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REVIEW ARTICLE

A systematic review on the adverse health effects of di-2-ethylhexyl phthalate

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Abstract Di (ethylhexyl) phthalate (DEHP) is a global environmental pollutant. This study aims to systematically review the literature on health effects of exposure to DEHP including effects on reproductive health, carcinogenesis, pregnancy outcome, and respiratory system. The literature search was done through Scopus, ISI Web of Science, Google Scholar, PubMed, Medline, and the reference lists of previous review articles to identify relevant articles published to June 2016 in each subject area. The inclusion criteria were as follows: original research, cross-sectional studies, case–control studies, cohort studies, interventional studies, and review articles. Both human and animal studies were included. The search was limited to English language papers. Conference papers, editorials, and letters were not included. The systematic review was conducted and reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement. Overall, 152 of the 407 papers met the inclusion criteria. We provided an up-to-date comprehensive and critical assessment of both human and animal studies undertaken to explore the effects of DEHP. It revealed that in experimental studies, exposure to DEHP mainly targeted the reproductive, neurodevelopment, and respiratory systems. Human studies reported that exposure to this contaminant had carcinogenic effects and influenced neurodevelopment in early life. This systematic review underscored the adverse health effects of DEHP for pregnant women and the pediatric age group. It summarizes different response of humans and experimental animals to DEHP exposure, and some suggested underlying mechanisms.

Keywords Phthalates · Environmental exposures · Health · Systematic review · Toxicity

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Introduction

In recent years, accumulating evidence exist on the health effects of environmental agents including plasticizers, pesticides, metals, and several industrial chemicals (Dalsenter et al. 2006). Endocrine disruptor substances (EDS) are a group of chemical environmental factors with deleterious effects on hormonal balance (Albert and Jegou 2014). Phthalates or phthalate esters are synthetic diesters of phthalic acid, first produced in 1920 (Kimber and Dearman 2010; North et al. 2014). Phthalates are used in a wide 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 goods including PVC interior surface coverings, food wrappings, shower curtains, nail polish, plastic goods, and kitchen plastic ware (Kimber and Dearman 2010). The most common

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phthalates can be loosely grouped into lower molecular weight (diethyl phthalate [DEP], dimethyl phthalate [DMP], and dibutyl phthalate [DBP]) and higher molecular weight (di(2-ethylhexyl) phthalate [DEHP], diisodecyl phthalate [DIDP], diisononyl phthalate [DINP], and benzyl butyl phthalate [BBP]) (North et al. 2014). Approximately 90 % of phthalates, particularly the higher molecular weight compounds, are used as plasticizers (DEHP, DIDP, and DiNP) and the lower molecular weight phthalates (DMP, DEP, and DBP) are used in solvents, inks, waxes, adhesives, cosmetics, insecticides, and pharmaceuticals. Human exposure to phthalates occurs through the ingestion of contaminated food and water, as well as other routes including inhalation, house dust, and dermal contact (North et al. 2014; Ventrice et al. 2013). Phthalate toxicity has been reported since 1950s. In recent years, the alleged harmful effects of phthalates on human health have been of concern of the media and populations (Albert and Jegou 2014). As in the case of other phthalates, DEHP, also known as dioctyl phthalate (DOP), is mainly used as a plasticizer in PVC products. The main concerns on the potential toxic effects of DEHP that are experimentally observed in various species are of substantial concern on the human endocrine system. Based on fertility studies in mice, DEHP is the most potent reproductive toxicant among the phthalate esters (Ventrice et al. 2013). Human exposure to DEHP can occur via the dermal contact, inhalation, oral, and intravenous routes of exposure, and when released from medical equipment to patients in the neonatal intensive care unit, its levels in the body can be high (Caldwell 2012). A study among Russian women revealed that chronic occupational exposure of phthalate esters was associated with increased rates of miscarriage, anovulation, and decreased rates of pregnancy (Hoyer 2001). A study on 84 newborns indicated that either DEHP or mono-(2-ethylhexyl) phthalate (MEHP) was present in 77.4 % of the examined samples Moreover, MEHP-positive newborns showed a significantly lower gestational age compared with other infants (Latini et al. 2003b). The toxicokinetics of DEHP in humans, experimental animals, and cellular systems might have important roles in discriminating the potential adverse effects (Caldwell 2012). DEHP is a lipophilic compound and it is not chemically combined to PVC; therefore, this phthalate can be released from plastic (Herreros et al. 2010). Pre- and postnatal oral exposure to DEHP may be related in animals with male reproductive regulation and function alteration (Carbone et al. 2010). It is well established that DEHP might produce intrauterine deaths, testicular atrophy, and teratogenicity in rodents. More specifically, its marked adverse effects are documented on the male reproductive development following in utero exposure (Saillenfait et al. 2011). In animal studies, repeated oral exposure to DEHP caused disruptions of reproductive performance (Hoyer 2001). An in utero study found that lactational exposure of pregnant rats to the DEHP showed effects on male offspring including reduced anogenital distance, areola and nipple retention, undescended testes, and permanently incomplete preputial separation (Moore et al. 2001). DEHP carcinogenic hazard and its reproductive hazard have been reviewed previously by the World Health Organization (WHO) International Agency for Research on Cancer (IARC) and expert panels convened by the National Toxicology Program (NTP) Center for the Evaluation of Risks to Human Reproduction (CERHR), a joint venture between the NTP and the National Institute of Environmental Health Sciences (NIEHS), respectively. Both the previously published CERHR and IARC efforts contained discussed DEHP exposure and absorption, distribution, metabolism, and elimination (ADME) (Caldwell 2012).

Given the increasing evidence about adverse health effects of exposure to phthalate and some controversies in current findings, the aim of this study is to systematically review and summarize the scientific literature on associations between exposure to DEHP and health effects including reproductive effects, carcinogenesis, pregnancy outcome, neurodevelopment, and respiratory effects.

Methods

The present systematic review was conducted and reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Liberati et al. 2009). Ethical approval was not required as this was a secondary data analysis.

Literature search

We searched Scopus, ISI Web of Science, Google Scholar, PubMed, and Medline. All databases were searched to June 2016. As we had different outputs, we used several separate keywords for each aim or each output of this study.

The following keywords and search strategy were used for reproductive system, carcinogenesis effects, effect on pregnancy outcome, and effect on respiratory system, respectively:

1. For reproductive system: ("bis(2-ethylhexyl) phthalate esterase" [Supplementary Concept] OR "Diethylhexyl Phthalate"[Mesh] OR "di-n-octyl phthalate" [Supplementary Concept] OR "phthalate ester hydrolase" [Supplementary Concept] OR "phthalic acid" [Supplementary Concept]) AND ("Reproduction"[Mesh] OR "Reproductive Health"[Mesh] OR "Infertility"[Mesh] OR "Pregnancy"[Mesh] OR "Fertility"[Mesh] OR "Infertility, Male"[Mesh] OR "Infertility, Female"[Mesh]).
2. For carcinogenesis effects: ("bis(2-ethylhexyl) phthalate esterase" [Supplementary Concept] OR "Diethylhexyl Phthalate"[Mesh] OR "di-n-octyl phthalate"

[Supplementary Concept] OR "phthalate ester hydrolase" [Supplementary Concept] OR "phthalic acid" [Supplementary Concept]) AND ("Neoplasms"[Mesh]).

3. For pregnancy outcome and neurodevelopment: ("bis(2-ethylhexyl) phthalate esterase" [Supplementary Concept] OR "Diethylhexyl Phthalate"[Mesh] OR "di-n-octyl phthalate" [Supplementary Concept] OR "phthalate ester hydrolase" [Supplementary Concept] OR "phthalic acid" [Supplementary Concept]) AND ("Premature Birth"[Mesh] OR "Birth Weight"[Mesh] OR "Infant, Low Birth Weight"[Mesh]), OR "development" OR "neurodevelopment". We also searched for ("bis(2-ethylhexyl)phthalate esterase" [Supplementary Concept] OR "Diethylhexyl Phthalate"[Mesh] OR "di-n-octyl phthalate" [Supplementary Concept] OR "phthalate ester hydrolase" [Supplementary Concept] OR "phthalic acid" [Supplementary Concept]) AND ("Abortion, Induced"[Mesh] OR "Abortion, Spontaneous"[Mesh] OR "Pregnancy Outcome"[Mesh] OR "Stillbirth"[Mesh]).
4. For respiratory system: ("bis(2-ethylhexyl) phthalate esterase" [Supplementary Concept] OR "Diethylhexyl Phthalate"[Mesh] OR "di-n-octyl phthalate" [Supplementary Concept] OR "phthalate ester hydrolase" [Supplementary Concept] OR "phthalic acid" [Supplementary Concept]) AND ("Asthma"[Mesh] OR "Asthma, Occupational"[Mesh] OR "Pulmonary Disease, Chronic Obstructive"[Mesh]).

