TOXICOLOGICAL PROFILE FOR DI-(2-ETHYLHEXYL) PHTHALATE (DEHP)

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Integrated Risk Assessment Branch
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

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Table of Contents

Executive Summary

This toxicological profile on **di-(2-ethylhexyl) phthalate (DEHP)** describes its effects on freshwater and marine life, humans, and laboratory animals. Because of its prevalence in the environment and the high likelihood of exposures to humans and other species, DEHP has been the subject of considerable toxicological research.

Use and Exposure

DEHP is the most commonly used phthalate plasticizer. Plasticizers are used to make rigid compounds such as polyvinyl chloride or PVC more flexible. PVC products containing DEHP include packaging film and sheets, wall coverings, floor tiles, furniture upholstery, shower curtains, garden hoses, swimming pool liners, rainwear, toys, shoes, sheathing for wire and cable, and medical tubing.

Due to its high volume use, DEHP is a ubiquitous contaminant—found at low concentrations—in air, water, soil and sediments. Since DEHP is not bound in plastic, it slowly leaches out of plastic materials and into the environment. Examples of how humans can be exposed to DEHP include ingestion of DEHP transferred to food from PVC films and plastic containers, and transfer of DEHP in PVC plastic disposable medical equipment to patients (i.e., into their blood).

Effects on Aquatic Life

In water, DEHP predominantly attaches to suspended particles and sediments, but a small amount remains dissolved in the water. DEHP in air binds to dust particles and is removed from the atmosphere by settling of dry particles and by being washed out by rain and snow. The toxicology of DEHP in aquatic animals has not been well studied. Reported toxic effects in aquatic organisms in laboratory studies generally occur at exposure levels higher than the concentration of DEHP that can dissolve in water (3 micrograms per liter). There is some evidence of reproductive effects in salmon. Because fish can break down DEHP, it does not accumulate in their tissues to any significant extent; invertebrates, however, are less able to break down DEHP. Two studies report effects in invertebrates at lower concentrations. Further environmental (field) and laboratory studies are needed to investigate the impacts of DEHP on freshwater and marine life.

Health Hazard and Toxicity in Humans and Laboratory Animals

- **Reproductive and Developmental Effects.** A major concern is DEHP's effect on the reproductive system. The male reproductive toxicity of DEHP is well established in laboratory animals including rats, mice, hamsters, and ferrets. Depending on the dose, duration of exposure, and age of animals, DEHP causes reduced fertility, decreased weights of male reproductive organs, and histopathological changes in the testes of juvenile and adult rats. Although it has not been studied as extensively, female reproductive toxicity in laboratory animals has been reported. DEHP also has been found to cause developmental toxicity, including intrauterine death, developmental delay, and structural malformations and variations; neurological developmental effects have also been reported. Evidence of DEHP's reproductive toxicity in humans is less conclusive.

- **Cancer.** According to U.S. EPA, there is sufficient evidence that DEHP causes cancer in laboratory animals. However, the International Agency for Research on Cancer considers the mechanism responsible for causing cancer in laboratory animals as unlikely to occur in humans; there are some who disagree with this finding.
- **Neurological Effects.** Alteration in brain function and behavioral changes has been observed in rodent studies. The results thus indicated that DEHP might have potential effects on neural development and behavior.
- **Other effects.** There is some evidence in laboratory animals indicating that DEHP may affect the thyroid and immune system. Little information is available on its effect on the human thyroid and immune system.

Summary Table

This table provides some idea of the availability of information on the toxicology of BPA for the endpoints and organisms identified. It also provides some sense of the evidence available in that information can be used to determine if the endpoint effect does or does not occur. If there is no information the evidence column will be marked with a "--."

Health Effect	Human		Lab Animal		Aquatic Life	
	Information	Evidence	Information	Evidence	Information	Evidence
Reproductive					S	S
male	S	E	Su	Su		
female	S	L	Su	Su		
Cancer	S	E	Su	Su	N	--
Developmental	L	E	Su	Su	N	--
Neurological	N	--	S	S		
Immunological	L	L	S	S	N	--
Other Chronic effects					S	S
Thyroid	L	L	S	S		
Other Sub-chronic effects					S	S
Acute					S	S

N = None S = Some
L = Little Su = Sufficient
E=Equivocal
These rating categories are qualitative in nature and designed to give the reader a general sense of the availability and strength of the information.

Abbreviations

ADHD	attention-deficit hyperactivity disorder
AGD	anogenital distance
ANOVA	analysis of variance
ATPase	adenosine triphosphatase
BBP	butyl benzyl phthalate
BCF	bioconcentration factor
CERHR	Center for the Evaluation of Risks to Human Reproduction
CG	chorionic gonadotropin
Con A	concanavalin A
DBP	Di-n-butyl phthalate
DEHP	di-(2-ethylhexyl) phthalate
DEP	diethyl phthalate
DINP	di-isononyl phthalate
DNA	deoxyribonucleic acid
DOP	dioctyl phthalate
2-EH	2-ethylhexanol
ERL	environmental risk limit
FSH	follicle-stimulating hormone
GABA	γ-aminobutyric acid
HPOA	hypothalamic/preoptic area
IL-4	interleukin-4
Insl3	insulin-like hormone 3
IARC	International Agency for Research on Cancer
LC_{50}	lethal concentration to 50 percent of the population
LH	luteinizing hormone
LOEC	lowest observed concentration
LOELs	lowest observed effect levels
MBP	mono-n-butyl phthalate
MBzP	mono-benzyl phthalate
MCSI	Mitsubishi Chemical Safety Institute Ltd.
MEHP	mono-ethylhexyl phthalate
MEP	mono-ethyl phthalate
MiNP	mono-isononyl phthalate
MIP-1α	macrophage inflammatory protein-1α
MMP	monomethyl phthalate
NIS	sodium/iodide symporter
NOEC	no observed effect concentration
NOELs	no observed effect levels
OEHHA	Office of Environmental Health Hazard Assessment
OPC	California Ocean Protection Council
PA	phthalic acid
PCBs	polychlorinated biphenols
PKC	Protein kinase C
PND	postnatal day

PPAR-γ	peroxisome proliferators-activated receptor-γ
PVC	polyvinyl chloride
RNA	ribonucleic acid
SEB	Staphylococcus enterotoxin B
SHBG	sex hormone-binding globulin
T3	triiodothyronine
T4	thyroxine
TSH	thyroid stimulating hormone

Introduction

On February 8, 2007, the California Ocean Protection Council (OPC) passed a resolution, "On Reducing and Preventing Marine Debris." Research is underway to determine whether these constituents leach out of plastic products in the marine environment, and as a result, present a threat to the health of humans and wildlife. The OPC has asked the Office of Environmental Health Hazard Assessment (OEHHA) to aid in this effort by preparing toxicity profiles characterizing certain chemical constituents of plastics that are thought to be harmful to marine life and humans. In preparing this profile, OEHHA reviewed reported information on the adverse effects of exposure to DEHP in aquatic organisms in the laboratory and in the natural environment, humans, and experimental laboratory animals.

Properties and Uses

Phthalate esters (esters of 1, 2-benzenedicarboxylic acid) are widely used as plasticizers to increase the flexibility and workability of high-molecular-weight polymers. Phthalates constitute up to 50 percent of the total weight of some plastics. Their low melting point and high boiling point make them useful as heat transfer fluids and carriers. The world-wide production of phthalates approximates 2.7 million metric tons a year, a quarter of which is di-(2-ethylhexyl) phthalate (DEHP) (CAS number, 117-81-7) (van Wezel et al., 2000).

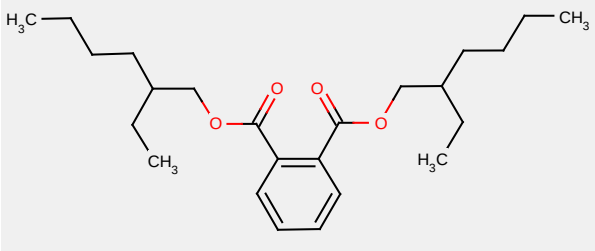

Figure 1: Di-(2-ethylhexyl) phthalate (DEHP)

Table 1: Physicochemical Properties of DEHP (NTP-CERHR, 2000)

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	Highly variable (see text)
Log K_{ow}	7.50

DEHP is the most commonly used phthalate plasticizer. Polyvinyl chloride (PVC) is by itself a hard and rigid plastic and is used to make products, such as pipes, but when a plasticizer like DEHP is added, polyvinyl chloride becomes soft and flexible and can be made into a variety of other products like packaging film and sheets, wall coverings, floor tiles, furniture upholstery,

shower curtains, garden hoses, swimming pool liners, rainwear, toys, shoes, sheathing for wire and cable, and medical tubing. DEHP does not bind with the polymer in the plastic. It mixes with the polymer and allows the polymer strands to move, providing the flexibility.

Since the DEHP is not bound in plastic, it slowly migrates or leaches out of the plastic and into the environment. DEHP in the PVC films and plastic containers used to cover and store food can transfer into the contents, thereby exposing people via ingestion of the food. DEHP is also used in PVC plastic disposable medical equipment and has been found to transfer to patients.

DEHP is a ubiquitous contaminant in today's environment. DEHP has been found in several kinds of food; monitoring data indicate that DEHP residues are generally low in U.S. foods, but the available data are in excess of 10 years old and might not be representative of current conditions. Fish and other seafood have been reported to be contaminated with concentrations ranging from 2 to 32,000 ppb. In the past and in addition to fish, DEHP has been detected in foods such as milk, cheese, meat, margarine, eggs, cereal products, baby food, and infant formula (ATSDR, 2002). In water, DEHP predominantly attaches to suspended particles and sediments, but a small amount remains dissolved in the water. DEHP in air binds to dust particles and is removed from the atmosphere by settling of dry particles and by being washed out by rain and snow. In view of the considerable direct and expected indirect emission of

Freshwater and Marine Laboratory and Environmental Studies

Environmental Contamination and Fate

The solubility of DEHP in distilled water has been determined both experimentally and theoretically and varied between 1.1 and 1,200 µg/L (Staples et al., 1997). The lowest experimentally derived value for the solubility of DEHP in distilled water was 41 µg/L; however, a value of 3 µg/L for the water solubility of DEHP has been recommended by Staples et al. (1997) "based on available evidence", rather than any one specific experimentally derived value (ATSDR, 2002). Staples et al. note that most published values exceed true water solubilities due to experimental difficulties associated with solubility determinations for these hydrophobic organic liquids. Laboratory and field studies show that partitioning to suspended solids, soils, sediments and aerosols increase as K~ increases and VP decreases. In seawater, Howard et al. (1985) and Giam et al. (1980) report DEHP solubilities of 160 and 1160 µg/L, respectively, using conventional techniques. Concentrations of DEHP in wastewater samples collected from Oakland, California, urban sources ranged from 0.99 to 2700 µg/L (Jackson and Sutton, 2008). DEHP concentrations in Japanese surface waters ranged from non-detect to58 µg/L (Naito et al., 2006).

Huang et al. (2008) investigated phthalate compounds in sediments and fishes in 17 Taiwan rivers and found DEHP in sediment at low-flow season was <0.05 to 46.5 mg/kg dry weight and at high-flow season was <0.05 to 13.1 mg/kg dry weight; they report a water solubility of 300 µg/L. The DEHP sediment concentrations reported by Huang et al. (2008) are within the range of those reported by other authors in other rivers (Fromme et al., 2002; Peijnenburg and Struijs, 2006; Sha et al., 2007; Tan, 1995). Naito et al. (2006) reported Japanese sediment concentrations ranging from non-detect to 210,000 µg/kg dry weight. Adsorption of DEHP to marine sediments might be greater than adsorption to freshwater sediments, due to reduced solubility of DEHP in saltwater; levels of DEHP in a marine environment ranged from 0.1 to 0.7 ppb in the water and from 280 to 640 ppb in the suspended particles (ATSDR, 2002).

Phthalates have several degradation pathways and therefore are not considered to be persistent chemicals. They are easily photodegraded in the atmosphere with predicted half-lives of approximately 1 day, and they can be biodegraded by bacteria and actinomycetes, which is likely the dominant loss mechanism in surface waters, soils and sediments (Staples et al., 1997). Primary degradation half-lives in surface and marine waters range from <1 day to 2 weeks and in soils from <1 week to several months; standardized aerobic biodegradation tests with sewage sludge inocula show that phthalate esters undergo ~ 50% ultimate degradation within 28 days (Staples et al., 1997).

Environmental Bio-uptake

DEHP experimentally has been shown to bioaccumulate in the aquatic plant *Elodea canadensis*, the snail *Physa* sp., the water flea *Daphnia magna* and mosquito (*Culex pipiens quinquefasciatus*) larvae and pupae (Metcalf et al., 1973), as well as in isolated rat liver (Jaeger and Rubin, 1970). Furthermore, Taborsky (1967) isolated DEHP from bovine pineal glands, and it has found it in mitochondria from the hearts of cattle, dogs, rabbit, and rat (Nazir et al., 1971). Questions from these earlier studies remain whether the phthalates are of physiological origin or are ingested by the animals from artificial sources and retained. Additionally, DEHP has been found in human spleen, liver, lung, and abdominal fat in quantities ranging from 25 ppm (dry weight) in spleen to 270 ppm in abdominal fat from patients who had received transfusions of blood stored in plastic bags (Jaeger and Rubin, 1970). DEHP, fluxes may be high, explaining the concentrations found in the environment and bio-uptake by organisms.

Despite its high tendency to accumulate in fatty tissues, fish do not extensively accumulate DEHP. Norman et al (2007) found tissue concentrations in Atlantic salmon at 0.3 percent of the dietary concentration. Experiments with rainbow trout fitted with an indwelling cannula showed that the majority of $^{[14C]}$DEHP did not reach the systemic circulation of the fish, but DEHP metabolites were present in the exposure water. Isolated perfused gill arches of the trout metabolized DEHP in the exposure bath to monoethylhexyl phthalate, demonstrating the ability of enzymes in the gill to break down DEHP, thus potentially limiting its entry into the fish (Barron et al., 1989). Bioconcentration factors for fish are not strongly related to the organic carbon absorption coefficient (Koc) of the pthalates; this is most likely due to metabolism. Bioconcentrations in aquatic organisms other than fish, (e.g., algae, mollusks) exceeds that in fish (Table 2), suggesting lower biotransformation capacities in these organisms. Bioaccumulation in the food chain is limited because of biotransformation. An extra risk related to accumulation in the food chain is therefore not expected (van Wezel et al., 2000).

Table 2: Bio-uptake and Bioconcentration

Compartment	Water (µg/L)	Tissue (µg/kg)	Bioconcentration Factor	Reference*
Rainbow trout	~100		42-113	(Barron et al., 1989)
Atlantic salmon	827 mg/kg diet	2551		(Norman et al., 2007)
Daphnia magna	3.2 10 32 100	0.00053 0.00140 0.00835 0.02677	166 140 261 268	(Brown and Thompson, 1982a)
Mytilus edulis	4	0.0097	2366	(Brown and

Compartment	Water (µg/L)	Tissue (µg/kg)	Bioconcentration Factor	Reference*
	42	0.1106	2627	Thompson, 1982b)
Algae	No data	No data	3173±3149	(Staples et al., 1997)
Molluscs	No data	No data	1469±949	
Crustacean	No data	No data	1164±1182	
Insects	No data	No data	1058±772	
Fish	No data	No data	280±230	
Amphibians	No data	No data	605	

Toxicology: Marine and Other Aquatic Organisms

Fish

Spawned eggs from a commercial orange-red variety of Japanese medaka were exposed to 0.01, 0.1, 1.0, and 10.0_µg/L until hatching; no differences in eyeing or hatching success were observed between the treatments and the control; however, intermediate levels of DEHP resulted in delayed hatching time (Chikae et al., 2004a). In the adult stage, mortality rates were higher and male body weights were lower for those individuals hatched from eggs exposed to DEHP, but no effects were seen in female body weights or the gonadosomatic index (GSI) of either sex (Chikae et al., 2004a). In a later study, declines were observed in female body weights at 0.1, 1.0, and 10.0_µg/L and the GSI was reduced in males at 0.01 – 10 µg/L (Chikae et al., 2004b) (Chikae et al., 2004b). Effects were observed in female medaka under chronic exposure conditions of 10 – 50 µg/L for 3 months (Kim et al., 2002). Conversely, no effect was observed in medaka exposed to higher concentrations for 14 days (Shioda and Wakabayashi, 2000). The potential risks of DEHP in Japanese water environments were characterized by comparing the margin of exposure (MOE) with a specified uncertainty multiplier (UM); Naito et al., (2006) concluded that the levels of DEHP were of little concern to aquatic life in the majority of Japanese surface waters and sediments.

Invertebrates

Kwak and Lee (2005) observed that exposure of the midge (*Chironomus riparius*) at concentrations ranging from 0.3 – 30 µg/L resulted reproductive effects (Kwak and Lee, 2005) , whereas Streufert et al. (1980) in work on the midge (*C. plumosus*) found no effect on egg hatchability or midge emergence from the larval stage. In *C. tentans* changes in gene expression (Lee et al., 2006) and increased mortality (Park and Choi, 2007) were observed at high concentrations.

Sanders et al. (1973) investigated the effect of DEHP on the reproduction of *Daphnia magna* and found that at the lowest concentration tested (3 µg/L) the number of young produced after 3 weeks of exposure was only 40% of the control and an 83% inhibition of reproduction at 30 µg/L. However, no differences were observed among treatments in the number of young produced and surviving parent *Daphnia* after 21 days exposure to 0 – 100 µg/L DEHP (Brown et al. 1982a). (Park and Choi, 2007)) reported a 96-hr LC_{50} for *Daphnia* at 1.82 µg/L.

