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Review

The Impact of Di-2-Ethylhexyl Phthalate (DEHP) in Focus on the Reproductive System and Toxicity in Rat or Mammalian Model

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Abstract: Endocrine disruptor pollution and its effects on the human reproductive system have garnered significant interest. Di-2-Ethylhexyl Phthalate (DEHP) is a reproductive toxin and environmental endocrine disruptor. Due to their widespread use in consumer goods and flexible plastics, phthalate diesters are now common environmental pollutants. One of the many industrial and consumer products that contain environmental endocrine disruptors is DEHP. Examples of these products include toys, medical supplies, cleaning products, cosmetics, interior design, and décor materials. Since DEHP reaches the human body in large concentrations and can have an enhanced effect through biological concentration, it has gained widespread attention. In addition to changing the molecular, endocrinological, cytological, and biochemical elements of female reproduction, it also affects the structural and functional features, leading to irregularities in the ovarian cycle and infertility. The literature search was done through Scopus, ISI Web of Science, Google Scholar, PubMed, and Medline. Up until now, studies on the reproductive toxicity of DEHP exposure in adult females have been conducted. It can result in illnesses including uterine hemorrhage, sexual precocity, and infertility, which worries academics and the general public. Moreover, research on animals indicates that exposure to phthalates may alter the levels of circulating hormones, which could have a negative impact on reproductive physiology and the growth of target tissues that are susceptible to estrogen. As a result, we carried out a thorough analysis of the experimental animal and epidemiological literature to determine the connection between phthalate exposure and unfavorable results for the reproductive health of women. The epidemiological literature does not provide evidence for a conclusion that exposure to phthalates has a negative impact because it is lacking for the majority of outcomes examined, has small sample sizes, and has methodological flaws. We have come to the conclusion that there is enough data to imply that phthalates are reproductive toxicants, notwithstanding the dearth of experimental animal investigations for numerous phthalates. We do point out that the quantities required to cause harmful health consequences are higher than those seen in recent human biomonitoring studies. We suggest that additional research is necessary because of the patchwork of existing studies, the possibility of additive effects, and the indication of negative consequences of phthalate exposure in later generations at lower concentrations than in the parent generation.

Keywords: Plasticisers, Di-2-Ethylhexyl Phthalate (DEHP), Endocrine Disruptor, Fertility, Reproductive System, Toxicity

Introduction

In recent years, evidence has increased on the health effects of environmental agents, including plasticizers, pesticides, metals, and many industrial chemicals (Dalsenter *et al*., 2006). Endocrine Disruptor Substances (EDS) are environmental chemicals that have a toxic effect on hormonal balance (Albert and Jegou, 2014).

Phthalates, or phthalate esters, are synthetic diesters of phthalic acid, first produced in 1920 (North *et al*., 2014). Phthalates are used in a variety of products and applications and are found in medical devices, gelling agents, adhesives, lubricants, cosmetics, dispersants, and emulsifying agents. They are also used in many household and consumer products, including PVC in roofing, food packaging, shower curtains, nail polish, plastic products, and kitchen plastics (Kimber and Dearman, 2010). DEHP, the most commonly used plasticizer in PVC products, has an interesting classification history. For some years, it has been suspected to be carcinogenic to humans and classified as a possible cause of cancer (category 2) by the United States Environmental Protection Agency (USEPA) and the International Cancer Archives (WHO, 1992). But recently, IARC Working Group on the Evaluation of Carcinogenic Risks to Humans (2018) re-evaluated DEHP, after which it was downgraded and declared unclassified in terms of carcinogenicity to humans (category 3). In addition, for many years, DEHP was suspected to be toxic to reproduction (Thomas *et al*., 1984), but without classification in this category, in the 1990s, DEHP was suspected of having estrogenic effects. However, new studies have supported the idea that DEHP may harm the reproductive system due to its antiandrogenic effects (Gray, 2000) and these results put the chemical on the EU list of dangerous substances, where it is now classified as a substance that can cause harm to reproduction and cause developmental toxicity in humans (type 2) (Sands and Galizzi, 2006). The effects of DEHP on the reproductive system are considered important due to their perceived role as endocrine disruptors (Latini *et al*., 2004). The possible exposure of humans and animals to many industrial chemicals and toxic drugs has been a growing concern in the last decade, both for scientists and the public. Many studies have shown that these environmental pollutants can harm the reproductive performance of various vertebrates (Foster *et al*., 2001). DEHP is one of the most commonly used phthalates and has been shown to cause developmental and reproductive toxicity in mouse models. The reproductive toxicity of DEHP in rats and mice is characterized by a decrease in fertility, size, sperm density and motility, and ovarian and testicular weight (Arcadi *et al*., 1998). Due to the use of DEHP in many products, it can be found in the air, water, and soil. DEHP can bind to particles in the air and be returned to the earth when released; DEHP can also bind strongly to soil and dissolve significantly in groundwater (Sai Sandeep and Jiaxiang, 2018). The toxicity of phthalates has been reported since the 1950s. In recent years, the perceived harmful effects of phthalates on human health have affected the media and the population (Albert and Jegou, 2014). Based on studies in mice, DEHP is the most toxic to children among phthalate esters (Ventrice *et al*., 2013). The toxicokinetics of DEHP in humans, laboratory animals, and cellular systems may play an important role in determining the extent of adverse effects (Caldwell, 2012). DEHP is a lipophilic compound and does not bind to PVC; therefore, this phthalate can be released from the plastic (Herreros *et al*., 2010). A previously published CERHR and IARC effort addressed the exposure and Absorption, Distribution, Metabolism, and Elimination (ADME) of DEHP (Caldwell, 2012). DEHP has generated public concern about its potential health risks due to the results of studies in animal models following oral exposure (Kavlock *et al*., 2002). However, there are few studies on its reproductive toxicity following inhalation exposure; In this case, Klimisch *et al*. (1992) evaluated general toxicity and Kurahashi *et al*. (2005) studied reproductive toxicity in males. In contrast, oral ingestion studies have shown that DEHP can have testicular toxicity in male rats (Akingbemi *et al*., 2001) and that it can also have ovarian toxicity in female rats (Lovekamp-Swan and Davis, 2003). *In vivo*, DEHP decreases serum estradiol levels and prolongs the estrous cycle in adult female rats (Davis *et al*., 1994). *In vitro*, Mono-2-Ethylhexyl Phthalate (MEHP), an active metabolite of DEHP, inhibits estradiol production by reducing both mRNA and protein levels as well as aromatase activity (Lovekamp and Davis, 2001). However, little is known about the *in vivo* effects of DEHP on the expression levels of steroidogenic enzymes. Several studies in humans suggest a link between exposure to phthalates and poor reproductive development in women. Exposure of female workers to high levels of phthalates was associated with reduced pregnancy rates and higher miscarriage rates (Aldyreva *et al*., 1975). Puerto Rican girls with premature breast development (Thelarche) have higher blood phthalate levels than normal girls (Colon *et al*., 2000). Endometriotic women had higher plasma DEHP concentrations than controls (Cobellis, 2003). However, there is no information on the effect of DEHP inhalation on the onset of puberty and reproductive activity in female animal models. Previous studies have shown that mature female rats exposed to Di-2-Ethylhexyl Phthalate (DEHP) and its metabolite, Mono-2-Ethylhexyl Phthalate (MEHP), have reduced serum progesterone, delayed ovulation, and smaller preovulatory follicles with high levels of serum Follicle-Stimulating Hormone (FSH). Several studies have shown that exposure to phthalates is associated with endometriosis. The effects of DEHP on endometrial cells include cell invasion, viability, proliferation, and oxidative stress through the Mitogen-Activated Protein Kinase (MAPK), Extracellular regulated protein kinase (Erk), and Nuclear Factor-κB (NF-κB) pathways. Endometriosis harms granulosa cells because it affects steroidogenesis and cell growth, reduces aromatase

activity, and changes mitochondrial gene expression in human granulosa cells (Chou and Tzeng, 2021).

