list the organ weights and histology findings, and DEHP clearly causes no change in histopathology or in testis weights: mean absolute weights for control and treated marmoset testis (unclear whether this is one testis or both testes) are 0.803 and 0.876 g, with a variance of 0.315. Based on histology and biochemical measures, liver peroxisomes were strongly induced in male rats, but not female rats and not in marmosets.

The alternate route, IP, effectively showed differences between species in a number of hepatic measures. However, the reader does not know the length of time between the last dose and death and the testes were fixed and stained inappropriately. When coupled with the fact that the authors presented no histologic findings of testes, the reader is uncertain of the quality of the histologic preparation and therefore the ability of the authors to detect any damage. The testis problems are particularly acute because histology is the most sensitive endpoint at this exposure duration, and poor histology could well mean that modest (or even marked) lesions could go undetected. Testis weight is presented, but a single graph is presented which apparently summarizes 2 experiments, although this is not clearly stated anywhere. The full data

are presented only in an EPA docket, which is difficult to access. The authors used a single dose in each route; there was no gestational or perinatal dosing, and no evaluation of endpoints of reproductive development.

Because of the limited confidence that can be placed in the testis evaluations and the use of a single dose, no meaningful LOAEL/NOAEL can be determined from these data.

Sjoberg et al. (*93*) looked at the effects of IV administration of DEHP on testicular histology. The DEHP was emulsified with egg yolk phosphatides and administered in a glycerol solution. Controls were administered vehicle that was exactly the same, but without the DEHP. Doses of DEHP were 5, 50, or 500 mg/kg bw. Male Sprague-Dawley rats (n=5–6/group) were 40 days of age. DEHP was administered every other day for 12 days for a total of 6 administrations. Therefore, the time weighted average doses were 2.5, 25, or 250 mg/kg bw/day. 2–3 Hours after the last infusion, a mL of blood was taken for clinical chemistry measures, and BSP was administered to measure renal clearance. Twenty minutes after BSP infusion, the animals were killed and various organs removed. Liver was fixed in glutaraldehyde. Testes were fixed in Bouin's (n=3–4/group) or perfused with glutaraldehyde in cacodylate buffer (n=2–3/group). Tissues were examined at the light and EM level for any abnormalities and for tubular diameter of 100 tubules. Tubular diameter is a moderately gross integrative measure of cell number.

Non-reproductive systemic effects are discussed in Section 2. Relative weights of reproductive organs were unchanged. The authors state that no abnormalities in testis structure were observed at the light microscope level. At the EM level, in 3/5 animals at the high dose there were slight enlargements of the smooth endoplasmic reticulum in Sertoli cells, the earliest hallmark of the DEHP testis lesion. There were also slight structural changes in early spermatocytes.

A group of 25-day-old Sprague-Dawley rats (n= 5) was also administered 6 intravenous doses of 500 mg DEHP/kg bw and testicular effects were compared to the 40 day-old-rats. Details of effects were not provided by Sjoberg et al. (*93*) but they reported that the 25-day-old rats were not more susceptible than the 40-day old rats.

Since the study used appropriate controls, excellent histology, sensitive measures, multiple dose levels, and an appropriate route, the Panel is confident in the findings.

However, the study protocol does not require measures of the developing reproductive system; neither does it require functional measures, but with these subtle effects, no changes in reproductive system function would be expected (i.e., fertility would be unchanged). In addition, the animals were older than the likely most sensitive developmental age.

The LOAEL for reproductive changes in the 40-day-old rats was 500 mg/kg bw (250 mg/kg bw/day) and the NOAEL was 50 mg/kg bw (25 mg/kg bw/day).

Confidence in this study is high, because of the care taken in the methods and measures, and the fact that the changes seen in the testis mimic those found by other investigators. The Panel is confident that the authors found any effects that were present in these animals. Confidence that these authors "found the real L/NOAEL's" is moderate because testicular development was not evaluated. Also, it should be noted that because the effects seen at the 500 mg/kg bw/day dose were quite subtle, it is likely that the NOAEL is nearer to 500 than to 50 mg/kg bw/day.

Agarwal et al. (*211*) report two studies. In the first, they dosed adult male ICR mice (n=8/group) subcutaneously with undiluted DEHP at 1, 2, 5, and 10 mL/kg; the authors translate this to doses of 0.99, 1.97, 4.93, and 9.86 g/kg. These mice were dosed on days 1, 5, and 10, and each male was cohabited with

1 female on day 21. The females were killed on gd 10–15, and live and dead implants and corpora lutea were counted. In the second study, the authors used groups of 10 adult male ICR mice, receiving the same doses on the same three days. In this experiment, 1 virgin female mouse was cohabited with each male in each successive 5- or 7-day interval until day 63. These females were killed as above, and uterine contents examined.

In the first study, the authors report a 10-fold increase in early fetal deaths that was not dose related. In the second study, statistical changes are seen primarily when summing over multiple time points, so that the n=22–36, or (more effectively) 54–74. In the case of the latter, the authors noted an approximate tripling in the rate of pre-implantation loss that was only poorly dose-related, early deaths that were increased by factors of 3, 4, 4, and 5 (as dose increased), and a slight reduction in the number of viable fetuses from a mean of 11.2, to 10.1, 9.6, 9.8, and 9.1 live fetuses per litter as dose increased. All were significant. The authors also calculate a mutagenicity index for DEHP, and found it to be mutagenic, based on the increases in early deaths and preimplantation losses.

A sufficient number of female animals were studied and an appropriate non-oral route was utilized. However, the use of undiluted DEHP raises concerns about local reactions and percent of dose that is absorbed; no histology was performed on the males to correlate fertility effects with structure; there were too few males or females used at each time point to get a clear picture of dose or time trends, requiring the authors to collapse data over time to obtain sufficient *n* to allow a stronger statistical evaluation. There were few clear dose or time trends at any given time or dose, respectively, making it difficult to believe the data. A LOAEL of 0.99 g/kg bw (administered on 3 non-consecutive days) was identified, but there was no NOAEL.

The Panel's confidence in these data is moderate-to-low because of the concentration of the test article administered, the relatively weak dose-responses, the low number of females per male and per dose level, and the different conclusion about DEHP's mutagenicity in this paper versus the rest of the literature. These data are not very useful to the CERHR process because they do not evaluate the endpoints of interest for determining a reproductive effect, and the dose duration is short (a total of 3 doses over 10 days), and the exposure is to adult males, not to gestating males.

A subsequent study by Agarwal et al. (*212*) used similar exposure conditions, and reported other measures. In this study, the authors dosed adult male ICR mice in groups of 25 (control) or 10–13 (treated) subcutaneously with undiluted DEHP at volumes of 1, 2, 5, 10, 15, 120, 40, 60, 80, and 100 mL/kg, on days 1, 5, 10, a regimen used in the previous study. Doses in g/kg were not provided; in the previous report, a dose of 10 mL/kg was equivalent to 9.86 g/kg. The animals were mated from day 21–28, and then sacrificed. Other animals were sacrificed on day 21; one testis and epididymis from 10 mice was fixed in formalin and evaluated histologically. The other testis was used for biochemical measures. The disposition of the animals was not clear from the Methods description in the prescribed study. The data were analyzed by ANOVA and then compared pairwise by t-test; this is an incorrect comparison, as it produces false positives.

The authors noted that doses of 20 mL/kg or more were incompletely absorbed, and the animals had fluid-filled pouches containing some DEHP and some apparent lymph. Testis weights (unclear when during the study these were collected) were reduced at and above 20 mL/kg. There were various biochemical changes (whose meaning is difficult to determine) at 10 mL/kg and higher. Testicular histology was affected at and above 10 mL/kg, with inflammation being most common at 10 mL/kg, and tubular changes appearing at 40 mL/kg (which might be calculated to be an administered dose of 39.4 g/kg). The testicular pathology (description of the changes and the doses at which they began to appear) was not tabulated, and the text description was cursory. The effect on fertility was limited to determining how many females per group were pregnant; this was 76% in the controls, and as the dose increased, the

proportions of pregnant females were: 50, 25, 33, 42, 25, 8, 20, 0, 8, and 0%. The authors did not perform statistical analyses on these data, and it can be seen that even at the lowest dose (which can be calculated to be 0.99 g/kg) there may be a decrease in percent pregnant females, and the next highest dose (2 mL/kg, wherein 25% of the females were pregnant), gave the fourth lowest pregnancy value, the same as that found with 15 mL/kg.

Pregnancy and histology were evaluated in this study, which is informative. However, the methods used for histologic evaluation were suboptimal; the time between treatment and mating and necropsy meant that there might have been some degree of recovery from any DEHP effect; the concentration and volume of the dosing solution meant that residual neat DEHP was in a depot at the site of injection, complicating the kinetics in vivo; the numbers of animals for the mating trials (10) were relatively small, and the measures of fertility (the % of females getting pregnant) are gross; the apparently most sensitive period of male development was not evaluated; the Panel has no idea what the kinetics of such an administration would be.

Because of the uncertainties surrounding this study, it is not possible to have confidence in a LOAEL or a NOAEL.

These data are of little use for the CERHR process, due to the idiosyncrasies of study design (and consequent uncertainties of recovery versus chemical damage), and the relatively low animal numbers.

Baxter Healthcare Corporation (*213*) submitted a summary describing testicular histology in neonatal rats and rabbits following IV exposure to 62/mg/kg bw/day DEHP in 4% Bovine Serum Albumin. Control rats were dosed with saline. Rats (n= 7 treated and 8 controls) were dosed on pnd 3–21 and rabbits (n=5 treated and 7 controls) were dosed on pnd 14–42. The animals were sacrificed on the day of or the day after the last treatment and testes were preserved in formalin. No histopathological effects were observed in the testes.

Curto and Thomas (*214*) studied the effects of parenteral DEHP exposure on organ weight and zinc levels in prostate, seminal vesicle and testis of Sprague-Dawley rats (200–300 g) and Swiss-Webster mice (35–40 g). Groups of 6 male mice were injected intraperitoneally IP with DEHP in peanut oil at levels of 0, 50, or 100 mg/kg bw/day for 5 days. In a second experiment, groups of 6 male mice and 6 male rats were injected IP with DEHP in peanut oil at 0, 50, or 100 mg/kg bw DEHP every other day for 20 days. Control rats and mice were injected with vehicle. There were no effects observed in mice. Reductions in prostate and testes zinc levels were observed in rats treated with DEHP at 100 mg/kg bw.

Of the above studies, Sjoberg et al. (*93*) engenders the greatest confidence because of the dosing and design idiosyncrasies of the Agarwal et al. studies (*211, 212*), and the relative lack of reproductive-related detail in the Rhodes (*85*) study. The lesions found by Sjoberg et al. (*93*) in 3/5 high-dose rats (administered 6 doses with 500 mg/kg bw/dose) are mild, but consistent with what others have reported, which lends credence. Since the lesions noted were quite subtle, it is quite likely that the real NOAEL is much closer to 500 mg/kg bw/dose than to the next lower dose of 50 mg/kg. In the absence of a data point between these doses, the default NOAEL is 50 mg/kg bw and because this is relatively close to the doses received by some humans undergoing medical treatment involving phthalate-containing devices, it increases the level of concern. Even though the Panel recognizes that this is, to some degree, artificial and based on Sjoberg's selection of doses, it is nonetheless where the process leads.

Mode of Action

Several other pertinent aspects of DEHP reproductive toxicity were reviewed and are summarized below.

Age Specificity in Rats. Two earlier studies (*215, 216*) illustrate the clear susceptibility of young (neonatal/pubertal) rats to testicular insult compared to their adult counterparts. This effect may be related to age-dependent differences in chemical disposition (*108*). Recently, these have been superceded by several studies (*202-204*) that clearly show that prenatal exposure to a related phthalate (di-n-butyl phthalate) produces significant adverse effects in the postnatal development of the reproductive system, effects that are not seen when adult animals are dosed and evaluated at necropsy. Because these effects occur at lower doses than are toxic to adult animals, current concern focuses on prenatal exposure leading to postnatal toxicity. Although young animals were found to be more susceptible to reproductive effects following oral DEHP exposure, Sjoberg et al. (*93*) did not find increased susceptibility in 25-day-old versus 40-day-old rats treated by IV exposure.

While the Sertoli cell is considered by the Panel as most clearly manifesting the adverse effects of DEHP and MEHP, this manifestation may take different forms at different ages. During the time of Sertoli cell divisions (before pnd 15 in rats), phthalate exposure apparently inhibits cell division (*217*). In animals older than pnd 15, toxicity is manifest as vacuoles, followed by germ cell sloughing. Whether the vacuoles and the inhibited cell division are mechanistically linked is unknown.

Active Agent in Rats. MEHP, not a further oxidative product, is the active agent responsible for testicular (and probably ovarian) toxicity (*218*). These findings were observed in both *in vivo* and *in vitro* studies using DEHP, MEHP, 2-EH, and three further oxidative metabolites of MEHP.