Mussels (*Mytilus edulis*) were exposed to 5 and 50 µg/L DEPH for 14 days; non-quantitative observations did not indicated evidence of any adverse effects of the phthalates on the mussels,

and all 5 groups appeared to be actively feeding throughout the study; throughout the test period there was 1 death in each of the test vessels receiving DEHP and in the solvent control (Brown and Thompson, 1982b). At concentrations exceeding 100 µg/L, cellular effects were observed in mussels (Table 3).

Table 3: Aquatic Toxicity Summary

Species	DEHP exposure	Effect	Reference
Salmon (Salmo salar) fry	16.5 mg/kg bw	Intersex fish	(Norman et al., 2007)
Medaka (Oryzias latipes)	Eggs exposed to 0.01, 0.1, 1.0, 10.0 µg/L until hatching	No effect on eyeing, hatching success, female body weight, or gonadosomatic index Significant effects on hatching time, overall mortality and male body weight	(Chikae et al., 2004a)
Medaka (Oryzias latipes)	Eggs exposed to 0.01, 0.1, 1.0, 10.0 µg/L until hatching	Reduced female body weight Reduced male gonadosomatic index	(Chikae et al., 2004b)
Medaka (Oryzias latipes) adults	10, 50, and 100 µg/L: 5 days	No significant effects	(Kim et al., 2002)
Medaka (Oryzias latipes) hatchlings	1, 10, and 50 µg/L: 3 months	Some evidence of lower vitellogenin levels in blood serum of females; Lower GSI, retarded oocyte development in females at 10 and 50 µg/l	
Medaka (Oryzias latipes) adults	0.1, 0.3, 1 µmol/L (~39, 117, 390 µg/L): 14 days	No effect	(Shioda and Wakabayashi, 2000)
Mussel (Mytilus edulis)	5, 50 µg/L: 14 days	No effect	(Brown and Thompson, 1982b)
Mussel (Mytilus galloprovincialis)	500 µg/L	Peroxisome proliferation	(Marigomez and Baybay-Villacorta, 2003; Orbea et al., 2002)
	100 µg/L	↑ neutral fat in digestive gland	
Copepod (Tigriopus japonicus)	7,000–10,000 µg/L	96-hr LC50	(Seo et al., 2006)
Estuarine copepod (Eurytemora affinis)	511 µg/L	96-hr LC50	(Forget-Leray et al., 2005)
	109 µg/L: 10 days	Failure to develop beyond nauplii. Survival NOEC	
	245 µg/L: 10 days	Survival 10-day LOEC	
Water flea (Daphnia magna)	1.82 µg/L	96-hr LC50	(Park and Choi, 2007)
Water flea (Daphnia magna)	0 – 100 µg/L: 21 days	No effect	(Brown and Thompson, 1982a)

Species	DEHP exposure	Effect	Reference
Dragonfly (Latin name?) larva	587-623 mg/kg sediment	↓ predation efficiency	(Woin and Larsson, 1987)
Midge (Chironomous tentans)	500 – 5000 µg/L	↑ Heat-shock Protein gene expression ↓ Hemoglobin gene expression	(Lee et al., 2006)
Midge (Chironomous tentans)	1124 µg/L	96-hr LC50	(Park and Choi, 2007)
Midge (Chironomus riparius)	>0.3 µg/L	↓ adult emergence (no dose/response)	(Kwak and Lee, 2005)
	0.3, 30 µg/L	Sex ratio Female > male	

Environmental Criteria

The California State Water Resources Control Board does not have ambient water quality criteria for the protection of freshwater or marine life. An environmental risk limit (ERL) is an estimated ecosystem no-effect concentration used as a basis for environmental quality standards. In order to derive a DEHP ERL for the Netherlands, van Wezel et al. (2000) reviewed the DEHP literature. They considered data on chronic and acute toxicity of DEHP to aquatic species along with effects on growth and reproduction. Their smallest aquatic lowest observed effect concentration (LOEC) was 5 µg/L, based on survival and reproduction in a 15-week study in rainbow trout. Because all identified aquatic toxicity no observed effect concentrations (NOEC) exceeded the 3 µg/L water solubility of DEHP recommended by Staples et al. (1997), an ERL based on aquatic toxicity was not developed. Instead, the ERL was based on sediment toxicity. The lowest NOEC was 10 mg/kg sediment based on hatching success of the moor frog, *Rana arvalis*. Based on equilibrium partitioning between solid and liquid phases, this sediment concentration was equivalent to 1.9 µg/L; a 10-fold safety factor made the ERL 0.19 µg/L.

Summary

Toxicology data are summarized in Table 3. Arthropods appear to be the most sensitive, with several reports of effects in the low or fractional µg/L range. Some fish, especially Medaka are also sensitive, with effects reported in the low µg/L range. Mollusks appear to be less sensitive with effects reported in the tens to hundreds of µg/L range. The relationship of the toxic concentrations to the solubility limit is unclear, since published solubility limits for DEHP vary widely. These disparate and conflicting findings indicate the need for further work to clarify the risk of adverse effects on invertebrates at realistic environmental concentrations. More work should focus on free-living organisms in marine and estuarine environments.

Human and Laboratory Studies

Reproductive and Developmental Toxicity

Introduction

Evidence on the developmental and reproductive toxicity of DEHP has been reviewed in several comprehensive reports (e.g., the National Toxicology Program-Center for the Evaluation of Risk to Human Reproduction (NTP-CERHR)(Kavlock et al., 2006; NTP-CERHR, 2000)). The current review relies heavily on the experimental data summarized in those reports as well as on major findings from numerous studies that were reported in the past few years. Because of the general concordance between reproductive toxicity of chemicals in humans and wildlife, as discussed in the endocrine disruption literature (Colborn, 1994; Hotchkiss et al., 2008), evidence of toxicity in laboratory animals and humans should be taken into account in evaluating the potential effects of exposure to DEHP on marine organisms.

Male Reproductive Toxicity in Animals

The male reproductive toxicity of DEHP is well established and has been studied in many species including rats, mice, hamsters, ferrets, and non-human primates. Findings from the majority of these studies have been well reviewed and summarized in many documents or review reports (e.g., NTP-CERHR, 2000; U.S. FDA, 2001). Therefore, detailed findings from each individual study are not discussed in this document. Instead, this document focuses on a number of key studies that can be potentially identified as the most sensitive study of sufficient quality for quantitative risk assessment.

Studies in Rats

The majority of studies on the male reproductive toxicity of DEHP were conducted in rats using oral administration (gavage, feed, or drinking water). A few studies used non-oral routes of exposure (e.g., intravenous injection or inhalation). The male reproductive toxicity of DEHP following intravenous injection was similar to that following oral administration, though the intravenous doses required to induce obvious testicular damage were higher than those by oral treatment (Cammack et al., 2003). One inhalation study that investigated the testicular effects of DEHP in prepubertal Wistar rats found that inhalation of DEHP at concentrations of 5 or 25 mg/m^3, six hours per day for four or eight weeks caused significant increases in the plasma level of testosterone and the weight of seminal vesicles. Although the underlying mechanism for this DEHP-induced increase in testosterone and its long-term functional consequence remain to be determined, these findings indicate that inhalation exposure to DEHP can alter testicular function. The findings are also consistent with those reported by Akinbemi et al (2004; 2001) who treated rats of similar ages by gavage.

Depending on the doses, dosing duration, age of animals, and endpoints included, it has been shown that oral treatment with DEHP causes reduced fertility, decreased weights of male reproductive organs, and histopathological changes in the testis of juvenile and adult rats (NTP-CERHR, 2000; U.S. FDA, 2001). Characteristics of histopathological changes include vacuolation and rarefaction of the cytoplasm, disruption of cytoskeletons, destruction of intercellular specializations (e.g., ectoplasmic specialization, occluding junctions) in Sertoli cells,

followed by degeneration of spermatocytes by apoptosis and/or sloughing of germ cells into the lumen of seminiferous tubules (e.g., Boekelheide, 2004; Park et al., 2002; Saitoh et al., 1997). Different groups of germ cells in the testis of rats are organized in an orderly manner along the length of seminiferous tubules. A defined group of germ cells is called a stage. Along the length of a seminiferous tubule, there is a distinct ordering of stages, namely from stage I to XIV. Sertoli cells undergo morphological and functional fluctuation from stages I to XIV. In the testis of young rats (8-week-old), Sertoli cells and the spermatocytes associated with them in seminiferous tubules at stages IX-XIV and I are most sensitive to the testicular effects of DEHP (Saitoh et al., 1997).

Oral administration of DEHP to rats during the perinatal period results in severe permanent abnormalities in the male reproductive system of male offspring (Arcadi et al., 1998; Gray et al., 1999b; Moore et al., 2001; Schilling et al., 1999; Tandon et al., 1991). Neonatal or young rats have been found to be more sensitive to the male reproductive effects of DEHP than are adults (Akingbemi et al., 2004; 2001; Borch et al., 2004; Cammack et al., 2003; Dostal et al., 1988; Gray and Butterworth, 1980; Li et al., 2000; NTP-CERHR, 2000; Sjoberg et al., 1986; 1985; U.S. FDA, 2001). The testis at early developmental stages (late gestation and early days after birth in rats) is more sensitive to DEHP than that of juvenile or adult animals (Gray et al., 1999a; 2000; Moore et al., 2001; NTP-CERHR, 2000). Thus, the no observed effect levels (NOELs) and/or lowest observed effect levels (LOELs) for the male reproductive effects of DEHP observed in studies that treated rats either perinatally or in the early weeks of the postnatal period are in general lower than those observed in young or adult animals. It should be emphasized that several recent studies have found that concurrent exposure to multiple phthalates can cause cumulative effects on the male reproductive system in a dose-additive manner (e.g., Howdeshell et al., 2007; 2008).

Table 4 summarizes a list of studies that observed relatively low values of LOELs and/or NOELs in rats. The animals used in these studies received DEHP treatment either perinatally (Arcadi et al., 1998) or as three-four week old juveniles (Akingbemi et al., 2004; 2001; David et al., 2000a; Poon et al., 1997). Manifestation of DEHP-caused testicular damage takes different forms, depending on the age of animals, dosing levels, and dosing durations. For example, as stated in the document by the Center for the Evaluation of Risks to Human Reproduction (NTP-CERHR, 2000), "during the time of Sertoli cell divisions (before pnd [post natal day] 15 in rats), phthalate exposure apparently inhibits cell division. In animals older than pnd 15, toxicity is manifest as vacuoles, followed by germ cell sloughing." Therefore, when comparing different studies to identify "the most sensitive study," OEHHA considered different endpoints used in different studies and attempted to compare different studies based on the same or similar endpoints. In addition, the clear difference in sensitivity to the testicular effects of DEHP between developing and adult rats suggests that a NOEL observed in adult animals should be compared to those observed in developing animals in order to determine if a NOEL in adult animals has no observable effects in developing animals.

The study by Arcadi (1998) observed the lowest LOEL (32.5 μl/L in drinking water) in rat dams for the male reproductive effects of DEHP in male offspring exposed to DEHP from gestational day 1 to postnatal day 21. The authors stated this dose was roughly equivalent to 3.0-3.5 mg/kg-day, but assumptions of body weights and water consumption for their estimate were not reported. This study has some limitations. For example, DEHP is essentially insoluble in water (3 μg/L or approximately 0.003 μl/L; (NTP-CERHR, 2000)). The concentrations of

DEHP used in the study were 32.5 and 325 μl/L. The authors stated that "the suspension was prepared daily by adding DEHP to mineral water and then sonicating for 30 min." However, actual concentrations of DEHP in the drinking water were not verified. Daily water consumption was not recorded. Maternal body weights were not reported. Therefore, although this study provided important evidence on the adverse effects of DEHP on rat testicular development during the perinatal period, this study report lacks detailed data that are critical for dose estimation.

Among other studies listed in Table 4, the studies by Akingbemi et al. (2004; 2001) observed an oral LOEL of 10 mg/kg-day, based on abnormal changes in testosterone production and altered Leydig cell proliferation in the testes of prepubertal rats. This LOEL is markedly lower than those based on histopathological changes in adult animals following long-term treatment with DEHP (29 mg/kg-day as observed by David et al. (2000a) or 38 mg/kg-day by Poon et al. (1997). It should be noted that the NOELs observed in adult animals by Poon et al. or David et al., respectively, are lower than the LOEL of 10 mg/kg-day observed in juvenile animals by Akingbemi et al. (2004; 2001). Therefore, based on endpoints indicative of morphological or functional changes, there is no observed effect of DEHP on the testis at doses lower than 10 mg/kg-day following oral administration, regardless of the age of rats used in the studies. The highest dose below 10 mg/kg-day used in the studies listed in Table 4 is the NOEL (5.8 mg/kg-day) observed by David et al. (2000a). Thus, this NOEL (5.8 mg/kg-day) has no observable testicular effects in rats of different ages.

Table 4. Oral studies that observed relatively low values of LOEL or NOEL for male reproductive toxicity of DEHP in rats.

Study Reference	**Animals**	**Treatment**	**General Toxicity**	**Male repro effects and LOEL**	**NOEL**
(Poon et al., 1997)	Sprague-Dawley rats, about 6 wks old at the beginning, 10 rats per group.	Feed, 0, 5, 50, 500, 5,000 ppm for 13 wks.	Increased liver and kidney weights, histopathological changes in the liver at 5,000 ppm.	Sertoli cell vacuolation and seminiferous tubular atrophy at 5000 ppm. Minimal Sertoli cell vacuolation in 7/10 rats at 500 ppm. LOEL: 500 ppm (38 mg/kg-day)	50 ppm (3.7 mg/kg-day).
(Arcadi et al., 1998)	Long-Evans rats, 12 pregnant rats per group	Drinking water, 0, 32.5, 325 μl/L DEHP, from gestational day 1 to postnatal day (PND) 21. Pups examined on PND 21, 28, 35, 42, and 56.	No effects on body weight gains of dams or pups. Changed weights and pathology in the kidney and liver of pups at both doses.	Reduced testis weights and histopathological changes in the testes of male pups at both doses. LOEL: 32.5 μl/L (3.0-3.5 mg/kg-day, estimated by the study authors; water consumption not reported)	Not observed.
(David et al., 2000a)	Fischer 344 rats, about six-wk-old at the start, 55-80 rats per group.	Feed, 0, 100, 500, 2,500, or 12,500 ppm DEHP for 104 wks.	Reduced survival rates, reduced body weights, adverse effects in the liver, kidney, and pituitary at ≥2,500 ppm.	Significantly increased incidence of aspermatogenesis at ≥ 500 ppm at Week 105. LOEL: 500 ppm (29 mg/kg-day)	100 ppm (5.8 mg/kg-day)
(Akingbemi et al., 2001)	**Male Long-Evans rats, 21, 35, or 62**	Gavage, 0, 1, 10, 100 or 200 mg/kg-day, PND	**No obvious general toxicity**.	Decreased testosterone (T) production by Leydig cells at ≥10 mg/kg-day at PND 21-34;	1 mg/kg-day

Study Reference	Animals	Treatment	General Toxicity	Male repro effects and LOEL	NOEL
	days of age; ten rats per group	21-34, 35-48, 21-48, or 62-89.		increased T production when exposed at PND 21-48. **LOEL: 10 mg/kg-day**	
(Akingbemi et al., 2004)	**Male Long-Evans rats, 21 day of age, ten rats per group**	Gavage, 0, 10, 100 mg/kg-day, from postnatal day (PND) 21 to PND 48, 90 or 120.	**No obvious general toxicity**.	Reduced T production in Leydig cells. Increased numbers and proliferating activity of Leydig cells at ≥ 10 mg/kg-day: **LOEL: 10 mg/kg-day**	Not found.

Studies in Other Species

The male reproductive effects of DEHP following oral administration have also been studied in mice, guinea pigs, hamster, ferrets, and non-human primates. There is clear evidence indicating that oral administration of DEHP causes adverse effects in the male reproductive systems of mice, guinea pigs, hamsters, and ferrets (e.g., David, 2000; Gangolli, 1982; Gray et al., 1982; Lake et al., 1976; Lamb et al., 1987; NTP-CERHR, 2000). The LOELs and/or NOELs for the male reproductive effects of DEHP observed in mice are generally higher than those in rats. Syrian hamsters are much less sensitive to the testicular effects of DEHP than are rats (David, 2000; Gangolli, 1982; Gray et al., 1982; Lake et al., 1976; Lamb et al., 1987). The LOEL for the testicular effects of DEHP administered in diet for 14 months in mature albino ferrets were 1200 mg/kg-day, which again is much higher than that in rats (e.g., David et al., 2000a). The studies in mice, hamsters, and ferrets clearly demonstrated that male reproductive effects occur in these species. However, these species are less sensitive to the testicular effects of DEHP than is the rat, based on similar endpoints indicative of testicular damage under similar treatment regimes.

In addition to rats, mice, hamsters, and ferrets, non-human primates have been used in several oral studies of the toxic effects of DEHP (Kurata et al., 1998; MCSI, 2003; Pugh et al., 2000; Rhodes et al., 1986). The studies by Rhodes et al. (1986), Kurata et al. (1998), and Mitsubishi Chemical Safety Institute Ltd. (MCSI, 2003), were conducted in common marmosets (*Callithrix jacchus*), a New World primate. In the study by Pugh et al. (2000), cynomolgus monkeys (*Macaca fascicularis*), an Old World primate, were used. Because results from these primate studies have been suggested as a basis for determining the relevance of rodent data to humans (e.g., McKee et al., 2004), details of these four primate studies are discussed below.

In the study by Rhodes et al. (1986), groups of five adult male marmosets (weighing 250-400 g) were treated by gavage with 0 or 2,000 mg/kg-day DEHP for 14 days. Body weight gain in the DEHP-treated group was significantly lower than that in the control (body weights in the DEHP-treated group were approximately 70 percent of those in the control group, $p<0.05$), but no effect on testicular weights was observed. The histopathological findings were not reported, although the authors reported that they conducted histopathological evaluation of the testes by light microscopy.