In particular, DEHP causes several developmental and reproductive toxicities in mammals, while its potential to produce toxicity in humans remains unclear. Limited studies in the population suggest an association between phthalate exposure and adverse reproductive health effects in both sexes. In fact, chronic occupational exposure to high levels of phthalates is associated with higher rates of pregnancy and higher rates of miscarriage and anovulation among female industrial workers (Latini *et al*., 2004). Due to low atmospheric pressure and low water solubility, the concentration of DEHP in outdoor air and water is low (Kavlock *et al*., 2002). However, the indoor environment is covered with PVC in household products, flooring, wall coverings, and electronic devices (Wensing *et al*., 2005). Phthalate esters have been recognized as an important indoor pollutant (Wensing *et al*., 2005) and, second only to food exposure, indoor air is considered the most common mechanism by which the public is exposed to phthalate esters (DEHP) (Kavlock *et al*., 2002). DEHP has been found in soil samples from several areas, which means that food and drinking water may include residues of this substance (Zhang *et al*., 2015). DEHP exposure in humans can happen through a number of different channels, including oral consumption, inhalation, and skin contact. Furthermore, DEHP in soil can infiltrate plants, which can then make its way up the food chain to humans or animals, where it may have mutagenic and carcinogenic effects (Li *et al*., 2020). DEHP has been found in a variety of human tissues and fluids recently, including the liver, blood, placenta, breast milk, and amniotic fluid during the early stages of pregnancy (Wójtowicz *et al*., 2019). Long-term exposure to plastic materials, such as DEHP, has been associated with reproductive malfunction and sexually premature puberty in children (Golestanzadeh *et al*., 2020). Adult exposure to DEHP has been linked to changes in the gut microbiome, reproductive abnormalities, testicular and ovarian cancer, hepatocyte apoptosis, lymphatic epithelium damage, and liver inflammatory responses (Liu *et al*., 2021). The heart, kidneys, and lungs of young and old rats have notable structural changes that offer an experimental basis for researching chronic diseases and comprehending organ malfunction in the elderly (Çoban *et al*., 2014). However, the possible harm brought about by varying DEHP exposure dosages to various organs in animals at various stages of life has received little consideration. Research that offers trustworthy data on the distribution of DEHP in human tissues other than blood was found in studies. Although DEHP has been found in human adipose tissues taken after autopsy (Mes *et al*., 1974), the amounts of DEHP found in this review may have been contaminated by plastics used during the handling and storage of the tissues. Exact measures of tissue distribution can be obtained from research carried out on animals administered labeled DEHP (e.g., ^{14}C-DEHP). In rats, dogs, pigs, and nonhuman primates, the tissue distribution of ^{14}C after intravenous, oral, inhalation, and cutaneous dosage with (^{14}C)-DEHP has been investigated (Kurata *et al*., 2012). For all of the aforementioned exposure modalities, the absorption of ^{14}C in the liver, colon, muscle, kidney, and fat (as well as in the lung during inhalation exposure) often dominates the initial distribution within 4 h of dose. Concentrations in the colon, lung, kidney, heart, liver, spleen, and adipose tissue can surpass those in the blood (Rhodes *et al*., 1986). Distribution to the colon happens after intravenous administration, demonstrating the intestinal transit of absorbed ^{14}C (Wallin *et al*., 1974). After a single dose of (^{14}C)-DEHP, the removal of ^{14}C from fat occurs more slowly than from other tissues. Consequently, over time, fat's contribution to the total ^{14}C body burden grows as ^{14}C is eliminated from other tissues (Ikeda *et al*., 1980). For 6 h, male Sprague-Dawley rats exposed to an aerosol of ^{14}C-DEHP (83 mg/m^3) with particle sizes ranging from 0.24-0.61 μm expelled almost half of the inhaled ^{14}C in urine, 40% in feces within 72 h and roughly 5-7% remained in the carcass. Still, it is not possible to compare the harmful health effects of various ingestion channels within the system. The distribution of PAEs in the body's organs and tissues after entering the human body by three pathways oral ingestion, cutaneous penetration, and inhalation also has to be studied in order to establish a connection between external exposure to DEHP and internal exposure. The primary worries about the possible harmful effects of DEHP on the human endocrine system arise from observations made in several species during experiments. Among the phthalate esters, DEHP is the most effective reproductive toxicant, according to mouse fertility tests (Ventrice *et al*., 2013). According to a study conducted on Russian women, long-term occupational exposure to phthalate esters was linked to higher rates of anovulation, and lower rates of pregnancy and miscarriages (Hoyer, 2001). Reproductive performance was disrupted in animal studies when animals were repeatedly exposed orally to DEHP (Hoyer, 2001). According to an in-utero investigation, male offspring of lactationally exposed pregnant rats to the DEHP had shortened anogenital distance, retained areola and nipple, undescended testes, and permanently incomplete preputial separation (Moore *et al*., 2001). The World Health Organization's (WHO) International Agency for Research on Cancer (IARC) and expert panels assembled by the National Toxicology Programme's (NTP) Centre for the Evaluation of Risks to Human Reproduction (CERHR), a joint venture between the NTP and the National Institute of Environmental Health Sciences (NIEHS), have

previously reviewed the carcinogenic hazard of DEHP and its reproductive hazard. DEHP exposure and Absorption, Distribution, Metabolism, and Elimination (ADME) were covered in both the previously published CERHR and IARC initiatives (Caldwell, 2012).

Female rats exposed to Di-2-Ethylhexyl Phthalate (DEHP) at a dose of 2,000 mg/kg also showed a lengthening of their cycles and anovulation due to a decrease in serum estradiol levels. Anovulation is associated with the absence of corpus luteum in the ovary and the occurrence of follicular cysts (Lovekamp-Swan and Davis, 2003). Therefore, we carefully discuss the findings of the recent research articles and provide an overview of the studies on the disruptive effects of phthalates. In addition, we review data on the reproductive toxicity of phthalates in some *in vitro* studies and on male and female reproductive systems in experimental and domestic animals. Finally, we outline some critical questions that should be addressed to clarify the implications of phthalates for human reproduction.

Methodology

Information about EDCs, mainly DEHP, was collected from different scientific search engines, viz., Google Scholar, PubMed, Science Direct, and Scopus. All the published information about the impact of DEHP in indexed journals was included and cited in the text.

Di-2-Ethylhexylphthalate (DEHP)

One of the most popular EDCs, DEHP, is frequently used to soften and increase the flexibility of vinyl polymers. One of the most widely produced compounds, DEHP, is expected to expand by 5% per year due to rising global manufacturing demand. Every year, more than 10 million metric tons of DEHP, an unbound chemical that resembles estrogen, are used globally to liquefy materials or make polymers softer (Zhang *et al*., 2013a). DEHP is present everywhere and exposes both humans and animals through their food supplies. According to Li *et al*. (2015), the general public is thought to be exposed to DEHP on a regular basis, mostly through their cuisine, as well as through drinking water and using plastic medical devices for feeding, medicating, and helping newborns breathe. Owing to the toxicological character of DEHP exposure, analyses of DEHP assimilation and disposal in several species have been conducted. According to a preliminary investigation, once the rats were given the drug intravenously, the 14C-labeled DEHP quickly vanished from circulation, with roughly 57% of the total dose found in the feces and 42% in the urine (Daniel and Bratt, 1974). According to estimates, the average person's daily exposure to DEHP varies from 0.21-2.1 mg/day (Tickner *et al*., 2001). Furthermore, DEHP urinary metabolite concentrations (3-30 mg/kg/day) (Koch *et al*., 2006) imply that even a minimal amount of daily exposure to DEHP from all sources could be a sign of a possible risk factor. The general population's consumption of food and water is the primary means of exposure to DEHP, making it a critical public health problem. However, information regarding the extent of DEHP absorption and its primary mechanism of action is not well understood.

Analysis

Detection and detection of very low levels of DEHP are extremely limited due to the presence of these compounds as contaminants in almost all laboratory equipment and reagents. It has been found that plastic, glass, aluminum foil, cork, rubber, glass wool, solvents, and Teflon® sheets are contaminated (ATSDR, 2002). Table 1 lists options for the analysis of DEHP in various matrices.

Table 1: Selected methods for the analysis of Di-2-ethylhexyl Phthalate

Sample matrix	Sample preparation	Assay Procedure [a]	Limit of detection
Air	Collect on cellulose ester membrane filter; desorb with carbon disulfide	GC/FID	10 µg/sample
Drinking-water, and source water	Extract in liquid-solid extractor; elute with dichloromethane; concentrated by evaporation	GC/MS	0.5 µg/L
Drinking-water	Extract in liquid-liquid extractor; isolate; dry; concentrate	GC/PID	2.25 µg/L
Wastewater, municipal and industrial	Extract with dichloromethane; dry; exchange to hexane and concentrate	GC/ECD	2.0 µg/L
	Extract with dichloromethane; dry; concentrate	GC/MS	2.5 µg/L
	Add isotope-labelled analogue; extract with dichloromethane; dry over sodium sulfate; concentrate	GC/MS	10 µg/L
Groundwater, leachate, soil, sludge, sediment	Aqueous sample: extract with dichloromethane; elute with acetonitrile; exchange to hexane; Solid sample: extract with dichloromethane/acetone (1:1) or hexane/acetone (1:1); clean-up	GC/ECD	0.27 µg/L (aqueous)

[a]bbreviations: GC, gas chromatography; ECD, electron capture detection; FID, flame ionization detection; MS, mass spectrometry; PID, photoionization detection

Use

As a plasticizer, DEHP's main function is to break down plastics and polymers. Plastic may contain 1-40% DEHP by weight. Approximately 90% of DEHP is used as a plasticizer in Polyvinyl Chloride (PVC) polymers. In the EU, 95% of DEHP is used as a plasticizer in polymer products. The use of DEHP falls into two categories: The use of polymeric materials (products such as shoes, bath towels and toys, medical devices, and commercial/commercial use) and non-polymeric applications (dielectric fluids, paints, adhesives, and inks). Nonpolymers account for less than 5% of all DEHP use in the United States. Approximately 45% of all DEHP consumption in the United States is used in various industrial and commercial products. Commercial and industrial uses of DEHP include flexible floor coverings, wall coverings, roofs, aluminum foil coverings and laminates, paper coverings, extrudable molds and profiles, electrical components wire and cable coatings, and sheathing. In the United States, medical devices account for approximately 25% of all DEHP production. Medical devices containing DEHP include PVC sheets such as intravenous bags and tubes used for a variety of medical purposes.

Toxic Effects of Di-2-Ethylhexyl Phthalate

Toxic effects of Di-2-Ethylhexyl Phthalate (DEHP), an environmental endocrine disruptor, is one of the organic chemicals that are used in a large variety of industrial and consumer applications. It is the most commonly used plasticizer worldwide (Cirillo *et al*., 2013). DEHP is an endocrine disruptor that alters the functions of endocrine systems and causes adverse effects by altering organisms (Mastorakos *et al*., 2007). In recent years, DEHP has raised much public concern about its potential health hazards because of its reproductive effects in an animal model following oral exposure or inhalation exposure (Wang and Qian, 2021). Several authors have reported the nature of toxicity in mammalian systems. DEHP significantly decreased libido (by increasing the reaction time), thiobarbutyric acid reactive substances and lactate dehydrogenase were significantly increased, while glutathione-s-transferase, transaminases, and phosphatases were significantly decreased in the seminal plasma of male rabbits (Fayrouz, 2021). The mRNA expression of LH receptors, FSH receptors androgen, estrogen, progesterone, peroxisome proliferator-activated receptors, 3β-hydroxysteroid dehydrogenase, aromatase, and steroidogenic acute regulatory proteins were significantly altered, as were the ovarian and uterine functions of the adult offspring rats (Somasundaram *et al*., 2016). DEHP exerts dual effects on the pituitary-gonadal axis, stimulating the hormonal function of the pituitary and, at the same time, inhibiting steroidogenesis by Granulosa cells in female rats (Svechnikova *et al*., 2007). Caused testicular toxicity in male rodents (Akingbemi *et al*., 2001). Ovarian toxicity in female rats (Lovekamp-Swan and Davis 2003). Decreases serum estradiol levels and prolongs estrus cycles in adult female rats (Davis *et al*., 1994). Advances the onset of puberty and alters postpubertal reproductive functions (Ma *et al*., 2006). Exposure may histologically alter the uterus and induce endometriosis, hyperplasia, myoma, and developmental and reproductive toxicity (Cheon, 2020). A review of the hazardous effects of EDCs, including DEHP, on reproductive health (Ghosh *et al*., 2022). Reproductive and hepatotoxicity in rats (Ambe *et al*., 2019). Effects on human health (Arrigo *et al*., 2023). Lead to disruption in pubertal female rats and imbalance of hypothalamus-uterus. Negative influence on the development and functioning of the reproductive system in pubertal female rats (Liu *et al*., 2016).