All elements were searched using both controlled vocabulary terms (Medical Subject Headings) and free text words, and the search was not limited to title and abstract for this reason that maybe our desired result or desired outcome being a secondary aim of the studies. Limits were applied to exclude conference papers, editorials, and letters. The search was refined to English language, and we did not consider any time limitation. We included both human and animal studies.

Hand searching

To increase the sensitivity and to select more studies, the reference list of the published studies was checked as well.

Selection criteria

Studies identified from the literature search were selected on the basis of the predefined selection criteria presented below.

Inclusion criteria

1. Cross-sectional studies, case–control studies, cohort studies, reviews, and interventional studies
2. Studies investigating the associations between di-2-ethylhexyl phthalate and effects on reproductive system, carcinogenesis effects, effect on pregnancy outcome, and effect on respiratory system

Exclusion criteria

1. Letters, conference abstracts, or editorials
2. Poor quality articles

Data extraction and abstraction

Titles and abstracts of papers were screened, and relevant papers were selected. Duplicates were removed. Then, full texts of relevant papers were read, and findings were rescreened. Two independent reviewers (MZ and MK) screened the titles and abstracts of papers, which were identified by the literature search, for their potential relevance or assessed the full text for inclusion in the review. In the case of disagreement, the discrepancy was resolved in consultation with an expert investigator (RK).

Two reviewers abstracted the data independently (PP and MZ). The required information that was extracted from all eligible papers was as follows: data on first author's family name, year of publication, country of the study, population studied, aim, and findings of studies.

Study selection strategy

In the systematic search, 3063 unique references were identified (Fig. 1). Of them, 2448 were excluded on the basis of the title and abstract. For the remaining 407 articles, the full text was retrieved and critically reviewed. After the selection process, 152 papers were included.

Results

The studies included in this review revealed different adverse health effects of exposure to phthalates; we categorized them to following effects:

Effects on the reproductive system

In animal studies, some phthalates induced reproductive tract developmental anomalies that consisted of epididymal malformations or absence of the epididymis, cryptorchidism, decreased anogenital distance (AGD), increased incidence of hypospadias, delayed preputial

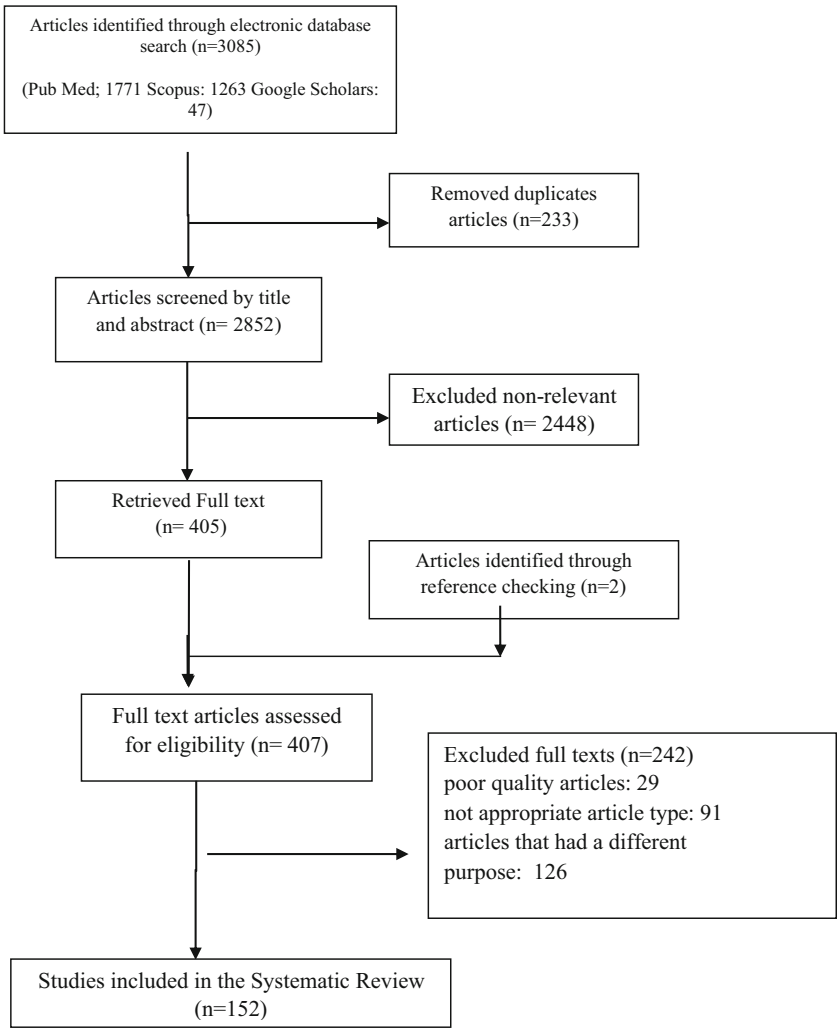

Fig. 1 Literature search and flowchart for selection of primary study

separation (pubertal milestone), retention of thoracic nipples, and testicular lesions (Hauser and Calafat 2005). Reproductive tract developmental anomalies were seen in rats dosed during gestation and/or lactation with DEHP (Gray et al. 2000). Therefore, few published human studies have explored their relationships with exposure to phthalates and potential risk factors. A study on the relationship between prenatal exposure to phthalate esters and AGD in 85 male infants revealed that maternal prenatal urinary concentrations of MEP, MBP, MBzP, and mono-isobutyl phthalate (MiBP) were inversely associated to age-adjusted anogenital index (AGI). However, MEHP is a DEHP oxidative metabolite, but it was unrelated to AGI, and MEOHP and MEHHP were of borderline significance with AGI (Swan et al. 2005). An increased proliferative activity of Leydig cells in the testes of DEHP-treated rats as well as the induction of Leydig cell hyperplasia are reported (Akingbemi et al. 2004). Summary of published literature of DEHP effects on reproductive system is presented in Table 1.

Carcinogenesis effects of DEHP

The Carcinogen Assessment Group of the USEPA classified DEHP as a probable human carcinogen (group B2) (Voss et al. 2005). Administration of some phthalates, e.g., DEHP, to rodents resulted in adverse liver effects including increased liver weights, histological changes, elevated liver enzyme levels, and, in some cases, tumors. These effects were related with peroxisomal proliferation, a process related to metabolism of cholesterol and fatty acids (McKee et al. 2002; Ward et al. 1998). Also, in adult rodents, DEHP showed a liver tumor-promoting effect, which is mediated by peroxisome proliferator-activated receptor α (PPARα). On the other hand, DEHP and other phthalates might hit the liver metabolic programming through different pathways, including perturbations of gap junctional intercellular communication

Table 1 Phthalates and reproductive system

Reference	Location	Population studied	Type of study	Aims	Finding
Nikonorow et al. (1973)	–	Wistar rats weighing 90–120 g	Experimental study	To investigate dealing with the effects of DEHP, di-*n*-butyl phthalate, di-(*n*-octyl)tin *S*,*S*′-*bis*-(isooctyl mercaptoacetate), dibenzyltin *S*,*S*′-bis-(isooctyl mercaptoacetate), on reproduction and fetal development have not been described. Some studies using chick embryos	Rats fed dietary levels 0.35 % of di-(2-ethylhexyl)phthalate and 0.02 % of di-(*n*-octyl)tin *S*,*S*′-*bis*-(isooctyl mercaptoacetate) for periods up to 12 months showed a significant decrease in body weight and an increase in kidney weight; furthermore, di-(2-ethylhexyl)phthalate caused liver enlargement. Red and white blood cell counts, hemoglobin concentration and histopathology of liver, kidneys, and spleen were within normal limits. Reproduction studies showed an increased number of resorptions and decreased fetal body weights
Curto and Thomas (1982)	–	Male Swiss-Webster mice weighing between 35 and 40 g	Experimental study	To examine changes in testes and sex accessory weight as well as gonadal zinc and compared these effects in the rat and in the mouse injected with varying doses of DEHP or MEHP	Rats injected with MEHP (50 mg/kg) showed a 37 % decrease in prostatic zinc; DEHP (100 mg/ kg) caused a 33 % decrease in prostatic zinc, and a 31 % decrease in gonadal zinc. These studies indicated that the reproductive system of the male rat is more sensitive to phthalate
Autian (1982)	–	Male mice	Experimental study	To explore the possibility that DEHP might be a cumulative toxic agent even at lower doses in regard to antifertility and mutagenic effects in mice	Preliminary results indicated that antifertility effects occurred with as little as three subcutaneous doses of 1 ml/kg each
Agarwal et al. (1985)	–	Male mice	Experimental study	The possibility that DEHP might be a cumulative toxic agent, even at lower doses, prompted our laboratories to initiate several preliminary experiments to clarify whether a possible cumulative effect can impose both an antifertility effect and mutagenic effects during an 8-week period	For DEHP, for example, the acute LD_{50} was 38.35 ml/kg, but after 10 weeks, the value fell to 1.37 ml/kg, suggesting that the ester was producing a cumulative toxicity. A more recent study explored the possibility that DEHP might be a cumulative toxic agent even at lower doses in regard to antifertility and mutagenic effects in mice. Preliminary results indicated that antifertility effects occurred with as little as three subcutaneous doses of 1 ml/kg each
Oishi (1986)	–	Young male Crj: Wistar rats	Experimental study	(1) To study the effects of DEHP on the selected enzyme activities in rat testis, (2) to identify whether testicular enzymes could serve as useful biochemical markers for evaluating toxic effects of cytotoxic chemicals such as DEHP on rat testis, and (3) to characterize the course of morphological changes in the testis of rats administered DEHP	The specific activities of enzymes associated with premeiotic spermatogenic cells, Sertoli cells, or interstitial cells (13-glucuronidase, y-glutamyl transpeptidase, and malate dehydrogenase) were higher than those of control by day 10. The specific activities of alcohol dehydrogenase and aldolase, zinc-containing enzymes, increased after DEHP treatment in spite of the decrease in zinc concentration in the testis
Parmar et al. (1986)	–		Experimental study		