Female reproductive effects. Davis et al. (*219*) found that 2,000 mg/kg bw/day DEHP (administered to 6-10 female Sprague-Dawley rats/group by gavage for 1–12 days) lowered circulating estradiol levels, which prevented the pre-ovulatory rise in LH and blocked ovulation. This was shown to be due to an MEHP suppression of aromatase, an important enzyme in the synthesis of estradiol (*220*).

Species sensitivity. Species differences have been noted for the induction of testicular toxicity. Gray et al. (*200*) reported that the hamster is largely resistant to short-term exposure to DEHP; mice, rats, and guinea pigs are the most sensitive species. A study in the marmoset (*85*) indicated that high doses of DEHP (2 g/kg bw/day) given by gavage for 14 days resulted in low absorption (about 2% of dose) and had no effect on testes weight. Testes morphology was evaluated but the results were not reported. Kurata et al. (*82*) found that up to 2.5 g/kg bw/day in marmosets produced no testicular effects; whether this is due to lack of sensitivity or to poor absorption is unclear.

In addition, Lake et al. (*87*) showed that consumption of diets with 1% DEHP by ferrets for 14 months (average: 1,200 mg/kg bw/day; range: 650–2,000 mg/kg bw/day) produced testicular histopathology in 3/7 animals. The inconsistent effect across different animals appeared similar to effects seen in rats receiving lower doses of DEHP, and so is judged to be treatment-related. Lake's concomitant metabolic studies indicated that the DEHP was metabolized to MEHP and then conjugated and excreted by the ferret, as in the rat. A recent abstract by Higuchi et al. (*221*) reports that the male offspring of pregnant Dutch Belted rabbits given 400 ppm/kg bw on gd 15–30 had reduced anogenital distance and accessory sex gland weights. One male pup had ambiguous genitalia, undescended testes, and hypospadias. These are hallmarks of anti-androgen action, as reported in rats (above). Although this is only published as an abstract at this time, the results are consistent with numerous previous data from rats. Thus, DEHP causes testicular and/or male reproductive toxicity in mice, rats, guinea pigs, ferrets, and possibly rabbits. The species differences or concordances in the developmental toxicities of DEHP are much less well documented.

Reversibility of testicular effects. The reversibility of the adverse testicular effects noted in rats has been examined in a number of studies, and most completely in Oishi (*222*) and Dostal et al. (*216*). All show at least partial recovery from testicular injury and, where tested, recovery of fertility.

Mechanisms of Effect. A recent *in vitro* study using co-cultures of Sertoli-gonocytes isolated from 2-day-old rats supports an MEHP-induced anti-proliferative effect in Sertoli cells as a sensitive endpoint (*209*). These data are important because 1) they evaluate a relevant response in a target tissue taken at the likely most vulnerable time, 2) this study effectively shows that MEHP, and not DEHP, is the active toxicant, and 3) the effect is observed at a low level of exposure (10^{-7} M MEHP for 24 hours). The challenges in using it for risk assessment are those of using *in vitro* data: the exposure level is constant *in vitro*, and is continuously variable i*n vivo*; the tissue *in vitro* is removed from other systemic inputs that exist *in vivo*, and the *in vitro* study uses rodent tissue. However, the endpoints measured are entirely relevant to what has been noted *in vivo* (i.e., germ cells/gonocyte loss *in vitro* would correlate with, or produce, reduced testes weights *in vivo*, which is an effect actually seen in the *in vivo* studies), which is a strength. The Expert Panel considers that the study by Li et al. (*209*) buttresses the effects seen *in vivo*, and prompts the call for toxicokinetic studies which actually measure levels of MEHP in tissues of fetuses from exposed dams.

All phthalates that cause testicular toxicity produce a common lesion characterized by alterations in Sertoli cell ultrastructure and function (*223-225*). It is known that some Sertoli cell functions are mediated by FSH interaction with membrane bound receptors. Lloyd and Foster (*226*) demonstrated that MEHP disturbs FSH interaction with the FSH receptor. Further studies with MEHP using primary rat Sertoli cell cultures revealed that the monoester of DEHP inhibited FSH-stimulated cAMP accumulation. The MEHP-induced inhibition was specific for FSH (*227*). The Panel was not able to reach agreement that interfering with FSH signaling function was the accepted mode or mechanism of action.

Factors affecting increased sensitivity to phthalate-induced testicular toxicity in young animals were studied for DBP, DEHP, DnHP, and dipentyl phthalate. The monoester derivatives of DBP and DEHP have been shown to cause similar testicular effects. Sjoberg et al. (*108*) demonstrated that gavage treatment with DEHP resulted in greater absorption of MEHP, and hence, a greater systemic dose to young, versus mature, rats. Further, *in vitro* studies did not find that FSH-stimulated cAMP accumulation and lactate secretion were age related (*228*). Lloyd and Foster (*226*) noted that initiation of spermatogenesis was dependent on FSH interaction with the Sertoli cell in young rats, but was not necessary for maintenance of spermatogenesis in adults. Their experiment in Sertoli cell cultures demonstrated that MEHP interferes with FSH interaction at the receptor level and provided a hypothesis for increased sensitivity to testicular toxicity in young animals.

Hormonal activity. Several studies have examined the ability of selected phthalate esters to compete with labeled estradiol (E2) for binding to the estrogen receptor (ER). Sources of ER protein included rat uterine cytosol (*229*), rainbow trout hepatic cytosol (*230*), recombinant human ERs (rhER) overexpressed in SF9 insect cells using the baculovirus system (*231, 232*) and rainbow trout ERs expressed in yeast (*233*). Tritiated E2 was used in the tissue cytosol binding assays while a high affinity fluorescent E2 derivative was used in the rhER binding assays. DEHP exhibited no or weak activity in an *in vitro* assay that measured binding of phthalates to the estrogen receptor (*229*), but MEHP did bind to the estrogen receptor in a second *in vitro* assay (*234*).

Selected phthalate esters have been examined in a number of in vitro gene expression assays systems. The assays have used stably transfected cells (*229*), transiently transfected cells (*229, 230*), yeast based assays (*229, 233, 235, 236*) and vitellogenin induction in rainbow trout hepatocyte cultures (*233*). No activity was noted for DEHP or MEHP in assays of estrogen-induced gene expression (*229, 233, 236*).

In vivo assays demonstrated that DEHP did not increase uterine wet weight or vaginal epithelial cell cornification in immature or mature ovariectomized rats that were treated by gavage with up to 2,000 mg/kg bw/day for 4 days (*229*).

DEHP was considered to have antiandrogenic effects in the male offspring of Sprague Dawley rats administered DEHP by gavage at one dose, 750 mg/kg bw/day, from gd 14 through pnd 3 (*202*). Effects in the offspring included reduced anogenital distance, retained nipples, hypospadias, and vaginal pouches.

Peroxisome Proliferation. The role of peroxisome proliferation in reproductive toxicity has been examined. In contrast to hepatic toxicity, testicular toxicity is noted in PPAR-alpha knockout mice exposed to DEHP, albeit that appearance of the testicular effects was delayed compared to wild-type mice. In addition, the guinea pig, a non-responding species to the peroxisomal-proliferating effects of DEHP, is susceptible to the testicular effects of this agent. Since the PPAR-alpha knockout mouse is susceptible to phthalate-induced testicular toxicity and expresses PPAR-gamma in the testis which can be activated by MEHP (*190*), PPAR-gamma may play a role in the reproductive toxicity of phthalates.

Overall, the Panel believes that the reproductive toxicity of DEHP appears independent of PPAR-alpha. However, other members of the PPAR family (beta or delta and gamma) have not been extensively studied with regard to activation by phthalates. PPAR-gamma has been found in human testis, ovary, placenta, and embryo. MEHP (but not DEHP, 2-EH, or 2-EHA) has been shown to activate PPAR-gamma receptor in a transcription reporter assay (*190*)

4.2.2 MEHP

Curto and Thomas (*214*) studied the effects of parenteral MEHP exposure on organ weight and zinc levels in prostate, seminal vesicle and testis of Sprague-Dawley rats (200–300 g) and Swiss-Webster mice (35–40 g). Groups of 6–12 mice were injected subcutaneously (sc) with MEHP at 0, 1, 5, or 10 mg/kg bw/day for 5 days or 5, 10, or 20 mg/kg bw/day for 10 days. Dosing regimens for IP exposures were 0, 50, or 100 mg/kg bw/day for 5 days in mice and 0, 50, or 100 mg/kg bw every other day for 20 days in mice and rats (6 animals/group in each experiment). Controls were exposed to the vehicle (0.9% sodium chloride). In mice there were no effects on reproductive organ weights or zinc levels, but death occurred in 3/6 mice exposed IP to 100 mg/kg bw/day for 5 days. Prostate zinc levels were reduced in rats exposed to 50 mg/kg bw MEHP over 20 days.

4.2.3 2-EH

In a 'prechronic' type study of 2-EH, Astill et al. (*98*) (WEB Table 8 and WEB Table 9) dosed (10/group) young adolescent F344 rats (42–43 days old) and $B6C3F_1$ mice (49–61 days old) with 2-EH by gavage in Cremophore at 25, 125, 250, and 500 mg/kg bw/day, 5 day/week for 13 weeks. Dosing solution was checked for 2-EH stability and concentration. At the end of the exposure period, organs were removed and weighed, then fixed in neutral buffered formalin, a fixative that can cause significant fixation artifacts in the testes.

Non-reproductive systemic effects are reported in Section 2. An increase in ovary weight in females was observed in the mid-dose group. Relative testes weight was increased at the high dose.

No histopathology was reported for the testes. The reproductive LOAEL is not calculable, because no adverse reproductive effects were seen. The NOAEL is 500 mg/kg bw/day, based on lack of effect on testes weight.

Confidence in the conduct of the study is moderate-to-high; the methods used in the study would probably have found any major effect. However, confidence that this study found the actual NOAEL is

low, because the design started with animals that were too mature and the most sensitive endpoints were not evaluated. Astill et al. (*99*) also conducted a chronic study in rats and mice that reported no effects on testicular histology.

In addition, four studies of the reproductive toxicity of 2-EH *in vitro* were reviewed. These studies did not report adverse effects (*218, 237-239*). 2-EH is metabolized under normal conditions to 2-EHA.

4.2.4 2-EHA

The effect of 2-EHA on reproduction and postnatal development was reported for Wistar rats(*180*) (WEB Table 49). The sodium salt of 2-EHA was mixed into drinking water to give 0, 100, 300, or 600 mg/kg bw/day. Twenty-four male rats per group (16 weeks old) were exposed to the chemical for 10 weeks, and 24 females per group (15–16 weeks old) for 2 weeks prior to mating, both sexes during the mating trial, and females throughout the gestation and lactation period. Food and body weights were measured weekly. Males were killed and necropsied at the end of the mating period. Reproductive organs were weighed and fixed in neutral buffered (NBF). Histopathology and sperm measures were taken for five males/group. Litters were culled to eight at pnd 4. Pups were weighed and evaluated for markers of general postnatal development until pnd 21. At pnd 21, all dams and litters were sacrificed. Histopathological examination was conducted on five randomly chosen dams/group, and on no pups.

No effects were observed on body weight, feed consumption, or water consumption, except for the period of pregnancy for females that received the 600 mg/kg bw/day dose. During this period, water consumption and rate of weight gain were decreased.

Absolute and relative reproductive organ weights were not lowered by 2-EHA exposure. Testes histology was unaffected by 2-EHA at any dose. Sperm motility was significantly reduced at 100 and 600 mg/kg bw/day but not at 300 mg/kg bw/day (mean % motile: 35, 22, 28, 27). The authors reported that they failed to observe statistically significant decreases in the quantity or quality of sperm in the cauda epididymis.

No effect on ability to conceive or pregnancy index was observed; however, there was a dose-dependent delay in fertilization as indicated by the number of estrous cycles required to effect pregnancy at the 600 mg/kg bw/day dose. Mean litter size at pnd 0 was also reduced by ~15%. In the growing pups, tooth eruption, pinna detachment, eye opening, and hair growth were delayed and an increased incidence of kinky tails was observed at 600 mg/kg bw/day, the dose group that had a mild (<10%) transient delay in body weight gain. A delay in pinna detachment and increased numbers of pups with kinky tails were also observed at the 300 mg/kg bw/day group. At necropsy, dam histology was unchanged by 2-EHA exposure.

In a separate study also reported in Pennanen et al. (*180*), a single administration of 600 mg/kg bw/day on a specific gd (4, 5, 6, or 7) resulted in fewer implants when 2-EHA was given on gd 6.

The LOAEL for reproductive effects is considered to be 600 mg/kg bw/day, based on changes in sperm motility, reduced litter size, and increased time-to-pregnancy. The litter size and time-to-pregnancy effects are probably female effects. Based on these same sperm and cyclicity effects, the NOAEL would be 300 mg/kg bw/day.

Confidence in the quality of the study is moderate-to-high, because of the redundancy of endpoints observed in the males. Confidence that this study found the real NOAEL is moderate-to-low, since the structure and function of the reproductive system after developmental exposure was not evaluated.