The Risk of DEHP

Three phases can be used to explain the fundamentals of risk assessment: (1) Identification of hazards, including evaluation of dosage response; (2) Evaluation of exposure; and (3) Characterization of risks. Determining the No Observed Adverse Effect Level (NOAEL) is a crucial step in the first step of the hazard identification process. The threshold is based on research conducted on animals with the most delicate species and effects. The greatest dose at which no effects were recorded is referred to as the value. Step 2 of the exposure assessment is quite involved, especially for a chemical like DEHP that is used so extensively. Nonetheless, a number of assessment programs have already evaluated DEHP exposure. The EU Risk Assessment Report on DEHP provides information on exposure for workers, adults, children, and medically treated individuals. These results are employed in step 3 of the risk characterization process, where the Margin of Safety (MoS) is calculated by comparing the NOAEL from animal studies with human exposure levels. Box 3 is an example of such a calculation. When a chemical's MOS value is less than 100, it indicates that there is a danger and that it will cause an event in that specific scenario. These risk estimates are of tremendous relevance to the industry. They have attempted to impose an extremely high NOAEL on the effects of DEHP on testicles. As per the press release issued by European Council for Plasticisers and Intermediates (ECPI) on March 6, 2002, the NOAEL for rats was estimated from a multigeneration study by Wolfe *et al*., (2002) and was stated to be 100 mg/kg body weight each day. Remarkably, based on the same Wolfe *et al*. (2002) study, the European Union Scientific Committee on Toxicology (CSTEE, 2004) recently determined that the NOAEL,

which should be used in the risk assessment of testicular toxicity, should be 4.8 mg/kg body weight per day. The chemical industry's suggested NOAEL of 100 mg/kg will result in an extremely high MOS and almost no risk of impacts on testes. Thus far, the industry has not suggested lowering the exposure levels for children compared to the values determined by EU experts. This is concerning because the EU experts' value is frequently referred to as the most uncertain component in the risk assessment.

Animal Studies

While epidemiological studies are able to uncover potential associations between environmental contaminant exposure and adverse health outcomes (Table 2), they alone cannot establish causal links. Animal experiments are important because they allow for controlled exposure to known quantities of test chemicals in genetically similar or identical animals and thus allow for mechanistic insight that cannot be achieved through epidemiological studies. Therefore, animal studies are important to understand the potential relationship between phthalate exposure and adverse health outcomes and to provide insight into the effects of species and dosage characteristics. Thus, the effect of phthalate exposure on reproductive health in experimental animals was evaluated. While there are few studies on more phthalate diesters, there are more studies on phthalates. Table 3 shows the Lowest Observed Adverse Effect Level (LOAEL), No Observed Adverse Effect Level (NOAEL), and maximum adverse effect for each phthalate.

Table 2: Summary of the epidemiological literature exploring the link between phthalate exposure and adverse reproductive health outcomes in women

Outcome	Associations
Puberty	96.5±134 ng/mL MMP in cases of premature thelarche versus 26.4±30 ng/mL MMP in controls 68% of premature thelarche cases are associated with high levels of phthalates in serum Association with delayed pubic hair growth (mean age 11.4 years (11.1–11.7) in the fourth quartile of exposure compared to 10.7 years (10.4–11.0) in the first), no association with breast development No association with precocious puberty No association between exposure to extracorporeal membrane oxygenation in infancy and the onset of puberty DBP and DEHP were detected in the serum of more girls with precocious puberty (27.3 and 22.7% of cases with DBP and DEHP respectively compared to 4 and 3% of controls) High molecular weight phthalates associated with decreased development of pubic hair (prevalence ratio = 0.94 (0.88–1.00) p = 0.04); Low molecular weight phthalates non-significantly associated with more rapid breast development (first to fifth quintile: prevalence ratio =1.06 (0.99–1.14), p = 0.087)
Endometriosis	Higher plasma DEHP in cases compared to controls (p = 0.0047), no difference in plasma MEHP No significant association, MBP and MEHP are non-significantly higher in endometriosis (MBP: OR = 2.77 (0.68–11.3), MEHP: OR = 2.03(0.62–6.69)) No association (p = 0.23-0.90) Plasma MEHP associated with endometriosis (OR = 1.020 (1.003-1.038) p = 0.020); plasma DEHP was not associated with endometriosis (OR = 1.001 (1.000–1.002) p = 0.161) Plasma DBP, BBP, DEHP, and DnOP are associated with endometriosis (p<0.01) and with the severity of endometriosis (p<0.01) Plasma DBP, BBP, DEHP, and DnOP associated with endometriosis (p<0.05) MBP is non-significantly associated with endometriosis (OR = 1.36 (0.77–2.41)), MEHP is higher in controls (OR = 0.44 (0.19-1.02))
Leiomyomas	MEHP associated with leiomyomas (OR = 2.90 (1.05-7.97); p = 0.04), no association with other Metabolites MBP non-significantly higher in cases (OR = 1.56 (0.93-2.61); MEHP non-significantly higher in controls (OR = 0.63 (0.35-1.12))
Time to pregnancy	Probable occupational exposure associated with increased time to pregnancy (OR = 2.16 (1.02-4.57))
Pregnancy loss	Phthalate exposure associated with increased risk of spontaneous abortion MEHP associated with early pregnancy loss (third to first tertile: OR = 2.87 (1.09-7.57)), MEHP associated with reduced late pregnancy loss (third to first tertile OR = 0.25 (0.05-1.8))
Gestational age	MEHP, MEOHP, and MEHHP are associated with increased gestational age at birth (MEHP: OR = 2.0 (1.1-3.5), MEOHP: OR = 2.2 (1.3-4.0), MEHHP: OR = 2.1 (1.3-3.7)) Absence of MEHP associated with increased gestational age at birth (OR = 1.50 (1.013-2.21)) MEHP associated with decreased gestational age at birth (5d shorter (2.1-8d), p = 0.001 between fourth and first quartiles) Low-molecular-weight phthalates (β = 0.14 week (0.01-0.27 week)) and MEHP (β = 0.15 weeks (0.02-0.29)) associated with increased gestational age at birth
Preterm birth	No association (OR = 1.28 (0.39-4.20)) MBP associated with preterm birth (OR = 5.4 (1.5-19.3))

Table 2: Continue

Birth size	Occupational exposure to phthalates associated with low birth weight (OR = 2.42 (1.10-5.34))
	DBP associated with increased birth weight and length (birth weight p = 0.031; length p = 0.018 between first and fourth quartiles)
	No association with birth weight, length, or head circumference
	No association with birth weight, length, or head circumference
	Low-molecular-weight phthalates associated with increased head circumference (β = 0.13 cm (0.01-0.24 cm) with increases in concentration), MBzP associated with increased length (β = 0.20 cm (0.00-0.40)), MEP associated with increased head circumference (β = 0.12 cm (0.01-0.23))
	DBP and MEHP associated with low birth weight (DBP: OR = 3.54 (1.54-6.15), p = 0.008; MEHP: OR = 2.05 (1.17-3.70), p¼0.05), no associations with DEP or DEHP
Breast cancer	No association with occupational exposure to BBP
	MEP and MECPP associated with breast cancer (MEP OR = 2.20 (1.33-3.63), p = 0.003), MECPP: OR = 1.68 (1.01-2.78), *p* = 0.047); MBP and MCPP associated with decreased risk of breast cancer (MBzP: OR = 0.46 (0.27-0.79), *p* = 0.008, MCPP OR = 0.44 (0.24-0.80)

Unless otherwise noted, phthalate exposure was estimated by measurement of phthalate metabolites in urine

Table 3: Summary of the NOAELs and LOAELs in mg/kg/d as well as the direction of change for each outcome, respectively, found in animal studies for each individual phthalate

Phthalate	Maternal weight gain	Infertility	Resorptions	Litter size	Pup weights	Irregular estrous cycle	Sexual maturation #	Circulating progesterone	Circulating estradiol	Ovarian weight	Ovarian histology references
DMP	NR *	2 mL/kg	2 mL/kg	2 mL/kg	NR	NR	NR	NR	NR	NR	NR
		–	–	–							
		NC **	NC	NC							
DEP ppm	600 ppm	15000 ppm	15000 ppm	1.25%	2.5%	15000 ppm	–	NR	NR	15000 ppm	15000
	–	–	–	2.5%	–	–	15000 ppm			–	–
	↑	NC	NC	NC	NC	NC	↑			NC	NC
DPrP	1000	1.25%	1500	1.25%	500	5%	NR	NR	NR	NR	5%
	1500	2.5%	–	2.5%	1000	–					–
	↓	↑	NC	↓	↓	NC					NC
DBP	12	12	250	250	–	1000	–	250	1500	1000	1500 ***
	50	50	500	500	12	–	12	500	–	1250	–
	↓	↑	↑	↓	↓	NC	↑	↓	NC	↓	NC
DiBP	250	1000	500	500	250	NR	NR	NR	NR	NR	NR
	500	–	750	750	500						
	↓	NC	↑	↓	↓						
BBP	167	250	375	500	250	500	120	–	500	100	1000 ***
	250?	500	500	625	375	–	500	2%	–	500	–
	↓	↑	↑	↓	↓	NC	↑	↓	NC	↓	NC
DPP	100	–	NR	–	300	2.5%	NR	NR	NR	NR	2.5%
	300	0.5%		0.5%	–	–					–
	↓	↑		↓	NC	NC					NC
DnHP	500	0.6%	500	–	250	NR	NR	NR	NR	NR	NR
	750	1.2%	625	0.3%	500						
	↓	↑	↑	↓	↓						
DcHP	240 ppm	6000 ppm	6000 ppm	6000 ppm	1200 ppm	1200 ppm	500	NR	6000 ppm	500	NR
	1200 ppm	–	–	–	6000 ppm	6000 ppm	–		–	–	
	↓	NC	NC	NC	↓	↑	NC		NC	NC	
DEHP	375	5	100	100	–	5 mg/m^3	5	–	–	–	–
	750	500	500	500	0.05	25 mg/m^3	15	500	500	0.05	300
	↓	↑	↑	↓	↓	↑	↑¶	↓	↓&	↑	↓@
DHPP	1000	1000	1000	1000	1000 $	NR	NR	NR	NR	NR	NR
	–	–	–	–	–						
	NC	NC	NC	NC	NC						
DiHP	300	4500 ppm	300	300	300	8000 ppm	8000 ppm	NR	NR	4500 ppm	8000 ppm
	750	8000 ppm	750	750	750	–	–			8000 ppm	–
	↓	↑	↑	↓	↓	NC	NC			↓	NC
DnOP	5%	5%	1000	5%	5%	5%	NR	NR	NR	NR	NR
	–	–	–	–	–	–					
	NC	NC	NC	NC	NC	NC					
DiNP	0.5%	900	900	–	600 ¥	NR	900	NR	NR	1%	NR
	1%	–	–	0.2%	750		–			1.5%	
	↓	NC	NC	↑	↓		NC			↓	
DiDP	0.4%	0.8%	NR	0.8%	0.4%	0.8%	0.2%	NR	NR	0.4%	0.8%
	0.8%	–		–	0.8%	–	0.4%			0.8%	–
	↓	NC		NC	↓	NC	↑			↓	NC
D79P	1000	1000	1000	1000	0.5%	1%	NR	NR	NR	0.5%	1%
	–	–	–	–	1%	–				1%	–
	NC	NC	NC	NC	↓	NC				↓	NC