Table 1 (continued)

Reference	Location	Population studied	Type of study	Aims	Finding
		Adult male albino rats (150–200 g)		To study the effect of different doses of DEHP on the activity of 7-glutamyl transpeptidase (TGT), lactate dehydrogenase (LDH), sorbitol dehydrogenase (SDH), β-glucuronidase, and acid phosphatase, related to the specific events of spermatogenesis	An increase in the activity of ~- glucuronidase and decrease in the activity of acid phosphatase were also observed at the highest dose of DEHP. The activity of sorbitol dehydrogenase (SDH) was found to be decreased in the animals exposed to 1000 and 2000 mg/kg of DEHP
Agarwal et al. (1986)	–	Sexually mature male (average age 15–16 weeks, 240 + 10 g) and female (average age 12–13 weeks, 200 + 10 g) F344 rats	Experimental study	To compare the reproductive performance of male rats at the dose levels which caused (or did not cause) measurable gonadotoxicity and to observe the patterns of recovery from toxicity upon discontinuance of exposure to DEHP	Degenerative changes were observed in testis, along with decreased testicular zinc content, reduced epididymal sperm density and motility, and increased occurrence of abnormal sperm at 20,000 ppm. There was a trend towards reduced testosterone and increased luteinizing hormone and follicle-stimulating hormone in serum at 5000 and 20,000 ppm. The mean percentage of fertile animals was unchanged and reduction in fertility parameters, although not marked in severity, was correlated with gonadal effects. Average litter size was reduced at 20,000 ppm, but initial pup weights and growth were unaffected. There were no grossly observed abnormalities in the offspring and the rate of neonatal deaths was similar in control and DEHP-treated groups. Characteristic toxicity manifestations of DEHP included dose-dependent enlargement of liver and reduced sperm triglycerides and cholesterol. Additionally, serum albumin and total proteins were dose dependently increased upon treatment with DEHP
Lamb et al. (1987)	–	COBS Crl: CD-1. (ICR)BR outbred albino mice (6 weeks of age)	Experimental study	To investigate reproductive toxicity of four phthalates by a continuous breeding protocol	DEHP (at 0.1 and 0.3 %) caused dose-dependent decreases in fertility and in the number and the proportion of pups born alive. A crossover mating trial showed that both sexes were affected by exposure to DEHP
Agarwal et al. (1989)	–	Sexually mature male and female mice (20–25 g, 8–10 weeks of age)	Experimental study	To examine possible toxicity of DEHP on reproductive parameters and to assess the gonadotoxic potential, from a dose–response relationship, on functional, biochemical, and histologic alterations of testes and ovaries	In both male and female treated mice, there was a reduction in incidence of pregnancy. There were biochemical suggestions of reduced anabolic activity in the gonads (as reflected by decreased ATPase activity and of RNA, DNA, and protein content) and of increased catabolic activity in the gonads (as reflected by an increase in lysosomal enzyme activity and histological damage). Testicular, but not ovzarian, weight was reduced in treated animals. Of the other parameters examined, the

Table 1 (continued)

Reference	Location	Population studied	Type of study	Aims	Finding
					ovaries exhibited histological injury at lower doses of DEHP than the testes, but unlike testes, there was not a significant dose-related increase in histopathology. Biochemical changes were dose-related, for the most part, in both ovaries and testes, with the changes being more pronounced in testes
Parmar et al. (1995)	–	Young (21-day-old) male Sprague–Dawley rats	Experimental study	To test the hypothesis that the testicular toxicity of DEHP is mediated by a decrease in Zn levels, possibly due to altered expression of ZnT-1	Body weight gain and testicular weight (absolute and relative) were significantly lower in DEHP-treated rats. DEHP produced morphological changes in the testis, including apoptosis, necrosis, and loss of spermatogenic cells, which resulted in testicular atrophy. Apoptotic index (AI: the percentage of apoptotic cells in seminiferous tubules), determined using the TUNEL technique, was markedly increased after 1 day (AI, 2.9 %; control AI, 0.1–0.3 %) followed by a peak at 3 days (AI, 11.5 %) and a gradual decrease till 10–14 days (AI, 7–9 %). Zinc content in testis was not changed 1 day after DEHP administration but decreased significantly at later time points. No difference was found in ZnT-1 mRNA expression between control and DEHP-treated animals until day 14. Our results suggest that apoptosis, along with necrosis, plays an important role in the mechanism of testicular atrophy by DEHP. In addition, ZnT-1 mRNA expression was not altered by DEHP, and therefore, it appears that ZnT-1 cannot account for the decrease in testicular Zn content
Peters et al. (1997)	–	Pregnant female mice, 10–14 weeks of age, F4 C57BL/6N × Sv/129, wild-type (+/+) or PPARa-null (−/−)	Experimental study	To investigate whether or not DEHP-induced reproductive toxicity and teratogenicity are mediated by PPARa and to evaluate the effect of DEHP on maternal and embryonic Zn metabolism	PPARa does not mediate the reproductive toxicity, teratogenicity, and alterations in maternal and embryonic Zn status induced by DEHP
Shioda and Wakabayashi (2000)	–	Adult medaka	Experimental study	To understand the effects of estrogenic chemicals [bisphenol A, nonylphenol (NP), and DEHP] on fish reproduction	The results indicated that exposure to 17 b-E2 caused a significant decrease in the number of eggs and hatchings as compared to the negative control group at and above 3 nmol/l. In the treatment using these chemicals, the decrease in egg numbers was not so much as in hatching numbers. When compared to other in vitro studies, concentrations observed to have adverse effects on

Table 1 (continued)

Reference	Location	Population studied	Type of study	Aims	Finding
					reproduction in this study are generally lower
Parks et al. (2000)	–	Pregnant Sprague–Dawley rats of approximately 90 days of age	Experimental study (in vitro and in vivo assays)	To elucidate the alterations in the testicular testosterone production and reproductive development with DEHP exposure during late gestation and neonatal life	Maternal DEHP treatment at 750 mg/kg/day from gestational day (GD) 14 to postnatal day (PND) 3 caused a reduction in T production and reduced testicular and whole-body T levels in fetal and neonatal male rats from GD 17 to PND 2. As a consequence, anogenital distance (AGD) on PND 2 was reduced by 36 % in exposed male, but not female, offspring. By GD 20, DEHP treatment also reduced testis weight. Histopathological evaluations revealed that testes in the DEHP treatment group displayed enhanced 3β-HSD staning and increased numbers of multifocal areas of Leyding cell hyperplasia as well as multinucleated gonocytes as compared to control at GD 20 and PND 3. In contrast to the effects of DEHP on T levels in vivo, neither DEHP nor its metabolite MEHP displayed affinity for the human androgen receptor at concentration up to 10 μM in vitro
Moore et al. (2001)	–	Pregnant Sprague–Dawley rats	Experimental study	To determine effects of in utero and lactational exposure to the most prevalent phthalate ester, DEHP, on male reproductive system development, and sexual behavior	Dose-related effects on male offspring included reduced anogenital distance, areola and nipple retention, undescended testes, and permanently incomplete preputial separation. Testis, epididymis, glans penis, ventral prostate, dorsolateral prostate, anterior prostate, and seminal vesicle weights were reduced at PND 21, 63, and/or 105–112. Additional dose-related effects included a high incidence of anterior prostate agenesis, a lower incidence of partial or complete ventral prostate agenesis, occasional dorsolateral prostate and seminal vesicle agenesis, reduced sperm counts, and testicular, epididymal, and penile malformations. No major abnormalities were found in any of eight control litters, but DEHP caused severe male reproductive system toxicity in 5 of 8 litters at 375 mg/kg/day, 7 of 8 litters at 750 mg/kg/day, and 5 of 5 litters at 1500 mg/kg/day
Akingbemi et al. (2001)	–	Long–Evans rats	Experimental study	To investigate the ability of DEHP to affect Leydig cell androgen biosynthesis	Serum testosterone (T) and LH levels were significantly reduced in male offspring, compared to control, at 21 and 35 days of age. Exposure of rats to 200 mg 21 kg 21 day 21 DEHP caused a 77 % decrease in the activity of the steroidogenic