In another study of 2-EHA, Juberg et al. (*97*) (WEB Table 6 and WEB Table 7) exposed groups (n=10) of Fisher 344 rats or $B6C3F_1$ mice to 2-EHA in the diet at 0, 0.1, 0.5, and 1.5% (w/w) for 13 weeks, starting at ~pnd 42. Body weight and food consumption were measured. Intake of 2-EHA for the male rats was calculated at 61, 303, and 917 mg/kg bw/day; male mouse intake was 180, 885, and 2,728 mg/kg bw/day. Female intake was approximately 10% higher. Feed was analyzed for 2-EHA content. After 13 weeks of exposure, the animals were sacrificed and necropsied. Tissues were fixed in NBF. Testes were weighed, but epididymides, prostate, and seminal vesicles were not.

Non-reproductive systemic effects are reported in Section 2. Testes weight was increased in high-dose animals of both species. While the methods do not indicate that histology was performed on the reproductive organs, the results state that "no testicular changes were noted."

There were no reproductive effects, so a LOAEL was not identified. The NOAELs for reproductive effects are 1.5% 2-EHA in feed, or 917 mg/kg bw/day (rats) and 2,728 mg/kg bw/day (mice)

Confidence in the quality of the study is moderate. Confidence that the authors found the real NOAEL for reproductive effects is low, because they did not expose the most vulnerable age or examine the most sensitive markers.

The summary for Section 4 is located in Section 5.1.4.

5.0 DATA SUMMARY & INTEGRATION

5.1 Summary

5.1.1 Human Exposure

Overview

DEHP is a plasticizer of polyvinyl chloride (PVC) and other flexible plastics. PVC that contains DEHP is used in the manufacture of a wide variety of consumer products. Examples of DEHP use include: building products (wallpaper, wire and cable insulation), car products (vinyl upholstery, car seats), clothing (footwear, raincoats), food packaging, children's products (toys, crib bumpers), and medical devices. According to Aristech Chemical company, the production volume of DEHP is approaching 2 million tons annually (*2*). Exposure to DEHP in children's products has declined in recent years. In the United States and Canada, DEHP is no longer used in toys intended for mouthing (nipples, teethers, pacifiers, rattles), but is still found in larger toys used by older children.

DEHP is widely dispersed in the environment and gains entry to the body during manufacture of products, migration from products during use, and during disposal in municipal and industrial landfills and waste incineration. The release of DEHP directly into the atmosphere is believed to be the most important mode of entry into the environment. Vapor-phase DEHP is degraded with the atmospheric photo-oxidation half-life reported to be 0.2–20 days (*6*). Because of its low vapor pressure and limited water solubility, concentrations of DEHP in outdoor air and water are low; they range from 10^0 to 10^2 ng/m^3 and 10^{-3} to 10^1 ppb, respectively. DEHP concentrations are often at or below detection limits.

DEHP sorbs strongly to sediments and aerosol particulates; it also bioaccumulates in invertebrates, fish, and plants. Biomagnification is not observed; biodispersal occurs at higher trophic levels in the food chain because of metabolism. Biodegradation proceeds well in aerobic, nutrient-rich environments over weeks, but may be extremely slow (many years) under anaerobic conditions (*5*). The aerobic biodegradation of dialkyl phthalates begins with hydrolysis to the monoester and alcohol (*6*). The monoester is converted to phthalic acid and then further metabolized to pyruvate and oxaloacetate or acetyl CoA and succinate.

General Population Exposure

Exposure of the general population to DEHP occurs via ingestion and inhalation. The largest source of general population exposure to DEHP is dietary. A few food surveys show a range of DEHP content with fatty foods, including dairy, fish, meat, and oils, containing the most (*7*, *8*). Discrepancies in food exposure estimates may be due to inherent variability of food eaten by individuals based on age, sex, ethnicity, time of sampling, and geographical locations. Few studies on indoor air exist, but DEHP was measured in levels of microgram per cubic meter ($\mu g/m^3$).

Infants and children should be considered as a separate subset of the general population. General assumptions about diet do not apply to infants and children who consume significantly different diets than do adults (*10*). Similarly, children have a higher minute ventilation than adults and tend to be more active. The Health Canada environmental exposures clearly show the differences in exposure to DEHP from all compartments (*7*). The Canadian estimates are presented below.

Table 14: Estimated Daily Intake of DEHP by the Population of Canada (Expressed in micrograms per kilogram body weight per day.)

	Age				
Substrate/ Medium	**0.0–0.5 yr**	**0.5–4 yr**	**5–11 yr**	**12–19 yr**	**20–70 yr**
Ambient Air: Great Lakes Region	0.00003–0.0003	0.00003–0.0003	0.00004–0.0004	0.00003– 0.0003	0.00003– 0.0003
Indoor Air	0.86	0.99	1.2	0.95	0.85
Drinking Water	0.13–0.38	0.06–0.18	0.03–0.10	0.02–0.07	0.02–0.06
Food	7.9	18	13	7.2	4.9
Soil	0.000064	0.000042	0.00014	0.000004	0.00003
Total Estimated Intake	8.9–9.1	19	14	8.2	5.8

Adapted from (*7*)

Total estimated daily intake is highest in children ages 0.5–4 years. The primary quantitative difference between each age group is associated with the food category. Levels of DEHP found in infant formula in the US are lower than those reported from other countries (*12-15*). Dietary exposures may vary from country to country because DEHP is introduced through various food processing and packaging techniques which differ internationally. There were no studies located that report levels of DEHP metabolites (MEHP, 2-EH, or 2-EHA) in foods.

DEHP is used in some children's toys (*18-20*). A recent Austrian risk assessment suggests that children's exposures to DEHP from sucking or chewing PVC articles could reach 85 μg/kg bw/day (*23*). Exposures to children's products may also vary internationally because of differences in manufacturing practices.

The range of exposure in the general population from all sources, excluding non-dietary ingestion, medical, and occupational, is estimated to be within the range of 3–30 µg/kg bw/day (*24*). Exposures in children may be higher due to non-dietary exposure resulting from mouthing of DEHP-containing objects.

Occupational Exposure

Occupational exposure occurs during the manufacture and processing of DEHP. Workers may be exposed to relatively high concentrations during the compounding of DEHP with PVC resins (*5*). The major route of exposure is inhalation (*24*). The OSHA standard is 5 mg/m^3, with actual workplace exposures typically reported to be below 1 mg/m^3 or 143 µg/kg bw/workday at phthalate manufacturing facilities and below 2 mg/m^3 or 286 µg/kg bw/workday at flexible PVC production facilities (*1*). Studies based upon workplace air measurements in Europe and the former USSR estimate occupational exposures to be from <2–6,600 µg/kg bw/day (*8*).

Medical Exposure

DEHP is currently the primary phthalate plasticizer used in PVC medical devices. The majority of medical exposures are intravenous through infusion of blood or blood products, hemodialysis, or bypass procedures. In these circumstances, patients are exposed to DEHP and its monoester metabolite, MEHP. DEHP migrates from the PVC bag during storage and from the tubing used during infusion. MEHP is formed through metabolism of DEHP by blood during storage. Exposure to DEHP may also occur by inhalation (e.g., ventilators) and by ingestion (e.g., nasogastric tubes).

Exposures can be short-term, moderate dose (e.g., single blood transfusion); short-term, high dose (e.g., double volume exchange transfusion); or chronic, moderate-to-high dose (e.g., hemodialysis). The magnitudes of the exposures are highly variable and have not been extensively studied. DEHP exposure ranges from tenths of milligrams per kilogram body weight, as with a single blood transfusion, to tens of grams per year for chronic hemodialysis patients (see Table 15). Very high exposures in neonates occur during exchange transfusion, cardiac surgery, and ECMO. Some exposure scenarios can be reasonably estimated within one or two orders of magnitude, but additional variability is possible depending on assumptions made and study design. The best studies of exposure measure delivered dose using area under the curve calculations for dialysis (*57-59*), and exchange transfusion (*63*). Most studies estimate dose based upon spot measurements of DEHP in blood, or calculated leaching rates from blood bags or perfusion circuits. Neither approach is as accurate as the AUC measurement approach.

Table 15. Comparison of DEHP Exposures – General & Medical Sources

Procedure Resulting in Exposure	DEHP Exposure (µg/kg bw/day)
General population — Primarily Ingestion	3–30 (*24*)
Maximum Allowable Occupational Exposure to DEHP—Inhalation	700 (*24*)
Selected Medical Exposure to DEHP-IV [1]	Average (range)
• Neonatal Replacement Transfusion-short-term, acute (10 cc/kg bw/tx)	300 (140–720) (*63*)[2]
• Double Volume Exchange Transfusion-short-term, acute (neonate)	1,800 (840–3,300) (*63*)[3]
• Adult Hemodialysis-long-term, averaged over 1 year (70 kg, 156 sessions/yr)	640 (150–2,200) (*58*)[4]
• Adult Hemodialysis—long-term, averaged over 1 year (70 kg, 156 sessions/yr)	100 (20–360) (*59*)[4]

[1]These numbers will not match those reported by the authors due to rounding and utilization of tabular data as discussed in Section 1 of the Background Document.
[2]Calculated from reported DEHP concentration in blood bags averaged (range) and rounded to nearest 10.
[3] Calculated from reported area under curve (AUC) dose to patients, averaged and (range) rounded to nearest 10.
[4] Calculated from reported AUC dose at average (range), 3 sessions per week, 52 weeks, 70 kg, for one year.

An alternative method of assessing dose to humans during medical procedures is to measure DEHP and MEHP serum or plasma levels before and after procedures. A number of authors have reported these measures and they are referenced in the full table found in Section 1 under 'Medical Exposures.' Two Sjoberg studies also measured MEHP using area under curve analysis and showed average delivered doses of 100 µg/kg bw/exchange transfusion and 360 µg/kg bw/exchange transfusion.

5.1.1.1 Utility of Data to the CERHR Evaluation

The Expert Panel believes the range 3–30 µg/kg bw/day intake for the general population is a reasonable estimate. Estimates of exposure associated with medical uses of DEHP are less reliable. More precision in exposure estimates is not currently possible because the number of patients actually studied is quite low and the variation within the small study populations is great. Accuracy of calculations based upon presumed DEHP/MEHP inputs from blood products or particular procedures is also limited by the age of the data, often gathered over decades during which both medical procedures and analytic measurement techniques changed dramatically. Furthermore, blood banking protocols have changed significantly since the initial studies, calling attention to the presence of DEHP and MEHP in blood products, and may render the use of older measurements unreliable. Several important exposures in the fetus and neonates have not been explored, including placental transfer of maternally-derived DEHP/MEHP from medical and/or dietary sources, and contributions from parenteral and enteral feeding, ventilators, IV fluids, or combinations of simultaneous exposures. It is likely that such investigation would yield higher exposures to small babies during a developmentally vulnerable time.

5.1.2 General Biological and Toxicological Data

5.1.2.1 General Toxicity

DEHP

General toxicity-oral

Human Data. No human oral toxicity data were available to the Expert Panel.

Experimental Animal Data. The toxic response is variable in animal species where there is repeated exposure to DEHP. This variability is seen in the dose required to cause a response and in the nature of the effect. For example, rats and mice are the most sensitive species with primary target organs being liver and testes; these effects are also observed in hamsters and guinea pigs. In contrast, the liver and testes of cynomolgus monkeys, marmosets, and dogs appear insensitive to repeated exposure to DEHP. Table 16 summarizes selected repeat dose studies in rodents and studies in other species. Other rodent studies and all summarized studies are described in Section 2.2

The oral repeat-dose studies in rats and mice consistently show that the primary targets for effect are liver, kidney, and testes. Effects were also observed in some studies on the pituitary, thyroid, thymus, ovaries, and blood. While the liver shows a biological response at the lowest doses of DEHP that cause effects, the testes' response at somewhat higher doses is a greater health hazard concern. Liver effects are primarily those associated with peroxisome proliferation and include increases in weight, hepatocellular hypertrophy and hyperplasia, and increases in enzyme markers relevant to peroxisome stimulation. Adverse effects in liver have been noted in subchronic studies of mice at oral doses of 1,200 mg/kg bw/day for 28 days (*70*), and in rats 260 mg/kg bw/day for 13 weeks (*76*). Tumor response in 104-week studies (hepatocellular adenomas and carcinoma) was observed in rats at 147 mg/kg bw/day and in mice at 292–354 mg/kg bw/day (*80*). A mechanism of action which integrates all of these hepatic effects has not been identified. However, it is believed that many of the hepatic effects are due to peroxisome proliferation. (See Mode of Action heading in this section.) This theory is supported by the findings that hepatic hyperplasia, hypertrophy, stimulation of peroxisomal oxidation enzymes, and decreases in plasma lipid levels are common effects among structurally unrelated chemicals and drugs inducing peroxisome proliferation. Testicular effects are discussed in Section 5.1.4.