In the study by Kurata et al. (1998), groups of four adult male marmosets (body weights at the end of 13-week treatment averaged about 330 g) were treated orally with 0, 100, 500, and 2,500 mg/kg-day DEHP for 13 weeks. Body weight gain was significantly reduced in males treated

with 2,500 mg/kg-day, but no significant effect on blood testosterone levels, testis weights or morphology at light and electron microscopic levels was observed.

In a recent study in juvenile marmosets, sponsored by the Japanese Plasticizer Industry Association, conducted by Kurata et al. at the Mitsubishi Chemical Safety Institute Ltd. (MCSI, 2003), groups of male marmosets (8-10 animals per group) aged from 90 to 110 days were treated by gavage for 65 weeks with 0, 100, 500, or 2,500 mg/kg-day DEHP. The authors stated that there was no treatment-related effect on body weights or weights of reproductive organs including testes and epididymides. No apparent histopathological changes in the testis were observed in DEHP-treated animals. Epididymal sperm count in DEHP-treated animals was not different from that in the control animals. There was no significant difference in mean levels of blood testosterone in blood samples collected at intervals during the treatment between DEHP-treated and control animals. No treatment-related changes in histochemical and biochemical examinations for testicular functions were observed.

The findings from three studies conducted in common marmosets indicate that DEHP, even at very high dose levels, does not cause testicular damage in this species. Because the seminiferous epithelium in the testis of common marmoset is organized similarly to that in humans, some have suggested the common marmoset to be a good model to predict the potential testicular effects of chemicals in humans (Millar et al., 2000; Sharpe et al., 2000), while others have noted fundamental species differences and have concluded otherwise (Li et al., 2005; Zuhlke and Weinbauer, 2003). The testis of the common marmoset has some unique characteristics that are dramatically different from other mammals including rats, cynomolgus macaques, and humans. For example, sperm production and androgen synthesis in humans, macaque monkeys, and rodents are regulated by hormones produced in the pituitary, such as FSH and LH. However, the pituitary of the common marmoset does not produce LH. Instead, it produces chorionic gonadotropin (CG), which is only produced in the placenta of humans or rodents (Muller et al., 2004). Both CG and LH in mammals use the same receptor, the LH receptor. The gene for this receptor in common marmoset is lacking one segment called exon 10. Lack of exon 10 in the LH receptor causes androgen deficiency and hypogonadism in humans (Gromoll et al., 2000; Zhang et al., 1998). Recent studies using transplanting techniques have also shown that the conditions needed for initiation of spermatogenesis in the marmoset are remarkably different from those present in most other mammals (e.g., Wistuba et al., 2004). Because of fundamental differences in the testis between common marmosets and humans, it has been suggested that "the use of this animal model cannot be recommended for reproductive toxicology assessment" (Zuhlke and Weinbauer, 2003). In addition, vitamins C and E are protective against the testicular effects of DEHP in rats or mice (Ablake et al., 2004; Ishihara et al., 2000). Common marmosets require high levels of dietary vitamin C so regular diets for this species usually contain high levels of vitamin C supplements (e.g., MCSI, 2003). Serum levels of vitamin C in common marmosets are markedly higher (2.56 mg/100ml in average) than most other mammals (0.63 mg/100 ml in average in humans; (Flurer and Zucker, 1987; 1989; Hampl et al., 2004)), creating the possibility of reduced sensitivity to DEHP in this species.

In addition to the three studies in common marmosets discussed above, there is one study in cynomolgus monkeys reported by Pugh et al. (2000). In this study, male monkeys of about two years of age (weighing 1977-2921 g), four animals per group, were treated by gavage with 0, 500 mg/kg-day DEHP, 500 mg/kg-day di-isononyl phthalate (DINP), or 250 mg/kg-day clofibrate for 14 days. The overall objective of this study was to assess the effects of DEHP, DINP, and

clofibrate on peroxisome proliferation in the cynomolgus monkey. The initial body weights for each group were not reported. The final body weight of monkeys in the DEHP-treated group (2378±194 g; mean ± standard deviation) was lower than that of the control group (2590±138 g), but the difference was not statistically significant (determined by analysis of variance (ANOVA) followed by a Dunnet's test, as reported by the authors). With regard to testicular effects of DEHP, absolute testis or epididymis weights were not reported. Relative weight (percent) of testes/epididymides in the DEHP group (0.069±0.005; mean ± standard deviation) was approximately 83 percent of that of the control animals (0.083±0.018), indicating a 17 percent decrease, but the difference is not statistically significant. It is unclear whether the relative weight of testes/epididymides as reported was a combined weight of testes and epididymides or testes only. The authors stated that there was no treatment-related histopathological change in the testes, but detailed information on histopathological observations was not reported. No effect on liver or kidney weight, hepatic peroxisomal beta-oxidation, or replicative DNA synthesis and gap junctional intercellular communication in the liver was observed. The authors concluded primates were unresponsive to the induction of DNA synthesis and peroxisomal beta-oxidation, but did not make any conclusion regarding their observations on the possible testicular effects of DEHP.

The study by Pugh et al. (2000) used four monkeys per group. The sample size is small and thus has limited statistical power to reveal treatment-related effects among DEHP-treated animals. Statistical power is the probability of detecting an effect if there really is one. It is highly influenced by the size of a study (the number of subjects per group). A statistical power of 0.8 or higher is generally used (Lenth, 2001; Schwetz, 2001). Based on reported means and standard deviations of relative testis/epididymis weights, the sample size only provides a statistical power of 0.2 – 0.3. Thus, the study by Pugh et al. (2000) has only approximately a 20-30 percent chance to detect a difference in testicular weights between the control and DEHP-treated monkeys if a real difference exists. In order to detect a statistically significant difference (at a significance level of 0.05) in body weights or relative testis/epididymis weight with a statistical power of 0.8 (i.e., an 80 percent likelihood of detecting the effect), at least 10-14 animals per group are required (Stata Corporation, 2003). Thus, the study by Pugh et al. (2000) does not have sufficient power to detect a statistically significant difference in the relative weight of testis/epididymis in cynomolgus monkeys between the control and treated group under the experimental designs used in the study.

Cynomolgus monkeys used in the Pugh et al. (2000) study were approximately two years of age and weighing 1977-2921 g. The testis in two-three year old cynomolgus monkeys is immature and relatively quiescent (e.g., (Cho et al., 1975; Kluin et al., 1983; Liang et al., 2001; Smedley et al., 2002). Tightly-packed, small-diameter seminiferous cords consist of Sertoli cells with few interspersed spermatogonia. There are no spermatocytes or spermatids since meiosis does not occur until puberty around 3.5-4 years of age (Kluin et al., 1983; Smedley et al., 2002). Therefore, degenerative changes in spermatocytes, which are seen in young or adult rat testis following DEHP treatment, may not be expected in the testis of cynomolgus monkeys two-three years of age. Sertoli cell proliferation remains at very low levels; with only approximately 0.3 percent of Sertoli cells in the S-phase of the cell cycle in cynomolgus monkeys two-three years of age, as compared to approximately 10-20 percent in rats during the first two weeks after birth (Kluin et al., 1983; Liang et al., 2001; Orth, 1982). This cellular event (i.e., Sertoli cell proliferation) is critical for establishing normal testis size in the adult (e.g., Orth et al., 1988) and

has been shown to be targeted by DEHP in the developing testis (Li and Kim, 2003; Li et al., 2000; 1998). Based on the physiological characteristics of the testis (e.g., slow growth in the testis, low proliferating activity in Sertoli cells, low testosterone production in Leydig cells) in two-to-three year old cynomolgus monkeys, it appears that the age of two to three years may represent a window of relatively low sensitivity to the testicular effects of DEHP. Because proliferative activity of Sertoli cells is low, any possible change in testis weight resulting from inhibition of Sertoli cell proliferation by DEHP treatment as seen in neonatal rat testis may not be dramatic in cynomolgus monkeys two or three years of age. Nevertheless, a decrease (by approximately 17 percent) in relative weight of testes/epdidymides (assuming combined weights) was observed in the DEHP-treated monkeys by Pugh et al. (2000).

Female Reproductive Toxicity in Animals

There are several studies showing sufficient evidence of female reproductive toxicity in laboratory animals, though this toxicity endpoint has not been studied as extensively as has the developmental or the male reproductive toxicity of DEHP. Critical findings in this area are summarized below.

Schilling et al. (1999) reported a two-generation reproductive study of DEHP in Wistar rats. Dietary exposure to DEHP at 9000 ppm (equivalent to approximately 1088 mg/kg-day) caused reduction in the numbers of corpora lutea and growing follicles in parent (F0) and the first generation female offspring (F1). Vaginal opening was also delayed in the female offspring, indicating adverse effects on the development of the female reproductive system. In a recent study by Grande et al. (2006), perinatal exposure to DEHP caused a significant delay in the age of vaginal opening at doses of 15 mg/kg-day and above and a trend for a delay in the age at first estrus at high doses. An increase in the number of ovarian atretic tertiary follicles was also observed in adult female offspring exposed to DEHP during the perinatal period (Grande et al., 2007).

Complete infertility in CD-1 female mice was observed in a National Toxicology Program reproductive toxicity study with continuous breeding and cross-over mating design (Lamb et al., 1987). Both male and female mice were treated with DEHP in feed at estimated doses from 14 to 425 mg/kg-day. Therefore, it is not clear if the complete infertility resulted from the reproductive effects of DEHP in females or males alone, or both. However, significantly reduced serum levels of estradiol and suppression of ovulation had been observed in female rats treated with 2000 mg/kg-day of DEHP by gavage for 12 days, clearly indicating the female reproductive toxicity of DEHP (Davis et al., 1994a). In addition, Davis et al. conducted a series of in vitro studies exploring the mechanism(s) underlying the ovarian effects of DEHP (Davis et al., 1994b; Lovekamp-Swan and Davis, 2003; Lovekamp-Swan et al., 2003; Lovekamp and Davis, 2001). They found that MEHP, the active metabolite of DEHP, can reduce the production of estradiol by suppressing transcription of the aromatase gene, which is mediated by activation of peroxisome proliferator- activated receptors alpha and gamma in the granulose cells.

A chronic study in common marmosets had found that treatment with DEHP at 500 and 2,500 mg/kg-day from 90-115 days of age (juvenile) to 18 months of age (young adult) caused an increase in serum 17-beta-estradiol, with a significant increase in ovarian and uterine weights, indicative of early onset of puberty (MCSI, 2003). This line of data is in contrast to delayed pubertal development following perinatal exposure, but is similar to the increased levels of

testosterone in juvenile male rats following prepubertal treatment with DEHP (Akingbemi et al., 2004; Akingbemi et al., 2001).

Developmental Toxicity in Animals

The developmental toxicity of DEHP in laboratory animals has been extensively studied and the results from numerous studies on the developmental toxicity of DEHP are remarkably consistent. In traditional developmental toxicity studies, DEHP has been found to cause intrauterine death, developmental delay, and structural malformations and variations (NTP-CERHR, 2000). The pattern of malformations include morphological abnormalities of the axial skeleton (including tail), cardiovascular system (heart and aortic arch), appendicular skeleton (missing bones, finger abnormalities), eye (including open eye), and neural tube (exencephaly).

Based on the relevant data available, the CD-1 mouse appears to be the species most sensitive to the developmental effects of DEHP following oral treatment. The lowest LOEL for the developmental toxicity of DEHP via the oral route of exposure was 0.05 percent in feed as observed in the studies reported by Tyl et al. (1988) and Price et al. (1988a). Major findings from these two studies were presented in Table 5. The estimated doses, expressed as mg/kg-day, of DEHP used in the study by Price et al. (1988a) were slightly higher (95 mg/kg-day for 0.05 percent; 48 mg/kg-day for 0.025 percent) than those in the study by Tyl et al., (1988); 91 mg/kg-day for 0.05 percent; 44 mg/kg-day for 0.025 percent). The NOEL (48 mg/kg-day) for the developmental toxicity of DEHP observed in the study by Price et al. (1988a) is slightly higher than that (44 mg/kg-day) in the study by Tyl et al. (1988) and is lower than the LOEL from either study (91 or 95 mg/kg-day). Therefore, endpoints for traditional developmental toxicity are not as sensitive as those for the development and/or function of the male reproductive system in rodents. Consequently, recent studies on the developmental toxicity of DEHP have focused on the adverse effects of DEHP on the development of the reproductive systems of both sexes.

Table 5. Major findings in the studies by Tyl et al. (1984; 1988) and by Price et al. (1988)

Study Reference	Animals	Treatment	Maternal Toxicity	Developmental or male repro effects and LOEL	NOEL
(Tyl et al., 1988)	CD-1 mice, 30-31 pregnant mice per group.	Diet, 0, 0.025, 0.05, 0.10, 0.15% DEHP, GD 0-17; examined on GD 17.	Reduced maternal body weights at ≥ 0.10%.	Increased number and percentage of resorptions; reduced live litter size; increased malformations. LOEL: 0.05% (91 mg/kg-day)	0.025% (44 mg/kg-day)
(Price et al., 1988a)	CD-1 mice, 28-29 pregnant mice per group.	Diet, 0, 0.01, 0.025, or 0.05% DEHP, GD 0-17. Examined postnatally.	No obvious maternal or general effects.	Increased prenatal mortality and reduced live litter size at 0.05% on PND 1. LOEL: 0.05% (95 mg/kg-day)	0.025% (48 mg/kg-day)

Findings from studies that investigated the effects of DEHP following gestational exposure on development of the reproductive systems are summarized above. Briefly, it has been found that DEHP administered during gestation causes adverse changes in testosterone production, Leydig cell proliferation, prostate development, or expression of genes for insulin-like hormone 3 (Insl3) in the testes of male fetuses or male offspring in rats (Akingbemi et al., 2004; 2001; Banerjee et al., 2002; Borch et al., 2004; Wilson et al., 2004). Insl3 is considered to be a biomarker of

Leydig cell maturation in fetal and pubertal rats; disruption in this gene causes cryptorchidism in mice (Ivell and Bathgate, 2002; Nef and Parada, 1999; Teerds et al., 1999). Thus, alteration in expression of Insl3 gene by DEHP may represent one of the potential molecular pathways underlying DEHP-caused damage in testicular development.

It should be noted that gestational exposure to DEHP at doses as low as 0.045 mg/kg-day can cause alterations in the activity of brain aromatase in male pups on postnatal day (PND) 1 (Andrade et al., 2006b). Although it is unclear if or how changes in aromatase activity could result in other neurological or reproductive damages in the offspring, this finding indicates that the developmental effects of DEHP may occur at very low dose levels.

Human Studies

There are a number of epidemiological studies in the past few years that investigated the potential developmental and reproductive effects of exposure to phthalates (including DEHP). Major findings from these studies are briefly summarized in Tables 6 and 7.

Di-esters of phthalates (parent compounds) are quickly metabolized by hydrolysis into mono-esters either in the gastrointestinal tract following oral exposure or in the circulation upon non-oral exposure (e.g., Kavlock et al., 2006; NTP-CERHR, 2003). Therefore, concentrations of mono-ethyl phthalate (MEP), mono-benzyl phthalate (MBzP), or mono-ethylhexyl phthalate (MEHP) in human biological samples (blood, urine, semen, breast milk, or amniotic fluids) indicate levels of human exposure to di-ethyl phthalate (DEP), benzylbutyl phthalate (BBP), or DEHP, respectively (e.g., Blount et al., 2000; Hoppin et al., 2002; Kohn et al., 2000; Silva et al., 2004b). In addition, oxidative metabolites of MEHP in biological samples have also been measured to assess human exposure to DEHP (Koch et al., 2003). Most studies listed in Tables 6 and 7 measured levels of phthalate monoesters – metabolites of these diesters - in biological samples for exposure evaluation. A few studies, for example, the studies by Reddy et al. (2006a; 2006b) or by Zang et al.(2006), analyzed concentrations of phthalate diesters (parent compounds) in blood samples.

Table 6. Epidemiological studies on the male reproductive toxicity of DEHP

Study Citation	Subjects	Exposure Assessment	Endpoints for Developmental or Reproductive Effects	Significant Findings related to DEHP
(Modigh et al., 2002)	326 pregnancies fathered by 193 men occupationally exposed to DEHP	Air concentration of DEHP in the range of <0.1 to 2.1 mg/m^3	Time to pregnancy	No apparent effect on time to pregnancy.
(Duty et al., 2003b)	168 male partners of sub-fertile couples	Urinary levels of phthalate monoesters	DNA integrity in sperm using the neutral comet assay	No association with parameters indicative of DNA damage in sperm.
(Duty et al., 2003a)	168 male partners of sub-fertile couples	Urinary levels of phthalate monoesters	Sperm concentration, motility, and morphology	No apparent increase in odds ratios for all three sperm parameters.
(Duty et al., 2004)	220 male partners of sub-fertile couples	Urinary levels of phthalate monoesters	Computer-aided sperm motion analysis	Possible reduction in straight-line velocity of sperm.

Study Citation	Subjects	Exposure Assessment	Endpoints for Developmental or Reproductive Effects	Significant Findings related to DEHP
(Duty et al., 2005a)	295 male volunteers (18-54 years of age) from the general population.	Urinary levels of phthalate monoesters	Levels of reproductive hormones in blood samples	No relevant association with blood hormone levels.
(Hauser et al., 2006)	463 male partners of sub-fertile couples (168 of them were subjects reported by Duty et al., 2003b).	Urinary levels of phthalate monoesters and MEHP oxidative metabolites	Semen quality (sperm concentration, motility, and morphology)	No apparent association between levels of DEHP metabolites and any sperm parameter.
(Hauser et al., 2007)	379 male partners of sub-fertile couples	Urinary levels of phthalate monoesters & MEHP oxidative metabolites	DNA integrity in sperm using the neutral comet assay	Significant association with comet assay parameters indicative of DNA damage in sperm.
(Pan et al., 2006)	74 male workers occupationally exposed to DBP and DEHP and 63 controls in China	Urinary levels of MBP and MEHP	Blood levels FSH, LH, testosterone, and estradiol.	Decreased level of free testosterone in the exposed group.
(Jonsson et al., 2005)	234 men 18-21 years of age from military recruits in Sweden	Urinary levels of phthalate monoesters including MEH	Semen quality, and blood levels of reproductive hormones	No clear pattern of association with male reproductive toxicity endpoints evaluated.
(Main et al., 2006)	Nursing mothers of 62 boys with cryptorchidism and of 68 healthy boys as controls.	Levels of monoesters of phthalates in breast milks from nursing mothers.	Blood levels of testosterone, FSH, LH, SHBG, inhibin B from all boys at about 3 months of age.	No apparent association of MEHP level in breast milk with any hormone endpoint.
(Zhang et al., 2006)	52 male patients attending a family planning clinic in Shanghai, China	Levels of DEP, DBP, and DEHP in seminal plasma.	Semen quality	No apparent association between DEHP levels and semen parameters.