Table 1 (continued)

Reference	Location	Population studied	Type of study	Aims	Finding
					enzyme 17b-hydroxysteroid dehydrogenase, and reduced Leydig cell T production to 50 % of control. Paradoxically, extending the period of DEHP exposure to 28 days (postnatal days 21–48) resulted in significant increases in Leydig cell T production capacity and in serum LH levels. The no-observed-effect-level and lowest-observed-effect-level were determined to be 1 mg 21 kg 21 day 21 and 10 mg 21 kg 21 day 21, respectively. In contrast to observations in prepubertal rats, exposure of young adult rats by gavage to 0, 1, 10, 100, or 200 mg 21 kg 21 day 21 DEHP for 28 days (postnatal days 62–89) induced no detectable changes in androgen biosynthesis
Tanaka (2002)	–	Male and female mice (Crj: CD-1, 4 weeks of age)	Experimental study	To evaluate reproductive and neurobehavioural effects of DEHP in mice throughout two generations	There were no adverse effects of DEHP on either litter size, litter weight, or sex ratio at birth. The average body weight of male offspring was significantly decreased in the low-dose group at birth. In behavioral developmental parameters, surface righting at PND 4 was significantly delayed in the low- and middle-dose group in female offspring, and those effects were slightly dose-related ($P < 0.05$). Surface righting at PND 7 was significantly depressed in the high-dose group of male offspring, and those effects were significantly dose-related ($P < 0.001$). That of female offspring was significantly depressed in the low-dose group
Kim et al. (2002)	–	Japanese medaka	Experimental study	To investigate the mechanism of the reproduction disorder caused by DEHP and to analyze the amount of vitellogenin production as a preliminary biomarker for the reproduction disorders	First, blood vitellogenin levels in all treated test subjects markedly decreased. Second, gonado-somatic index (GSI) decreased to 33 and 38 % of the control GSI in 10- and 50 Ag/l-treated female fish, respectively. Third, 54 % of female fish in the control treatment had completely matured oocytes in their ovaries, but only 37, 0, and 22 % of female fish matured to the last stage in the 1, 10, and 50 Ag/l-treated test subjects, respectively. Unlike female fish, no change or adverse effects were observed in the male fish
Sekiguchi et al. (2003)	–	25 immature female rats of F344/DuCrj strain	Experimental study	Investigated the influence of DEHP on ovulation induced by equine chorionic gonadotropin (eCG)	When rats received 4 daily doses of DEHP at 500 mg/kg, ovulation occurred in 4 of 6 rats in the 15 iu eCG group and in 1 of 3 rats in the 30-iu group. Mean numbers

Table 1 (continued)

Reference	Location	Population studied	Type of study	Aims	Finding
					of ovulated ova were 2.50 and 0.33 ova in the 15 and 30 iu groups, respectively. Changes in ovarian and uterine weights were not found. Inhibition of ovulation by the injection of DEHP indicated the utility of induced ovulation in immature rats to detect reproductive toxicity in females
Cammack et al. (2003)	–	3- to 5-day-old male Sprague–Dawley rats	Experimental study	To ascertain potential permanent effects at the time of sexual maturity, a histopathological evaluation of the testis was conducted and to perform an assessment of sperm count, sperm morphology, and sperm motility	No effects of any type were observed in animals treated intravenously with 60 mg/kg/day. Testicular changes, consisting of a partial depletion of the germinal epithelium and/or decrease in diameter of seminiferous tubules, were present in all animals of the 300- and 600-mg/kg/day groups after the 21-day dosing period. Testes weight decreased and liver weight increased in these animals. Testes changes were dose-related and generally more severe among animals dosed orally vs. intravenously. In the recovery animals, a residual DEHP-induced decrease in seminiferous tubule diameter was present in the testis of several animals dosed orally at 300 and 600 mg/kg/day but not in animals dosed intravenously. There was no germinal cell depletion or Sertoli cell alteration observed in any dose group at any time. Notably, no effects on sperm count, sperm morphology, or sperm motility were observed at 90 days of age in any of the groups
Wilson et al. (2004)	–	10 timed pregnant Sprague–Dawley rats	Experimental study	To report that phthalate-induced lesions of the gubernacular ligaments, which are critical for testis descent	Compared to chemicals like vinclozolin, linuron, and prochloraz that act as AR antagonists and/or inhibit fetal Leydig cell testosterone production, only the three phthalates significantly reduced ex both vivo testosterone production and insl3 gene expression when quantified by real-time RT-PCR
Tanaka (2005)	–	Male and female mice (Crj: CD-1, 4 weeks of age)	Experimental study	To evaluate reproductive and neurobehavioural effects of DEHP in a cross-mating method of mice	There were no adverse effects of DEHP on either litter size, litter weight, and sex ratio at birth. The average body weight of female offspring was significantly affected in-group IV (T/T) at PND 14. In behavioral developmental parameters, swimming direction at PND 4 was significantly accelerated in-group III (C/T) in female offspring. In movement activity of exploratory behavior at 3 weeks of age, number of movement of

Table 1 (continued)

Reference	Location	Population studied	Type of study	Aims	Finding
					male offspring was significantly affected in-group IV (T/T)
Shirota et al. (2005)	–	Adults rates of Sprague-Dewley strain (Crj: CD IGS)	Experimental study	To study influence of in utero exposure to DEHP on development of testes in rats	Fetal deaths averaging 20–36 % were observed at every examination in the group receiving 1000 mg/kg of DEHP. Increases of fetal deaths over 50 % were also observed in the reference group that received 0.5 mg/kg of EE. Microscopic examination of the fetal testis in groups treated with DEHP revealed degradation of germ cells in G16 fetuses and localized proliferation or hyperplasia of interstitial cells in G18 and 20 fetuses. Germ cells having more than two nuclei were observed in a few cases including the control testes of G14 fetuses
Andrade et al. (2006)	–	Female Wistar rats (HsdCpb:WU), weighing 200 ± 15 g	Experimental study	To investigate the reproductive effects of in utero and lactational exposure to DEHP in adult male offspring rats	Quantitation of specific cell types shows that the observed effects in daily sperm production are not related to changes in the number of Sertoli cells or their capability to support early stages spermatocytes. A low incidence of cryptorchidism was observed in DEHP-exposed groups with a lowest observed adverse effect level of 5 mg/kg/day. Serum testosterone concentration was similar to control at most doses but was significantly increased at 0.045, 0.405, and 405 mg DEHP/kg/day. In spite of this effect, the weight of seminal vesicle with coagulating glands was significantly reduced at 405 mg/kg/day. Testis, epididymis, and prostate weights were similar among groups. Fertility and sexual behavior were not affected by DEHP treatment at any dose
Dalsenter et al. (2006)	–	Wistar rats	Experimental study	To determine whether in utero and lactational exposure to lower doses of DEHP could induce permanent alterations in the reproductive function and behavior of male offspring rats	The effects produced clearly demonstrate the ability of DEHP to disrupt the androgen-regulated development of the male reproductive tract. Absolute and relative weights of androgen-dependent tissue organs (ventral prostate and seminal vesicle) were significantly reduced at the highest dose level tested (500 mg/kg/day). Impairment of male sexual behavior (500 mg/kg/day) was also observed. Moreover, the reduction in daily sperm production and epididymal sperm counts observed after administration of the highest dose suggests an impairment of the spermatogenic processes
Stroheker et al. (2006)	–	Timed pregnant female Wistar rats	Experimental study		

Table 1 (continued)