Studies of 14-months' duration where ferrets were fed a diet containing 1% DEHP (average 1,200 mg/kg bw/day; range 650–2,000 mg/kg bw/day) also demonstrated a liver response indicative of peroxisome proliferation (*87*). Repeat oral dose studies of 14–25 days duration in cynomolgus monkeys and marmosets, and a 13-week study with marmosets at doses as high as 2,500 mg/kg bw/day, had no effects on liver or testes, with the exception of increased peroxisomal volume in marmosets (*82-85*). While specifically examined, there was little or no evidence of peroxisome stimulation in these species; limited studies in dogs at doses of ~ 40 mg/kg bw/day for 1 year gave similar results (*88*).

Table 16. Summaries of DEHP Effects in General Toxicity Studies with Oral Exposure

Protocol and DEHP Doses (mg/kg bw/day)	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day) and Effects	Major Effects at Higher Doses.
21-day dietary study in Fischer 344 rats. 6 weeks old at start of study, 5 rats/sex/group. Doses – M: 0, 11, 105, 667, 1,224, 2,101; F: 0, 12, 109, 643, 1,197, 1,892. (*69*)	M: 105 F: 109	M: 643 F: 667 ↑ Liver weight with histological changes (M). Peroxisomal proliferation.	↓ Testes weight. Testicular atrophy. ↑ Liver weight with histological changes. ↓ Kidney weight. Peroxisomal proliferation.
90-day dietary study in Sprague-Dawley rats. Estimated to be 4–6 weeks old at start of study, 10 rats/sex/group. Doses – M: 0, 0.4, 3.7, 38, 375 F: 0, 0.4, 4.2, 42, 419. (*75*)	M: 3.7 F: 4.2	M: 38, F: 42 Mild testicular lesions. Liver enzyme changes.	Testicular lesions. Mild liver hypertrophy and necrosis. ↑ Kidney weight. Anemia (M). Mild thyroid histological changes.
13-week dietary study in Fischer 344 rats. 8 weeks old at start of study, 10 rats/sex/group. Doses – M: 0, 63, 261, 850, 1,724 F: 0, 73, 302, 918, 1,858. (*76*)	None	M: 63 F: 73 ↑ Liver weight (M). ↑ Kidney weight (F).	↓ Testes weight with testicular atrophy. ↑ Aspermia. ↓ Uterine weight. Liver hypertrophy. Anemia. ↑ Kidney weight with pigmentation changes. ↑ Pituitary effects.
4 week dietary study in $B6C3F_1$ mice. 6 weeks old at start of study, 10 mice/sex/group. Doses – M: 0, 245, 1,209, 2,579, 6,992 F: 0, 270, 1,427, 2,897, 7,899. (*70*)	M: 245 F: 270	M: 1,209 F: 1,427 ↑ Liver weight with necrosis. ↓ Kidney weight with inflammation (M). Anemia (M).	↓ Testes weight with atrophy. Absence of corpora lutea. ↑ Liver weight with necrosis. ↓ Kidney weight (M) and ↑ kidney weight (F) with inflammation. Anemia. Thymus atrophy.
13 week dietary study in B6C3F1 mice. 5–6 weeks old at start of study, 10 mice/sex/group. Doses – M: 0, 144, 289, 578, 1,156, 2,311 F: 0, 157, 314, 629, 1,258, 2,516. (*77*)	M: 289 F: 314	M: 578 F: 629 ↓ Weight gain.	↓ Weight gain. No histological changes.
14-month gavage study in male ferrets.	None	1,200 ↓ Weight gain.	No higher doses.

18 months old at start of study, 6-7/group. Doses : 0, 1,200 (*87*)		↑ Liver weight with histological changes. Testicular lesions.	
25-day gavage study in cynomolgus monkeys (age not specified). 2 male monkeys/group. Doses: 0, 100, or 500. Study only examined liver effects, especially peroxisomal proliferation . (*84*)	500	No peroxisomal proliferation at any dose.	No peroxisomal proliferation at any dose.
13-week gavage study in marmosets. 12-15 months old at start of study, 4 marmosets/sex/group. Doses: 0, 100, 500, or 2,500. (*82*)	100	500 Increased peroxisomal volume (M).	Increased peroxisomal volume (M). ↓ Body weight. No testicular lesions.

General toxicity – inhalation

Human Data. There is limited information about DEHP toxicity in humans exposed by inhalation. No controlled epidemiological studies are available. Increased incidence of neuropathy was reported in groups occupationally exposed to several phthalates, including DEHP. However, the association is considered questionable due to the lack of a control group and presence of other exposure components (phthalic anhydride and respective alcohols) that could have caused this effect (*89*). Exposure to DEHP-containing fumes in the thermo-plastic industry has been associated with decreased erythrocyte and platelet counts and increased isotransferrin ratio (*90*).

A series of case studies describing lung disorders resembling hyaline membrane disease in three pre-term infants ventilated with PVC respiratory tubes for 4 weeks has been published (*66*).

Experimental Animal Data. Klimisch et al. (*91*) found an increase in lung and liver weights of rats after inhalation exposure to 1 mg/L, 6 hours/day, 5 days/week for 28 days. Histological lung changes were noted, but there was no histologic evidence of peroxisomes, no adverse effects on fertility, and no histopathology in testes.

General toxicity – parenteral

Human Data. A single report of 29 infants undergoing ECMO found a statistically significant positive association between both free hemoglobin and DEHP serum levels and the degree of cholestasis. A causal link between DEHP and either increased hemolysis or cholestasis is speculative (*92*).

Experimental Animal Data. The literature is sparse in this area. Sjoberg et al. (*93*) reported that 6 treatments with DEHP administered intravenously to male rats increased liver weight by 35%, and increased peroxisomal content by ~41% after 500 mg/kg bw (time weighted average of 250 mg/kg bw/day), while changes were not detectable at the next lower dose (50 mg/kg bw; time weighted average of 25 mg/kg bw/day). There were no changes in serum liver enzymes. The relatively small response after

these exposure routes is probably related to the limited conversion of DEHP to MEHP, which occurs much more slowly when exposure to the gut lipases and esterases is bypassed (*107*).

Table 17: Summaries of DEHP Effects in General Toxicity Studies with Parenteral Exposure

Protocol and DEHP Doses	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day) and Effects	Major effects at higher doses
12-day study with 6 IV administrations every other day. Male Sprague-Dawley rats 40 days old at start of study, 5–6 rats/group. Doses: 0, 5, 50, or 500 mg/kg bw (0, 2.5, 25, or 250 mg/kg bw/day). (*93*)	25	250 ↑ Liver weight. Peroxisomal proliferation. Slight testicular changes.	No higher doses.

2- Ethylhexanol (2-EH) & 2- Ethylhexanoic acid (2-EHA)

The Expert Panel did not review human data for 2-EH or 2-EHA.

The liver was the target organ for the two DEHP metabolites, 2-EH and 2-EHA. Hepatic hypertrophy was reported in F344 rats and $B63CF_1$ mice fed diets with 2-EHA at doses of 303 and 885 mg/kg bw/day, respectively, for 13 weeks (*97*). Peroxisomal proliferation and reduced hepatic fatty infiltration were observed in F344 rats but not $B6C3F_1$ mice gavaged with 500 mg 2-EH for 13 weeks (*98*). In 2-year gavage studies, Astill et al. (*99*) found that 2-EH is a weak carcinogen in mice at a dose of 750 mg 2-EH/kg bw/day, but the compound was not carcinogenic in rats treated with up to 500 mg 2-EH/kg bw/day.

Table 18: Summary of 2-EH Effects in General Toxicity Studies with Oral Exposure

Protocol and 2-EH Doses (mg/kg bw/day)	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day) and Effects	Major effects at higher doses
13-week repeated dose gavage study in Fischer 344 rats. 6 weeks old at the start of the study, 10 rats/sex/group. Doses – 0, 25, 125, 250, 500. (*98*)	125	250 Changes in liver enzyme activities (F). ↑ Kidney, liver, and ovary weight.	↑ Liver weight with changes in liver enzyme activities (F) and mild histological changes. ↑ Testes, and kidney weight. Peroxisomal proliferation. No lesions in testes or ovaries.
13-week repeated dose gavage study in $B6C3F_1$ mice.	125	250	↑ Liver weight (M). No lesions in testes or

49–61 days old at the start of the study, 10 mice/sex/group. Doses – 0, 25, 125, 250, 500. (*98*)		↑ Liver weight (M).	ovaries.

Table 19: Summary of 2-EHA Effects in General Toxicity with Oral Exposure

Protocol and 2-EHA Doses (mg/kg bw/day)	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day) and Effects	Major effects at higher doses
13-week repeated dose dietary study in Fischer 344 rats. 6 weeks old at start of study, 10 rats/sex/group. Doses – M: 0, 61, 303, 917 F: 0, 71, 360, 1,068 (*97*)	M: 61; F: 71	M: 303; F: 360 Liver hypertrophy (M). ↑ Cholesterol.	↑Cholesterol. Liver hypertrophy. No testicular effects.
13-week repeated dose dietary study in $B6C3F_1$ mice. 6 weeks old at start of study, 10 mice/sex/group. Doses – M: 0, 180, 885, 2,728, F: 0, 205, 1,038, 3,139 (*97*)	M: 180; F: 205	M: 885; F: 1,038 Liver hypertrophy (M). ↑ Cholesterol.	↑ Liver hypertrophy. ↑ Cholesterol. Mild histological kidney changes. No testicular effects.

Mode of Action

Liver. It is well understood that DEHP produces a range of hepatic effects (induction of peroxisomes; increased Cyp4A1; PCoA) and hepatocellular adenoma and carcinoma in rat and muse studies. The induction of these effects is believed due to activation of PPAR-alpha. In PPAR-alpha knockout mice, administration of DEHP does not result in the induction of hepatic effects or tumors, unlike the tumor response in wild type control animals. Peroxisomal proliferation was not observed in marmosets exposed with up to 2,500 mg/kg bw/day or in cynomolgus monkeys dosed with up to 500 mg/kg bw/day (*82*, *84*). However an increase in peroxisomal volume was noted in the marmosets. In humans, PPAR-alpha, is activated upstream of, and regulates different enzymes from, those noted in the rat. Subsequently, peroxisomal proliferation does not occur and induction of these enzymes does not appear to produce toxic responses in humans. Taking note of the large body of research data in this area, IARC recently re-evaluated DEHP and changed its classification to "not classifiable as to carcinogenicity in humans" from the previous classification of "possibly carcinogenic to humans." In contrast to hepatic effects, renal toxicities are noted in PPAR-alpha knockout mice. The panel is aware that a Consumer Product Safety Commission (CPSC) review of DEHP is in progress.

The modes of action for developmental and reproductive effects are addressed in Sections 5.1.3 and 5.1.4, respectively.

5.1.2.2 Toxicokinetics

After oral administration in rats, DEHP is rapidly hydrolyzed to MEHP by lipases in the gut and absorbed as the monoester and 2-EH (*105-108*). Plasma levels peak in 1–3 hours. In primates, including humans and marmosets, a smaller proportion of DEHP is hydrolyzed and absorbed as the monoester, apparently because of less lipase activity in primate intestine (*85, 114, 116, 119, 240*). Rats metabolize DEHP by oxidation to diacids while primates, including humans, predominately form MEHP and metabolite IX (a secondary alcohol). Adult humans and other primates excrete the monoester as a glucuronide, whereas rodents further metabolize these intermediates. The toxicological significance, if any, of this species difference is unclear. Oral doses of DEHP are metabolized quickly and the metabolites are rapidly excreted via urine and feces (*126-130*). However, the parent compound, DEHP, has not been detected in the urine of any species studied, but is detected in feces in amounts inversely related to the degree of absorption. No significant retention in organs and tissues was observed in any species studied, with less than 1% dose retained a few days after administration.

Inhaled DEHP is absorbed as parent compound from the lung (*110*); dermal absorption of DEHP is slow (*101, 102*). Once absorbed into the blood stream, DEHP and its metabolites are widely distributed throughout the body without accumulation in tissues. While DEHP can be hydrolyzed to the monoester and alcohol by lipases in the liver and lung, plasma MEHP levels after parenteral DEHP exposure are only ~1–5% of those seen after oral DEHP exposure (*107*).

DEHP and its monoester, MEHP, can cross the placenta (*111*). It is also secreted in the milk of lactating rats (*112, 113*). Metabolism to MEHP, and it persistence, is relevant to evaluation of hazard to human infants. Pancreatic lipase appears in the latter third of gestation but is at low levels at birth. It approaches adult levels at several months of age (*241, 242*). However, lingual lipase is present at birth and is responsible for 60-70% of lipid hydrolysis in the early neonatal period (*243*). Gastric lipase is also present at birth and reaches adult levels in the infant at 3 months of age (*244*). Thus, it is reasonable to assume that the neonate is able to hydrolyze DEHP to MEHP. Children do not have mature glucuronidation pathways until they are 3 months old. Thus, this important clearance mechanism is not fully available to neonates and young infants (*134*). Higher levels of DEHP in plasma were seen in infants in the early course of ECMO therapy; it was not determined if DEHP became more efficiently metabolized during the treatment or that it was redistributed to various tissues (*54*). DEHP levels were higher at necropsy in premature neonates who had received varying levels of blood products in comparison to infants who had not received blood products (*122*).