Table 7. Epidemiological studies on the developmental and female reproductive toxicity of DEHP

Study Citation	Subjects	Exposure Assessment	Endpoints for Developmental or Reproductive Effects	Significant Findings related to DEHP
(Colon et al., 2000)	41 Puerto Rican girls with premature breast development and 35 controls	Levels of DBP, DEP, BBP, DEHP, and MEHP in blood samples	Case-control study to compare levels of phthalates between the two groups.	High levels of phthalates including DEHP and MEHP in thelarche patients
(Latini et al., 2003)	82 pregnancies with live newborns	DEHP and MEHP concentrations in the cord blood samples	Birth weights, gestational age	Significantly lower gestational age among MEHP-positive newborns than that in MEHP-negative ones.

Study Citation	Subjects	Exposure Assessment	Endpoints for Developmental or Reproductive Effects	Significant Findings related to DEHP
(Reddy et al., 2006b)	85 infertile female patients with endometriosis and 135 controls with proven fertility	Levels of DBP, BBP, DEHP, Di-noctyl phthalate and polychlorinated biphenyls (PCBs) in blood samples	Case-control study to compare levels of phthalates and PCBs between the case and the control group.	Significantly increased levels of all phthalates and PCBs in female patients with endometriosis
(Reddy et al., 2006a)	49 infertile female patients with endometriosis as cases; 38 infertile female patients without endometriosis as control group I and 21 with proven fertility as control group II	Levels of DBP, BBP, DEHP, DiOP in blood samples	Case-control study to compare levels of phthalates between the case and control groups.	Significantly increased levels of all phthalates measured in the case group as compared to the control groups.
(Qiao et al., 2007)	110 precocious and 100 normal girls	Concentrations of DBP and DEHP in blood samples	Case-control study to compare levels of phthalates, volume of uterus and ovaries between the two control groups.	Precocious girls have higher concentrations of DBP, DEHP, and larger volumes of the uterus and ovary.
(Swan et al., 2005)	134 boys 2-36 months of age	Maternal urinary levels of phthalate metabolites including MEHP and its oxidative metabolites during pregnancy	Anogenital distance measured at 15.9 months of age (mean)	No apparent association between levels of DEHP metabolites and alterations in the anogenital distance.

MMP: mono-methyl phthalate; MiNP: mono-iso-nonyl phthalate; DiOP: Di-iso-octyl phthalate; FSH: follicle-stimulating hormone; SHBG: Sex hormone binding globulin; LH: Luteinizing hormone.

Epidemiological Studies of Male Reproductive Endpoints

A few studies listed in Table 6 indicate possible associations between elevated exposure to DEHP and abnormal changes in endpoints indicative of male reproductive functions, though no such association was observed in other studies.

Modigh et al. (2002) found no effect of DEHP at a mean exposure level of <0.5 mg/m^3 on time to pregnancy among partners of 193 men who were occupationally exposed to DEHP in air at three plants either producing DEHP or processing polyvinyl chloride (PVC) plastic.

A series of studies reported by Duty et al. (2004; 2005b; 2003a; 2003b) and Hauser et al. (2006), respectively, investigated the relationship between urinary levels of phthalate monoesters and semen quality or blood sex hormone levels among male partners of subfertile couples presented to an andrology laboratory the Massachusetts General Hospital in Boston. The authors found no association between urinary levels of MEHP or other phthalate monoesters and sperm DNA

damage (Duty et al., 2003a) or blood sex hormone levels (Duty et al., 2005b). However, there was a significant association between parameters indicative of sperm DNA damage and MEHP or its oxidative metabolites in the recent study that included a larger study population and measurement of multiple oxidative metabolites of DEHP (Hauser et al. 2007; 2008). A possible reduction in the straight-line velocity of sperm among male partners with increased urinary level of MEHP was observed in the 2004 study by Duty et al (2004). No other apparent changes in parameters for semen quality or reproductive hormone levels were observed in the studies by Duty et al. or Hauser et al.

A significantly decreased blood level of free testosterone has been observed in a cross-sectional study among workers occupationally exposed to high levels of di-n-butyl phthalate *(*DBP) and DEHP in China (Pan et al., 2006). In this study, urinary concentrations of mono-n-butyl phthalate (MBP) and MEHP and levels of reproductive hormones were measured among 74 male workers at a factory producing PVC flooring products (exposed group) and 63 workers working in a construction company as controls. The two groups were matched to age (mean age 33.9 years), history of cigarette-smoking (60-62 percent smokers in both groups), and alcohol consumption (54-64 percent consumers in both groups). The exposed group had one year (mean, SD = 0.8) of employment time at the factory surveyed, suggesting that the exposed workers had a limited time of exposure. Urinary concentrations of MBP and MEHP in exposed workers were significantly higher than those in the control group (644.3 and 565.7 μg/g creatinine in the exposed group vs. 129.6 and 5.7 μg/g creatinine in the control group for MBP and MEHP, respectively, geometric means). The median concentrations of MBP and MEHP in the control group were about two to seven fold higher than those in the general population in the U.S. (Silva et al., 2004a), but were similar to those observed in German males (Koch et al., 2003). The mean concentration of free testosterone in exposed workers was significantly lower than that of the controls (8.4±1.5 vs. 9.7±1.4, $\log_{10}$-transformed blood levels of free testosterone: actual concentrations of reproductive hormones were not reported). The authors reported no significant difference in blood levels of other reproductive hormones, follicle-stimulating hormone (FSH), luteinizing hormone (LH), and estradiol, between the control and exposed groups. Statistical correlation and regression analysis conducted by the authors found that both MBP and MEHP were responsible for the decreased level of free testosterone in blood. Thus, data from this study clearly show that occupational exposure to DBP and DEHP is associated with decreased levels of free testosterone, indicative of male reproductive toxicity of DBP and DEHP in men. However, the data presented by the authors are not sufficient to quantitatively distinguish among the effects attributable to DBP and those attributable to concurrent DEHP exposure, or to interaction between the two phthalates.

Jonsson et al. (2005) found no association between urinary levels of MBP, MBzP, or MEHP and parameters for semen quality or reproductive hormone levels among 234 Swedish men 18-21 years of age. All the subjects in this study were participants in the conscript examination before military service in Sweden. Each subject received a comprehensive evaluation of parameters for semen quality (e.g., semen volume, sperm count, motility, morphology, etc.). Serum levels of reproductive hormones (FSH, LH, testosterone, sex hormone-binding globulin (SHBG), estradiol) and urinary levels of phthalate metabolites (MEP, MBP, MBzP, MEHP and phthalic acid) were analyzed. Urinary levels of MBP in this study were in the same order of magnitude as those previously reported in the U.S. populations (e.g., Blount et al., 2000). The reason(s) for the inconsistent findings between this study and those reported by Duty et al. (2003b) and Hauser et al. (2006) is unclear. Jonsson et al. (2005) noted that the subjects in their study were from the

general population in Sweden and were healthy young (18-21 years old) males, whereas the studies by Duty et al. (2003a; 2003b) or Hauser et al (2006) were based on male partners of subfertile couples with a wide range of age (20-54 years). Thus, the U.S. studies may have been more sensitive in design. Because of increased age and possible poor status of spermatogenesis, subjects in the U.S. studies may have been more susceptible to the adverse effects of phthalates than those who participated in the Swedish study.

Two epidemiological studies investigated potential male reproductive effects of phthalates in boys who were exposed to phthalates through their pregnant or nursing mothers (Main et al., 2006, respectively; Swan et al., 2005). Major findings from the study by Swan et al. (2005) are discussed below in the subsections on developmental effects of DEHP. In the study by Main et al. (2006), the authors evaluated the association between phthalate levels in breast milk of nursing mothers and blood levels of reproductive hormones among 62 boys (29 from Denmark and 33 from Finland) with cryptorchidism, and 68 healthy boys without cryptorchidism (36 from Denmark and 32 from Finland) as controls. Aliquots of breast milk samples were collected and pooled by each nursing mother between one and three months after birth. Blood samples from all the boys were collected at 3 months of age. Concentrations of six monoesters of phthalates, including monomethyl phthalate (MMP), MEP, MBP, MBzP, MEHP, and mono-isononyl phthalate (MiNP) were measured in breast milk samples. Milk samples from Finland showed significantly higher values for MBP, MBzP, and MEHP than were present in samples from Denmark. The authors found no significant difference in any phthalate monoester concentration in the mothers' breast milk between children with or without cryptorchidism. However, significant negative effects on milk quality, whether measured directly or reflected in impaired development of young, is generally considered an adverse female reproductive effect, (e.g., U. S. Environmental Protection Agency Guidelines for Reproductive Toxicity Risk Assessment (U.S. EPA, 1996)). Thus, this study by Main et al. (2006) provided evidence on both the male and female reproductive effects of DEHP in humans.

Epidemiological Studies of Female Reproductive Endpoints

In addition to the study by Main et al. (2006) as discussed above, there are a few studies providing limited evidence on the female reproductive toxicity of DEHP in women.

Colon et al. (2000) found higher levels of phthalates, including DEHP and MEHP in 41 Puerto Rican girls who had premature breast development (thelarche patients). Similar findings have also been reported by Qiao et al. (2007) among 110 precocious Chinese girls in Shanghai.

Latini et al. (2003) investigated the possible association of concentrations of DEHP and MEHP, in the cord blood of 84 newborns to birth outcomes including weight, gestational age, and other endpoints. All 84 newborns were born at a general-practice hospital in Italy; there were 82 singleton births, one set of twins and 39 male and 45 female offspring. Eleven were preterm, and three of them had very low birth weight. The maternal age range was from 18 to 24 years. The authors found that DEHP or MEHP were present in 74 (88.1 percent) of the 84 examined cord serum samples at mean concentrations of 1.19±1.15 μg/ml and 0.52±0.61 μg/ml (mean ±standard deviation), respectively. MEHP-positive newborns (65 or 77.4 percent) had a significantly lower gestational age (38.16±2.34 weeks) compared with MEHP-negative infants (39.35±1.35 weeks, $p<0.05$). Logistic regression analysis also indicated a positive correlation between absence of MEHP in cord blood and gestational age at delivery (odds ratio = 1.50, 95 percent confidence interval = 1.013-2.21). No other statistically significant relationships were observed between

DEHP or MEHP concentrations and other birth outcomes (e.g., birth weight). The authors concluded that phthalate exposure is significantly associated with shortened pregnancy duration. Altered gestation length associated with maternal exposure to a chemical can be an indicator of female reproductive toxicity (U.S. EPA, 1996).

Two study reports by Reddy et al. (2006a; 2006b) also reported possible associations between exposure to DBP and female reproductive effects. In these two studies, Reddy et al. (2006a; 2006b) measured blood levels of polychlorinated biphenols (PCBs) and/or phthalate di-esters in female patients with endometriosis (cases) or without endometriosis (controls) from a south Indian region. It is unclear if there is an overlap between the subjects in two studies, which were reported separately. The authors of both studies found that blood levels of all the compounds measured in endometriosis patients, including four PCB congeners, DBP, BBP, DEHP, and dioctyl phthalate (DOP), were significantly higher than those in controls ($p<0.05$). The authors concluded that phthalates may have an etiological association with endometriosis in women. However, it has been shown that exposure to PCBs is associated with endometriosis in laboratory animals and in women (e.g., Arnold et al., 1996; Louis et al., 2005; Rier, 2002); high concentrations of DBP have also been found in women with endometriosis (Cobellis et al., 2003). Because the authors did not control for this confounding factor, the data from this study are not sufficient to determine if or how much DEHP per se contributes to development of endometriosis in women.

Epidemiological Studies of Developmental Endpoints

The study by Swan et al. is the only epidemiological study that provided relevant data on the developmental effects of DEHP in humans. In this study, Swan et al. (2005) analyzed levels of MEHP and eight other phthalate monoesters in urine samples from 85 pregnant women at a mean gestational time of 28.3 weeks. The authors also performed genital examinations and measured anogenital distance (AGD) among a total of 134 boys at 2-30 months of age. Data from boys whose mother's urine samples had been analyzed for phthalate levels were included in the statistical regression analysis. The authors found that increased levels of MEP, MBP, MBzP, and mono-isobutyl phthalate in prenatal urine samples in mothers was associated with decreased AGD in boys after birth. However, there was no apparent association between MEHP levels and the AGD.

Summary

The male reproductive toxicity of DEHP has been extensively studied in laboratory animals including rats, mice, hamsters, and ferrets. Depending on the dose, duration of exposure, and age of animals, DEHP causes reduced fertility, decreased weights of male reproductive organs, and histopathological changes in the testes of juvenile and adult rats. Although it has not been studied as extensively, female reproductive toxicity in laboratory animals has been reported. DEHP also has been found to cause developmental toxicity, including intrauterine death, developmental delay, and structural malformations and variations; neurological developmental effects have also been reported. Evidence of DEHP's reproductive toxicity in humans is less conclusive. Concordant effects of exposure to DEHP may be anticipated in marine organisms, particularly mammalian species.

Cancer

Introduction

In 2001, the International Agency for Research on Cancer (IARC) changed its classification of DEHP from Group 2B (sufficient evidence for carcinogenicity to laboratory animals) to Group C (not classifiable as to its carcinogenicity to humans). This change was based on agreement that the mechanism of DEHP carcinogenesis in laboratory animals involves peroxisome proliferation and that this mechanism is not likely to occur when humans are exposed to DEHP. Several letters and articles expressing disagreement with the evaluation of IARC have been published (Brody et al., 2003; Huff, 2003; Melnick, 2001).

Laboratory Rodent Studies

Chronic exposure of mice or rats to DEHP produces carcinomas of the liver and testicular tumors (Fay et al., 1999). DEHP also is a promoter of liver carcinomas when given to rats following exposure to the tumor initiator diethylnitrosamine (Sano et al., 1999).

Studies published since 2000 are consistent with earlier studies demonstrating that DEHP is carcinogenic to rodents. Male Sprague-Dawley rats treated with 300 mg DEHP per kg body weight per day for until death (up to 159 weeks) developed tumors of the liver and testes (Toyosawa et al., 2001). DEHP exposure produced liver tumors in transgenic mice carrying the human protooncogene c-Ha-ras (Toyosawa et al., 2001) in a 26 week carcinogenicity test.

Effects on Cultured Mammalian Cells

DEHP caused an increase in cell proliferation and a decrease in nuclear fragmentation, a sign of apoptosis, in cultured mouse Sertoli cells (Kang et al., 2002). DEHP transformed mouse embryo fibroblast T1 cells carrying bovine papillomavirus DNA (Kowalski et al., 2000).

Summary

There is sufficient evidence to conclude DEHP causes cancer in rats. The evidence suggest it is caused by the formation of peroxisomes in the rat liver from exposure to DEHP. Since humans are unlikely to form peroxisomes from DEHP exposure, it is unlikely humans will get cancer from DEHP, although there is still debate on this point.

Obesity

Stemp-Morlock (2007) observed that the obesity rate has greatly increased over the past 20 years. An estimated one-third of U.S. adults are overweight and more than one-third of U.S. children are overweight or at risk for being overweight. There is a strong association between obesity and a number of health issues such as diabetes, coronary heart disease, hypertension, and gall bladder disease. Traditionally, obesity has been viewed as a result of reduced physical activities and increased caloric intake. Data from recent studies, however, suggest that exposure to chemicals that perturb the critical pathways in adipogenesis, lipid metabolism or energy balance could also initiate or exacerbate obesity. Phthalates were suggested as chemicals that might possess obesogenic properties (Grun and Blumberg, 2007).

OEHHA's literature search did not turn up specific information on DEHP with regard to obesity. However, MEHP was shown to activate peroxisome proliferators-activated receptor-γ (PPAR-γ) in adipocyte differentiation of 3T3-L1 preadipocytes (Feige et al., 2007). The role of PPAR-γ in lipogenesis and adipose differentiation is well established (Kersten, 2002). Thus, DEHP via

metabolic activation could cause obesity. A low dose of DEHP (7.5 mg /kg) on alternate days for 14 days caused a decrease in serum insulin and cortisol and an increase in serum glucose (Gayathri et al., 2004). Incubation of Chang liver cells with 200 or 400 micromoles DEHP per liter for 24 hours resulted in a decrease in insulin receptor activity and a decrease in glucose oxidation (Rengarajan et al., 2007).

Other circumstantial evidence also suggests that additional research should be performed to further characterize the obesogenic properties of DEHP. DEHP is also known to be anti-androgenic. Androgen deprivation in prostate cancer therapy resulted in a higher incidence of hyperlipidemia, hyperglycemia, and metabolic syndrome (Braga-Basaria et al., 2006; Sharifi et al., 2005). Stahlhut et al. (2007) observed an association between urinary phthalate metabolites to increased waist circumference and insulin resistance. This led them to suggest an obesogenic outcome via the anti-androgenic pathway.