Reference	Location	Population studied	Type of study	Aims	Finding
				To analyze the distribution of DEHP in various organs of the Wistar rat fetus and newborn rat after in utero exposure at 750 mg/kg, a dose which affects the rat male offspring testes	The radioactivity concentration recovered in the fetuses was 1 or 2 order of magnitude lower than the concentration found in the dam plasma. A low proportion of radioactivity was present in fetal gonads, ca. 2, 5, and 3.6 % on GD18, GD21, and PND4, respectively. The effect on testosterone production of DEHP and its metabolites (MEHP, metabolites VI and IX) was assessed in fetal testis cultures using a dose range, which included the maximal exposure observed in vivo. None of the compounds affected testosterone production
Kang et al. (2006)	–	60, 5-week-old male F344 rats	Experimental study	To investigate the effects of DEHP and di(2-ethylhexyl) adipate (DEHA) in a thioacetamide (TAA)-induced rat liver damage model	Significant decrease in sperm numbers and motility and increase in morphology abnormalities were evident in-group 1 as compared to groups 5 and 6 ($P < 0.01$). However, DEHA treatment was not associated with any apparent testicular toxicity in either TAA- or vehicle-treated animals
Borch et al. (2006)	–	40 time-matedWistar rats (HanTac:WH, Taconic M&B, Denmark, body weight approx. 200 g)	Experimental study	To investigate the effects of four different doses of DEHP on fetal testicular histopathology, testosterone production, and expression of proteins and genes involved in steroid synthesis in fetal testes	Immunohistochemistry showed clear reductions of StAR, PBR, P450scc, and PPAR_ protein levels in fetal Leydig cells, indicating that DEHP affects regulation of certain steps in cholesterol transport and steroid synthesis. The suppression of testosterone levels observed in phthalate-exposed fetal rats was likely caused by the low expression of these receptors and enzymes involved in steroidogenesis
Grande et al. (2006)	–	Female Wistar rats, weighing 200 ± 15 g	Experimental study	To evaluate the possible reproductive effects of low (human relevant) and high doses of DEHP on female offspring rats exposed in utero and during lactation	At the dose levels tested, no signs of maternal toxicity were observed. A significant delay in the age at vaginal opening (approximately 2 days) at 15 mg DEHP/kg bw/day and above, as well as a trend for a delay in the age at first estrus at 135 and 405 mg DEHP/kg bw/day (approximately 2 days), was observed. Liver enlargement (characteristic of phthalate exposure in rats) was limited to the 135- and 405-mg DEHP/kg bw/day doses. Anogenital distance and nipple development were unaffected
Ma et al. (2006)	–	Female Wistar-Imamichi strain rats, 21 days old	Experimental study	To evaluate the effects of inhaled DEHP on the onset of puberty and on postpubertal reproductive functions in prepubertal female rats	Upon completion of exposure, the rats were sacrificed at PND 42 and PNDs 85–88 during the diestrous stage. DEHP exposure advanced the age of vaginal opening (VO) and first estrous cycle, and serum cholesterol, luteinizing hormone,

Table 1 (continued)

Reference	Location	Population studied	Type of study	Aims	Finding
					and estradiol levels were significantly elevated in the 25-mg/m3 DEHP group. Irregular estrous cycles were observed more frequently in DEHP-exposed rats, and serum cholesterol decreased in DEHP-exposed rats in adulthood; RT-PCR showed that the expression of aromatase mRNA, encoding a rate-limiting enzyme, that catalyzes the conversion of testosterone to estradiol was elevated in the 25-mg/m^3 DEHP group
Pan et al. (2006)	China	74 male workers at a factory producing unfoamed polyvinyl chloride flooring	Cross-sectional study	To assess the effect of occupational exposure to high levels of phthalate esters on the balance of gonadotropin and gonadal hormones including luteinizing hormone, follicle-stimulating hormone, free testosterone (fT), and estradiol	Compared to the unexposed workers, the exposed workers had substantially and significantly elevated concentrations of mono-*n*-butyl phthalate (MBP; 644.3 vs. 129.6 μg/g creatinine, $P < 0.001$) and mono-2-ethylhexyl phthalate (MEHP; 565.7 vs. 5.7 μg/g creatinine, $P < 0.001$). fT was significantly lower (8.4 vs. 9.7 μg/g creatinine, $P = 0.019$) in exposed workers than in unexposed workers. fT was negatively correlated to MBP ($r = -0.25$, $P = 0.03$) and MEHP ($r = -0.19$, $P = 0.095$) in the exposed worker group. Regression analyses revealed that fT decreases significantly with increasing total phthalate ester score (the sum of quartiles of MBP and MEHP; $r = -0.26$, $pP = 0.002$)
Spjuth et al. (2006)	–	20 male piglets (Swedish Yorkshire Swedish Landrace)	Experimental study	To determine the potential late effects of prepubertal oral exposure to DEHP on semen quality in young pigs	Total sperm motility tended to be lower while local motility was higher in the DEHP-exposed group compared with controls ($P = 0.07$) when assessed by computer-assisted sperm analysis. The DEHP-exposed group had a significantly ($P < 0.05$) lower percentage of spermatozoa with tailless, defective heads (at 7–8 months of age) and double-folded tails (at 6–7, 7–8, and 6–9 months of age), compared with controls (albeit always under 5 %)
Reddy et al. (2006)	India	Blood samples were collected from 49 infertile women with endometriosis (study group); 38 age-matched women without endometriosis (control group I) but with infertility related to tubal defects, fibroids, polycystic ovaries, idiopathic infertility, and pelvic inflammatory diseases diagnosed by laparoscopy and	Case–control study	To evaluate the possible association between phthalate esters (PEs) and the occurrence of endometriosis	Women with endometriosis showed significantly higher concentrations of di-*n*-butyl phthalate (DnBP), butyl benzyl phthalate (BBP), di-*n*-octyl phthalate (DnOP), and diethyl hexyl phthalate (DEHP) (mean 0.44 [SD 0.41]; 0.66 [SD 0.61]; 3.32 [SD 2.17]; 2.44 [SD 2.17] μg/ml) compared with control group I (mean 0.08 [SD 0.14]; 0.12 [SD 0.20]; 0; 0.50 [SD 0.80] micrograms/ml) and control group II (mean 0.15 [SD 0.21]; 0.11 [SD

Table 1 (continued)

Reference	Location	Population studied	Type of study	Aims	Finding
		a further group of 21 age-matched women (control group II) with proven fertility and no evidence of endometriosis and other gynecological disorders during laparoscopic sterilization			0.22]; 0; 0.45 [SD 0.68] μg/ml). The correlation between the concentrations of PEs and different severity of endometriosis was strong and statistically significant at $P < 0.05$ for all four compounds (DnBP: $r = +0.73$, $P < 0.0001$; BBP: $r = +0.78$, $P < 0.0001$; DnOP: $r = +0.57$, $P < 0.0001$; and DEHP: $r = +0.44$, $P < 0.0014$)
Luisi et al. (2006)		Women with uterine fibromatosis undergoing surgical menopause (n¼15) and (ii) healthy women (n¼20)	Cross-sectional	To test serum concentrations of DEHP and/or MEHP in women with uterine fibromatosis	Serum MEHP distribution was found to be non-Gaussian (p¼0.001), while serum DEHP distribution was compatible with a normal curve (p¼0.141). Patients with uterine fibromatosis showed significantly lower serum MEHP concentrations (median [interquartile range] 0 [0–0] mg/ml, range 0–0.57 mg/(60)ml) than controls (0.42 [0–0.51] mg/ml, range 0–1.20 mg/ml, z¼72.93, p¼0.0034). Likewise, serum DEHP concentrations in women with fibromatosis were found to be significantly lower than in controls (patients 0.27 + 0.096 mg/ml (mean + standard deviation (SD)), range 0.14–0.59 mg/ml vs. controls 0.30 + 0.14 mg/ml (mean + SD), range 0–0.63 mg/ml; t¼3.212, df¼33, difference 70.325 (95 % confidence interval 70.5309, 70.1191), p¼0.0029)
Roh et al. (2007)	–	The wild-type *C. elegans* Bristol strain N2	Experimental study	To identify a suitable tool to develop a screening system for ecotoxicity monitoring and to investigate DEHP toxicities to *C. elegans* using multiple toxic endpoints, such as mortality, growth, reproduction, and stress-related gene expression, focusing on the identification of chemical-induced gene expression as a sensitive biomarker for DEHP toxicity	Decreases in body length and egg number per worm observed after 24 h of DEHP exposure may induce long-term alteration in the growth and reproduction of the nematode population. Based on the result from the *C. elegans* genome array and indicated in the literatures, stress proteins, metallothionein, vitellogenin, xenobiotic metabolism enzymes, apoptosis-related proteins, and antioxidant enzyme genes were selected as stress-related genes and their expression in *C. elegans* by DEHP exposure was analyzed semi-quantitatively. Expression of heat shock protein (hsp)-16.1 and hsp-16.2 genes was decreased by DEHP exposure. Expression of cytochrome P450 (cyp) 35a2 and glutathione-*S*-transferease (gst)-4, phase I and phase II of xenobiotic metabolism enzymes, was increased by DEHP exposure in a concentration-dependent manner. An increase in stress-

Table 1 (continued)