A PBPK model of the tissue distribution of DEHP and MEHP in rats has been developed by Keys et al. (*136*) and includes pH trapping as a mechanism for uptake of MEHP into tissue. The model should provide a valuable tool for extrapolating rat data to predicted tissue dosimetry in humans for use in human risk assessments. The model has been used to develop a benchmark dose risk assessment for testicular toxicity of MEHP in humans with appropriate modifying factors (*245*). The Expert Panel believes the model, with additional refinements, could be used to extrapolate dosimetry data between different species and ages, genders, and pregnant versus non-pregnant individuals. With modifications, the model can eventually be used to determine dosimetry of MEHP following IV administration.

5.1.2.3 Genetic Toxicity

DEHP is not known to be genotoxic. DEHP has tested negative in the majority of genetic toxicity assays conducted and is considered non-mutagenic by KEMI (*137*). Gene mutation studies were conducted in

bacteria, yeast, mammalian cells, and rodents. Other genetic endpoints examined included sister chromatid exchange, micronucleus effects, unscheduled DNA synthesis, Rec assay, and cell transformation. There was no evidence of chromosomal aberrations in workers exposed to DEHP, but the study was considered inadequate because of the small sample size and low exposure to DEHP (*137*).

5.1.2.4 Utility of Data for the CERHR Evaluation

The dataset is adequate for the evaluation of general toxicity induced by DEHP. There were several studies conducted according to GLP standards with relevant exposure routes, study duration, and dose levels. Numerous species including rats, mice, monkeys, hamsters, and ferrets were evaluated, thus allowing a comparison of interspecies sensitivity. Testicular pathology was also evaluated in many of these studies. A limitation is that in most studies, testes were preserved in formalin and embedded in paraffin, which can lead to histopathological artifacts. These artifacts can confound interpretation of early and subtle changes, but do not impair the identification of severe lesions.

Peroxisomal proliferation was found in rats and mice exposed for 3 months or 2 years and in ferrets exposed for 14 months. Peroxisomal proliferation was not observed in marmosets exposed for 3 months but an increase in peroxisomal volume was noted. Peroxisomal proliferation was not observed in dogs or guinea pigs exposed to relatively low doses (approximately 40–60 mg/kg bw/day) for 1 year. These data show species differences in this response.

The dataset provides limited information about the role of DEHP metabolites in DEHP-induced toxicity. The available studies for 2-EH and 2-EHA were conducted according to GLP standards with the appropriate exposure routes, doses, and duration. However, there are no known studies that evaluate the general toxicity following MEHP exposure. Peroxisomal proliferation was not examined for 2-EHA. In 2-EH studies in rats and mice, peroxisomal proliferation was elevated in rats following subchronic exposure. In 2-year studies, 2-EH was not carcinogenic in rats and produced weak responses in female mice at the highest dose. Testicular histopathology was evaluated in 2-EH by Astill et al. (*98, 99*).

There is a significant amount of toxicokinetic data relevant to estimating exposure of DEHP and major metabolites at tissue sites of toxicological interest in adult individuals. The data on metabolism and clearance of DEHP and metabolites through medical treatment involving neonates is incomplete. Similar data on patients undergoing long term treatments, such as kidney dialysis, is not robust. Recent publication of PBPK models for DEHP and other phthalates have the potential to provide a sound scientific basis for refining estimates of exposure.

5.1.3 Developmental Toxicity

There were no studies located on the developmental toxicity of DEHP or its metabolites in humans.

Given the rapid metabolism of DEHP to the monoester, MEHP, and 2-EH this review included an evaluation of the developmental toxicity of the parent chemical and the two initial metabolites. In addition, secondary metabolites of MEHP and 2-EH, phthalic acid (PA) and 2-EHA, respectively, were evaluated.

More than 40 developmental toxicity studies of DEHP and its metabolites were reviewed. Individual studies are not described in this section as they are reviewed in Section 3 of this document. Key aspects of the evaluation of that data set are discussed below. Terse summaries of the large number of available laboratory animal studies are presented below.

DEHP

Developmental toxicity findings were remarkably consistent. DEHP was found to produce malformations, as well as intrauterine death and developmental delay. The pattern of malformations seen in fetuses is consistent across studies. It included morphological abnormalities of the axial skeleton (including tail), cardiovascular system (heart and aortic arch), appendicular skeleton (missing limb bones, finger abnormalities), eye (including open eye), and neural tube (exencephaly). In many cases, the type and detail of teratological examination would lead to differences in the observed incidences and types of malformations. For example, detailed examination of the aortic arch using the Staples dissection method led to detection of a high incidence of cardiovascular abnormalities. Also, some studies used bolus doses late in embryogenesis, after neural tube closure had already taken place; therefore exencephaly would not be an expected outcome.

In terms of maternal toxicity, some differences were seen across studies and species. In rats, increased liver weight occurred along with an increase in water consumption and decrease in food consumption. Lower maternal body weight gain appeared as early as 4 days after initiation of exposure. In contrast, mice administered DEHP in diet showed no effect on water consumption, and their food consumption was increased, although maternal body weights were markedly lower than controls. No changes in liver weight were seen in mouse dams up to doses that resulted in 85% resorption. In general, across studies there was not a strong relationship between the type and amount of maternal toxicity and developmental toxicity.

In addition to the studies of developmental toxicity with post-conception exposure discussed above, developmental toxicity was also manifested in reproductive toxicity studies, as discussed in Section 5.1.4 (Reproductive Toxicity). The database as a whole identified CD-1 mice as the most sensitive species for DEHP developmental toxicity via the oral route. The critical papers are Tyl et al. (*149*), Huntingdon (*150*), Reel et al. (*186*), and Lamb et al. (*168*). LOAELs and NOAELs for some relevant developmental toxicity studies are presented in Table 20. Developmental effects in reproduction studies are listed in Table 25 in Section 5.1.4. Studies that address developmental toxicity are consistent in identifying the lower effective range of oral exposure, taking into account differences in duration of treatment.

The DEHP database contains four rat studies conducted by a route other than oral: an IV study, two IP studies, and an inhalation study. These studies provide valuable information but do not contain enough data for separate route-specific hazard identification and NOAEL/LOAEL selection. DEHP was extracted from plastics used to make blood storage bags and given intravenously to pregnant rabbits (*163*). No maternal or developmental toxicity was reported. The exposure (1–5 mg/kg bw/day) was low relative to doses used in oral studies that show fetal effects. With IP administration to mice and rats, the lowest dose reported to produce fetal effects was 1,972 mg/kg bw/day (*165*). Authors of an inhalation study with a maximum dose of 84 mg/kg bw/day to pregnant rats concluded that no fetal or maternal toxicity was evident (*162*).

The tabular study summaries in support of Section 3.2 suggest that developmental toxicity was sometimes reported at doses for which maternal toxicity was absent or minimal and thus did not interfere with interpretation. In studies that include both a maternal and fetal NOAEL and LOAEL, there is not a consistent relationship between these values. When maternal toxicity was reported, no mechanistic link between developmental toxicity endpoints and maternal toxicity was suggested by the authors, with the exception of the studies of zinc deficiency and acute phase proteins (*152, 246*). Thus, the possibility that fetal toxicity can occur without maternal toxicity in humans cannot be excluded.

The Panel is not confident that the lowest dose has been established at which developmental toxicity (the development of the male reproductive system) occurs.

Table 20. Summary of DEHP Effects in Developmental Toxicity Studies with Oral Exposure

Protocol & Doses	NOAEL (mg/kg bw/day)	Maternal LOAEL (mg/kg bw/day)	Developmental LOAEL (mg/kg bw/day)	Fetal Effects at Higher Doses
Prenatal feeding study in CD-1 mice. 30/group received 0, 44, 91, 191, or 293 mg/kg bw/day on gd 0–17. Dams and pups examined in late gestation. (*149, 185*)*	44 for maternal 44 for developmental	91 Clinical signs. ↓ Weight gain.	91 ↑ Skeletal, visceral, and external malformations.	↑ Skeletal, visceral, and external malformations. ↑ Prenatal mortality. ↓ Fetal weight.
Prenatal gavage study in CD-1 mice. 14 /group received 0, 40, 200, or 1,000 mg/kg bw/day on gd 6–15. Dams and pups examined in late gestation. (*150*)*	200 for maternal. 40 for developmental.	1000 ↑ Liver weight. ↓ Weight gain.	200 ↑ Visceral and external variations and malformations.	↑ Skeletal, visceral, and external variations and malformations. ↑ Prenatal mortality.
Prenatal feeding study in Fischer 344 rats. 20/group received 0, 164, 313, or 573 mg/kg bw/day on gd 0–20. Pups evaluated postnatally. (*156*)*	164 for maternal 164 for developmental	313 ↓ Food Intake.	313 ↑ Prenatal mortality.	↑ Prenatal mortality. ↓ Pup body weight on pnd 1 only.
Prenatal feeding study in CD-1 mice. 28/group received 0, 19, 48, or 95 mg/kg bw/day from gd 0–17. Pups evaluated postnatally. (*146*)*	95 for maternal 48 for developmental	No higher doses.	95 ↑ Prenatal mortality. ↓ Pup survival on pnd 4.	No higher doses.

*Doses calculated by study authors.

MEHP

MEHP, like DEHP, is a teratogen and developmental toxicant. The developmental toxicity of MEHP administered in diet to mice was found to be qualitatively similar to that of DEHP (*247*). However, resorption, rather than malformation was the most sensitive endpoint with a LOAEL of 35 mg/kg bw/day. DEHP and MEHP also had comparable effects when administered to mice by gavage during pregnancy (*154*). Effective MEHP doses were the same or less than those of DEHP. When administered intravenously to rabbits, MEHP was not found to be developmentally toxic at doses up to 11.4 mg/kg bw/day (*172*).

Table 21: Summary of MEHP Developmental Toxicity Studies with Oral Exposure

Protocol & Study	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)		Fetal Effects Observed at Higher Dose Levels.
		Maternal	Developmental	
Prenatal feeding study in CD-1 mice. 25 per group received 0, 35, 73, 134, or 269 mg/kg bw/day on gd 0–17. Dams and pups examined late in gestation. (*171, 247*)	134 for maternal None for developmental	269 ↓Weight gain. ↑ Liver weight.	35 ↑Prenatal mortality.	↑Prenatal mortality. ↑ Skeletal and visceral malformations.

2-EH & 2-EHA

2-EH is a developmental toxicant in rats. A mouse study with administration in diet found no effect at 191 mg/kg bw/day (*175, 176*). Two rat studies with administration by gavage found effects at 650 and 814 mg/kg bw/day (*166, 196*), and a rat inhalation study found effects at 262 mg/kg bw/day, the developmental LOAEL (*173*). Cutaneous administration at doses up to 2,520 mg/kg bw/day had no fetal effects in rabbits. 2-EHA is a developmental toxicant in rats. Four rat studies with administration by gavage and two rat studies with administration in drinking water found developmental toxicity. The NOAEL was 100 mg/kg bw/day (*182*). A gavage study in rabbits found no effects at doses as high as 250 mg/kg bw/day (*183*).

Table 22: Summary of 2-EH Developmental Toxicity Studies with Oral Exposure

Protocol & Study	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)		Fetal Effects Observed at Higher Dose Levels
		Maternal	Developmental	
Prenatal feeding study in CD-1 mice. 27 mice per group received 0, 17, 59 or 191 mg/kg bw/day on gd 0–17. Dams and pups examined late in gestation. (*175, 176*)	191 for maternal and developmental.	None	None	No higher doses.
Prenatal gavage study in Wistar rats. 10 animals per group received 0, 130, 650, or 1,300 mg/kg bw/day on gd 6–15. Dams and pups examined late in gestation. (*196*)	130 for maternal and developmental	650 "Slight maternal toxicity" according to authors.	650 ↓Fetal weight.	↓Fetal weight. ↑ Prenatal mortality. ↑Skeletal variations and malformations.

Table 23: Summary of 2-EHA Developmental Toxicity Studies with Oral Exposure

Protocol & Study	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)		Offspring Effects Observed at Higher Dose Levels
		Maternal	Developmental	
Prenatal gavage study in Fischer 344 rats. 25 animals per group received 0, 100, 250, or 500 mg/kg bw/day on gd 6–15. Dams and pups examined late in gestation. (*182*)	250 for maternal 100 for developmental	500 Clinical signs.	250 ↓ Ossification.	↓ Fetal weight. ↑ Skeletal variations.
Prenatal gavage study in New Zealand White rabbits. 15 animals per group received 0, 25, 125, or 250 mg/kg bw/day on gd 6–18. Does and pups examined late in gestation. (*183*)	25 for maternal 250 for developmental	125 Death in 1 doe. Abortion in 1 doe.	None.	No higher doses.
Prenatal drinking water study in Wistar rats. 20 animals per group received 0, 100, 300, or 600 mg/kg bw/day on gd 6–19. Dams and pups examined late in gestation. (*179*)	300 for maternal 100 for developmental	600 ↓Weight gain and water intake.	300 ↓Fetal weight. ↑Skeletal malformations.	↓Fetal weight. ↑External malformations. ↓Ossification

One-generation reproductive drinking water study in Wistar rats. 24 dams per group received 0, 100, 300, or 600 mg/kg bw/day for 2 weeks prior to mating through gestation and lactation. (*180*)*	100 for maternal 100 for developmental	300 Delayed pregnancy.	300 ↑External malformations. Developmental delays.	↑External malformations. Developmental delays. ↓ Litter size. ↓ Postnatal pup body weight.