Thyroid

Introduction

A limited number of animal studies indicate that exposure to DEHP may be associated with altered thyroid function. Thyroid hormones such as thyroxine (T4) and triiodothyronine (T3) play a key role in many physiological events and are important for normal brain development. Alterations in thyroid hormone levels and thyroid function may lead to adverse clinical conditions. Environmental contaminants such as phthalates may bind to thyroid hormone receptors and alter its signaling (Zoeller, 2005).

Laboratory Rodent Studies

Thyroid histopathology

DEHP-induced thyroid hyperactivity has been shown in some animal studies. Rats were given DEHP in the diet over periods from 3 days to 9 months. DEHP induced hyperactivity and changes in the colloid in thyroid. Rats treated with DEHP showed ultrastructural changes in the thyroid, such as increase in the number and size of lysosomes, enlarged Golgi apparatus, dilation of the rough endoplasmic reticulum, and damaged mitochondria (Hinton et al., 1986; Price et al., 1988b). In a separate study, male rats were given DEHP in diet for 14 days. The thyroid of rats receiving diet containing DEHP showed signs for hyperactivity, as indicated by the decrease in follicular size and increase in the proportion of follicular cells with a columnar appearance (Howarth et al., 2001). The subchronic oral toxicity of DEHP was also studied. DEHP was given to rats in the diet for 13 weeks. DEHP again altered thyroid histopathology. Histological changes in the thyroid include reduced follicle size and decreased colloid density (Poon et al., 1997).

Thyroid function

DEHP was given to rats on alternate days for 14 days. Changes in the levels of T3, T4 and thyroid stimulating hormone (TSH) in the serum were studied. Increases in serum T3 and T4 were seen in rats exposed to DEHP, while there was no change in TSH. Thus, the results indicated that low levels of DEHP exposure in rats may alter thyroid function (Gayathri et al., 2004). Iodide uptake is also important for the normal function of thyroid. Effects of DEHP on iodide uptake were studied *in vitro* in the rat thyroid cell line FRTL-5. The results showed that DEHP significantly enhanced iodide uptake at the concentrations that do not cause toxicity to the

cells. The key molecule in iodide transportation is the sodium/iodide symporter (NIS). Additional experiments demonstrated that the increase in iodide uptake is due to NIS (Wenzel et al., 2005). A separate study in a rat thyroid cell line PC C13 cells showed increased transcriptional activity of NIS induced by DEHP. This led the authors to suggest that the increase in iodide uptake in thyroid is a consequence of NIS transcriptional activation by DEHP (Breous et al., 2005).

Human Studies

Human studies investigating the association between DEHP exposure and thyroid function are limited. A decrease in serum levels of thyroid hormones such as T4 and T3 were observed in human blood stored in DEHP plasticized blood bags (Gayathri et al., 2004). Meeker et al. (2007) studied the potential association between DEHP exposure and serum levels of free T4, total T3, and TSH in adult men. Samples of urine and blood were collected from 408 men. Meeker et al. measured urinary concentrations of MEHP along with serum levels of T4, T3, and TSH. An inverse association between MEHP urinary concentrations and free T4 and T3 serum levels were observed. The results thus indicated that urinary MEHP concentrations may be associated with altered T4 and/or T3 levels in adult men. The authors indicated in the paper that the associations between MEHP and thyroid hormones observed in this human study are at certain levels consistent with animal studies, and the urinary MEHP concentrations are comparable with those found in the general human population in the United States (Meeker et al., 2007).

Summary

Thyroid as one of the largest endocrine glands in the body plays an important role in maintaining the homeostasis and normal function of many organs and systems. Only a limited number of studies are available to demonstrate the potential effects of DEHP on thyroid, but they do show effects on the morphology and function of the gland. Considering the importance of the thyroid in regulating the normal physiology of multiple organs and systems, the evidence should not be ignored and further studies are necessary.

Immune System

Introduction

The prevalence of allergic diseases has been increasing dramatically for the past several decades. The exposure to environmental contaminants may contribute to the increased sensitivity of the human immune system, leading to elevated allergic responses. Alteration of immune function induced by environmental contaminants may result in hypersensitivity, autoimmune diseases, and changes in the ability to resist infectious diseases. Despite the fact that the number of people who had been diagnosed with autoimmune or allergic diseases is on rise, the etiology still remains unknown for most of these diseases. Both inherent and environmental factors may be involved in the development of the disease. Foreign antigens may act as an immune modulator to initiate autoimmune responses and cause allergy-related diseases such as asthma. There are concerns that DEHP may compromise the immune system; however, limited studies are available to elucidate the potential mechanism of the immunological effects of DEHP.

Laboratory Rodent Studies

The association between DEHP and immunological disorders was also studied in laboratory animals. Mice were injected intraperitoneally with DEHP three times at 10-day intervals. High levels of serum anti-DNA antibodies were developed in mice injected with DEHP in a dose-dependent manner. This led the authors to suggest that DEHP might be involved in the induction of autoimmune response (Lim and Ghosh, 2005). Takano et al. (2006) studies the effects of DEHP exposure on the allergic reactions in mice to a dust mite allergen. Seven-week-old mice were exposed to mite allergen and also received DEHP in olive oil by intraperitoneal injection. DEHP enhances atopic dermatitis-like skin lesions induced by mite allergen. These changes were consistent with the effects of DEHP on the protein expression of chemokines macrophage inflammatory protein-1α (MIP-1α) and eotaxin (Takano et al., 2006). Male mice fed DEHP in the diet had lower levels of lactoferrin, a protein with antimicrobial activity, in liver tissue than did control mice (Hasmall et al., 2002). DEHP was given to 4-week-old mice in drinking water for 2 weeks. DEHP has been shown to enhance the proliferative responses of thymus cells as indicated by increased responses to mitogens concanavalin A (Con A) and Staphylococcus enterotoxin B (SEB) (Yamashita et al., 2003). The thymus and spleen are important organs for the normal function of immune system and they are also highly sensitive to xenobiotics. In a separate study, atrophy of thymus and spleen was seen in mice exposed to DEHP. Yang et al. (2000) treated adult male mice (8 weeks old) with DEHP in diet for 10 days. Decreased thymus and spleen weight, decreased DNA content in thymus and spleen were observed in DEHP-treated mice (Yang et al., 2000). The effects of DEHP on immune system were also studied in rats. Young male albino rats were given DEHP by intraperitoneal injection for up to 90 days (long-term exposure), or for 5 days (short-term exposure). In the long-term exposure groups, depletion of cortical cells and hyperplasia of medulla in the thymus, cellular depletion of peripheral follicles of white pulp in the spleen, and reduction in the number of follicles in cortical region of lymph nodes were observed in DEHP-treated rats. In the short duration exposure groups, DEHP caused dose-dependent reduction in the weight of lymphoid organs and their cellular population, the spleen cell response to B-lymphocyte mitogen lipopolysaccharide is also reduced in DEHP-treated rats, which led the authors to suggest that DEHP effect on the B-lymphocyte population is how it modulates the immune system (Dogra et al., 1985). These animal studies indicate that DEHP may have immunomodulating effects *in vivo*.

Inhalation of MEHP for 60 minutes by BALB/c mice resulted in an increase in the number of pulmonary macrophages. The NOEL for this effect was estimated to be 1.7 mg per cubic meter (Larsen et al., 2004). Inhalation exposure of BALB/c mice to a mixture of ovalbumin and DEHP did not result in an increase in IgE (immunoglobulin E) production above the amount produced in response to ovalbumin alone. However, DEHP exposure did increase the number of macrophages in bronchi (Larsen et al., 2004).

In vitro Studies

The effects of DEHP on immune responses were also studied *in vitro* DEHP and MEHP produced a significant increase in hydrogen peroxide production in isolated rat hepatocytes (Kambia et al., 2004). MEHP reduced the production of inflammatory cytokines IL6 and IL8 in A549 cells, a human epithelial cell line (Jepsen et al., 2004). DEHP has been shown to trigger allergic responses by enhancement of cytokine interleukin-4 (IL-4) production in CD4+ T cells in a concentration-dependent manner (Lee et al., 2004). In a separate study, blood leukocytes from healthy volunteers were studied *in vitro* for the effects of DEHP and its metabolites MEHP,

2-ethylhexanol (2-EH), and phthalic acid (PA) on immune functions. The suppressed oxidative respiratory metabolism and decreased superoxide (O_2^-) generation was observed in leukocytes that were incubated with DEHP or its metabolites. The impairment of leukocyte oxidative metabolism may imply the potential inhibition of leukocyte function by DEHP and its metabolites (Fischer et al., 1998). The effects of DEHP on murine thymus and spleen cells were also studied *in vitro*. DEHP has been shown to induce proliferative responses and enhance cytokine (a group of inflammatory cell proteins) production in murine thymus and spleen cells (Yamashita et al., 2002) (Yamashita et al., 2003). The findings of these studies raise concerns regarding the possibility of DEHP to alter immune system and its potential role in immunological disorders.

Human Studies

The potential link between DEHP and allergic diseases has been studied in humans. The use of PVC plastics and other plasticizer-containing surface materials in the home was associated with the development of bronchial obstruction in young children in Norway (Jaakkola et al., 1999). The presence of plastic materials was associated with increased risks of asthma and pneumonia and higher incidence of lower respiratory tract symptoms such as wheezing, cough, and phlegm in children studied in Finland (Jaakkola et al., 2000). Another study from Finland also linked plastic materials with the risk of asthma (Kavlock et al., 2006). The concentration of DEHP in indoor dust was associated with asthma in children in Sweden (Bornehag et al., 2005). The concentration of DEHP in indoor dust was also shown to be associated with allergic symptoms such as wheezing in preschool children from children's homes in Bulgaria (Kolarik et al., 2008).

Summary

Limited studies in humans and laboratory animals raised concerns regarding the potential link between DEHP and immunological disorders. Despite the limitation of available studies, attention should be made to the potential effects of DEHP on immune system and its involvement in immunological disorders.

Nervous System

Introduction

The central nervous system and brain function has been demonstrated to be a target of many environmental contaminants. Rodent animals as a model system have been heavily used for the research on the nervous system. The following paragraphs will discuss the effects of DEHP on the central nervous system based on currently available studies.

Laboratory Rodent Studies

The effects of DEHP on the central nervous system and brain function have been studied in laboratory animals. Rodent studies showed behavioral changes caused by DEHP exposure during development. For example, Tanaka (2002) studied neurobehavioral effects of DEHP in male and female mice offspring following *in utero* and lactational DEHP exposures. DEHP was given in the diet. The dose level used in the study did not produce significant adverse effects in reproductive and neurobehavioral parameters examined. However, surface righting, an indicator of the development of coordinated movement, was significantly depressed in both male and female offspring during postnatal development (Tanaka, 2002). DEHP has also been shown to cause motor hyperactivities in rats. Masuo et al. (2004a; 2004b) exposed 5-day-old rats with

DEHP in olive oil by intracisternal injection. The brain cell development such as differentiation and synaptogenesis are still incomplete in rats at 5 days of age, which makes them more vulnerable to environmental pollutants such as endocrine disrupters. The neonatal exposure is thus useful in understanding the neurodevelopmental effects of DEHP. The results showed that DEHP caused a significant increase in spontaneous motor activities at 4-5 weeks of age, a juvenile stage. It is worth mentioning that the motor behavior was studied during a 24-hour period under a 12-hour light (day) and 12-hour dark (night) condition. DEHP caused hyperactivity in both the dark and light phases. This prompted the authors to suggest that endocrine disrupters such as DEHP may cause deficits in sleep. These studies indicated that DEHP can induce motor hyperactivity and other behavioral changes in laboratory animals. Motor hyperactivity is associated with developmental disorders in humans, such as autism and attention-deficit hyperactivity disorder (ADHD). To study the mechanisms underlying motor hyperactivity, Masuo et al. also conducted gene-expression profiling using a cDNA (complimentary DNA) membrane array in DEHP-treated rats. In order to understand the long-lasting effects of neonatal exposure, the gene expression was evaluated at 8 weeks of age, long after the cessation of DEHP exposure. The results demonstrated alteration of expression of genes in the midbrain and striatum, which include those related to neuropeptides, growth factors, and the transmission of important neurotransmitters such as dopamine, γ-aminobutyric acid (GABA), and glutamate. These results suggest that further studies are necessary in order to elucidate the mechanisms of the neurological effects of DEHP (Masuo et al., 2004a; 2004b).

G protein-coupled receptors

DEHP has been shown to alter gene expression of G-protein-coupled receptors in rat brain. Ishido et al. (2005) conducted a study in rats. Single intracisternal administration of DEHP into male Wistar rats at 5 days of age induced significant hyperactivity at 4-5 weeks of age. DNA macroarray analyses were conducted in the midbrain at 8 weeks of age. DEHP changed the levels of gene expression of G protein-coupled receptors that were important for the transmission of dopamine and other neurotransmitters. For example, the gene expression of dopamine receptor D_{1A} was decreased by DEHP. The expression of galanin receptor 2 was also reduced by DEHP. Dopamine and its transporters might be associated with the pathogenesis of ADHD. Galanin, as a neuromodulator, plays an important role in cognition, learning and memory. Galanin can also regulate midbrain dopamine activity (Ishido et al., 2005).

The membrane Na^+-K^+ ATPase

The membrane Na^+-K^+ adenosine triphosphatase (ATPase) of the brain plays an important role in brain development and function, a decrease in this enzyme activity has been associated with a number of neurodegenerative and psychiatric diseases. Dhanya et al. (2004; 2003) studied the effects of DEHP on the activity of membrane Na^+-K^+ ATPase in brain. DEHP was given to rats by intraperitoneal injection, the histopathology of the brain and the activity of membrane Na^+-K^+ ATPase in brain were studied. Histopathology examination showed neuronal degeneration in rats exposed to DEHP. Significant inhibition of membrane Na^+-K^+ ATPase was also observed in the brain. Additional experiments demonstrated that the inhibition of membrane Na^+-K^+ ATPase is a direct effect of DEHP or its metabolites (Dhanya et al., 2004; Dhanya et al., 2003). The authors indicated in the paper that Na^+-K^+ ATPase is an important enzyme for cation transportation and inhibition of this enzyme may lead to an increase in intracellular calcium, and thus cause disruption of ion homeostasis. The increased intracellular calcium may elicit apoptosis and cell death, while apoptosis may be involved in neuronal degeneration. A study on

cultured cortical neurons showed that the inhibition of Na^+-K^+ ATPase resulted in neuronal cell death. Electron microscopy confirmed the existence of apoptosis and necrosis in individual cells. The effects were considered to be mediated by the disruption of ion homeostasis such as the accumulation of intracellular calcium (Xiao et al., 2002).

Intracellular calcium

Intracellular calcium has been shown to play an important role in the neurotransmitter release, modulation of excitability and gene expression of the nerve cells (Berridge, 1998). The alteration in intracellular calcium level may, in part, be involved in the pathogenesis of a number of neurological disorders. Studies have been conducted with a focus on the effects of DEHP on intracellular calcium. DEHP has been shown to increase the intracellular calcium levels in mammalian neurosecretory terminals and in cultured pheochromocytoma cells, a neuronotypic cell line. Alterations in intracellular calcium in the nanomolar concentration range within minutes of exposure led the authors to suggest the potential adverse effects in situations where prolonged exposure occurs (Tully et al., 2000).

Protein kinase C (PKC)

Protein kinase C (PKC) is a family of enzymes that is considered to play a key role in signal transduction in response to hormones or neurotransmitters. *In vitro* study showed that DEHP inhibits the activity of PKC from rat brain in a concentration-dependent manner. The effect of DEHP is noncompetitive and the mechanism of the inhibition may involve the interference with the interaction between calcium ion and the regulatory domain of PKC (Shukla et al., 1989).

Brain aromatase enzyme

It has been suggested that DEHP may interfere with estrogen metabolism through inhibition of aromatase enzyme activity. Brain aromatase enzyme controls the conversion of testosterone to estradiol and plays an important role in the sexual differentiation of neural structures, neuroendocrine functions, and sexual behaviors (Lephart, 1996). Andrade et al. (2006) studied the effects of DEHP on brain aromatase activity of male and female rat offspring following *in utero* and lactational DEHP exposures. Aromatase activity was examined in hypothalamic/preoptic area (HPOA) from pups on two different developmental stages: perinatal stage (postnatal day 1) and weanling stage (postnatal day 22). The results demonstrated that DEHP alters brain aromatase activity in both male and female offspring following *in utero* and lactational exposures, though males and females respond differently to DEHP (Andrade et al., 2006a). Further study indicated that DEHP may exert its effects on aromatase at the transcription level. A study showed that the decrease in aromatase mRNA (messenger RNA) and protein can be induced by DEHP metabolite MEHP in cultured rat granulosa cells (Lovekamp and Davis, 2001). Considering the importance of brain aromatase in the development and function of the central nervous system, more attention should be given to the alteration of aromatase induced by DEHP and its potential effect on neural development and behavior.

Brain peroxisomes

Peroxisomes are cytoplasmic organelles that participate in the metabolism of fatty acids. In mammals, over half of the peroxisomal enzymes are related to lipid metabolism. Peroxisomes contain oxidative enzymes such as catalase, D-amino acid oxidase, etc. It may also play a role in protecting cells from oxygen toxicity and peroxidation. The effects of DEHP on brain

peroxisomes were also studied. Female rats exposed to DEHP during lactation showed DEHP-induced modifications of the peroxisomal enzymatic pattern in brain of both dams and offsprings. Biochemical analysis showed alterations in the activities of catalase, D-amino acid oxidase, palmitoyl-CoA oxidase. Electron microscopy showed high catalase-like immunoreactivity in the cytosol of neurons (Cimini et al., 1994). In a separate study, Peroxisomes were identified in the neurons of the cerebral cortex of lactating pups at 12 days of age from DEHP fed mothers. Electron microscope study indicated that the diameters of peroxisomes in the neurons and the number of neurons that contain peroxisomes are both increased in brain of the DEHP-treated group. DEHP may therefore elicit peroxisomal proliferation in developing neurons (Dabholkar, 1988).