Table 3: Continue

D911P	1000	1000	1000	1000	0.5%	1%	1%	NR	NR	1%	1%
	–	–	–	–	1%	–	–			–	–
	NC	NC	NC	NC	↓	NC	NC			NC	NC
DAP	150	405	405	405	150	NR	NR	NR	NR	NR	NR
	200	–	–	–	200						
	↓	NC	NC	NC	↓						

* - Not Reported;
** - No Change
# - Where ↑ is a delay in the onset of vaginal opening
*** - Based on the number of corpora lutea
? - MBzP, the metabolite of BBP
¶ - Another study reported earlier vaginal opening after inhalation of 5 and 25 mg/m^3
& - Another study reported increased estradiol after inhalation of 5 and 25 mg/m^3
@ - Vacuolization of stromal cells
$ - A trend for decreased pup weight at 1000 mg/kg/d
¥ - Another study reported an increase in male pup weights at a dose of 250 mg/kg/d

Effect of Phthalate on the Female Reproductive System

It is widely believed that the female reproductive system is far less responsive to phthalates than the male reproductive system. However, new research indicates that phthalates may potentially cause negative effects in females after exposure during pregnancy and lactation (Grande *et al*., 2006). Early research showed that the ovary is one of DEHP's target organs. According to Davis *et al*. (1994), adult rats given a high dose of DEHP (2,000 mg/kg/day) had longer estrous cycles, lower blood estradiol levels, and no ovulation. According to Hirosawa *et al*. (2006), long-term exposure to DEHP caused chronic diestrous with lower levels of Follicular Stimulating Hormone (FSH), pituitary FSH, Luteinizing Hormone (LH), and serum estradiol. Active phthalates-like DEHP have been shown in multiple fertility studies using crossover breeding to decrease rat and mouse fertility through effects mediated by both sexes.

DEHP as a Developmental and Reproductive Toxicant

DEHP is a well-known developmental and reproductive toxin that affects both sexes equally and appears to elicit toxicity in females at a later age than in males (Hoyer, 2001). DEHP and its primary metabolite, Mono-2-Ethylhexyl Phthalate (MEHP), seem to target similar locations in the ovary (granulosa cells) and testis (Sertoli cells). DEHP inhibits the transmission of the Follicle-Stimulating Hormone (FSH) signal, making it a testicular toxicant in males (Park *et al*., 2002). Its target is most likely the Sertoli cell membrane (Heindel and Chapin, 1989). Hormonal disruption of the developing fetal testis is one potentially harmful consequence of the male reproductive system (NCP, 2003). Testis development and hormone synthesis by the fetal testis are the two main factors that determine whether a fetus develops into a phenotypic male (Sharpe, 2001). For instance, chemical exposures that may interfere with the development or action of these hormones can partially or totally inhibit masculinization. These exposures must affect the production and activity of hormones, specifically androgens and anti-mullerian hormones. Testicular abnormalities during fetal or neonatal life can have long-term effects on all facets of adult reproductive function, including sperm counts (Sharpe, 2001). Furthermore, the developmental stage of exposure affects the effects of DEHP on Leydig cell steroidogenesis (Akingbemi *et al*., 2001). While development along this pathway is still subject to hormonal disturbance, such as the masculinization of the female fetus by exposure to androgens, the development of a fetus into a female is essentially hormone-independent, in contrast to that of a male (Sharpe, 2001). DEHP modifies the ovary's synthesis of estradiol via a receptor-mediated signaling route via its metabolite MEHP (Lovekamp-Swan and Davis, 2003). Consequently, DEHP is the most potent phthalate that disrupts hormones and the most significant phthalate in terms of manufacture, use, incidence, and omnipresence.

Sexual Hormone Alterations

The effects of DEHP at a dose of 500 mg kg^1 on sex hormones in rats from the day of gestation (day 7) to the day of lactation (day 2) were investigated. The results showed that DEHP had no effect on testosterone in offspring (Botelho *et al*., 2009).

Mechanisms of Steroid Action

The main steroid effect on cells is mediated by genomic action (Sever and Glass, 2013). However, there is also a known rapid non-genomic mechanism of action by activating membrane receptors and protein kinases in signaling pathways (Wierman, 2007) and by epigenetic processes as well (Piferrer, 2013).

Genomic Action of Steroid Hormones

Nuclear Receptors (NRs) are the intermediates in the genomic mechanism of steroid action. Nuclear NRs fall into two primary categories: Type I and Type II. The term "type I" or "steroid hormone receptors" refers to the mineralocorticoid, glucocorticoid androgen, progesterone and its three isoforms (PR-A, PR-B, and PR-C), as well as the two types of Estrogen Receptors (ERβ and ERβ) (Liu *et al*., 2017b). Thyroid hormone, vitamin D, retinoic

acid, Peroxisome Proliferator-Activated Receptor (PPAR), and other type II or non-steroid hormone receptors are among them (Liu *et al*., 2017b). Estrogen-related receptors (ERRα, ERRβ, and ERRγ) are located between orphan receptors receptors with an unidentified ligand (Giguère, 2002). The NR signaling pathway is made up of the following particular steps: The ligand attaches to the Ligand-Binding Domain (LBD) of NR in the cytoplasm or nucleus after passing through the cytoplasmic membrane. The receptor is associated with Heat-Shock Proteins (HSPs) in an inactive state prior to the ligand binding to the NR. This complex is activated and dimerizes when the ligand attaches to the receptor. Without HSPs, the ligand and receptor go into the nucleus. They attach themselves to a DNA sequence found in particular gene promoters called the Hormone Response Elements (HREs). When coactivators attach to the promoter regions, gene expression begins (Puzianowska-Kuznicka *et al*., 2013).

Non-Genomic Action of Steroid Hormones

When paired with NRs, steroid hormones can trigger the activation of cytoplasmic protein kinases linked to many signaling pathways, Including Phosphoinositide 3-kinase (PI3k/Akt), calcium, MAPK, Nuclear Factor kappa B (NF-kB) and cyclic Adenosine Monophosphate (cAMP). Steroids can occasionally bind to membrane receptors that are not intended for steroid binding, such as cytokine receptors and G Protein-Coupled Receptors (GPCRs) (Heinlein and Chang, 2002). In certain instances, steroids attach themselves to membrane receptors that are intended to bind steroid hormones as well. Examples of these receptors are membrane Progesterone Receptors (mPRα-Σ) (Valadez-Cosmes *et al*., 2016), membrane estrogen receptors (mERα, mERβ, GPER, ER-X, ERx and Gq-mER) (Soltysik and Czekaj, 2013) and membrane androgen receptors (GPCR6 and ZIP) (Kamińska *et al*., 2020). Membrane androgen receptors GPCR6 and ZIP9 control the function of Sertoli cells in men by non-classical testosterone signaling; additionally, they are linked to the initiation of breast and prostate cancer (Kamińska *et al*., 2020). In males, GPER and ER control the activity of Leydig cells (Kotula-Balak *et al*., 2018). However, according to Luo and Liu (2020), GPER is linked to cancers of the testicles, breast, ovarian, endometrial, and prostate, among other reproductive tissues. According to Valadez-Cosmes *et al*. (2016), the membrane progesterone receptor controls the development of oocytes, labor, sperm motility, and the start of cancer in the reproductive organs. The following describes how membrane steroid receptors primarily mediate the steroid effect through cellular proliferation, death, and metabolic processes. GPCRs are the primary stimulators of the cAMP pathway. Critical physiological functions such as muscular contraction, calcium homeostasis, metabolism, secretion, and gene transcription are all regulated by it (Podda and Grassi, 2014). Calcium is one of the signal molecules that control a wide range of cell processes, including myosin ATPase activation during muscle contraction (Wakabayashi, 2015), sperm capacitation during fertilization (Navarrete *et al*., 2015), the release of neurotransmitters to synapses (Südhof, 2012) and the control of the apoptotic process linked to calpains, which are proteases that depend on Ca^{2+} levels (Momeni, 2011). Cell division and apoptosis are controlled by the PI3k/Akt signaling pathway. According to Aksamitiene *et al*., (2012), Akt stimulates a number of target proteins that are involved in protein synthesis, metabolism, and cell determination. Serine-threonine kinases, known as MAPKs, control a number of cellular functions related to cell determination. The MAPK signaling pathway is activated by receptors for cytokines and growth factors, including Transforming Growth Factor (TGF). According to Plotnikov *et al*. (2011), the majority of the signaling pathways linked to steroid signaling are related to metabolism and the control of cell division and proliferation. Biological processes such as immunological response, inflammation, cell proliferation, survival, and development are all impacted by the transcription factors that NF-B generates (Williams *et al*., 2014).