Reference	Location	Population studied	Type of study	Aims	Finding
					related gene expressions occurred concomitantly with the deterioration on the physiological level, which suggests an increase in expression of those genes may not be considered as a homeostatic response but as a toxicity that might have physiological consequences
Baek et al. (2007)	–	5-week-old Sprague–Dawley male rats	Experimental study	To examine the potential antiandrogenic or estrogenic EDCs affect spermatogenesis, by analyzing PHGPx mRNA expression and histopathological changes in testes of rats exposed to various EDCs	Mild proliferation of germ cells and hyperplasia of interstitial cells were observed in the testes of the flutamide-treated group and deletion of the germinal epithelium and sloughing of germ cells were observed in testes of the diethylstilbestrol-treated group. Treatment with testosterone was shown to slightly decrease PHGPx mRNA levels in testes by the reverse transcription polymerase chain reaction. However, antiandrogenic compounds (flutamide, ketoconazole, and diethylhexyl phthalate) and estrogenic compounds (nonylphenol, octylphenol, and diethylstilbestrol) significantly upregulated PHGPx mRNA in the testes ($P < 0.05$)
Spjuth et al. (2007a, b)	–	One pair of male piglet siblings (Swedish Yorkshire, Swedish Landrace	Experimental study	To determine whether prepubertal exposure in boars to DEHP	The spermatozoa were cryopreserved and examined post-thaw by flow cytometry for their ability to capacitate in vitro when exposed to the effector bicarbonate and to acrosome-react when exposed to calcium ionophores, using the lipid stain Merocyanine-540 (m-540) and peanut agglutinin-fluorescein isothiocyanate, respectively, as probes. The ability of the DNA to sustain denaturation in vitro was tested using a sperm chromatin structure assay (SCSA). No significant differences between the DEHP-exposed group and controls were found for any of the sperm attributes examined. Frozen–thawed spermatozoa showed similar rates of non-capacitated cells between groups and were capacitated at similar rates. Rates of induced ARs were also similar. Values of DNA denaturation were low and showed no differences between groups
Svechnikova et al. (2007)	–	Immature (20-day-old) Sprague–Dawley female rats	Experimental study	To investigate the effects of DEHP on the hypothalamic–pituitary–gonadal axis of young developing female rats, as well as on ex vivo steroidogenesis by granulosa cells (GCs) and secretion of LH by gonadotropes	Exposure of 20-day-old female rats to 500 mg DEHP by oral gavage once daily for 10 days reduced their serum levels of progesterone and estradiol while tending to enhance levels of LH. Furthermore, primary cultures of GCs isolated from these rats exhibited an attenuated

Table 1 (continued)

Reference	Location	Population studied	Type of study	Aims	Finding
					capacity to produce progesterone in response to stimulation by LH and FSH, as well as a lower degree of transport of endogenous cholesterol into mitochondria. Moreover, the ability of primary cultures of pituitary cells isolated from DEHP-treated rats to produce and secrete LH in response to GnRH was significantly enhanced. In addition, 2-ethylhexanoic acid, ametabolite of DEHP, significantly potentiated GnRH-stimulated production of LHby cultures of pituitary cells isolated from untreated 20-day-old female rats
Spjuth et al. (2007a, b)	–	Young boars	Experimental study	To assess the effects of prepubertal DEHP exposure on the ability of spermatozoa to penetrate homologous oocytes in vitro	Both the penetration rate and the number of spermatozoa per oocyte were considered within expected ranges for frozen boar semen of good quality. Penetration rate did not significantly differ ($P > 0.05$) between the groups with DEHP-exposed (50 %) and control (59 %), which could be owing to a large variation between boars, and between replicates. The number of spermatozoa in the ooplasm was low and similar ($p > 0.05$) between the groups with DEHP-exposed: 1.5 and the control: 1.7.
Lin et al. (2008)	–	Pregnant Long–Evans female rats	Experimental study	To investigate effects of DEHP on FLC function	The percentage of FLC clusters containing 6–30 cells increased in all treatment groups, with 29–2 % in control vs. 37 ± 3, 35 ± 3, and 56 ± 4 % in rats receiving 10, 100, and 750 mg/kg DEHP, respectively. In contrast, fetal Leydig cell (FLC) numbers were 33 and 39 % lower than control after exposures to 100 and 750 mg/kg DEHP, respectively. At these doses, mRNA levels of leukemia inhibitory factor (LIF) increased. LIF was found to induce cell aggregation in FLCs in vitro, consistent with the hypothesis that DEHP induced FLC aggregation. Testicular testosterone (T) levels were doubled by the 10-mg/kg dose and halved at 750 mg/kg. The mRNA levels of IGF-1 and c-Kit ligand (KITL) were induced by 10 mg/kg DEHP
Culty et al. (2008)	–	Timed pregnant Sprague–Dawley rats	Experimental study	To determine the effect of in utero exposure to a wide range of DEHP doses on steroid production from fetal life to adulthood and to relate functional, morphological and molecular changes	Exposure to 234 to 1250 mg/kg/day DEHP resulted in increases in the absolute volumes of Leydig cells per adult testis. Despite this, adult serum testosterone levels were reduced significantly compared to controls at all DEHP 38 doses. Organ cultures of testes from

Table 1 (continued)

Reference	Location	Population studied	Type of study	Aims	Finding
					GD20 rats exposed in utero to DEHP showed dose-dependent reductions in basal testosterone production. Surprisingly, however, there was no significant effect of DEHP on hCG-induced testosterone production by GD20 testes, suggesting that the inhibition of basal steroidogenesis was due to the alteration of molecular events upstream of the steroidogenic enzymes. Reduced fetal and adult testosterone production in response to in utero DEHP exposure appeared unrelated to changes in testosterone metabolism. In view of the DEHP-induced reductions in adult testosterone levels, a decrease in the expression of steroidogenesis-related genes was anticipated
Rozati et al. (2008)	Region of Andhra Pradesh, India	99 infertile women with endometriosis (study group); 135 age-matched women without endometriosis (control group) but with infertility related to tubal defects, fibroids, polycystic ovaries, idiopathic infertility and pelvic inflammatory diseases	Case–control study	To evaluate the possible association between phthalate esters (PEs) and the occurrence of endometriosis	Women with endometriosis showed significantly higher concentrations of phthalate esters (dimethyl phthalate (DMP), diethyl phthalate (DEP), di-*n*-butyl phthalate (DnBP), butyl benzyl phthalate (BBP), and bis(2-ethylhexyl) phthalate (BEHP)) compared with control group. We found 38 % of the cases with endometriosis and 21 % of the control group. The correlation between the concentrations of phthalate esters and different severity of endometriosis was strong and statistically significant at $P < 0.05$ for all five compounds (DMP: $r = +0.57$, $P < 0.0001$; DnBP $r = +0.39$, $P < 0.0001$; BBP: $r = +0.89$, $P < 0.0001$; DnOP: $r = +0.66$, $P < 0.0001$; and BEHP: $r = +0.33$, $P < 0.0014$
Liu et al. (2008)	–	Mature C57BL/6 mice	Experimental study	To examine the effects of DEHP on the expression of transforming growth factor-b1 (TGF-b1) in fetal mice, as genital tubercles (GT) development is dependent upon this factor	Data showed a significant inhibition of male fetal GT development following DEHP treatment. Hypospadic-like urethral orifice and abnormal urethra were evaluated macroscopically and by histology at ED19. By using reverse-transcription polymerase chain reaction (RT-PCR) and Western blot, the expression of TGF-b1 was upregulated in DEHP-treated mice
Yanagisawa et al. (2008)	–	Male and female mice (8 weeks of age)	Experimental study	To examine whether maternal exposure to DEHP during fetal and/or neonatal periods in NC/Nga mice affects atopic dermatitis-like skin lesions related to mite allergen in offspring	Maternal exposure to a 100-μg dose of DEHP during neonatal periods, but not during fetal periods, enhanced atopic dermatitis-like skin lesions related to mite allergen in males. The results were concomitant with the enhancement of

Table 1 (continued)

Reference	Location	Population studied	Type of study	Aims	Finding
					eosinophilic inflammation, mast cell degranulation, and protein expression of eotaxin in overall trend
Han et al. (2009)	Seoul	Semen specimens (n = 99) were obtained from healthy volunteers at the Department of Urology, Yonsei University HealthSystem (young men (age 20–25 years))	Cross-sectional	Measurements of the levels of DEHP and DBP metabolites (mono(2-ethylhexyl) phthalate (MEHP), mono-nbutyl phthalate (MBP), and phthalic acid (PA) are often included in exposure assessment of DEHP and DBP and DEHP, DBP, and PA	UPLC/MS/MS showed that mean concentrations in semen samples were 1.07 mg/ml for MEHP, 0.61 mg/ml for DEHP, 0.39 mg/ml for PA, 0.06 mg/ml for MBP, and 0.003 mg/ml for DBP. The concentration of MEHP (the metabolite of DEHP) was highest, and the concentrations of the metabolites including MEHP, MBP, and PA were higher than actual concentrations of parent DEHP and DBP
Botelho et al. (2009)	–	60 male Wistar rats aged 21 days	Experimental study	To assess possible alterations induced by the plasticizer DEHP on cholesterol, testosterone, and thyroxine (total T4) levels, as well as to discuss the significance of these data in global changes observed in the reproductive tract of pubertal animals	At the end of the treatment, significant decreases in relative weight of testosterone dependent organs, delayed preputial separation, and low serum testosterone were observed at the highest DEHP dose. The plot of the relationship between DEHP dose and serum cholesterol revealed a biphasic effect. The concentration of cholesterol in serum was significantly reduced at 250 mg/kg/day DEHP but returned to control values at 750 mg/kg/day. Cholesterol levels measured in testicular tissue increased with DEHP treatment. Serum T4 levels were not affected by DEHP at any dose, indicating the absence of a link between total thyroxin concentration and phthalate effects on cholesterol levels
Martino-Andrade et al. (2009)	–	Wistar rats	Experimental study	To investigate the changes produced by the coadministration of DEHP and DBP on fetal testis histology and testosterone content as well as postnatal endpoints	A significant decrease in fetal testicular testosterone levels was observed in animals exposed to 500 mg DBP/kg/day or the phthalate mixture. Similarly, histological analysis of the fetal testis revealed that the coadministration of DEHP and DBP was able to increase the diameter of seminiferous cords and induce gonocyte multinucleation at doses that individually had no significant effects on these variables. However, in the phthalate mixture group, no significant changes were observed in anogenital distance and nipple retention, variables that are used to indicate possible antiandrogenic effects. Also, the adult endpoints investigated that included reproductive organ weights and the number of spermatids per testis were unaffected by any treatment regimen
Martinez-Arguelles et al. (2009)	–		Experimental study		