Summary

Alterations in brain function and behavioral changes have been observed in rodent studies. DEHP has also been shown to alter gene and protein expression in the brain. The results thus indicated that DEHP might have potential effects on neural development and behavior. However, further studies are necessary in order to elucidate the mechanisms of the neurological effects of DEHP.

Liver and Kidney Effects

Administration of 10,000 mg per kg body weight by gavage to male Wistar rats for four weeks resulted in an increase in the relative weight of the liver, kidney, brain and adrenal glands compared with the relative weights of these organs in controls not given DEHP (Dalgaard et al., 2000). Male and female Fischer rats given 12,500 ppm DEHP in the diet for 104 weeks had increased weights of the liver and kidneys, increased concentrations of urea and albumin in blood but decreased concentrations of erythrocytes and hemoglobin compared to control rats (David et al., 2000b). Male Fischer 344 rats fed a diet containing DEHP for 360 days had decreased concentrations of ceruloplasm in blood and had increased concentrations of copper deposited in liver tissue (Eagon et al., 1999). Examination of livers of prepubertal rabbits given an intravenous infusion of lipid emulsion for one week revealed peroxisome proliferation when the infusion line was PVC that contained DEHP. Peroxisome proliferation was not seen when a polyethylene infusion line that did not contain DEHP was used (Loff et al., 2007). No histological effects and no effects on organ weights were seen in the liver, kidneys or testes of adult male cynomolgus monkeys given 500 mg per kg DEHP for 14 days (Pugh et al., 2000).

Conclusions

Findings

This toxicological profile on DEHP describes its effects on freshwater and marine life, humans, and laboratory animals.

- Arthropods appear to be the most sensitive, with several reports of effects in the low or fractional µg/L range. Some fish, especially Medaka are also sensitive, with effects reported in the low µg/L range. Mollusks appear to be less sensitive with effects reported in the tens to hundreds of µg/L range. The relationship of the toxic concentrations to the solubility limit is unclear, since published solubility limits for DEHP vary widely. These disparate and conflicting findings indicate the need for further work to clarify the risk of

adverse effects on invertebrates at realistic environmental concentrations. More work should focus on free-living organisms in marine and estuarine environments.

- The male reproductive toxicity of DEHP has been extensively studied in laboratory animals including rats, mice, hamsters, and ferrets. DEHP causes reduced fertility, decreased weights of male reproductive organs, and histopathological changes in the testes of juvenile and adult rats. Female reproductive toxicity in laboratory animals has been reported. DEHP also has been found to cause developmental toxicity. Evidence of DEHP's reproductive toxicity in humans is less conclusive
- There is sufficient evidence to conclude DEHP causes cancer in rats and mice, but evidence suggest that the same mechanism of action does not occur in humans. However, there is still debate on whether the mechanism of action is the only important one..
- Only a limited number of studies are available to demonstrate the potential effects of DEHP on thyroid, but they do show effects on the morphology and function of the gland. Considering the importance of the thyroid in regulating the normal physiology of multiple organs and systems, the evidence should not be ignored and further studies are necessary.
- Limited studies in humans and laboratory animals raised concerns regarding the potential link between DEHP and immunological disorders.
- Alterations in brain function and behavioral changes have been observed in rodent studies. The findings indicated that DEHP might have potential effects on neural development and behavior. Further studies are necessary in order to elucidate the mechanisms of the neurological effects of DEHP.

Data Gaps

- There are very few studies on DEHP toxicity to marine and freshwater organisms and even fewer outside the laboratory.
- Only two studies were found that showed toxicity in marine organisms at or below the level of DEHP's water solubility.
- There is little information on freshwater and marine DEHP concentrations in the water column and sediment.
- The current studies in laboratory animals on DEHP's adverse effects other than reproduction and development raise concern but are very limited.
- DEHP is only one of many phthalates used in large quantities in plastics and more information is needed about the others.

Recommendations

- Further study needs to be done on the potential toxicity of DEHP and other common phthalates on aquatic organisms. These need to investigate if low concentrations cause toxicity over subchronic and chronic timeframes.

- Studies on environmental concentrations of DEHP and other phthlates in the water column and sediment need to be conducted.
- Because of DEHP and other phthalates widespread use, further work needs to be done on the adverse effects to the edorine, immune and nervous systems.

References

Ablake M., Itoh M., Terayama H., Hayashi S., Shoji S., Naito M., Takahashi K., Suna S. and Jitsunari F. (2004) Di-(2-ethylhexyl) phthalate induces severe aspermatogenesis in mice, however, subsequent antioxidant vitamins supplementation accelerates regeneration of the seminiferous epithelium. *Int J Androl* **27**, 274-81.

Akingbemi B. T., Ge R., Klinefelter G. R., Zirkin B. R. and Hardy M. P. (2004) Phthalate-induced Leydig cell hyperplasia is associated with multiple endocrine disturbances. *Proc Natl Acad Sci U S A* **101**, 775-80.

Akingbemi B. T., Youker R. T., Sottas C. M., Ge R., Katz E., Klinefelter G. R., Zirkin B. R. and Hardy M. P. (2001) Modulation of rat Leydig cell steroidogenic function by di(2-ethylhexyl)phthalate. *Biol Reprod* **65**, 1252-9.

Andrade A. J., Grande S. W., Talsness C. E., Gericke C., Grote K., Golombiewski A., Sterner-Kock A. and Chahoud I. (2006a) A dose response study following in utero and lactational exposure to di-(2-ethylhexyl) phthalate (DEHP): reproductive effects on adult male offspring rats. *Toxicology* **228**, 85-97.

Andrade A. J., Grande S. W., Talsness C. E., Grote K. and Chahoud I. (2006b) A dose-response study following in utero and lactational exposure to di-(2-ethylhexyl)-phthalate (DEHP): Non-monotonic dose-response and low dose effects on rat brain aromatase activity. *Toxicology* **227**, 185-92.

Arcadi F. A., Costa C., Imperatore C., Marchese A., Rapisarda A., Salemi M., Trimarchi G. R. and Costa G. (1998) Oral toxicity of bis(2-ethylhexyl) phthalate during pregnancy and suckling in the Long-Evans rat. *Food Chem Toxicol* **36**, 963-70.

Arnold D. L., Nera E. A., Stapley R., Tolnai G., Claman P., Hayward S., Tryphonas H. and Bryce F. (1996) Prevalence of endometriosis in rhesus (Macaca mulatta) monkeys ingesting PCB (Aroclor 1254): review and evaluation. *Fundam Appl Toxicol* **31**, 42-55.

ATSDR. (2002) Agency for Toxic Substances and Disease Registry: Toxicological profile for Di(2-ethylhexyl)phthalate (DEHP). U.S. Department of Health and Human Services, Public Health Services, Alanta, GA.

Banerjee S., Thuillier R., Culty M., Papadopoulos V., Brown T. R. and Banerjee P. P. (2002) In utero exposure to di(2-ethylhexyl) phthalate alters growth, tissue organization, and the expression of androgen receptor protein of rat prostate. *Biol Reprod* **66(Suppl 1)**, 200.

Barron M. G., Schultz I. R. and Hayton W. L. (1989) Presystemic branchial metabolism limits di-2-ethylhexyl phthalate accumulation in fish. *Toxicol Appl Pharmacol* **98**, 49-57.

Berridge M. J. (1998) Neuronal calcium signaling. *Neuron* **21**, 13-26.

Blount B. C., Silva M. J., Caudill S. P., Needham L. L., Pirkle J. L., Sampson E. J., Lucier G. W., Jackson R. J. and Brock J. W. (2000) Levels of seven urinary phthalate metabolites in a human reference population. *Environ Health Perspect* **108**, 979-82.

Boekelheide K. (2004) Cracking the nut. *Toxicol Sci* **81**, 1-2.

Borch J., Ladefoged O., Hass U. and Vinggaard A. M. (2004) Steroidogenesis in fetal male rats is reduced by DEHP and DINP, but endocrine effects of DEHP are not modulated by DEHA in fetal, prepubertal and adult male rats. *Reprod Toxicol* **18**, 53-61.

Bornehag C. G., Sundell J., Weschler C. J. and Sigsgaard T. (2005) Correspondence: potential selection biases. *Environ Health Perspect* **113**, a152-3.

Braga-Basaria M., Dobs A. S., Muller D. C., Carducci M. A., John M., Egan J. and Basaria S. (2006) Metabolic syndrome in men with prostate cancer undergoing long-term androgen-deprivation therapy. *J Clin Oncol* **24**, 3979-83.

Breous E., Wenzel A. and Loos U. (2005) The promoter of the human sodium/iodide symporter responds to certain phthalate plasticisers. *Mol Cell Endocrinol* **244**, 75-8.

Brody C., DiGangi J., Easthope T., Rossi M. and Schettler T. (2003) IARC downgrading of DEHP. *Int J Occup Environ Health* **9**, 399-400.

Brown D. and Thompson R. E. (1982a) Phthalates and the Aquatic Environment: Part I. The effect of di-2-ethylhexyl phthalate and di-isodecyl phthalate on the reproduction of Daphnia magna and observations on their bioconcentrations. *Chemosphere* **11**, 417-426.

Brown D. and Thompson R. S. (1982b) Phthalates and the Aquatic Environment: Part II. The bioconcentration and depuration of di-2-ethylhexyl phthalate (DEHP) and di-isodecyl phthalate (DIDP) in mussels (Mytilus edulis). *Chemosphere* **11**, 427-435.

Cammack J. N., White R. D., Gordon D., Gass J., Hecker L., Conine D., Bruen U. S., Friedman M., Echols C., Yeh T. Y. and Wilson D. M. (2003) Evaluation of reproductive development following intravenous and oral exposure to DEHP in male neonatal rats. *Int J Toxicol* **22**, 159-74.

Chikae M., Hatano Y., Ikeda R., Morita Y., Hasan H. and E. T. (2004a) Effects of bis(2-ethylhexyl) phthalate and benzo[a]pyrene on the embryos of Japanese medaka (Oryzias latipes). *Environmental Toxicology and Pharmacology* **16**, 141-145.

Chikae M., Ikeda R., Hatano Y., Hasan Q., Morita Y. and Tamiya E. (2004b) Effects of bis(2-ethylhexyl) phthalate, γ-hexachlorocyclohexane, and 17β-estradiol on the fry stage of medaka (Oryzias latipes). Environmental Toxicology and Pharmacology. *Environmental Toxicology and Pharmacology* **18**, 9-12.

Cho F., Yabe M. and Honjo S. (1975) [The weight of the reproductive organs, hypophysis and thyroid of male cynomolgus monkeys. (Macaca fascicularis) (author's transl)]. *Jikken Dobutsu* **24**, 173-5.

Cimini A. M., Sulli A., Stefanini S., Serafini B., Moreno S., Rossi L., Giorgi M. and Ceru M. P. (1994) Effects of Di-(2-ethylhexyl)phthalate on peroxisomes of liver, kidney and brain of lactating rats and their pups. *Cell Mol Biol (Noisy-le-grand)* **40**, 1063-76.

Cobellis L., Latini G., De Felice C., Razzi S., Paris I., Ruggieri F., Mazzeo P. and Petraglia F. (2003) High plasma concentrations of di-(2-ethylhexyl)-phthalate in women with endometriosis. *Hum Reprod* **18**, 1512-5.

Colborn T. (1994) The wildlife/human connection: modernizing risk decisions. *Environ Health Perspect* **102 Suppl 12**, 55-9.

Colon I., Caro D., Bourdony C. J. and Rosario O. (2000) Identification of phthalate esters in the serum of young Puerto Rican girls with premature breast development. *Environ Health Perspect* **108**, 895-900.

Dabholkar A. S. (1988) Peroxisomes in the rat brain and the effects of di-(2-ethylhexyl) phthalate during postnatal development. An electron-microscopic study. *Acta Anat (Basel)* **131**, 218-21.

Dalgaard M., Ostergaard G., Lam H. R., Hansen E. V. and Ladefoged O. (2000) Toxicity study of di(2-ethylhexyl)phthalate (DEHP) in combination with acetone in rats. *Pharmacol Toxicol* **86**, 92-100.

David R. M. (2000) Exposure to phthalate esters. *Environ Health Perspect* **108**, A440.

David R. M., Moore M. R., Finney D. C. and Guest D. (2000a) Chronic toxicity of di(2-ethylhexyl)phthalate in mice. *Toxicol Sci* **58**, 377-85.
David R. M., Moore M. R., Finney D. C. and Guest D. (2000b) Chronic toxicity of di(2-ethylhexyl)phthalate in rats. *Toxicol Sci* **55**, 433-43.
Davis B. J., Maronpot R. R. and Heindel J. J. (1994a) Di-(2-ethylhexyl) phthalate suppresses estradiol and ovulation in cycling rats. *Toxicol Appl Pharmacol* **128**, 216-23.
Davis B. J., Weaver R., Gaines L. J. and Heindel J. J. (1994b) Mono-(2-ethylhexyl) phthalate suppresses estradiol production independent of FSH-cAMP stimulation in rat granulosa cells. *Toxicol Appl Pharmacol* **128**, 224-8.
Dhanya C. R., Gayathri N. S., Mithra K., Nair K. V. and Kurup P. A. (2004) Vitamin E prevents deleterious effects of di (2-ethyl hexyl) phthalate, a plasticizer used in PVC blood storage bags. *Indian J Exp Biol* **42**, 871-5.
Dhanya C. R., Indu A. R., Deepadevi K. V. and Kurup P. A. (2003) Inhibition of membrane Na(+)-K+ Atpase of the brain, liver and RBC in rats administered di(2-ethyl hexyl) phthalate (DEHP) a plasticizer used in polyvinyl chloride (PVC) blood storage bags. *Indian J Exp Biol* **41**, 814-20.
Dogra R. K., Khanna S., Nagale S. L., Shukla L. J., Srivastava S. N., Bhatnagar M. C., Gupta P. K. and Shanker R. (1985) Effect of dioctyl phthalate on immune system of rat. *Indian J Exp Biol* **23**, 315-9.
Dostal L. A., Chapin R. E., Stefanski S. A., Harris M. W. and Schwetz B. A. (1988) Testicular toxicity and reduced Sertoli cell numbers in neonatal rats by di(2-ethylhexyl)phthalate and the recovery of fertility as adults. *Toxicol Appl Pharmacol* **95**, 104-21.
Duty S. M., Ackerman R. M., Calafat A. M. and Hauser R. (2005a) Personal care product use predicts urinary concentrations of some phthalate monoesters. *Environ Health Perspect* **113**, 1530-5.
Duty S. M., Calafat A. M., Silva M. J., Brock J. W., Ryan L., Chen Z., Overstreet J. and Hauser R. (2004) The relationship between environmental exposure to phthalates and computer-aided sperm analysis motion parameters. *J Androl* **25**, 293-302.
Duty S. M., Calafat A. M., Silva M. J., Ryan L. and Hauser R. (2005b) Phthalate exposure and reproductive hormones in adult men. *Hum Reprod* **20**, 604-610.
Duty S. M., Silva M. J., Barr D. B., Brock J. W., Ryan L., Chen Z., Herrick R. F., Christiani D. C. and Hauser R. (2003a) Phthalate exposure and human semen parameters. *Epidemiology* **14**, 269-77.
Duty S. M., Singh N. P., Silva M. J., Barr D. B., Brock J. W., Ryan L., Herrick R. F., Christiani D. C. and Hauser R. (2003b) The relationship between environmental exposures to phthalates and DNA damage in human sperm using the neutral comet assay. *Environ Health Perspect* **111**, 1164-9.
Eagon P. K., Teepe A. G., Elm M. S., Tadic S. D., Epley M. J., Beiler B. E., Shinozuka H. and Rao K. N. (1999) Hepatic hyperplasia and cancer in rats: alterations in copper metabolism. *Carcinogenesis* **20**, 1091-6.
Fay M., Donohue J. M. and De Rosa C. (1999) ATSDR evaluation of health effects of chemicals. VI. Di(2-ethylhexyl)phthalate. Agency for Toxic Substances and Disease Registry. *Toxicol Ind Health* **15**, 651-746.
Feige J. N., Gelman L., Rossi D., Zoete V., Metivier R., Tudor C., Anghel S. I., Grosdidier A., Lathion C., Engelborghs Y., Michielin O., Wahli W. and Desvergne B. (2007) The Endocrine Disruptor Monoethyl-hexyl-phthalate Is a Selective Peroxisome Proliferator-

activated Receptor {gamma} Modulator That Promotes Adipogenesis. *J Biol Chem* **282**, 19152-66.
Fischer F. P., Machleidt C., Rettenmeier A. W., Kuhlmann U. and Mettang T. (1998) Plasticizers and inhibition of leukocyte function in vitro. *Perit Dial Int* **18**, 620-5.
Flurer C. I. and Zucker H. (1987) Difference in serum ascorbate in two species of Callithricidae. *Int J Vitam Nutr Res* **57**, 297-8.
Flurer C. I. and Zucker H. (1989) Ascorbic acid in a New World monkey family: species difference and influence of stressors on ascorbic acid metabolism. *Z Ernahrungswiss* **28**, 49-55.
Forget-Leray J., Landriau I., Minier C. and Leboulenger F. (2005) Impact of endocrine toxicants on survival, development, and reproduction of the estuarine copepod Eurytemora affinis (Poppe). *Ecotoxicol Environ Saf* **60**, 288-94.
Fromme H., Kuchler T., Otto T., Pilz K., Muller J. and Wenzel A. (2002) Occurrence of phthalates and bisphenol A and F in the environment. *Water Res* **36**, 1429-38.
Gangolli S. D. (1982) Testicular effects of phthalate esters. *Environ Health Perspect* **45**, 77-84.
Gayathri N. S., Dhanya C. R., Indu A. R. and Kurup P. A. (2004) Changes in some hormones by low doses of di (2-ethyl hexyl) phthalate (DEHP), a commonly used plasticizer in PVC blood storage bags & medical tubing. *Indian J Med Res* **119**, 139-44.
Giam C. S., Atlas E., Chart H. S. and Neff G. S. (1980) Phthalate Ester Plasticisers, PCB, DDT Residues in the Gulf of Mexico Atmosphere. *Atmos. Environ.* **14**, 65-69.
Grande S. W., Andrade A. J., Talsness C. E., Grote K. and Chahoud I. (2006) A dose-response study following in utero and lactational exposure to di(2-ethylhexyl)phthalate: effects on female rat reproductive development. *Toxicol Sci* **91**, 247-54.
Grande S. W., Andrade A. J., Talsness C. E., Grote K., Golombiewski A., Sterner-Kock A. and Chahoud I. (2007) A dose-response study following in utero and lactational exposure to di-(2-ethylhexyl) phthalate (DEHP): reproductive effects on adult female offspring rats. *Toxicology* **229**, 114-22.
Gray L. E., Jr., Wolf C., Lambright C., Mann P., Price M., Cooper R. L. and Ostby J. (1999a) Administration of potentially antiandrogenic pesticides (procymidone, linuron, iprodione, chlozolinate, p,p'-DDE, and ketoconazole) and toxic substances (dibutyl- and diethylhexyl phthalate, PCB 169, and ethane dimethane sulphonate) during sexual differentiation produces diverse profiles of reproductive malformations in the male rat. *Toxicol Ind Health* **15**, 94-118.
Gray L. E. J., Ostby J., Furr J., Price M., Veeramachaneni D. N. and Parks L. (2000) Perinatal exposure to the phthalates DEHP, BBP, and DINP, but not DEP, DMP, or DOTP, alters sexual differentiation of the male rat. *Toxicol Sci* **58**, 350-65.
Gray L. E. J., Price M., Lambright C., Wolf C., Hotchkiss A., Parks L. and Ostby J. (1999b) Environmental antiandrogens: the malformation pattern varies with the mechanism of antiandrogenic action. *Biol Reprod ;60(Suppl 1):201*.
Gray T. J. and Butterworth K. R. (1980) Testicular atrophy produced by phthalate esters. *Arch Toxicol Suppl* **4**, 452-5.
Gray T. J., Rowland I. R., Foster P. M. and Gangolli S. D. (1982) Species differences in the testicular toxicity of phthalate esters. *Toxicol Lett* **11**, 141-7.
Gromoll J., Eiholzer U., Nieschlag E. and Simoni M. (2000) Male hypogonadism caused by homozygous deletion of exon 10 of the luteinizing hormone (LH) receptor: differential action of human chorionic gonadotropin and LH. *J Clin Endocrinol Metab* **85**, 2281-6.