Changing the Oxidative Stress Parameters

A recent study investigated the effect of DEHP on antioxidant enzyme activity. Initially, DEHP (10 μg mL^{-1}) increased SOD activity at 24 h, but its level was lower at 72 and 96 h. GPX and CAT activities did not change (Wang *et al*., 2012a). Additionally, the effect of MEHP at 0.1, 1, 10, and 100 μg mL1 on antioxidant activity was also evaluated by the test. GPX activity was found to decrease after 96 h at 1, 10, and 100 μg/mL and at 0.1 μg/mL. CAT activity increased at two doses: 0.1 and 1 μg/mL. In addition, when a dose of 100 μg mL1 was given, SOD activity decreased and when a dose of 10 μg mL1 was given, it increased (Wang *et al*., 2012b). Additionally, the effect of DEHP on MA-10 was examined and the antioxidant enzyme was measured in Leydig cells. A decrease in GPX, TrxR, and GST activities was observed in both DEHPs (Erkekoglu *et al*., 2010). Total GSH levels also decreased. On the other hand, the toxicity of DEHP in rats was evaluated (Botelho *et al*., 2009). The animals were fed with a dose of 500 mg/kg DEHP from the day of gestation (day seven) until the day of lactation (day two) and the antioxidant enzyme levels of the offspring were measured. An increase in CAT activity and a decrease in GST activity were noted. Evidence shows that the effects of DEHP have been evaluated in rats (Kasahara *et al*., 2002). Animals were administered 1 and 2 g/kg DEHP per day in drinking water for 7 days. Decreases in free thiols, GSH, and ascorbic acid were reported at all doses. Additionally, CAT and GPX activities increased at all doses.

Alterations in Gene Expression of the Antioxidant Enzymes

Data showing the effect of DEHP on the anti-inflammatory enzyme were examined (Wang *et al*., 2012a). It was noted that SOD1 expression decreased. The results showed that SOD1 and GPX expression decreased at 100 µg/mL. A decrease in antiapoptotic factors (Bcl-2) and an increase in proapoptotic factors (Bax) were observed at three doses: 1, 10, and 100 µg mL^{1}. Moreover, at the same dose, the expression of the cell cycle genes also decreases.

Regulators of Reproduction

The development and functions of the reproductive system are under the control of numerous genes. Hormones produced by the Hypothalamic Pituitary Gonadal (HPG) axis regulate their expression (Acevedo-Rodriguez *et al*., 2018).

Hypothalamic-Pituitary-Gonadal (HPG) Axis

Hypothalamic neurons produce neural signals as well as neurohormones like kisspeptin, leptin, and others in the arcuate nucleus and periventricular region (Harter *et al*., 2018). They control the synthesis of Gonadotropin-Releasing Hormone (GnRH). GnRH released from the hypothalamic preoptic area stimulates the anterior pituitary GnRH receptors (GnRHRs). These receptors promote the secretion of anterior pituitary gonadotropins, Luteinizing Hormone (LH), and Follicle-Stimulating Hormone (FSH) via the Mitogen-Activated Protein Kinase (MAPK) and cyclic Adenosine Monophosphate (cAMP) signaling pathways (Casarini and Crépieux, 2019). FSH triggers the growth and development of ovarian follicles in the ovaries and sperm production in the testis. FSH and LH stimulate the secretion of sex steroids and protein hormones, such as anti-Müllerian hormone and insulin-like peptide 3, as well (Jones and Lopez, 2006). The anti-Müllerian hormone is essential for the inhibition of Müllerian duct development in males (Xu *et al*., 2016) and the inhibition of multiple ovarian follicles' development in females (Grynnerup *et al*., 2012). Insulin-like peptide 3 is vital for testicle descent (Bay *et al*., 2011). The production and function of steroid hormones will be discussed in the next chapter. The HPG axis regulates the levels of reproductive hormones via a positive and negative feedback loop. Higher levels of hormones from gonads inhibit the secretion of GnRH from the hypothalamus and gonadotropins from the adenohypophysis. The positive feedback loop is needed before ovulation when high levels of estradiol stimulate LH secretion from the adenohypophysis (Jones and Lopez, 2006). One of the main functions of the HPG axis is the regulation of steroidogenesis. The next chapter is focused on the characterization of the steroidogenic process.

Steroidogenesis

The first step in the process of steroidogenesis is cholesterol synthesis. Cholesterol is the precursor molecule for all steroidogenic hormones, including sex hormones. The essential enzymes for steroidogenesis include P450 and HSD enzymes from the mitochondria and endoplasmic reticulum. These enzymes can convert steroidal precursors to final hormones such as testosterone, dihydrotestosterone, progesterone, and estradiol (Miller and Auchus, 2011). LH binds to the LH Receptor (LHR) of thecal cells (in the case of ovarian follicles) and Leydig cells (in the case of the testis). FSH binds to the FSH Receptor (FSHR) of granulosa cells in the ovarian follicles. In these cells, FSH and LH activate a broad spectrum of signaling pathways leading to the gene expression of steroidogenic enzymes and the conversion of steroids to final products (Casarini and Crépieux, 2019). Testosterone and dihydrotestosterone are the leading male masculinization hormones. Testosterone is essential for the morphological differentiation of the internal genital organs and maintaining spermatogenesis. Dihydrotestosterone is an androgen that is 10-fold more potent than testosterone and is associated with the differentiation of male external genitals and male secondary sexual characteristics. However, androgens are vital for follicle maturation in females. Estradiol and progesterone are essential hormones for postnatal female reproductive system development and enable the ovarian and menstrual cycles, pregnancy, and labor. Estradiol is needed to maintain spermatogenesis in males as well (Jones and Lopez, 2006). In general, steroid hormones have proliferative features. They are crucial for germ cell proliferation, leading to sperm production and ovarian follicles' development (Knapczyk-Stwora *et al*., 2018). Moreover, sex steroids induce cell proliferation in non-reproductive tissue, such as the bladder (Liang *et al*., 2013), precursors of myotubes (Lee *et al*., 2010), or neural stem cells (Bramble *et al*., 2019). Furthermore, sex steroids have anti-apoptotic properties. Data show that after castration and during hormone insufficiency, the apoptotic process occurs. This process occurs mainly in hormone-dependent tissues such as the prostate gland, uterus, oviduct, and mammary gland (Thompson, 1994). Steroid hormones need to be transported with transport proteins in the bloodstream to reach this hormone-dependent tissue (Hammond, 2016). Thus, the next chapter is focused on the characterization of the most significant transport protein for sex steroids.

Sex Hormone-Binding Protein (SHBG)

Most of the sex steroid hormones are transported in an inactive bind with transport proteins, such as SHBG. The relative binding affinity of some sex steroids for SHBG is as follows: Dihydrotestosterone, testosterone, androstenediol, estradiol, and estrone. SHBG levels elevate when testosterone decreases and when estradiol

increases (Somboonporn and Davis, 2004). SHBG transports steroids to target tissues, where they can act through numerous mechanisms. The next chapter is focused on the definitions of the primary mechanisms of steroid action.

Hormonal Mechanisms of Phthalates' Action on Reproductive Functions and Health

Reproductive disorders caused by phthalates are associated with HPG axis dysregulation at different regulatory levels. At the hormonal level, phthalates interact with steroidogenic enzymes and hormones (Hannon and Flaws, 2015) as well as with SHBG (Sheikh *et al*., 2016). Phthalates' Effect on the Hypothalamic Pituitary Gonadal (HPG) Axis and Steroidogenesis Phthalates interfere with the regulation of the HPG axis. They alter the levels of GnRH, LH, and FSH. This leads to the disturbing activity of steroidogenic enzymes and their subsequent effects on steroid hormones. The results from *in vitro* and *in vivo* studies are shown in Table 4.

Effects on Reproduction

In earlier studies, the reported dose-related deleterious effects of DEHP on the developing rat embryo and fetus. Such effects included resorption, gross abnormalities, skeletal malformations, and fetal growth retardation. Shortly thereafter, it was reported that DEHP, administered early in gestation in rats, reduced the number of implantations and increased morbidity and mortality at parturition. At parturition, DEHP-treated rats were noted to experience excessive bleeding and fetal retention when compared with controls. Nikonorow showed a significant reduction in placental weights in treated rats. Dosage levels used approximate administration of pure DEHP 1.5 L (an equivalent total dosage of 1.48 kg) to a 50 kg woman. The maximum dose of plasma solubilized DEHP that can be achieved in humans by a total exchange transfusion of 21-day-old banked blood is approximately 4 mg/kg. In fact, the studies showed that normal saline solution and cottonseed oil produced a higher percentage of skeletal abnormalities' than did DEHP. A lack of teratogenicity using plasma-soluble extracts (vs. pure DEHP) of PVC plastics injected intravenously into pregnant rats. Compared with controls, no differences were observed in the growth rate of dams, litter size, pup weight, or viability. Observed incidences of gross external, skeletal, and visceral defects were similar in all groups. Shiota demonstrated that high doses of DEHP (~190 mg/kg/d) could be embryotoxic and possibly teratogenic in mice. The maximum "no effect" level of DEHP on mouse fetuses was 70 mg/kg/d. Lewandowski administered DEHP intravenously to pregnant rats at levels predicted to be received by a 60-kg human undergoing an exchange transfusion of 21-day-old blood.