* Reproductive effects listed in Table 27.

PHTHALIC ACID

Ema et al. (*184*) reported developmental toxicity (decreased fetal weight and ossification) following oral exposure of Wistar rats to 2,981 mg/kg bw/day phthalic acid. Maternal systemic toxicity was noted at this and a lower dose.

Table 24: Summary of Phthalic Acid Developmental Toxicity Studies with Oral Exposure

Protocol & Study	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)		Fetal Effects Observed at Higher Dose Levels
		Maternal	Developmental	
Prenatal dietary study in Wistar rats. 11 animals per group received 0, 1,021, 1,763, or 2,981 mg/kg bw/day on gd 7–16. Dams and pups examined late in gestation. (*184*)	1,021 for maternal 1,763 for developmental	1,763 ↓ Weight gain.	2,981 ↓Fetal weight. ↓Ossification.	No higher doses.

Mode of Action of DEHP and Metabolites

The mode of action of DEHP for developmental toxicity has not been investigated to the extent it has for male reproductive toxicity. Although Peters et al. (*152*) found DEHP-induced developmental toxicity in the PPAR-alpha knock out mouse, recent studies indicate that rat embryos express three PPAR isotypes (alpha, beta or delta, or gamma) (*188, 189*). MEHP, but not 2-EHA, has recently been found to activate PPAR- gamma in an *in vitro* assay (*190*). Further, valproic acid, a structural isomer of 2-EHA, was found to activate PPAR-delta *in vitro* (*191*). Involvement of PPAR with retinoid X receptor (RXR) heterodimers suggests that interesting, development-specific roles for embryonic PPAR may be discovered (*192, 193*). It may be premature to rule out a role of PPAR activation in DEHP-induced toxicity to the rodent embryo. Since the PPAR-alpha knockout mouse is susceptible to phthalate-induced testicular toxicity and expresses PPAR-gamma in the testis which can be activated by MEHP (*190*), PPAR-gamma may play a role in the reproductive toxicity of phthalates.

Studies with 2-EHA exposure suggest that the mechanism of action for DEHP-induced toxicity (assuming that 2-EHA is the active agent) is induction of zinc deficiency secondary to an acute phase response (*152, 246*). The studies of Bui et al. with 2-EHA are supportive of the hypothesis that zinc deficiency can be a risk factor for phthalate developmental toxicity, but are not adequate to support the hypothesis that phthalate developmental toxicity is necessarily secondary to elicitation of an acute phase response.

MEHP, a principal metabolite of DEHP, is a teratogen and developmental toxicant. The developmental toxicity of MEHP administered in diet to mice was found to be qualitatively similar to that of DEHP

(*247*). However, resorption, rather than malformation, was the most sensitive endpoint with a LOAEL of 35 mg/kg bw/day. DEHP and MEHP also had comparable effects when administered to mice by gavage during pregnancy (*154*). Effective MEHP doses were the same or lower than those of DEHP.

In considering extrapolation of animal findings on developmental toxicity to humans, the similarity in developmental toxicity actions between the DEHP metabolite 2-EHA and that of valproic acid may be relevant. Valproic acid (VPA, 2-pentylpropanoic acid) is used clinically as an anticonvulsant and is a known human and animal teratogen. 2-EHA and VPA are structural isomers; they are both carboxylic acids with eight-carbon alkyl chains. Thus, the comparable actions of 2-EHA and VPA in animal models as reviewed earlier (*166, 181, 198*) strengthen a generalization of the relevance of animal findings on 2-EHA to humans.

5.1.3.1 Utility of Data for the CERHR Evaluation

The database provides adequate information to identify DEHP as a developmental toxicant by the oral route and for identification of NOAELs and LOAELs for dose-response assessment. The data are also sufficient to identify the metabolites (MEHP, 2-EH, 2-EHA) as developmental toxicants. However, there are not enough studies for independent hazard identification and dose-response assessment for the parenteral route. Because of the known role of intestinal lipase in DEHP metabolism, it is not possible to readily generalize dose-response assessment from the oral to intravenous route. Existing PBPK models do not include fetal compartments; and hence have limited use at present.

5.1.4 Reproductive Toxicity

There are no data on the reproductive toxicity of DEHP or its major metabolites in humans.

Given the rapid metabolism of DEHP to the monoester (MEHP) and 2-EH, this review included an evaluation of the reproductive toxicity of the parent chemical and the two initial metabolites. In addition, secondary metabolites of the MEHP and 2-EH, phthalic acid (PA) and 2-EHA, respectively, were also evaluated. Approximately 70 reproductive toxicity studies of DEHP and its metabolites were reviewed.

Individual studies are not described in this section as they are reviewed in Section 3 of this document. Key aspects of the evaluation of that data set are discussed below. Terse summaries of the large number of available laboratory animal studies are presented below.

DEHP

There are experimental animal data on the effects of DEHP on reproduction following exposure during development *in utero* and the nursing period. There are also data on reproductive effects following exposure subsequent to weaning and during the adult phase of life. Studies that assess fertility and fecundity are discussed below, as are developmental studies where the principal effect is the testes or accessory sex organs. The distinction between reproductive toxicity and developmental toxicity, while necessary to avoid excessive repetition, is artificial from a biological and toxicological perspective. Readers are urged to study both sections to understand completely the data relevant to the broad area of reproduction.

Table 25: Summary of DEHP Reproductive Toxicity Studies with Oral Exposure

Protocol & Study	Reproductive NOAEL (mg /kg bw/day)	Reproductive LOAEL (mg/kg bw/day) and Effects	Systemic LOAEL (mg/kg bw/day) and Effects	Reproductive Effects Observed at Higher Dose Levels
Continuous breeding and cross-over mating study in 11-week- old CD-1 mice. 20 pairs of mice were fed diets with DEHP (0, 14, 141, and 425 mg/kg/day) for 7 days prior to mating and during a continuous 98-day mating period. (*168, 186*)	Reproductive: 14 Systemic: Not known since lower dose groups not examined.	141 Reduced fertility. ↓ Live pups per litter.	425 ↑Liver weight.	Complete infertility in females and reduced fertility in males. Seminiferous tubule damage with adverse effects on sperm numbers, motility, and morphology. ↓ Male reproductive organ weights.
Dose-setting two-generation dietary study in Wistar rats. 10 pairs per group received 0, 110, 339, or 1,060 mg/kg/day for 70 days prior to mating through gestation and lactation. (*169*)	Reproductive: 339 Systemic: None	1,060 ↓Anogenital distance in F_2 males. ↓Ovary weight in F_0. ↓Testes and epididymis weight in F_1. Testicular lesions. ↑ Nipple development in F_1 males. Spermatocyte loss in F_1. ↓ Postnatal survival in F_1 pups and prenatal survival in F_1 and F_2 pups.	110 ↑Liver weight in F_0 females.	No higher doses.
90 day repeat-dose dietary study in Sprague-Dawley rats. 10 rats/sex/group (4–6 weeks old) received doses of: M: 0, 0.4, 3.7, 38, 375 F: 0, 0.4, 4.2, 42, 419. (*75*)	Reproductive: 3.7(M). Systemic: 3.7–4.2 (M and F).	38 (M) Mild Sertoli cell vacuolation.	38(M)–42(F) Liver enzyme effects.	Atrophy of seminiferous tubules, loss of spermatogenesis, and Sertoli cell vacuolation.
1–12 day repeat-dose gavage study in female Sprague-Dawley rats. 6–10 rats per group (60–70 days old) received doses of 0 or 2,000 mg/kg/day. (*219*)	Reproductive: Not known since only one dose administered. Systemic: Not examined.	2,000 ↓Estradiol levels and suppression of ovulation.	Not examined.	No higher doses.

Table 26: Summary of DEHP Reproductive Toxicity Studies with Parenteral Exposure

Protocol & Study	Reproductive NOAEL (mg /kg bw/day)	Reproductive LOAEL (mg/kg bw/day) and Effects	Systemic LOAEL (mg/kg bw/day) and Effects	Reproductive Effects Observed at Higher Dose Levels

12-day study with 6 intravenous administrations on every other day. Male Sprague-Dawley Rats (40-days-old; 5–6 rats/group) received Doses (time-weighted): 0, 2.5, 25, or 50 mg/kg. (*93*)	Reproductive: 25 Systemic: 25	250 Enlargement of Sertoli cell endoplasmic reticulum and slight changes in spermatocyte structure.	250 ↑Liver weight. Peroxisome proliferation.	No higher doses.

Females. There are data that indicate that oral exposure to DEHP can affect reproductive processes in rats and mice. The Lamb et al. (*168*) data clearly show adverse functional effects at a dietary dose of 425 mg/kg bw/day where complete infertility was observed, although the design did not allow conclusions as to whether males, females, or both sexes contributed to reduced number of pups and pup viability. Only the data from Davis et al. (*219*) come close to evaluating broadly the effects on the female tract, and this study examined only a high dose. Davis et al. (*219*) showed clear effects on estradiol synthesis and ovulation in rats at 2,000 mg/kg bw/day. No histopathological structural changes were seen in the uterus or vagina. Further, there are no studies that have evaluated adult female reproductive structure and function after prenatal exposure. Current data are not adequate to confidently ascribe a NOAEL or LOAEL for female reproduction.

Data on non-oral administration (IV, inhalation) are extremely limited and are not adequate for characterization of adult or developmental reproductive toxicity or identification of NOAELs or LOAELs by non-oral routes.

Males. The oral exposure studies of Lamb et al. (*168*) and Schilling et al. (*169*) are sufficient to conclude that DEHP is a reproductive toxicant in male rats and mice. In the Lamb et al. study (*168*), only the control and high-dose F_0 mice were necropsied; thus, it is not known if the reduced fertility at ~141 mg/kg bw/day (the middle dose) is partly male-mediated resulting from testicular damage. Schilling et al. (*169*) reported effects (androgen mediated and testicular lesions) at 1,060 mg/kg bw/day in rats, and no effects (i.e., a NOAEL) at ~339 mg/kg bw/day. Gray et al. (*202*) reported testicular atrophy and anti-androgenic effects (discussed under Steroid Hormone Activity) in F_1 Sprague-Dawley rats whose mothers were exposed to 750 mg/kg bw/day from gd 14 to pnd 3. The Lamb et al. (*168*) study has a NOAEL of ~14 mg/kg bw/day.

Arcadi et al. (*161*) reported a LOAEL of ~3 mg/kg bw/day, based on 'young adult" rat testicular weight after gestational/lactational exposure of dams. The methods used to verify and characterize the administered dose were not clearly described or completely reported. The Expert Panel considered the study of Arcadi et al. (*161*) but had concerns about the actual exposure conditions that were not resolved by contacting the authors. Therefore, this study is not used to identify NOAELs and LOAELs. Poon et al. (*75*) found a NOAEL of ~3.7 mg/kg bw/day in adult rats after 13 weeks of exposure that commenced when they were young adults. The lowest adverse effect, mild focal Sertoli cell vacuolation, was observed in 7/10 rats at 38 mg/kg bw/day.

Weight of evidence is important when reaching conclusions about adverse effects. The lack of real replicates of design in this database limits the confidence of any conclusion. However, it is clear from the existing data that testicular pathology and reduced sperm numbers are consistent effects. The data are sufficient to conclude that DEHP is a reproductive toxicant in male rats, mice, ferrets, and guinea pigs when administered orally. There is greater uncertainty when determining lowest-adverse and no-adverse effect levels of exposure. The two studies (*169*) (*75*) that used peripubertal dosing (believed to be the

most sensitive period for causing adverse effects) and evaluated the rats when at, or close to, maturity (believed to be the most sensitive period for observing adverse effects) present markedly different NOAELs. The Expert Panel could not confidently reconcile these differences (~339 vs 3.7 mg/kg bw/day). Confidence in results observed at a given dose in the Schilling et al. (*169*) study is eroded slightly by the small group size. The study of Poon et al. (*75*) was thorough in its design and execution, including verification of dose. While there were 20 animals per dose group, only 10 were males; the study design did not incorporate measures of reproductive function. The authors were clear in asserting that ". . . the mild Sertoli cell vacuolation at 500 ppm (~38 mg/kg bw/day) should be considered an adverse effect." The Expert Panel finds there is a reasonable basis for such a conclusion (i.e., a LOAEL). The NOAEL from this study is therefore 3.7 mg/kg bw/day. When comparing the NOAELs from the Poon et al. and the Lamb et al. studies, 3.7 mg/kg bw/day vs 14 mg/kg bw/day, it is reasonable to conclude that these values are indistinguishable given the wide dose spacing and inherent biological variability in the endpoints. It is the Panel's view that the existing data support a NOAEL within the range of 3.7–14 mg/kg bw/day for oral exposure in rats.