Grun F. and Blumberg B. (2007) Perturbed nuclear receptor signaling by environmental obesogens as emerging factors in the obesity crisis. *Rev Endocr Metab Disord* **8**, 161-71.
Hampl J. S., Taylor C. A. and Johnston C. S. (2004) Vitamin C deficiency and depletion in the United States: the Third National Health and Nutrition Examination Survey, 1988 to 1994. *Am J Public Health* **94**, 870-5.
Hasmall S., Orphanides G., James N., Pennie W., Hedley K., Soames A., Kimber I. and Roberts R. (2002) Downregulation of lactoferrin by PPARalpha ligands: role in perturbation of hepatocyte proliferation and apoptosis. *Toxicol Sci* **68**, 304-13.
Hauser R., Meeker J. D., Duty S., Silva M. J. and Calafat A. M. (2006) Altered Semen Quality in Relation to Urinary Concentrations of Phthalate Monoester and Oxidative Metabolites. *Epidemiology* **17**, 682-91.
Hauser R., Meeker J. D., Singh N. P., Silva M. J., Ryan L., Duty S. and Calafat A. M. (2007) DNA damage in human sperm is related to urinary levels of phthalate monoester and oxidative metabolites. *Hum Reprod* **22**, 688-95.
Hinton R. H., Mitchell F. E., Mann A., Chescoe D., Price S. C., Nunn A., Grasso P. and Bridges J. W. (1986) Effects of phthalic acid esters on the liver and thyroid. *Environ Health Perspect* **70**, 195-210.
Hoppin J. A., Brock J. W., Davis B. J. and Baird D. D. (2002) Reproducibility of urinary phthalate metabolites in first morning urine samples. *Environ Health Perspect* **110**, 515-8.
Hotchkiss A. K., Rider C. V., Blystone C. R., Wilson V. S., Hartig P. C., Ankley G. T., Foster P. M., Gray C. L. and Gray L. E. (2008) Fifteen years after "Wingspread"--environmental endocrine disrupters and human and wildlife health: where we are today and where we need to go. *Toxicol Sci* **105**, 235-59.
Howard P. H., Banerjee S. and Robillard K. H. (1985) Measirement of Water Solubilities, Octanol/Water Coefficients and Vapor Pressures of Commercial Phthalate Esters. *Environ Tox. Chem.* **4**, 653-661.
Howarth J. A., Price S. C., Dobrota M., Kentish P. A. and Hinton R. H. (2001) Effects on male rats of di-(2-ethylhexyl) phthalate and di-n-hexylphthalate administered alone or in combination. *Toxicol Lett* **121**, 35-43.
Howdeshell K. L., Furr J., Lambright C. R., Rider C. V., Wilson V. S. and Gray L. E. J. (2007) Cumulative effects of dibutyl phthalate and diethylhexyl phthalate on male rat reproductive tract development: altered fetal steroid hormones and genes. *Toxicol Sci* **99**, 190-202.
Howdeshell K. L., Wilson V. S., Furr J., Lambright C. R., Rider C. V., Blystone C. R., Hotchkiss A. K. and Gray L. E., Jr. (2008) A mixture of five phthalate esters inhibits fetal testicular testosterone production in the Sprague Dawley rat in a cumulative, dose additive manner. *Toxicol Sci* **105**, 153-65.
Huang P. C., Tien C. J., Sun Y. M., Hsieh C. Y. and Lee C. C. (2008) Occurrence of phthalates in sediment and biota: relationship to aquatic factors and the biota-sediment accumulation factor. *Chemosphere* **73**, 539-44.
Huff J. (2003) IARC and the DEHP quagmire. *Int J Occup Environ Health* **9**, 402-4.
Ishido M., Morita M., Oka S. and Masuo Y. (2005) Alteration of gene expression of G protein-coupled receptors in endocrine disruptors-caused hyperactive rats. *Regul Pept* **126**, 145-53.

Ishihara M., Itoh M., Miyamoto K., Suna S., Takeuchi Y., Takenaka I. and Jitsunari F. (2000) Spermatogenic disturbance induced by di-(2-ethylhexyl) phthalate is significantly prevented by treatment with antioxidant vitamins in the rat. *Int J Androl* **23**, 85-94.

Ivell R. and Bathgate R. A. (2002) Reproductive biology of the relaxin-like factor (RLF/INSL3). *Biol Reprod* **67**, 699-705.

Jaakkola J. J., Oie L., Nafstad P., Botten G., Samuelsen S. O. and Magnus P. (1999) Interior surface materials in the home and the development of bronchial obstruction in young children in Oslo, Norway. *Am J Public Health* **89**, 188-92.

Jaakkola J. J., Verkasalo P. K. and Jaakkola N. (2000) Plastic wall materials in the home and respiratory health in young children. *Am J Public Health* **90**, 797-9.

Jackson J. and Sutton R. (2008) Sources of endocrine-disrupting chemicals in urban wastewater, Oakland, CA. *Sci Total Environ* **405**, 153-60.

Jaeger R. J. and Rubin R. J. (1970) Plasticizers from plastic devices extraction, metabolism, and accumulation by biological systems. *Science* **170**, 460-2.

Jepsen K. F., Abildtrup A. and Larsen S. T. (2004) Monophthalates promote IL-6 and IL-8 production in the human epithelial cell line A549. *Toxicol In Vitro* **18**, 265-9.

Jonsson B. A., Richthoff J., Rylander L., Giwercman A. and Hagmar L. (2005) Urinary phthalate metabolites and biomarkers of reproductive function in young men. *Epidemiology* **16**, 487-93.

Kambia K., Dine T., Gressier B., Dupin-Spriet T., Luyckx M. and Brunet C. (2004) Evaluation of the direct toxicity of trioctyltrimellitate (TOTM), di(2-ethylhexyl) phthalate (DEHP) and their hydrolysis products on isolated rat hepatocytes. *Int J Artif Organs* **27**, 971-8.

Kang K. S., Lee Y. S., Kim H. S. and Kim S. H. (2002) DI-(2-ethylhexyl) phthalate-induced cell proliferation is involved in the inhibition of gap junctional intercellular communication and blockage of apoptosis in mouse Sertoli cells. *J Toxicol Environ Health A* **65**, 447-59.

Kavlock R., Barr D., Boekelheide K., Breslin W., Breysse P., Chapin R., Gaido K., Hodgson E., Marcus M., Shea K. and Williams P. (2006) NTP-CERHR Expert Panel Update on the Reproductive and Developmental Toxicity of di(2-ethylhexyl) phthalate. *Reprod Toxicol* **22**, 291-399.

Kersten S. (2002) Peroxisome proliferator activated receptors and obesity. *Eur J Pharmacol* **440**, 223-34.

Kim E. J., Kim J. W. and Lee S. K. (2002) Inhibition of oocyte development in Japanese medaka (Oryzias latipes) exposed to di-2-ethylhexyl phthalate. *Environ Int* **28**, 359-65.

Kluin P. M., Kramer M. F. and de Rooij D. G. (1983) Testicular development in Macaca irus after birth. *Int J Androl* **6**, 25-43.

Koch H. M., Rossbach B., Drexler H. and Angerer J. (2003) Internal exposure of the general population to DEHP and other phthalates--determination of secondary and primary phthalate monoester metabolites in urine. *Environ Res* **93**, 177-85.

Kohn M. C., Parham F., Masten S. A., Portier C. J., Shelby M. D., Brock J. W. and Needham L. L. (2000) Human exposure estimates for phthalates. *Environ Health Perspect* **108**, A440-2.

Kolarik B., Naydenov K., Larsson M., Bornehag C. G. and Sundell J. (2008) The association between phthalates in dust and allergic diseases among Bulgarian children. *Environ Health Perspect* **116**, 98-103.

Kowalski L. A., Laitinen A. M., Mortazavi-Asl B., Wee R. K., Erb H. E., Assi K. P. and Madden Z. (2000) In vitro determination of carcinogenicity of sixty-four compounds using a

bovine papillomavirus DNA-carrying C3H/10T(1/2) cell line. *Environ Mol Mutagen* **35**, 300-11.

Kurata Y., Kidachi F., Yokoyama M., Toyota N., Tsuchitani M. and Katoh M. (1998) Subchronic toxicity of Di(2-ethylhexyl)phthalate in common marmosets: lack of hepatic peroxisome proliferation, testicular atrophy, or pancreatic acinar cell hyperplasia. *Toxicol Sci* **42**, 49-56.

Kwak I. S. and Lee W. (2005) Endpoint for DEHP exposure assessment in Chironomus riparius. *Bull Environ Contam Toxicol* **74**, 1179-85.

Lake B. G., Brantom P. G., Gangolli S. D., Butterworth K. R. and Grasso P. (1976) Studies on the effects of orally administered Di-(2-ethylhexyl) phthalate in the ferret. *Toxicology* **6**, 341-56.

Lamb J. C. t., Chapin R. E., Teague J., Lawton A. D. and Reel J. R. (1987) Reproductive effects of four phthalic acid esters in the mouse. *Toxicol Appl Pharmacol* **88**, 255-69.

Larsen S. T., Hansen J. S., Hammer M., Alarie Y. and Nielsen G. D. (2004) Effects of mono-2-ethylhexyl phthalate on the respiratory tract in BALB/c mice. *Hum Exp Toxicol* **23**, 537-45.

Latini G., De Felice C., Presta G., Del Vecchio A., Paris I., Ruggieri F. and Mazzeo P. (2003) In utero exposure to di-(2-ethylhexyl)phthalate and duration of human pregnancy. *Environ Health Perspect* **111**, 1783-5.

Lee M. H., Park J., Chung S. W., Kang B. Y., Kim S. H. and Kim T. S. (2004) Enhancement of interleukin-4 production in activated CD4+ T cells by diphthalate plasticizers via increased NF-AT binding activity. *Int Arch Allergy Immunol* **134**, 213-22.

Lee S. M., Lee S. B., Park C. H. and Choi J. (2006) Expression of heat shock protein and hemoglobin genes in Chironomus tentans (Diptera, chironomidae) larvae exposed to various environmental pollutants: a potential biomarker of freshwater monitoring. *Chemosphere* **65**, 1074-81.

Lenth R. V. (2001) Some Practical Guidelines for Effective Sample Size Determination. *Am Statist* **55**, 187-193.

Lephart E. D. (1996) A review of brain aromatase cytochrome P450. *Brain Res Brain Res Rev* **22**, 1-26.

Li H. and Kim K. H. (2003) Effects of mono-(2-ethylhexyl) phthalate on fetal and neonatal rat testis organ cultures. *Biol Reprod* **69**, 1964-72.

Li L. H., Donald J. M. and Golub M. S. (2005) Review on testicular development, structure, function, and regulation in common marmoset. *Birth Defects Res B Dev Reprod Toxicol* **74**, 450-69.

Li L. H., Jester W. F. J., Laslett A. L. and Orth J. M. (2000) A single dose of Di-(2-ethylhexyl) phthalate in neonatal rats alters gonocytes, reduces sertoli cell proliferation, and decreases cyclin D2 expression. *Toxicol Appl Pharmacol* **166**, 222-9.

Li L. H., Jester W. F. J. and Orth J. M. (1998) Effects of relatively low levels of mono-(2-ethylhexyl) phthalate on cocultured Sertoli cells and gonocytes from neonatal rats. *Toxicol Appl Pharmacol* **153**, 258-65.

Liang J. H., Sankai T., Yoshida T. and Yoshikawa Y. (2001) Immunolocalization of proliferating cell nuclear antigen (PCNA) in cynomolgus monkey (Macaca fascicularis) testes during postnatal development. *J Med Primatol* **30**, 107-11.

Lim S. Y. and Ghosh S. K. (2005) Autoreactive responses to environmental factors: 3. Mouse strain-specific differences in induction and regulation of anti-DNA antibody responses due to phthalate-isomers. *J Autoimmun* **25**, 33-45.

Loff P. D., Subotic U., Oulmi-Kagermann J., Kranzlin B., Reinecke M. F. and Staude C. (2007) Diethylhexylphthalate extracted by typical newborn lipid emulsions from polyvinylchloride infusion systems causes significant changes in histology of rabbit liver. *JPEN J Parenter Enteral Nutr* **31**, 188-93.

Louis G. M., Weiner J. M., Whitcomb B. W., Sperrazza R., Schisterman E. F., Lobdell D. T., Crickard K., Greizerstein H. and Kostyniak P. J. (2005) Environmental PCB exposure and risk of endometriosis. *Hum Reprod* **20**, 279-85.

Lovekamp-Swan T. and Davis B. J. (2003) Mechanisms of phthalate ester toxicity in the female reproductive system. *Environ Health Perspect* **111**, 139-45.

Lovekamp-Swan T., Jetten A. M. and Davis B. J. (2003) Dual activation of PPARalpha and PPARgamma by mono-(2-ethylhexyl) phthalate in rat ovarian granulosa cells. *Mol Cell Endocrinol* **201**, 133-41.

Lovekamp T. N. and Davis B. J. (2001) Mono-(2-ethylhexyl) phthalate suppresses aromatase transcript levels and estradiol production in cultured rat granulosa cells. *Toxicol Appl Pharmacol* **172**, 217-24.

Main K. M., Mortensen G. K., Kaleva M. M., Boisen K. A., Damgaard I. N., Chellakooty M., Schmidt I. M., Suomi A. M., Virtanen H. E., Petersen D. V., Andersson A. M., Toppari J. and Skakkebaek N. E. (2006) Human breast milk contamination with phthalates and alterations of endogenous reproductive hormones in infants three months of age. *Environ Health Perspect* **114**, 270-6.

Marigomez I. and Baybay-Villacorta L. (2003) Pollutant-specific and general lysosomal responses in digestive cells of mussels exposed to model organic chemicals. *Aquat Toxicol* **64**, 235-57.

Masuo Y., Ishido M., Morita M. and Oka S. (2004a) Effects of neonatal treatment with 6-hydroxydopamine and endocrine disruptors on motor activity and gene expression in rats. *Neural Plast* **11**, 59-76.

Masuo Y., Morita M., Oka S. and Ishido M. (2004b) Motor hyperactivity caused by a deficit in dopaminergic neurons and the effects of endocrine disruptors: a study inspired by the physiological roles of PACAP in the brain. *Regul Pept* **123**, 225-34.

McKee R. H., Butala J. H., David R. M. and Gans G. (2004) NTP center for the evaluation of risks to human reproduction reports on phthalates: addressing the data gaps. *Reprod Toxicol* **18**, 1-22.

MCSI. (2003) Sixty-five week repeated oral dose toxicity study of di(2-ethylhexyl) phthalate (DEHP) in juvenile common marmosets. Mitsubishi Chemical Safety Institute Ltd.

Meeker J. D., Calafat A. M. and Hauser R. (2007) Di(2-ethylhexyl) phthalate metabolites may alter thyroid hormone levels in men. *Environ Health Perspect* **115**, 1029-34.

Melnick R. L. (2001) Is peroxisome proliferation an obligatory precursor step in the carcinogenicity of di(2-ethylhexyl)phthalate (DEHP)? *Environ Health Perspect* **109**, 437-42.

Metcalf R. L., Booth G. M., Schuth C. K., Hansen D. J. and Lu P. Y. (1973) Uptake and fate of Di-2-ethylhexyl phthalate in aquatic organisms and in a model ecosystem. *Environ Health Perspect* **4**, 27-34.