Table 1 (continued)

Reference	Location	Population studied	Type of study	Aims	Finding
		Timed pregnant Sprague–Dawley rats		To investigate in utero exposure to DEHP decreases mineralocorticoid receptor expression in the adult testis	Among the nuclear receptors studied, the mineralocorticoid receptor (MR) mRNA and protein levels were reduced in PND60 interstitial Leydig cells, accompanied by reduced mRNA expression of MR-regulated genes. Methylation-sensitive PCR showed effects on the nuclear receptor subfamilies NR3A and -3C, but only MR was affected at PND60. Pyrosequencing of two CpG islands within the MR gene promoter revealed a loss of methylation in DEHP-treated animals that was correlated with reduced MR
Saillenfait et al. (2009a, b)	-	Nulliparous female (180–200 g) Sprague–Dawley rats	Experimental study	To determine the long-term effects of an in utero exposure to DnHP on the reproductive development of the male offspring	DnHP had no significant effect on maternal body weight gain and pup weights during lactation. The proportion of live pups on postnatal day 1 was slightly, but not significantly, lower than control at 250 and 500 mg DnHP/kg/day. Male offspring displayed reduced anogenital distance on postnatal day 1 (PND) at 125 mg DnHP/kg/day and above and areola/nipple retention before weaning and at adulthood at 250 and 500 mg DnHP/kg/day. At necropsy on PNDs 70–78 or PNDs 111–120, severe malformations of the reproductive tract were observed in young adult males at 125 mg DnHP/kg/day and higher doses. They mainly consisted of hypospadias, underdeveloped testis, and undescended testis. Additionally, histopathological examination revealed seminiferous tubule degeneration at the two high doses
Saillenfait et al. (2009a, b)	–	Female (180–200 g) Sprague–Dawley rats	Experimental study	To evaluate the developmental toxic potential of di-*n*-hexyl phthalate (DnHP) and dicyclohexyl phthalate (DCHP) in rats	Slight changes in live weight associated with peroxisomal enzyme induction were seen in dams treated with DnHP or DCHP. DnHP caused dose-related developmental toxic effects, including marked embryo mortality at 750 mg kg^{-1} per day and presence of malformations (mainly cleft palate, eye defects, and axial skeleton abnormalities) and significant decreases in fetal weight at 500 and 750 mg kg^{-1} day. Significant delay of ossification and increase in the incidence of skeletal variants (e.g., supernumerary lumbar ribs) also appeared at 250 mg kg^{-1} day. DCHP produced fetal growth retardation at 750 mg kg^{-1} day, as evidenced by significant reduction of fetal weight.

Table 1 (continued)

Reference	Location	Population studied	Type of study	Aims	Finding
					DnHP and DCHP induced a significant and dose-related decrease in the anogenital distance of male fetuses at all doses, and there was a significant increase in the incidence of male fetuses with undescended testis at 500 and 750 mg kg^{-1} day of DnHP
Gray et al. (2009)	–	Timed pregnant Sprague–Dawley (CR:CD(SD)IGSBR) rats (approximately 90 days of age)	Experimental study	To define the dose–response relationship between DEHP and the phthalate syndrome of reproductive alterations	Anogenital distance, sperm counts, and reproductive organ weights were reduced in F1 males in the 300-mg/kg/day group, and they displayed retained nipples. In the IUL cohort, seminal vesicle weight also was reduced at 100 mg/kg/day. In contrast, serum testosterone and estradiol levels were unaffected in either the PUB or IUL cohorts at necropsy. A significant percentage of F1 males displayed one or more phthalate syndrome lesions at 11 mg/kg/day DEHP and above
Carnevali et al. (2010)	–	Adult *Danio* (zebrafish) females	Experimental study	To assess the impacts of DEHP on zebrafish oogenesis and embryo production	A significant reduction of fecundity in fish exposed to DEHP was observed. The reduced reproductive capacity was associated with an increase in ovarian protein-15 (BMP15) levels. This rise, in turn, was concomitant with a significant reduction in luteinizing hormone receptor (LHR) and membrane progesterone receptor (mPR) levels. Finally, ptgs2 expression, the final trigger of ovulation, was also decreased by DEHP
Carbone et al. (2010)	–	Wistar rats	Experimental study	To investigate the effect of the pre- and perinatal exposure to DEHP on the neuroendocrine parameters that regulate reproduction in peripubertal male rats	No changes in gonadotropin, aspartate, and gamma-aminobutyric acid levels were detected at the low dose. DEHP 30 mg/kg bw/day reduced testis weight and serum FSH, in correlation with a significant increase in the inhibitory GABAergic tone and a reduction in the stimulatory effect of aspartate on gonadotropin level
Wu et al. (2010)	–	Kunming mice	Experimental study	To analyze epigenetic (specifically, DNA methylation) change in testes induced by maternal exposure to DEHP	DEHP significantly had more than 10 % relative increase in the global DNA methylation and also increased DNA methyltransferases' expression
Uren-Webster et al. (2010)	–	Adult zebrafish	Experimental study	To investigate the effects of exposure to a range of concentrations of DEHP, including those occurring in the aquatic environment, on the reproductive health of malezebrafish (*D. rerio*)	Significant increase in the hepatosomatic index and levels of hepatic vitellogenin transcript were observed following exposure to 5000 mg DEHP kg^{-1}. Exposure to 5000 mg DEHP kg^{-1} also resulted in a reduction in fertilization success of oocytes spawned by untreated females. However, survival and development of the resulting embryos were unaffected by all treatments, and no

Table 1 (continued)

Reference	Location	Population studied	Type of study	Aims	Finding
					evidence of DEHP-induced sperm DNA damage was observed. Exposure to 50 and 5000 mg DEHP kg^{-1} caused alterations in the proportion of germ cells at specific stages of spermatogenesis in the testis, including a reduction in the proportion of spermatozoa and an increase in the proportion of spermatocytes, suggesting that DEHP may inhibit the progression of meiosis. In parallel, exposure to 5000 mg DEHP kg^{-1} increased the levels of two peroxisome proliferator-activated receptor (PPAR) responsive genes (acyl-coenzyme A oxidase 1 (acox1) and enoyl-coenzyme A, hydratase/3-hydroxyacyl coenzyme A dehydrogenase (ehhadh)
Gupta et al. (2010)	–	Cycling female CD-1 (Charles River Laboratories, Charles River, CA) mice (32–35 days old)	Experimental study	To investigate whether PEs (specifically DEHP and MEHP) directly inhibit antral follicle growth by reducing E2 levels	We found that DEHP and MEHP inhibited growth of follicles and decreased estradiol production compared to controls at the highest doses. DEHP and MEHP also decreased mRNA expression of Ccnd2, Cdk4, and Arom at the highest dose. Addition of estradiol to the culture medium prevented the follicles from DEHP- and MEHP-induced inhibition of growth, reduction in estradiol levels, and decreased Ccnd2 and Cdk4 expression
Martinez-Arguelleset al. (2011)	–	Timed pregnant Sprague–Dawley rats	Experimental study	To investigate in utero exposure to the antiandrogen DEHP decreases adrenal aldosterone production	There was no effect on circulating levels of potassium, angiotensin II, or adrenocorticotropin hormone (ACTH). However, there was reduced expression of AT receptor Agtr1a, Agtr1b, and Agtr2 mRNAs. The mRNA levels of proteins and enzymes implicated in aldosterone biosynthesis were not affected by in utero DEHP treatment except for Cyp11b2, which was decreased at high (500 mg kg^{-1} day^{-1}) doses
Ambruosi et al. (2011)	–	Horse	Experimental study	To analyze the effects of in vitro acute exposure to DEHP on oocyte maturation, energy and oxidative status in the horse, a large animal model, cumulus cell (CC), apoptosis, and oxidative status	DEHP significantly inhibited oocyte maturation when added at low doses (0.12 mM; $P < 0.05$). This effect was related to increased CC apoptosis ($P < 0.001$) and reduced ROS levels ($P < 0.0001$). At higher doses (12 and 1200 mM), DEHP-induced apoptosis ($P < 0.0001$) and ROS increase ($P < 0.0001$) in CCs without affecting oocyte maturation. In DEHP-exposed MII oocytes, mitochondrial distribution patterns, apparent energy status (MitoTracker fluorescence intensity),