Table 4: Phthalates effects on Hypothalamic Pituitary Gonadal (HPG) axis and steroidogenesis (*in vivo, in vitro* experiments)

Phthalates	Dose effect (mg kg day)	Animal/cell model	Time of exposure	Effect
DEHP	300	Sprague-Dawley rats	Prenatal	↓ estradiol
DEHP	1,50, or 300	Sprague-Dawley rats	Prenatal	↑ FSH
	300			↓ estradiol
DEHP	300	Sprague-Dawley rats	Postnatal	↓ pregnenolone, progesterone
DEHP	30	Wistar rats	Prepubertal	↑ LH
DEHP	1000 and 3000	Wistar rats	Postnatal	↑ GnRH
DEHP	3000			↓FSH, LH, estradiol, progesterone, testosterone
DEHP	1000 and 500	Wistar rats	Postnatal	↑ GnRH
DEHP	1,10,100 µg/mL	Mouse antral follicles (CD-1 mice)	Postnatal	↓Progesterone, dehydroepiandrosterone, androstenedione, testosterone, estradiol
	1,10,100 µg/mL			↓ Cyp11a1
	100 µg/mL			↓ Cyp17a1
	10 µg/mL			↑ Cyp19a1, Hsd17b1
	100 µg/mL			↓ Cyp19a1, Hsd17b1
	100 µg/mL			↑ Hsd3b1
DEHP	500 and 750	CD-1 mice	Prenatal	↑ estradiol (F1 generation)
	20			↑ estradiol (F3)
	500			↓ testosterone (F1)
	20			↓ testosterone (F2)
	20 and 500			↓ testosterone (F3)
	200			↓ progesterone (F2)
	500			↓ FSH (F1)
	500			↑ FSH (F3)
	20			↑ LH (F1)

No significant differences were demonstrated between the control and treated groups. Although animal data using dosage levels of DEHP equivalent to those in the clinical setting imply that accumulation in human embryonic tissues is not likely, the final evaluation will depend on the actual determination of tissue levels of DEHP in human embryos and fetuses and on the comparison of human fetal tissue levels with those found in experimental animals treated with "no effect" dosages. Currently, the acceptable level of in-utero exposure in humans is unknown. In addition, it is important to note that Shaffer, Lake, and Seth have shown DEHP-induced testicular degeneration in orally fed rats and ferrets, respectively. This effect was not observed in mice (Kaul *et al*., 1982).

Phthalates Action on Female Reproductive Health

The phthalate's effect on female reproductive health is mostly during the prenatal period but also during postnatal ontogenesis. This effect will be discussed in the next chapters.

Phthalates Can Influence Ovarian Function

In vivo and *in vitro* studies have pointed to the endocrine-disrupting properties of phthalates in females. These studies observed phthalates' effects on ovarian function in female rats and mice. Phthalates modified the follicular development by inhibiting antral follicle development and decreasing the number of antral follicles in mice (mixture of EDs-DEHP, DBP, BBzP, and two alkylphenols at 1 and 10 mg/kg) (Patiño-García *et al*., 2018), stimulating follicle development in mice (MEHP at 500 and 1000 mg/kg) (Moyer and Hixon, 2012) and decreasing the number of follicles in mice (DEHP at 200 and 500 g/kg) (Hannon *et al*., 2015b). Moreover, exposure to DEHP at environmentally relevant doses affected oocyte growth, maturation, and ovulation in females of Danio rerio (Carnevali *et al*., 2010). In Caenorhabditis elegans, exposure to DEHP at 10 mg/L decreased the number of oocytes and induced DNA damage in oocytes (Yin *et al*., 2018). *In vitro* experiments showed that oocytes from DEHP-exposed female mice at 20 and 40 µg/kg/day induced defects in oocyte meiosis (Zhang *et al*., 2013b). Then, an *in vitro* experiment showed similar results, with exposure to 10 and 100 µM of DEHP inhibiting meiotic progression in mice (Liu *et al*., 2017a). Oocyte meiosis was altered in female mice's progeny (DEHP at 20 µg/kg/day) (Mirihagalle *et al*., 2019). Zhang *et al*. (2014) showed that *in vitro* exposure to 10 and 100 µM of DEHP decreased germ cell nest breakdown in newborn mouse ovaries. A review by Zhang *et al*. (2016) summarized the effects of DEHP exposure on oogenesis and folliculogenesis. Data showed that DEHP induced altered development of the primordial germ cells, germ cell survival, meiotic progression, and increased follicle atresia. In addition, DEHP disturbed the maturation and activation of oocytes before fertilization via meiotic maturation inhibition and oxidative stress (Lu *et al*., 2019). Exposure to DEHP at 25 mg/m^3 by inhalation (Ma *et al*., 2006) and prenatal exposure to a mixture of DEP, DEHP, DnBP, DiNP, DiBP, and BBzP at 20, 200, and 500 mg/kg impaired the estral cycle, particularly ovulation and estradiol synthesis in rats and mice (Zhou *et al*., 2017). The imbalance of the hypothalamic-pituitary-ovarian axis further negatively affected the development and function of the reproductive system of female progeny (Liu *et al*., 2014). These effects of exposure to the mixture of phthalates were observed in the second and third generations of the progeny of mice (Zhou *et al*., 2017). Similar results occurred in the progeny of Caenorhabditis elegans after prenatal exposure to DEHP at 20 mg/L (Li *et al*., 2018). Therefore, we can assume that phthalate exposure has a transgenerational as well as a multigenerational effect on fertility in female animal models (Zhou *et al*., 2017).

Phthalates Can Induce Premature Ovarian Failure (POF)

POF in women is the condition when ovarian function terminates before 40 years of age. POF consists of symptoms such as amenorrhea, increased gonadotropin levels, and decreased estradiol (Ayesha and Goswami, 2016). *In vivo* studies noticed that exposure to DEHP and mixtures of EDs induced POF in mice (Patiño-García *et al*., 2018). Epidemiological studies have noticed consistent results in women. The anti-estrogenic activity of phthalates acts by inhibiting estradiol production in the ovary, leading to anovulation and premature ovarian insufficiency (Vabre *et al*., 2017). Higher DEHP exposure was associated with a higher risk of decreased ovarian reserve in 215 women visiting the Fertility Center at the Massachusetts General Hospital, USA (Messerlian *et al*., 2016a). The study by Gallicchio *et al*. (2009) observed that occupational exposure to EDs led to a five-fold higher risk of POF in hairdressers compared with control subjects limited to Caucasian women only. The possible mechanism of phthalates' action on developing POF is that DEHP can increase the FSH level (Meltzer *et al*., 2015), which is associated with a high rate of follicle growth and subsequent premature ovarian depletion (Jankowska, 2017).

Phthalates Can Induce Dysfunctions of Pregnancy

Phthalates affect the length and process of pregnancy. *In vivo*, studies observed that DEHP exposure at 250 and 500 mg/kg (Zong *et al*., 2015), at 50 and 200 mg/kg (Yu *et al*., 2018), and at 20, 200 and 500 mg/kg (130) during pregnancy in mice inhibited placental angiogenesis (Yu *et al*., 2018) and induced miscarriage and obstructed labor in the next generation, respectively (Ma *et al*., 2006). Results from *in vivo* studies are consistent with epidemiological studies. Toft *et al*. (2012); Messerlian *et al*., (2016a) noticed that high exposure to DEHP was associated with the spontaneous abortion of 303 pregnancies from a

fertility center in the USA (Messerlian *et al*., 2016b) and 430 pregnancies from Denmark (Toft *et al*., 2012). A similar pattern was observed in the case-control studies of Yi *et al*., (2016); Liao *et al*., (2018). A study by Yi *et al*. (2016) included women aged 22-35 from Shanghai in 150 matched pairs of case-controls. This study reported significantly higher levels of monomethyl phthalate (MMP) and MEHP among women with pregnancy loss (Yi *et al*., 2016). A study by Liao *et al*. (2018) involving women aged 20-49 from Taiwan was divided into case (n = 103) and control groups (n = 76). This study reported significantly higher levels of P-DBP among women with recurrent miscarriage (Liao *et al*., 2018). In the late stage of pregnancy, increased levels of P-DEHP had a protective effect against miscarriage (Toft *et al*., 2012). A US study of 283 pregnant women showed that urinary levels of P DEHP during pregnancy caused birth after the 41st gestational week or increased the probability of the section (Adibi *et al*., 2009). Then, a US nested case-control study by Ferguson *et al*. (2014) involved 130 pregnancies with preterm birth and 352 controls from Brigham and Women's Hospital, Boston, Massachusetts. This study reported the opposite phenomenon: Exposure to DEHP was associated with a risk of preterm birth. Similarly, Latini *et al*. (2003) conducted a study involving 84 newborns (39 males and 45 females) with an average gestational age of 38.4±2.2 weeks. This study observed that higher levels of MEHP in cord blood were associated with decreased gestational age at delivery. The reason for the conflicting results is that phthalates and their metabolites, such as DEHP and mono-2-ethylhexyl phthalate MEHP, can modulate both PPAR (Shoaito *et al*., 2019) and prostaglandins (Adibi *et al*., 2009). PPARs are necessary for maintaining a pregnancy. DEHP and its metabolites could bind to PPAR and prevent the maternal-fetal communication that allows birth to be initiated (Shoaito *et al*., 2019). Prostaglandins are signaling molecules; EHP would, thus, be classified as "practically nontoxic" by the toxicity rating system of Gleason and her colleagues. That induces contractions of the uterus, leading to birth or abortion. DEHP stimulates the secretion of prostaglandins, which could induce spontaneous abortion or preterm birth (Pinkas *et al*., 2017).

Toxicology

The toxicity of DEHP could be due to: (1) The alcohol; (2) Phthalic acid; (3) Ester; or (4) A combination of these factors. DEHP has an extremely low order of toxicity; in several animal models, doses of ~50 g/kg are required to produce an LD50. DEHP would, thus, be classified as "practically nontoxic" by the toxicity rating system of Gleason and her colleagues. This toxicity data cannot be extrapolated to human infant use. Studies of the toxicity of DEHP in rats, mice, guinea pigs, ferrets, and dogs have revealed toxic effects, as summarized in Table 5 (Kaul *et al*., 1982). Comprehensive reports have been issued recently that provide in-depth evaluations of the toxicity of DEHP, in particular, the European Union risk assessment report and the NTP-CERHR expert panel update on the reproductive and developmental toxicity published in 2006. SCENIHR has carefully considered these summary documents along with new pertinent original publications.

Animal Studies

Acute toxicity Acute toxicity studies of good quality indicate low acute toxicity of DEHP, with an LD50 of >25 g/kg in rats and mice. The intravenous acute toxicity of DEHP is higher, with an LD50 in the region of 200-250 mg/kg in rats. The acute toxicity of MEHP is about five times higher than that of DEHP (Kavlock *et al*., 2006).