Millar M. R., Sharpe R. M., Weinbauer G. F., Fraser H. M. and Saunders P. T. (2000) Marmoset spermatogenesis: organizational similarities to the human. *Int J Androl* **23**, 266-77.
Modigh C. M., Bodin S. L., Lillienberg L., Dahlman-Hoglund A., Akesson B. and Axelsson G. (2002) Time to pregnancy among partners of men exposed to di(2-ethylhexyl)phthalate. *Scand J Work Environ Health* **28**, 418-28.
Moore R. W., Rudy T. A., Lin T. M., Ko K. and Peterson R. E. (2001) Abnormalities of sexual development in male rats with in utero and lactational exposure to the antiandrogenic plasticizer Di(2-ethylhexyl) phthalate. *Environ Health Perspect* **109**, 229-37.
Muller T., Simoni M., Pekel E., Luetjens C. M., Chandolia R., Amato F., Norman R. J. and Gromoll J. (2004) Chorionic gonadotrophin beta subunit mRNA but not luteinising hormone beta subunit mRNA is expressed in the pituitary of the common marmoset (Callithrix jacchus). *J Mol Endocrinol* **32**, 115-28.
Naito W., Gamo Y. and Yoshida K. (2006) Screening-level risk assessment of Di(2-ethylhexyl)phthalate for aquatic organisms using monitoring data in Japan. *Environ Monit Assess* **115**, 451-71.
Nazir D. J., Alcaraz A. P., Bierl B. A., Beroza M. and Nair P. P. (1971) Isolation, identification, and specific localization of di-2-ethylhexyl phthalate in bovine heart muscle mitochondria. *Biochemistry* **10**, 4228-32.
Nef S. and Parada L. F. (1999) Cryptorchidism in mice mutant for Insl3. *Nat Genet* **22**, 295-9.
Norman A., Borjeson H., David F., Tienpont B. and Norrgren L. (2007) Studies of uptake, elimination, and late effects in Atlantic salmon (Salmo salar) dietary exposed to Di-2-ethylhexyl phthalate (DEHP) during early life. *Arch Environ Contam Toxicol* **52**, 235-42.
NTP-CERHR. (2000) NTP-CERHR Expert Panel Report on Di(2-Ethylhexyl)Phthalate. In *A Report of the CERHR Phthalates Expert Panel*, p. 115. Center for the Evaluation of Risks to Human Reproduction/National Toxicology Program-National Institute of Environmental Health Sciences-, Research Triangle Park, NC.
NTP-CERHR. (2003) NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Di-n-Butyl Phthalate (DBP). In *Expert Panel Reports and NTP-CERHR Monographs*, p. 169. Center for the Evaluation of Risks to Human Reproduction/National Toxicology Program-National Institute of Environmental Health Sciences, Research Triangle Park, NC.
Orbea A., Ortiz-Zarragoitia M. and Cajaraville M. P. (2002) Interactive effects of benzo(a)pyrene and cadmium and effects of di(2-ethylhexyl) phthalate on antioxidant and peroxisomal enzymes and peroxisomal volume density in the digestive gland of mussel Mytilus galloprovincialis Lmk. *Biomarkers* **7**, 33-48.
Orth J. M. (1982) Proliferation of Sertoli cells in fetal and postnatal rats: a quantitative autoradiographic study. *Anat Rec* **203**, 485-92.
Orth J. M., Gunsalus G. L. and Lamperti A. A. (1988) Evidence from Sertoli cell-depleted rats indicates that spermatid number in adults depends on numbers of Sertoli cells produced during perinatal development. *Endocrinology* **122**, 787-94.
Pan G., Hanaoka T., Yoshimura M., Zhang S., Wang P., Tsukino H., Inoue K., Nakazawa H., Tsugane S. and Takahashi K. (2006) Decreased serum free testosterone in workers exposed to high levels of di-n-butyl phthalate (DBP) and di-2-ethylhexyl phthalate (DEHP): a cross-sectional study in China. *Environ Health Perspect* **114**, 1643-8.
Park J. D., Habeebu S. S. and Klaassen C. D. (2002) Testicular toxicity of di-(2-ethylhexyl)phthalate in young Sprague-Dawley rats. *Toxicology* **171**, 105-15.

Park S. Y. and Choi J. (2007) Cytotoxicity, genotoxicity and ecotoxicity assay using human cell and environmental species for the screening of the risk from pollutant exposure. *Environ Int* **33**, 817-22.

Peijnenburg W. J. G. M. and Struijs J. (2006) Occurance of phthalate esters in the environment of the Netherlands. *Ecotoxicol. Environ. Safe.* **63**, 204-215.

Poon R., Lecavalier P., Mueller R., Valli V. E., Procter B. G. and Chu I. (1997) Subchronic oral toxicity of di-n-octyl phthalate and di(2-Ethylhexyl) phthalate in the rat. *Food Chem Toxicol* **35**, 225-39.

Price C. J., Tyl R. W., Marr M. C., Myers C. B., Sadler B. M. and Kimmel C. A. (1988a) REPRODUCTION AND FERTILITY EVALUATION OF DIETHYLHEXYL PHTHALATE (CAS NO. 117-81-7) IN CD-1 MICE EXPOSED DURING GESTATION. *Report (Ntp 88-092):286 Pp,.*

Price S. C., Chescoe D., Grasso P., Wright M. and Hinton R. H. (1988b) Alterations in the thyroids of rats treated for long periods with di-(2-ethylhexyl) phthalate or with hypolipidaemic agents. *Toxicol Lett* **40**, 37-46.

Pugh G. J., Isenberg J. S., Kamendulis L. M., Ackley D. C., Clare L. J., Brown R., Lington A. W., Smith J. H. and Klaunig J. E. (2000) Effects of di-isononyl phthalate, di-2-ethylhexyl phthalate, and clofibrate in cynomolgus monkeys. *Toxicol Sci* **56**, 181-8.

Qiao L., Zheng L. and Cai D. (2007) [Study on the di-n-butyl phthalate and di-2-ethylhexyl phthalate level of girl serum related with precocious puberty in Shanghai]. *Wei Sheng Yan Jiu* **36**, 93-5.

Reddy B. S., Rozati R., Reddy B. V. and Raman N. V. (2006a) Association of phthalate esters with endometriosis in Indian women. *BJOG* **113**, 515-20.

Reddy B. S., Rozati R., Reddy S., Kodampur S., Reddy P. and Reddy R. (2006b) High plasma concentrations of polychlorinated biphenyls and phthalate esters in women with endometriosis: a prospective case control study. *Fertil Steril* **85**, 775-9.

Rengarajan S., Parthasarathy C., Anitha M. and Balasubramanian K. (2007) Diethylhexyl phthalate impairs insulin binding and glucose oxidation in Chang liver cells. *Toxicol In Vitro* **21**, 99-102.

Rhodes C., Orton T. C., Pratt I. S., Batten P. L., Bratt H., Jackson S. J. and Elcombe C. R. (1986) Comparative pharmacokinetics and subacute toxicity of di(2-ethylhexyl) phthalate (DEHP) in rats and marmosets: extrapolation of effects in rodents to man. *Environ Health Perspect* **65**, 299-307.

Rier S. E. (2002) The potential role of exposure to environmental toxicants in the pathophysiology of endometriosis. *Ann N Y Acad Sci* **955**, 201-12; discussion 230-2, 396-406.

Saitoh Y., Usumi K., Nagata T., Marumo H., Imai K. and Katoh M. (1997) Early Changes in the Rat Testis Induced by Di-(2-Ethylhexyl) Phthalate and 2,5-Hexanedione: Ultrastructure and Lanthanum Trace Study. *J Toxicol Pathol* **10**, 51-57.

Sanders H. O., Mayer F. L., Jr. and Walsh D. F. (1973) Toxicity, residue dynamics, and reproductive effects of phthalate esters in aquatic invertebrates. *Environ Res* **6**, 84-90.

Sano M., Hagiwara A., Tamano S., Hasegawa R., Imaida K., Ito N. and Shirai T. (1999) Dose-dependent induction of carcinomas and glutathione S-transferase placental form negative eosinophilic foci in the rat liver by di(2-ethylhexyl)phthalate after diethylnitrosamine initiation. *J Toxicol Sci* **24**, 177-86.

Schilling K., Gembardt C. and Hellwig J. (1999) Reproduction toxicity of di-2-ethylhexyl phthalate (DEHP). *Toxicologist* **48**, :147-8.
Schwetz B. A. (2001) From the Food and Drug Administration. *JAMA* **286**, 2085.
Seo J. S., Park T. J., Lee Y. M., Park H. G., Yoon Y. D. and Lee J. S. (2006) Small heat shock protein 20 gene (Hsp20) of the intertidal copepod Tigriopus japonicus as a possible biomarker for exposure to endocrine disruptors. *Bull Environ Contam Toxicol* **76**, 566-72.
Sha Y., Xia X., Yang Z. and Huang G. H. (2007) Distribution of PAEs in the middle and lower reaches of the Yellow River, China. *Environ Monit Assess* **124**, 277-87.
Sharifi N., Gulley J. L. and Dahut W. L. (2005) Androgen deprivation therapy for prostate cancer. *JAMA* **294**, 238-44.
Sharpe R. M., Walker M., Millar M. R., Atanassova N., Morris K., McKinnell C., Saunders P. T. and Fraser H. M. (2000) Effect of neonatal gonadotropin-releasing hormone antagonist administration on sertoli cell number and testicular development in the marmoset: comparison with the rat. *Biol Reprod* **62**, 1685-93.
Shioda T. and Wakabayashi M. (2000) Effect of certain chemicals on the reproduction of medaka (Oryzias latipes). *Chemosphere* **40**, 239-43.
Shukla R. R., Albro P. W., Corbett J. T. and Schroeder J. L. (1989) In vitro studies of the inhibition of protein kinase C from rat brain by di-(2-ethylhexyl)phthalate. *Chem Biol Interact* **69**, 73-85.
Silva M. J., Barr D. B., Reidy J. A., Malek N. A., Hodge C. C., Caudill S. P., Brock J. W., Needham L. L. and Calafat A. M. (2004a) Urinary levels of seven phthalate metabolites in the U.S. population from the National Health and Nutrition Examination Survey (NHANES) 1999-2000. *Environ Health Perspect* **112**, 331-8.
Silva M. J., Reidy J. A., Herbert A. R., Preau J. L. J., Needham L. L. and Calafat A. M. (2004b) Detection of phthalate metabolites in human amniotic fluid. *Bull Environ Contam Toxicol* **72**, 1226-31.
Sjoberg P., Bondesson U., Gray T. J. and Ploen L. (1986) Effects of di-(2-ethylhexyl) phthalate and five of its metabolites on rat testis in vivo and in in vitro. *Acta Pharmacol Toxicol (Copenh)* **58**, 225-33.
Sjoberg P., Bondesson U., Kjellen L., Lindquist N. G., Montin G. and Ploen L. (1985) Kinetics of di-(2-ethylhexyl) phthalate in immature and mature rats and effect on testis. *Acta Pharmacol Toxicol (Copenh)* **56**, 30-7.
Smedley J. V., Bailey S. A., Perry R. W. and O Rourke C. M. (2002) Methods for predicting sexual maturity in male cynomolgus macaques on the basis of age, body weight, and histologic evaluation of the testes. *Contemp Top Lab Anim Sci* **41**, 18-20.
Stahlhut R. W., van Wijngaarden E., Dye T. D., Cook S. and Swan S. H. (2007) Concentrations of urinary phthalate metabolites are associated with increased waist circumference and insulin resistance in adult U.S. males. *Environ Health Perspect* **115**, 876-82.
Staples C. A., Peterson D. R., F. P. T. and Adams W. J. (1997) THE ENVIRONMENTAL FATE OF PHTHALATE ESTERS: A LITERATURE REVIEW. *Chemosphere* **35**, 667-749.
Stata Corporation. (2003) Small Stata 8.0 for Windows. Stata Corporation, College Station, TX.
Stemp-Morlock G. (2007) Exploring developmental origins of obesity. *Environ Health Perspect* **115**, A242.
Swan S. H., Main K. M., Liu F., Stewart S. L., Kruse R. L., Calafat A. M., Mao C. S., Redmon J. B., Ternand C. L., Sullivan S. and Teague J. L. (2005) Decrease in anogenital distance

among male infants with prenatal phthalate exposure. *Environ Health Perspect* **113**, 1056-61.
Taborsky R. G. (1967) Isolation studies on a lipidal portion of the bovine pinel gland. *J. Agr. Food. Chem.* **15**, 1073-1076.
Takano H., Yanagisawa R., Inoue K., Ichinose T., Sadakane K. and Yoshikawa T. (2006) Di-(2-ethylhexyl) phthalate enhances atopic dermatitis-like skin lesions in mice. *Environ Health Perspect* **114**, 1266-9.
Tan G. H. (1995) Residue levels of phthalate esters in water and sediment samples from the Klang River basin. *Bull Environ Contam Toxicol* **54**, 171-6.
Tanaka T. (2002) Reproductive and neurobehavioural toxicity study of bis(2-ethylhexyl) phthalate (DEHP) administered to mice in the diet. *Food Chem Toxicol* **40**, 1499-506.
Tandon R., Seth P. K. and Srivastava S. P. (1991) Effect of in utero exposure to di(2-ethylhexyl)phthalate on rat testes. *Indian J Exp Biol* **29**, 1044-6.
Teerds K. J., de Boer-Brouwer M., Dorrington J. H., Balvers M. and Ivell R. (1999) Identification of markers for precursor and leydig cell differentiation in the adult rat testis following ethane dimethyl sulphonate administration. *Biol Reprod* **60**, 1437-45.
Toyosawa K., Okimoto K., Kobayashi I., Kijima K., Kikawa E., Kohchi M., Koujitani T., Tanaka K. and Matsuoka N. (2001) Di(2-ethylhexyl)phthalate induces hepatocellular adenoma in transgenic mice carrying a human prototype c-Ha-ras gene in a 26-week carcinogenicity study. *Toxicol Pathol* **29**, 458-66.
Tully K., Kupfer D., Dopico A. M. and Treistman S. N. (2000) A plasticizer released from IV drip chambers elevates calcium levels in neurosecretory terminals. *Toxicol Appl Pharmacol* **168**, 183-8.
Tyl R. W., Price C. J., Marr M. C. and Kimmel C. A. (1988) Developmental toxicity evaluation of dietary di(2-ethylhexyl)phthalate in Fischer 344 rats and CD-1 mice. *Fundam Appl Toxicol* **10**, 395-412.
U.S. EPA. (1996) Guidelines for Reproductive Toxicity Risk Assessment. In *Risk Assessment Forum*, p. 132. United States Environmental Protection Agency, Office of Research and Development, Washington, DC.
U.S. FDA. (2001) Safety Assessment of Di (2-ethylhexyl)phthalate (DEHP) Released from PVC Medical Devices, p. 119. United States Food and Drug Administration, Centers for Devices and Radiological Health, Rockville, MD.
van Wezel A. P., van Vlaardingen P., Posthumus R., Crommentuijn G. H. and Sijm D. T. (2000) Environmental risk limits for two phthalates, with special emphasis on endocrine disruptive properties. *Ecotoxicol Environ Saf* **46**, 305-21.
Wenzel A., Franz C., Breous E. and Loos U. (2005) Modulation of iodide uptake by dialkyl phthalate plasticisers in FRTL-5 rat thyroid follicular cells. *Mol Cell Endocrinol* **244**, 63-71.
Wilson V. S., Lambright C., Furr J., Ostby J., Wood C., Held G. and Gray L. E. J. (2004) Phthalate ester-induced gubernacular lesions are associated with reduced insl3 gene expression in the fetal rat testis. *Toxicol Lett* **146**, 207-15.
Wistuba J., Mundry M., Luetjens C. M. and Schlatt S. (2004) Cografting of hamster (Phodopus sungorus) and marmoset (Callithrix jacchus) testicular tissues into nude mice does not overcome blockade of early spermatogenic differentiation in primate grafts. *Biol Reprod* **71**, 2087-91.

Woin P. and Larsson P. (1987) Phthalate esters reduce predation efficiency of dragonfly larvae, Odonata; Aeshna. *Bull Environ Contam Toxicol* **38**, 220-5.
Xiao A. Y., Wei L., Xia S., Rothman S. and Yu S. P. (2002) Ionic mechanism of ouabain-induced concurrent apoptosis and necrosis in individual cultured cortical neurons. *J Neurosci* **22**, 1350-62.
Yamashita U., Kuroda E., Yoshida Y. and Sugiura T. (2003) Effect of endocrine disrupters on immune responses in vivo. *J UOEH* **25**, 365-74.
Yamashita U., Sugiura T. and Kuroda E. (2002) Effect of endocrine disrupters on immune responses in vitro. *J UOEH* **24**, 1-10.
Yang Q., Xie Y. and Depierre J. W. (2000) Effects of peroxisome proliferators on the thymus and spleen of mice. *Clin Exp Immunol* **122**, 219-26.
Zhang F. P., Kero J. and Huhtaniemi I. (1998) The unique exon 10 of the human luteinizing hormone receptor is necessary for expression of the receptor protein at the plasma membrane in the human luteinizing hormone receptor, but deleterious when inserted into the human follicle-stimulating hormone receptor. *Mol Cell Endocrinol* **142**, 165-74.
Zhang Y. H., Zheng L. X. and Chen B. H. (2006) Phthalate exposure and human semen quality in Shanghai: a cross-sectional study. *Biomed Environ Sci* **19**, 205-9.
Zoeller R. T. (2005) Environmental chemicals as thyroid hormone analogues: new studies indicate that thyroid hormone receptors are targets of industrial chemicals? *Mol Cell Endocrinol* **242**, 10-5.
Zuhlke U. and Weinbauer G. (2003) The common marmoset (Callithrix jacchus) as a model in toxicology. *Toxicol Pathol* **31 Suppl**, 123-7.