The dose-selection used in Schilling et al. (*169*) and Gray et al. (*202*) is worthy of comment. The former was designed to select a dose for a subsequent study, whereas Gray et al. (*202*) primarily compared effects of an array of chemical types and used only a single dose. As such, data from these studies do not lend themselves to confident judgments for a NOAEL or LOAEL. They do, however, provide context for the consideration of results from other studies. Results from the ongoing additional multigeneration studies by Schilling et al. and the National Toxicology Program should provide additional data from which to establish a LOAEL and NOAEL.

Finally, the non-rodent data, while not voluminous, provide contrasting results from data derived from rats and mice. Kurata et al. (*82*) exposed adult marmosets to oral DEHP doses of up to 2,500 mg/kg bw/day for 13 weeks and did not detect effects on testes, liver, or kidney. There was also no evidence of peroxisome proliferation or decrease in testes zinc concentration; the latter is an effect seen in rats. The data of Rhodes et al. (*85*) are consistent with the data of Pollack et al. (*107*) and model predictions of Keys et al. (*136*) that absorption limits the amount of circulating MEHP at high exposure levels. More recently, Pugh et al. (*83*) found no evidence of testicular lesions or peroxisomal proliferation in prepubertal cynomolgus monkeys that were dosed with 500 mg/kg bw/day for 14 days.

The data from non-oral routes are extremely limited. The data engendering the greatest confidence in the Panel were those of Sjoberg et al. (*93*), who injected DEHP IV into male rats every other day for 12 days. Sertoli cell vacuoles and germ cell structural changes were observed at 500 mg/kg bw (250 mg/kg bw/day) and are similar to effects reported by other authors The endpoints were histological, and are thus sensitive. However, the limited exposure duration and the lack of functional evaluation severely limit the utility of these data, and they are not valuable for setting a NOAEL or LOAEL. No other reviewed studies were found to be useful in this regard, although the PBPK model of Keys et al. (*136*) should be useful.

Postnatal Consequences of Prenatal Exposures. Schilling et al. (*169*) found reduced pup viability and lactational weight gain, as well as abnormalities in the development of the reproductive system in males at 9,000 ppm (~1,060 mg/kg bw/day), effects which were not consistently seen at 3,000 ppm (~339 mg/kg bw/day). Poon et al. (*75*) has no relevant data. Thus, it appears that there are developmental effects that can be manifested postnatally, although these do not necessarily appear more sensitive than the reproductive effects in the current studies.

2-EH & 2-EHA

The data for 2-EH are insufficient to conclude whether it has any potential reproductive toxicity. Based on the rapid *in vivo* conversion to the acid, the Panel believes that it is unlikely that 2-EH will act directly. Because it is rapidly converted to 2-EHA, exposure *in vivo* is to 2-EHA. In subchronic and chronic gavage studies with 2-EH, no histopathological effects on reproductive organs were reported in adult rats dosed with up to 500 mg/kg bw/day and adult mice dosed with up to 750 mg/kg bw/day (*98, 99*).

The data from an oral (drinking water) one-generation reproduction study in rats (*180*) suggest that 2-EHA may alter reproduction following adult exposure. There are data to indicate a decrease in sperm motility, but there is inconsistency as to dose response; all other male parameters were similar to controls. While there was no effect on ability to conceive or pregnancy index there was an increase in the number of estrous cycles required to achieve pregnancy at the ~600 mg/kg bw/day dose. Whether this was male- or female-mediated could not be determined. The LOAEL and NOAEL from this study were 600 and 300 mg/kg bw/day, respectively. Stronger conclusions, including confidence in a NOAEL, can only come from additional studies (i.e., a multigeneration study). In subchronic dietary studies, there were no histopathological effects in reproductive organs of adult rats dosed with up to 917(M)–1,068(F) mg/kg bw/day and adult mice treated with up to 2,728(M)–3139(F) mg/kg bw/day (*97*).

Table 27: Summary of 2-EHA Reproductive Toxicity Studies with Oral Exposure

Protocol & Study	Reproductive NOAEL (mg /kg bw/day)	Reproductive LOAEL (mg/kg bw/day) and Effects	Systemic LOAEL (mg/kg bw/day) and Effects	Reproductive Effects Observed at Higher Dose Levels
One-generation reproductive drinking water study in Wistar rats. 24 pairs per group received 0, 100, 300, or 600 mg/kg bw/day for 2 weeks prior to mating through gestation and lactation in females and 10 weeks prior to mating in males. (*180*)*	Reproductive: 300 Systemic: 300	600 ↓Sperm motility. Delayed conception in females.	600 ↓ Water intake. ↓ Weight gain during pregnancy.	No higher doses.

* Developmental effects listed in table 23.

Mode of Action for DEHP and Metabolites

A recent *in vitro* study using co-cultures of Sertoli cells- gonocytes isolated from 2-day-old rats supports an MEHP-induced anti-proliferative effect in Sertoli cells as a sensitive endpoint (*209*). These data are important because they: (1) evaluate a relevant response in a target tissue taken at the likely most vulnerable time; (2) effectively show that MEHP, and not DEHP, is the active toxicant; and (3) show the effect is observed at a low level of exposure (10^{-7} M MEHP for 24 hours). The challenges in using it for risk assessment are those of using *in vitro* data. The exposure level is constant *in vitro*, and is continuously variable *in vivo*; the tissue *in vitro* is removed from other systemic inputs that exist *in vivo*, and the *in vitro* study uses rat tissue. However, the endpoints measured are entirely relevant to what has been noted *in vivo* (i.e., germ cells/gonocyte loss *in vitro* would correlate with, or produce reduced testes weights *in vivo*, which is an effect actually seen in the *in vivo* studies), which is a strength. The Expert Panel considers that the study by Li et al. buttresses the effects seen *in vivo*, and prompts the call for toxicokinetics studies which actually measure levels of MEHP in tissues of fetuses from exposed dams.

All phthalates that cause testicular toxicity produce a common lesion characterized by alterations in Sertoli cell ultrastructure and function (*223-225*). It is known that some Sertoli cell functions are mediated by FSH interaction with membrane bound receptors. Lloyd and Foster (*226*) demonstrated that MEHP disturbs FSH interaction with the FSH receptor. Further studies with MEHP using primary rat Sertoli cell cultures revealed that the monoester of DEHP inhibited FSH-stimulated cAMP accumulation. The MEHP-induced inhibition was specific for FSH (*227*). The Panel was not able to reach agreement that interfering with FSH signaling function was the accepted mode or mechanism of action.

Factors affecting increased sensitivity to phthalate-induced testicular toxicity in young animals were studied for DBP, DEHP, DnHP, and dipentyl phthalate. The monoester derivatives of DBP and DEHP have been shown to cause similar testicular effects. Sjoberg et al. (*108*) demonstrated that gavage treatment with DEHP resulted in greater absorption of MEHP, and hence, a greater systemic dose to young versus mature rats. Further, *in vitro* studies did not find that FSH-stimulated cAMP accumulation and lactate secretion were age related (*228*). Lloyd and Foster (*226*) noted that initiation of spermatogenesis was dependent on FSH interaction with the Sertoli cell in young rats, but was not necessary for maintenance of spermatogenesis in adults. Their experiment in Sertoli cell cultures demonstrated that MEHP interferes with FSH interaction at the receptor level and provided a hypothesis for increased sensitivity to testicular toxicity in young animals.

The role of peroxisomal proliferation in reproductive toxicity has been examined. In contrast to hepatic toxicity, testicular toxicity is noted in PPAR-alpha knockout mice exposed to DEHP, albeit that appearance of the testicular effects was delayed compared to wild-type mice. In addition, the guinea pig, a non-responding species to the peroxisomal-proliferating effects of DEHP, is susceptible to the testicular effects of this agent. Since the PPAR-alpha knockout mouse is susceptible to phthalate-induced testicular toxicity and expresses PPAR-gamma in the testis which can be activated by MEHP (*190*), PPAR-gamma may play a role in the reproductive toxicity of phthalates.

Overall, the Panel believes that the reproductive toxicity of DEHP appears independent of PPAR-alpha. However, other members of the PPAR family (beta or delta and gamma) have not been extensively studied with regard to activation by phthalates. PPAR-gamma has been found in human testis, ovary, placenta, and embryo. MEHP (but not DEHP, 2-EH, or 2-EHA) has been shown to activate PPAR-gamma receptor in a transcription reporter assay (*190*)

Based on morphological, functional, and biochemical endpoints, the Sertoli cell is a cellular target for neonatal, pubertal, and adult exposures. Testicular toxicity in the postnatal phase of life resulting from DEHP exposure occurs because of metabolism to MEHP, the proximate toxicant. The cellular target and proximate toxicant for reproductive effects arising from gestational exposure to DEHP are currently unknown.

DEHP exhibited no or weak activity in an *in vitro* assay that measured binding of phthalates to the rat uterine cytosol estrogen receptor (*229*), but MEHP did bind to the human estrogen receptor in a second *in vitro* assay (*234*). No activity was noted for DEHP or MEHP in assays of estrogen-induced gene expression (*229, 233, 236*). *In vivo* assays demonstrated that DEHP did not increase uterine wet weight or vaginal epithelial cell cornification in immature or mature ovariectomized rats (*229*). DEHP was administered by gavage at one dose level, 750 mg/kg bw/day, to female SD rats from gd 14 through pnd 3; male offspring were assessed for reproductive malformations and abnormal development (*202*). DEHP was considered to have significant antiandrogenic activity based on the spectrum of effects produced.

5.1.4.1 Utility of Data for the CERHR Evaluation

The data are adequate to identify male reproductive toxicity from exposure of several species of adult animals. Testes and specifically the Sertoli cell have been identified as a target. Mechanistic data are of value in judging utility of rodent data for human hazard assessment. A more limited data set has identified reproductive toxicity in adult female rats and mice; other species have not been tested. Toxicity to female reproductive organs has not been defined, so non-target tissues have not been confidently identified.

The current data are insufficient to determine the NOAEL for postnatal developmental effects on reproductive organs. Gray et al. (*202*) found retained nipples and areolae, reproductive tract malformations, testicular degeneration, and decreased weights for seminal vesicle, prostate, epididymides, and testes in offspring from dams treated with the single dose level of 750 mg/kg bw/day. The authors report that the response to DEHP was of a greater incidence and severity than those observed with molar equivalent doses of DBP in the same study. The adult oral data are consistent with the reproductive system being a target for DEHP effects; the parenteral exposure data, although sparse, are also consistent with this. Metabolite studies show that MEHP is the active metabolite, and toxicokinetic studies help relate data from one exposure route to another. Collectively, the data are mutually supportive and consistent, and can be used in the CERHR process.

5.2 Integrated Evaluation

DEHP is a general purpose plasticizer in many PVC consumer products. Through these uses, DEHP has a ubiquitous presence in our environment. In the United States it is no longer used in baby bottle nipples, teethers, or infant's toys. Exposure of the general population to DEHP occurs from food, water, and air, via inhalation and ingestion. The largest general population exposure to DEHP is dietary, followed by indoor air. In general, fatty foods including dairy, fish, meat, and oils contain DEHP. The range of exposure in the general population from all sources, excluding non-dietary ingestion, medical and occupational, is estimated to be 3–30 µg/kg bw/day.

Exposure to both DEHP and MEHP occur routinely during infusion of blood and blood products. DEHP alone is infused with IV fluids, medication, and lipids. The majority of medical exposures are intravenous, but inhalation (e.g., ventilators), and ingestion (e.g., nasogastric tubes) exposures can also occur. Precision in determining levels of exposure is not currently possible because the number of patients actually studied is quite small and the variation within those small study populations is great. Based upon the limited available data, infants undergoing routine replacement blood transfusions may be exposed to doses of DEHP 1–2 orders of magnitude above general population exposures and have concomitant MEHP exposure. Infants undergoing intensive therapies may be exposed to levels up to 3 orders of magnitude above general exposures. Chronic exposures in adults undergoing hemodialysis can be 1–2 orders of magnitude above average exposure to DEHP. It is important to note that medical exposures from simultaneous interventions in the same patient (e.g., ventilation, nasogastric tubes, transfusion, and parenteral feeding) have not been quantified. Such exposures may result in dose levels considerably above those documented for single medical procedures.