Repeated Dose Toxicity

Numerous studies investigated the toxicity of DEHP upon short-term and repeated administration to experimental animals, mostly rats, and with application by the oral route. Many of these studies are comparable to guideline studies and conducted in conformity with GLP. The target organs for DEHP-induced toxicity in rodents were the kidney, liver, and testis. The effects on the kidneys included increased absolute and relative organ weights, increased incidence and severity of mineralization of the renal papilla, increased incidence and/or severity of tubule cell pigment, and increased incidence and/or severity of chronic progressive nephropathy. In long-term studies in rats and mice, there was no indication that DEHP-related changes in the kidney were reversible upon cessation of DEHP exposure. The lowest NOAEL for kidney toxicity is 500 mg/kg DEHP in the feed (corresponding to 28.9 mg/kg/day in the males and 36.1 mg/kg/day in the females), derived from a well-performed 104-week study in rats (David, 2000) and based on increased absolute and relative kidney weight in both sexes at the next higher dose level (LOAEL = 146.6 mg/kg bw/day). More severe kidney lesions were observed at the highest dose level. The most striking effects observed in the liver are hepatomegaly due to hepatocyte proliferation (characterized by increased replicative DNA synthesis/cell division and hypertrophy), peroxisome proliferation, and hepatocellular tumors. The effects on the liver (hepatomegaly) are apparently mediated by the peroxisome-proliferator-activated receptor (PPARα) and the agonistic interaction of DEHP and its metabolite MEHP with the receptor. There are, however, marked species differences in the PPARα-mediated effects of DEHP, such that the hepatotoxic effects of DEHP in rodents are not judged to be relevant for humans (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2018). In a repeated exposure study, 16 rats were pre-treated with 100 mg/m^3 of aerosol for 2 weeks (6 h per day, 5 days per week). The study indicates that following repeated inhalation exposure, long-term retention does not occur. There are no other relevant studies in rodents investigating the health effects of the respiratory tract.

Table 5: Possible toxic effects of DEHP on reproduction

Species	Route of administration	Dose	Age	Day of gestation	Duration of study	Effects
Rat	po	1.5-3% concentration (0.4 g/kg/d)		_	90 d	Slight growth retardation, tubular atrophy, degeneration of testes
Rat	po	0.4% concentration	60 days	_	up to 2 years	Reduction in body weight, increase in liver and kidney weight
Guinea pig	po	0.13% concentration	49 days	_	1-year	Increasing liver weight of females
Pregnant rat	Ip*	9.85 mg/kg/d ×3 ¥		5,10,15	_	Fetal resorption, twisted hind legs
Adult virgin mice	Ip	25.18 mg/kg ×1 prior to Mating ¥	8-10 week	_	_	Reduced number of fertilizations and implantations, increased fetal deaths
Pregnant rat	Ip	1.97 and 3.94 mg/kg/d ×3 ¥		3,6,9	_	reduced number of implantations, retained fetuses, maternal death, and/or excessive bleeding at parturition, reduced number of pups weaned per litter
Pregnant rat	iv	1 and 3.7 mg/kg/d		6-15	10 d	None observed
Rat	po	0.34 and 1.7 g/kg/d		1-21	_	Decreased fetal weight 22 increased fetal resorption, endometritis, increased liver weight of dams
Ferret	po	1% concentration (weight/weight)	18 months	_	14 mo	Testicular degeneration, increased liver weight, reduced body weight
Adult male rats	ip	4.93 mg/kg on experiment days l,5,10 ¥		_	_	Focal degeneration of seminiferous tubules and edema of interstitium in testes
Prepubescent female rats	ip	4.93 mg/kg on experiment days l,5,10 ¥	4-6 weeks	_	_	No alterations in the ovary
Mouse	po	0.4% and 1% (weight/ weight) ('~ 830 and 2200 mg/kg/d)	_	0-18 d	_	All implanted ova died in utero
		0.2% ('~ 400 mg/kg/d)	_	0-18 d	_	Increased malformations (neural tube defects)
		0.1% ('~ 190 mg/kg/d)	_	0-18 d	_	More fetal resorptions and dead fetuses vs. control
		0.05% ('~ 70 mg/kg/d)	_	0-18 d	_	No effect
Pregnant rats	iv	1.3 mg/kg/d	_	6-15 d	_	No effect on numbers of live and resorbed
		4.7 mg/kg/d		6-15 d		foetuses nor on fetal weights and sizes

*Intraperitoneal
¥ tconverted from ml of pure DEHP (specific gravity 0.985 at 20°C)

DEHP and Endocrine Toxicity

DEHP is best known as an Endocrine Disruptor (ED). An endocrine disrupter is an exogenous substance or mixture that alters the functions of the endocrine system and consequently causes adverse health effects in an intact organism, its progeny, or subpopulations (Nohynek *et al*., 2013). In utero DEHP exposure diminishes Mineralocorticoid Receptor (MR) expression in adult rat Leydig cells, which affects aldosterone-induced androgen formation, which probably decreases testosterone production (Martinez-Arguelles *et al*., 2009). Further investigations revealed that a 50% decrease in aldosterone and testosterone concentrations in male rats was due to inutero exposure to DEHP at doses of 100, 300, and 750 mg kg^1 day1, but corticosterone levels did not change (Martinez-Arguelles *et al*., 2009). This could be explained by a significant decrease in adrenal tissue weight following 750 mg kg^1 day1 DEHP exposure due to diminished Angiotensin II (AT) receptor levels in adrenal tissue. Interestingly, components of the renin-angiotensin-aldosterone system (RAAS) and stimulants of aldosterone show no change in the serum (Martinez-Arguelles *et al*., 2011). DEHP is highly toxic, with an LC50 of 0.50 ppm, and leads to embryo mortality and typical toxicity symptoms, such as tail curvature, necrosis, cardiac edema, and no-touch response, in zebrafish. DEHP can enhance estrogenic activity at concentrations of 1.50 ppm *in vitro* and *in vivo*, suggesting that DEHP induces the transactivation of ER in an addictive manner. DEHP decreases the expression of Steroidogenic Acute Regulatory protein (StAR) mRNA in pregnant mice, which reduces steroidogenesis catastrophically in both mice and humans. DEHP also lowers the in-utero fetal testicular RNA levels of 17β-hydroxylase and cytochrome P450 17A1, which are key enzymes in the steroidogenic pathway (Kariyazono *et al*., 2015). The above two conditions can occur from either direct exposure to fetal testis or indirect maternal exposure. Aldosterone can induce or activate MR in rat Leydig cells, which enhances testosterone production by an aldosterone-mediated MR mechanism (Ge *et al*., 2005). Based on all the factors explained above, the reduced MR expression in Leydig cells (Martinez-Arguelles *et al*., 2009) and decreased aldosterone serum levels (Martinez-Arguelles *et al*., 2013), both provoked by DEHP in utero exposure, can probably reduce testosterone levels in adult rats.

DEHP and Ovarian Toxicity

The ovaries are a significant primary reproductive organ that plays an important role in female gamete production and the release of sex hormones. Functional disturbances of the ovaries can cause many reproductive problems, such as anovulation, irregular estrogen secretion, and sterility (Li *et al*., 2016). The antral follicle is an ovarian reserve and an important supplier of sex steroid hormones in females and Estradiol (E2) is necessary for follicle growth. DEHP affects follicle growth through a reduction of E2 levels *in vitro* (Kalo *et al*., 2015). A reduction of estradiol could decrease the expression of the Arom gene, which produces the aromatase enzyme, which converts testosterone to estradiol. Hence, decreased expression of the Arom gene can decrease serum E2 levels (Gupta *et al*., 2010). Usually, follicle function requires proper regulation of steroidogenesis and survival from atresia (Hannon *et al*., 2015a). Atresia is a natural apoptotic occurrence in which follicles undergo death and has a harmful effect on ovarian and reproductive health. Ovarian follicular atresia is controlled by proapoptotic factors (such as Bad, Bax, and Bok) and antiapoptotic factors (such as Bcl2 and Bcl2l10), which are generally dysregulated by DEHP exposure (Hannon *et al*., 2015b). Induction of oxidative stress by DEHP is another cause leading to follicular atresia (Agarwal *et al*., 2005). DEHP can also inhibit follicle growth via oxidative stress (Wang *et al*., 2012a). Mice treated with DEHP at a dosage of 20 μg/kg/day 750 mg/kg/day had altered estrous cyclicity and accelerated primordial follicle recruitment due to the dysregulation of ovarian mRNA and altered levels of proteins in the Phosphatidylinositol 3-Kinase (PI3K) signaling pathway, which is associated with early folliculogenesis. There was a decrease in the percentage of primordial follicles after DEHP exposure. It is evident that low doses of DEHP can interfere with normal reproductive functions (Hannon *et al*., 2014). *In vitro* studies on neonatal ovaries from mice exposed to DEHP (0.2-20 μg/mL) revealed that DEH caused a decrease in steroidogenic enzyme levels that led to a decrease in testosterone, estrone, and E2 levels. MEHP accelerated primordial follicle recruitment, potentially via overactivation of ovarian PI3K signaling (Hannon *et al*., 2015a). Oral administration of DEHP has been shown to have negative impacts on oocyte meiotic maturation and development *in vivo*. The reduced developmental ability could be due to lowered expression levels of the Pou5f1, Asah1, and Ccna1 genes. This suggests that DEHP-induced alterations in gene expression activity could explain how DEHP compromises fertility (Absalan *et al*., 2016).

Fertility and Pregnancy Loss

Several studies were found that revealed no effect of DEHP exposure on pregnancy based on maternal weight, food consumption, litter size, and pup weights in mice and rats (Gray *et al*., 2009). In these studies, the doses used ranged from 0.05% in the diet to 405 mg/kg/d orally (Gray *et al*., 2009). Interestingly, Wistar rat pup weights were increased at doses less than 5 mg/kg/d (Grande *et al*., 2006). Also of interest, one study reported increased litter sizes after administering oral doses of 1000 mg/kg/d to female mice (Moyer and Hixon, 2012). Other studies using high doses of DEHP found negative effects on pregnancy. Maternal weight and food consumption, as well as pup weight, the number of pups born, and the rate of post-implantation loss, were variably affected at oral doses ranging from 500-1500 mg/kg/d as well as 10 mL/kg/d and 1% in diet in mice and rats (Pocar *et al*., 2012). One study reported a 100% pregnancy loss rate after exposure to 500 mg/kg/d DEHP in C3H/N mice (Schmidt *et al*., 2012). Decreased pup weight in CD-1 mice was also found at a relatively low dose of 5 mg/kg/d in diet, although resorptions did not increase until 500 mg/kg/d (Pocar *et al*., 2012). While this study did not correct for a possible litter effect, it suggests that adverse effects may also occur at lower doses. Interestingly, the effects of DEHP exposure on pregnancy in PPARa knockout mice as well as in wild-type mice were similar at high doses in both groups of mice (Peters *et al*., 1997). PPARa is thought to be at least partially responsible for toxicity in rodents, particularly in the liver. However, the human PPARa has a lower affinity for phthalates and is expressed at a lower level (Rusyn and Corton, 2012). As a result, the consequences of phthalate exposure in rats may be due to mechanisms that are not applicable in humans. However, there may be PPARa-independent pathways leading to reproductive toxicity (Ito and Nakajima, 2008). Therefore, the effects of DEHP in rats and mice may be biologically relevant to humans, despite the lesser importance of the PPARa pathway in humans. Studies conducted *in vivo* have also examined the effects of DEHP on steroidogenesis. However, the results of these studies are mixed. One study found that serum estradiol and progesterone concentrations were unaffected by exposure to maximum oral doses of 405 and 2500 mg/kg DEHP in Wistar rats and marmosets, respectively (Grande *et al*., 2007). In contrast, circulating concentrations of estrogen were decreased, FSH was increased and surges in progesterone and LH were suppressed in adult Sprague-Dawley rats at 2000 mg/kg/d, a dose that also prevented ovulation (Davis *et al*., 1994). Furthermore, estradiol and progesterone concentrations were decreased in immature Sprague-Dawley rats treated orally with 500 mg/kg/d DEHP, potentially the result of decreased transport of cholesterol across cell membranes (Svechnikova *et al*., 2007). Additionally, Sprague-Dawley rats treated with 1400 mg/kg DEHP twice a week had lower serum concentrations of estradiol and FSH, as well as lower levels of FSH and LH in the pituitary (Hirosawa *et al*., 2006). This study also examined protein expression in the pituitary and concluded that secretion of

LH and FSH could be changed by DEHP treatment (Hirosawa *et al*., 2006). On the other hand, increased levels of estradiol and increased expression of aromatase in the ovaries after low doses administered by inhalation (around 1 mg/kg/d) have also been reported (Ma *et al*., 2006). Similarly, increases in estradiol and FSH, as well as decreases in LH, were reported in mice after oral exposure to 1000 mg/kg/d (Moyer and Hixon, 2012). Finally, one study reported increases in estradiol and testosterone production from ovaries taken from mice treated with 1500 mg/kg/d DEHP and cultured during the diestrus stage of the estrous cycle (Laskey and Berman, 1993). Taken together, these data suggest that DEHP can adversely affect ovarian function and steroidogenesis, although the mechanisms have yet to be defined.

DEHP Effect on Pregnancy Outcome

Once DEHP is absorbed into the human body, the DEHP action might be influenced by time, age, and dose and because the effects of DEHP are influenced by the stage of development at exposure among animals, the DEHP-related exposure risk is higher for the developing fetus and new-born, particularly preterm (Latini *et al*., 2003). Also, reduced anogenital distances in male infants, a potential early marker of reproductive toxicity in humans, have been reported (Arbuckle *et al*., 2014).

Future Directions of Phthalate Research

A review of the existing research reveals certain discrepancies or gaps in our understanding of the specific mechanisms by which phthalates affect reproduction. For instance, there is conflicting data about the relationship between exposure to phthalates and the beginning of puberty in both males and girls. According to the articles that are now available, phthalates can cause or postpone the development of premature puberty. The other instance might be a lack of knowledge regarding the incidence of cancer in men exposed to phthalates. There are only oblique signs that phthalates may have an impact on the development of human cancer: Male neonates with cryptorchidism and people who have worked in the plastics industry are at a higher risk of developing cancer. Therefore, in order to determine whether and how phthalates can cause cancer of the male reproductive system, further epidemiological and experimental research must be done. To effectively prevent and cure phthalates' harmful effects on human and animal reproduction, more understanding of the expression and intracellular mechanisms of these effects on the male and female HPG systems is required. The previous sections' discussions have made it clear that while there are numerous solutions available for remediating phthalate pollution, there aren't as many practical approaches to putting those strategies into practice for a variety of reasons. The main obstacle to addressing the remediation elements of phthalates is their widespread use and, consequently, pervasive presence in the environment. Despite having a shorter half-life in the environment than other resistant substances like dioxins, chlorinated phenols, etc., phthalates are extensively used and as a result, their concentration is consistently and noticeably high in a variety of environmental matrixes. Even though microbial biodegradation is a key factor in the phthalate's natural mineralization process, its potential as a remedial method is constrained in indoor environments where phthalates are the primary contaminant. Research on tactics like photo-catalysis, adsorption technology, and phytoremediation is more important in this situation. Microbial remediation is still a viable approach, nevertheless, particularly for cleaning up contaminated soil and water in outdoor settings. Even though a great number of phthalate-degrading microorganisms have been found and studied, there is still a dearth of information regarding their practical use. Therefore, future research on microbial remediation should concentrate on some of the new characteristics that are listed below, in addition to looking for powerful strains. The competitive survivability of these microorganisms presents the main obstacle to their field trials. This makes bio-stimulation of native phthalate-degrading microbial strains sound like a superior idea, but there is currently a dearth of field evidence in this field of study. Another difficulty is frequently lowering the biodegradation efficiencies of the microorganisms that were characterized in the lab to break down phthalates in the natural environment. The primary cause of this is the adsorption of phthalate esters on the organic matrix of sediments and soil, among numerous other variables. In order to create an effective and comprehensive remediation approach, scientific data on the fate of phthalates in various environmental matrixes, their translocation among various environmental components, and their interaction with other pollutants are still being collected. Furthermore, scientific approaches to creating genetically modified microorganisms capable of breaking down phthalates are still insufficient. The study of metagenomics has made it possible to investigate the possibility of using unculturable bacterial strains to effectively degrade phthalates.

Results and Discussion

DEHP is a plasticizer that is frequently used in many consumer goods. Animal studies provided enough evidence to support the Federal Hazardous Substances Act's (FHSA) regulations' conclusion that DEHP did not become acutely hazardous after a single oral dose. The conclusion that DEHP was not corrosive or the main cause of ocular or cutaneous irritation was also supported by sufficient evidence from animals and little data from humans. DEHP could not be classified as a toxicant for

acute skin or respiratory exposure due to insufficient data. Likewise, insufficient data supported the designation of DEHP as a sensitizer. DEHP was found to have acute, subchronic, and chronic toxicity in a number of organs based on adequate animal studies. Numerous published studies have documented the harmful effects of DEHP on the thyroid, liver, kidney, and reproductive organs of animal test subjects. The finding that DEHP was a carcinogen and a toxin for reproduction and development was also supported by sufficient data pertaining to animals. There have been reports of DEHP-induced cancer in animal tests, blood, and liver. There have been reports of DEHP-induced reproductive effects in the reproductive tissues of male and female animals. Animals developed differently after receiving doses of DEHP that were not harmful to mothers. The conclusion that DEHP was a direct-acting genotoxicant, a respiratory irritant, or a neurotoxicant was not well supported by the available data. All things considered, the findings point to the conclusion that DEHP can be classified as "toxic" under the FHSA because of its toxicity after exposure over short, medium, and long terms. The animals' ample evidence of DEHP-induced damage to the liver, kidney, testes, uterine, ovary, fetus, and thyroid served as the foundation for this finding. If short-, intermediate-, or long-term exposures to the general public during "reasonably foreseeable handling and use" surpass the short-, intermediate-, or long-term ADIs for the general public (0.1, 0.024 and 0.058 mg DEHP/kg bwday, respectively), then products containing DEHP may be classified as "hazardous" under the FHSA criteria. Furthermore, if male populations are exposed to DEHP-containing products for intermediate or long periods of time during "reasonably foreseeable handling and use," the intermediate-duration or long-term ADIs for reproduction (0.037 and 0.0058 mg DEHP/kg bw-day, respectively) may be exceeded, making the product "hazardous".

Furthermore, exposure to reproductively viable female populations (aged 13-49) during "reasonably foreseeable handling and use" of DEHP-containing items may render them "hazardous" if the DEHP ADI for development (0.011 mg DEHP/kg bw-day) is exceeded.

Conclusion

A low order of toxicity has been suggested by previously published research on the toxicology of phthalate esters, particularly DEHP. Nonetheless, the findings provided here suggest that DEHP can be shown to have substantial effects on a range of biological systems when the right circumstances are met. Most of these effects would not have been noted in the traditional toxicologic analyses that were used to determine the relative safety of plasticizers in the past. DEHP is a common ingredient in polythene and is used to package food products. Increased levels of DEHP in the bloodstream have been linked to a number of health issues that negatively impact ovarian physiology. These issues include anovulation, infertility, early ovarian failure, and reduced steroidogenesis. In conclusion, despite the fact that these substances have been demonstrated to be endocrine disruptors in experimental animal models, it is yet unknown if exposure to DEHP has negative health consequences for the endocrine system in the general population. Further toxicological and epidemiological research is required to ascertain the degree to which phthalate exposure affects human health and reproductive function. However, there is grave worry that DEHP exposure may be harmful to human reproduction and fertility because of the existing toxicity data and the sparse but suggestive human exposure data.

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Author's Contributions

Venkataramanaiah Poli: Conceptualization, study designed, manuscript written, Data acquisition and analysis.

Srinivasulu Reddy Motireddy: Supervision, review, and edited.

All authors read and approve the final manuscript.

Ethics

The study did not involve any human or animal testing.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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