There are very few human data from which to characterize the reproductive and developmental toxicity of DEHP. Therefore, evaluation of human reproductive risk must be extrapolated from studies in experimental animals where species differences in metabolism and dynamics of PPAR-alpha are important considerations. Uncertainties exist in the performance of such extrapolations. Animal species vary in the rate of absorption of metabolites following orally administered DEHP, but use of the PBPK model of Keys et al. with additional development and validation, should reduce the uncertainties in extrapolation of effective dosimetry among species, particularly primates. This is because DEHP is

absorbed from the digestive tract as the monoester, MEHP, and 2-EH or 2-EHA. The formation of MEHP from DEHP is dependent on lipases, which are present at much higher levels in the intestines of rodents than in primates, and at higher levels in adults than in children younger than 6 months of age. As a consequence, higher absorption of DEHP-derived compounds occurs in rodents than in primates. DEHP exposure in mice and rats of all ages commonly produces effects in liver and testes. In contrast, repeat-dose oral studies in adult or prepubertal cynomolgus monkeys and marmosets have not shown effects on liver or testes. Liver effects from exposure to DEHP are primarily those associated with peroxisome proliferation, whereas peroxisome proliferation in cynomolgus monkeys and marmosets does not occur or only a weak response is noted and only at high dose levels. DEHP in rodents and primates is absorbed from the digestive tract as the monoester MEHP and 2-EH or 2-EHA, little intact DEHP is absorbed. There is rapid distribution of metabolites in these same species to body tissues; subsequent excretion is also rapid. Adult humans and other primates excrete the monoester as a glucuronide, whereas rodents further metabolize these intermediates. The glucuronidation pathways of children do not mature until they are 3 months old. Thus, this important clearance mechanism is not fully available to neonates and young infants. Certain medical practices, such as hemodialysis and blood transfusions, result in intravenous exposure to DEHP in addition to the metabolites. DEHP in stored blood (and in the circulation) does undergo slow metabolism to MEHP, 2-EH, and 2-EHA due to lipases that are present in this fluid. The significance of the effect of DEHP *per se* in blood and body tissues on human reproduction and development is uncertain, but in *in vitro* studies, using rat testis cell cultures, DEHP is inactive.

As will be discussed below, there are sufficient data in rodents to conclude confidently that oral exposure to DEHP can cause reproductive and developmental toxicity in rats and mice. Further, an effect observed in rats involves adverse effects on the development, structure, and function of the male reproductive tract. Thus, for DEHP, the effects on reproduction and development are intertwined.

The developmental toxicity database contains well-conducted and reported studies, many available as full GLP study reports, and additional, more restricted studies that provide supplemental and supportive information. The database is somewhat limited in that it consists almost entirely of studies in rats and mice orally exposed during gestation where effects are seen by examining physical development of rodent pups just prior to birth (i.e., prenatal assessment). These studies indicate that a range of effects may occur, including malformations (tail malformations, axial and appendicular skeletal abnormalities, cardiovascular malformations, and neural tube closure defects), developmental delays, and intrauterine death. The NOAEL based on malformations in rodents was ~ 40 mg/kg bw/day and a NOAEL of 3.7-14 mg/kg bw/day was identified for testicular development/effects in rodents. In contrast, functional reproductive endpoints that are evaluated through postnatal observation have not been adequately studied. This is a significant data limitation. There are a limited number of studies by the inhalation, dermal, and intravenous administration routes. It was noted that results are consistent across studies, taking into account doses, route, species, timing, chronicity, study protocol (power of detection), and type of evaluation.

The examination of effects during the late gestational and neonatal periods is quite recent and incomplete. Despite the general belief among Expert Panel members that this represents a time of potentially high sensitivity to DEHP-induced disruption of the reproductive system, the dose-response relationships for reproductive effects following exposures in gestational versus postnatal ages are unknown. Low-dose studies examining sensitive endpoints following late gestational exposure are a critical data need.

Species differences in the sensitivity to peroxisome proliferator agents are not believed to be relevant to extrapolation of developmental toxicity to humans. There is a study that demonstrated the same spectrum of developmental toxicity (as seen in 'normal' mice) in mice that were genetically incapable of expressing peroxisome proliferation due to lack of PPAR-alpha. The review and tables provided in this document

demonstrate that developmental toxicity has been elicited with DEHP, MEHP, 2-EH, and 2-EHA. In general, similar patterns of effects were reported and metabolites were as potent, or more potent, than the parent compound when compared in the same study. DEHP data from rats and mice are assumed relevant to judging hazard to human reproduction and development; they are the standard mammalian test systems used.

In considering extrapolation of animal findings on developmental toxicity to humans, the similarity in developmental toxicity actions between the DEHP metabolite 2-EHA and that of valproic acid may be relevant. Valproic acid (VPA, 2-pentylpropanoic acid) is used clinically as an anticonvulsant and is a known human and animal teratogen. 2-EHA and VPA are structural isomers; they are both carboxylic acids with eight-carbon alkyl chains. Thus, the comparable actions of 2-EHA and VPA in animal models as reviewed earlier strengthen a generalization of the relevance of animal findings on 2-EHA to humans.

The reproductive toxicity database for DEHP holds evidence of adverse effects in rats, mice, guinea pigs, and ferrets. Historically, most studies have focused on the pubertal and adult male rodent, identifying primarily structural changes in the testis and, at higher doses, reduced fertility and altered sperm measures. High-dose exposure of adult female rats has shown decreased hormone production and ovarian dysfunction. Crossover matings after high-dose exposure of mice indicates that females are more affected than males, although both are severely affected. Recently, perturbations in the male reproductive system due to exposures during development have been documented. As discussed below, important issues relevant to the interpretation of these adverse effects include: differences in sensitivity of animals exposed at different developmental time points; differences in response across species; and the nature of the target site and proximate toxicant.

The absence of testicular effects following 13 weeks of oral dosing with 2,500 mg/kg bw/day DEHP in young adult marmosets, a primate, is distinctly different from the dramatic testicular effects produced by such high doses in adult mice, rats, guinea pigs, and ferrets. The data in cynomolgus monkeys partially support this observed difference between members of the Order Primates and other phylogenetic Orders. The absence of effect in marmosets can be explained, at least in part, by species differences in rate of MEHP absorption from the gut. Absorption studies, as well as PBPK modeling, suggest that DEHP metabolism to MEHP and its absorption from the human and marmoset gut saturates at a low dose relative to that of the rat. Consequently, for oral exposures, the blood level of MEHP, the proximate toxicant (see below), is lower in marmosets than in rats after high doses. Given these pharmacokinetic considerations, the rodent data are assumed relevant to predicting that DEHP has the potential to produce adverse reproductive system effects in humans. However, in terms of risk assessment, route of exposure and dose are important parameters which must be incorporated when extrapolating across species. In particular, these considerations strongly suggest that blood-MEHP levels associated with the ability of high-dose oral exposure to induce reproductive toxicity in rodents may never be achieved from oral exposures in most humans.

The molecular target is unknown for any of these exposures; however, based on morphological, functional, and biochemical endpoints, the Sertoli cell is a cellular target for neonatal, pubertal, and adult male exposures. The cellular target for gestational exposure is unknown. The presence of testicular effects in PPAR-alpha knockout mice and in guinea pigs exposed to DEHP indicates that the mechanism of action does not involve peroxisome proliferation. Testicular toxicity in the postnatal phase of life resulting from DEHP exposure occurs because of metabolism to MEHP, the proximate toxicant. Recent *in vitro* studies using co-cultures of Sertoli-gonocytes isolated from 2-day-old rats support an MEHP-induced anti-proliferative effect in Sertoli cells as a sensitive endpoint. The nature of the proximate toxicant for reproductive effects arising from gestational exposure to DEHP is currently unknown due to the limited database.

While there is sufficient data to conclude that oral exposure to DEHP can cause reproductive toxicity in rats and mice, there are significant gaps in the dose-response data. The effects described in mice include reduced fertility, aberrant sperm counts, and histologic evidence of testicular degeneration. The data in rats, while lacking definition in terms of breeding performance, clearly indicate testicular and ovarian dysfunction of biologic significance. Based on these studies, it would appear from the current data set that the LOAEL is ~38 mg/kg bw/day and the NOAEL is ~3.7–14 mg/kg bw/day for reproductive effects in rodents by the oral route.

5.3 Expert Panel Conclusions

While the Panel recognizes the variability and uncertainties in exposure estimates, it appears that for the general adult human population, ambient exposures may be on the order of 3–30 μg/kg bw/day. Non-dietary mouthing behaviors in infants and toddlers may result in exposures that are several-fold higher. The 3–30 μg/kg/d range may be increased by 2–3 orders of magnitude for infants undergoing intensive therapeutic interventions.

General Adult Population. DEHP conversion to active MEHP involves intestinal lipases that appear to be at significantly greater levels in rodents than in primates; adult rodents require 1–2 orders of magnitude more DEHP than is required in juvenile rodents to produce testicular effects; and adult marmosets (primates) showed no testis effects when exposed to DEHP at oral doses (2,500 mg/kg bw/day) for 13 weeks, conditions which produce testicular toxicity in juvenile rodents. Based on these data, the Panel has a minimal concern that ambient human exposures adversely affect adult human reproduction. This level of concern is not appreciably altered for adults medically exposed to DEHP or MEHP.

Healthy Infants and Toddlers. DEHP produces testicular toxicity at lower doses in juvenile rodents than in adults; the reproductive system is (and specifically, the Sertoli cells are) still in proliferative mode until puberty, and reproductive system development has been shown to be sensitive to MEHP in rodents; intestinal lipase activity is found at adult levels in babies older than 6 months of age. All of these points increase the level of concern. While adult marmosets showed no testicular toxicity at doses that produced toxicity in adult rats, no data are available for infant primates. If healthy human infant/toddler exposure is several-fold higher than adults, the Panel has concern that exposure may adversely affect male reproductive tract development.

Critically Ill Infants. Documented parenteral medical exposure to DEHP of critically ill infants can exceed general population exposures by several orders of magnitude; parenteral exposures to DEHP involving blood and blood products include concurrent exposure to MEHP; the most sensitive process (reproductive system development) is still occurring; human parenteral exposures can approach the rat parenteral NOAEL. On the other hand, concern is lowered by the fact that there is less conversion of DEHP to MEHP by the parenteral route of exposure, although the exact degree of reduction is not known. It is not known if primate Sertoli cells are more, equally, or less sensitive than rodent Sertoli cells to the effects of MEHP. The available reproductive and developmental toxicity data and the limited but suggestive human exposure data indicate that exposures of intensively-treated infants/children can approach toxic doses in rodents, which causes the Panel serious concern that exposure may adversely affect male reproductive tract development. The Panel recognizes that benefits of medical procedures can outweigh any risks.

Pregnancy and Lactation. In utero development is a life stage of particular vulnerability; exposures may be on the order of 3–30 μg/kg bw/day; the most relevant rodent data suggest a NOAEL for testis/developmental effects of 3.7–14 mg/kg bw/day; DEHP produces malformations in rodents, with a

NOAEL of ~40 mg/kg bw/day; even time-limited exposures are effective at producing irreversible effects; the active toxicant MEHP passes into breast milk and crosses the placenta. On the other hand, absorption from the primate gut appears to be less effective than from the rodent gut, which reduces the level of concern for oral exposure. Given that oral exposure is <30 μg/kg bw/day for humans and toxic effects are seen in rodents at >3 mg/kg bw/day in rodents, even in the face of significant species differences in absorption, the Panel has concern that ambient oral DEHP exposures to pregnant or lactating women may adversely affect the development of their offspring.

5.3 Critical Data Needs

1. Identification and follow-up studies of humans populations (e.g., premature infants) who were heavily exposed to DEHP. This would directly address the issue of whether there are functional effects in the most heavily and simultaneously the most vulnerable, human population. This would consist primarily of follow-up evaluation of reproductive system development and function.

2. Obtain better medical exposure data. Common clinical research designs with unified analysis approaches across centers, as are often used in the large group cooperative studies of cancer therapy, would be one approach to acquiring better data. Potential toxicity from medical exposures could also be evaluated using the multi-center model to study DEHP/MEHP exposed neonates and adults longitudinally over decades to capture the reproductive, developmental, and other outcomes of concern based upon animal toxicity studies. Finally, discussions with the manufacturers of the medical devices used in these procedures would be helpful to determine whether and how much the formulations of PVC blood bags, ECMO circuits, hemodialysis machines, and other medical devices that contain DEHP or MEHP have changed over time.

3. Significance of perinatal exposure:
    - Dose-Response of male and female reproductive tract malformations. There is a need to gather dose-response data across a wider range of lower exposures in dam and pups in order to correlate blood levels of MEHP with reproductive effects.
    - Relevant animal model (in utero reproductive tract maturation) in guinea pig or nonhuman primate to correlate dose with effects, if any, and compare these doses with those of rodents where adverse effects do occur.
    - Timing, PPAR, Metabolism. [A change was suggested by R. Chapin, but we are waiting to see what other comments we get from panel members]

4. Extension of PBPK model to
    - Pregnancy, because this is the human group thought to be most at risk.
    - Species (marmoset/human), as humans are the species of interest. The marmoset data provide a positive control to show that the PBPK model works as advertised.
    - ADME, specifically phase I and phase II metabolism extended across species, and into pregnant humans.
    - In order to acquire better data on primate/human toxicokinetics, including immature animals and humans, there is a need for a fetal compartment in the PBPK model and rate constants for fetuses and newborns for absorption, metabolism, and excretion.
    - Model DEHP/MEHP dose for IV exposure. This is another route of exposure for a great many people who may have reduced capability to clear the compound.

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7.0 WEB TABLES