Table 1 (continued)

Reference	Location	Population studied	Type of study	Aims	Finding
					intracellular ROS localization and levels, mt/ROS colocalization ,and total SOD activity did not vary, whereas increased ATP content ($P < 0.05$), possibly of glycolytic origin, was found
Xi et al. (2011)	–	Male and female mice (CD-1 strain, 8 weeks of age)	Experimental study	To investigate the effects of maternal transfer of bisphenol A (BPA) and DEHP during gestational and weaning periods on gonadal development of male offspring	The data indicated that the exposure significantly reduced the male-to-female sex ratio and the sizes of the gonads of male pups as recorded at PND15. The testes of the perinatally exposed male pups were developed less and the expression levels of testicular antimullerian hormone, androgen receptor, cyclin A, and StAR were significantly lesser than the control male pups. The less developed testes were accompanied with significant reductions in the expression levels of Gnrh and Fsh at the hypothalamic–pituitary levels
Hayashi et al. (2011)	–	Three genotyped male and female mice, i.e., wild-type (mPPAR), Ppar-null and hPPAR,Tet-Off with a Sv/129 genetic background	Experimental study	To clarify the mechanism of DEHP-induced adverse effects on offspring in relation to maternal mouse and human PPAR	Dams were killed on gestational day 18 and postnatal day (PND) 2. High-dose DEHP decreased the number of total and live fetuses and increased resorptions in mPPAR (peroxisome proliferator-activated receptor) mice. In hPPAR_ mice, resorptions were increased above the medium dose, and the number of births was decreased at the high dose. The number of live pups on PND2 was decreased over the medium dose in mPPAR_ and at the high dose in hPPAR mice. No such findings were observed in Ppar_-null mice. High-dose DEHP decreased plasma triglyceride in pregnant mPPAR mice but not in Ppar_-null and hPPAR_ ones. Above the medium dose in mPPAR, mice significantly reduced hepatic microsomal triglyceride transfer protein (MTP) expression. Mediumand/or high-dose DEHP increased the levels of maternal PPAR_ target genes in mPPAR and hPPAR mice
Li et al. (2011)	Shanghai	118 men who were suspected of infertility	Cross-sectional	To investigate the associations of hormone circulation with phthalate exposure in adult men	Serum PRL was positively associated with serum DBP and DEHP and semen DEHP in all models of Spearman correlation, linear regression, and binary logistic regression. In linear regression models adjusted for potential confounders and excluding subjects with undetectable phthalates, a 10-fold increase in semen DEHP was associated with a 23 % increase in serum PRL, as well as a 26 % increase in serum DBP and a 20 %

Table 1 (continued)

Reference	Location	Population studied	Type of study	Aims	Finding
					increase in serum DEHP. In logistic regression models, all subjects demonstrated a dose–response relationship between above reference value PRL and semen DEHP (odds ratio per tertile adjusted for potential confounders = 1.0, 1.70, 3.50; P for trend = 0.01), and serum DBP (1.0, 1.10, 2.62; P for trend = 0.04), and serum DEHP (1.0, 1.46, 4.69; P for trend < 0.01). A positive correlation between serum estradiol and semen DEHP (linear regression) and an inverse correlation between semen DBP and serum testosterone and T/E2 ratio (Spearman correlation) were also established
Huang et al. (2012)	–	8–10-week-old female B6D2F1 (C57BL/66DBA/2) strain mice were used as oocyte donors and 10–12-week-old male B6D2F1 mice were used as semen donors	Experimental study	To determine the reproductive hazards of DEHP on mouse spermatozoa and embryos in vitro and genomic changes in vivo	DEHP-treated spermatozoa (1 mg/ml, 30 min) presented reduced fertilization ability ($P < 0.05$) and the resultant embryos had decreased developmental potential compared to DMSO controls ($P < 0.05$). Meanwhile, the transferred 2-cell stage embryos derived from treated spermatozoa also exhibited decreased birth rate than that of control ($P < 0.05$). When fertilized oocytes or 2-cell stage embryos were recovered by in vivo fertilization (without treatment) and then exposed to DEHP, the subsequent development that proceeded to blastocysts was different, fertilized oocytes were significantly affected ($P < 0.05$) whereas developmental progression of 2-cell stage embryos was similar to controls ($P < 0.05$). Testes of the Big Blue® transgenic mice treated with DEHP for 4 weeks indicated an approximately 3-fold increase in genomic DNA mutation frequency compared with controls ($P < 0.05$)
Carbone et al. (2012)	–	Wistar rats	Experimental study	To investigate whether DEHP modifies gonadotropin levels and the hypothalamic content of the amino acid neurotransmitters ASP, glutamate (GLU), and GABA	No changes in gonadotropin levels and amino acid neurotransmitters were detected at the low dose in both sexes. However, DEHP administered at high dose (30 mg/kg bw/day) to dams produced a significant decrease in the inhibitory neurotransmitter GABA and an increase in the stimulatory neurotransmitter aspartate in prepubertal male offspring rats. These modifications were accompanied by gonadotropin serum level increase. On the contrary, in

Table 1 (continued)

Reference	Location	Population studied	Type of study	Aims	Finding
					treated female rats, this chemical increased both, aspartate and GABA, which exert a characteristic stimulatory action on gonadotropin in 15-day-old normal females
Suzuki et al. (2012)	Japan	340 pregnant women	Cohort study	To examine the relationship between prenatal exposure to phthalate esters and the anogenital distance (AGD) as a reproductive endpoint in human male newborns	In a multiple regression model, the log-transformed mono-2-ethylhexyl phthalate concentration (specific gravity-corrected) was negatively significant, and maternal smoking status was positively significant, in explaining anogenital index (AGI) when potential covariates were controlled for. Urinary isoflavones did not significantly contribute to AGI in any models
Klinefelter et al. (2012)	–	Pregnant dams	Experimental study	To examine the relationship between dysgenesis and steroidogenic capacity in the fetal rat testis more stringently by incorporating lower exposures than those typically used, conducting a comprehensive, non-targeted quantitative evaluation of the fetal testis proteome, and relating alterations in individual proteins to thecapacity of the fetal Leydig cell to produce testosterone and histopathology of the fetal testis	Each endpoint was represented by 16 l. Clustering of Leydig cells occurred before any significant decrease in the capacity of the GD19 Leydig cell to produce testosterone. At 100 mg DEHP/kg, testosterone production was reduced significantly, Leydig cell clusters became quite large, and additional dysgenetic changes were observed in the fetal testis. Of 23 proteins, whose expression was altered significantly at both DEHP exposure levels, seven were found to be correlated with and predictive of the quantified endpoints. None of these proteins have been previously implicated with DEHP exposure
Dorostghoal et al. (2012)	–	Wistar rats	Experimental study	To assess long-term effects of maternal exposure to DEHP on reproductive ability of both neonatal and adult male offspring	Mean testis weight decreased significantly ($P < 0.05$) in 500 mg/kg/day dose group from 28 to 150 days after birth. Significant decreases were seen in total volumes of testis in 100 ($P < 0.05$) and 500 ($P < 0.01$) mg/kg/day dose groups until 150 days after birth. Seminiferous tubule diameter and germinal epithelium height decreased significantly in 100 ($P < 0.05$) and 500 ($P < 0.01$) mg/kg/day dose groups during postnatal development. Also, mean sperm density in 100 mg/kg/day ($P < 0.05$) and 500 mg/kg/day ($P < 0.01$) dose groups and percent of morphologically normal sperm in highest dose group ($P < 0.05$) decreased significantly until 150 days after birth
Dobrzyńska et al. (2012)	–	Outbreed Pzh: SFIS mice	Experimental study	To investigate of potential genotoxic effects induced by subchronic exposure to DEHP in germ cells	A slight increase in the frequency of prenatal deaths and dominant lethal mutations, as well as a significantly increased percentage of abnormal

Table 1 (continued)

Reference	Location	Population studied	Type of study	Aims	Finding
					skeletons among the F1 offspring of males exposed to 8000 mg/kg of DEHP, were observed. Exposure of the fathers did not cause a delay in the postnatal development of the offspring, except for fur development in the group of 8000 mg/kg of DEHP. Gametes of male offspring of exposed fathers showed reduced motility
Schmidt et al. (2012)	–	Female C3H/N mice	Experimental study	To examine the effects of dietary DEHP exposure on metabolism and fertility in female mice	In study I, DEHP-exposed F0 females (all dose groups) had a significant increase in body weight, food intake, and visceral adipose tissue compared with controls. In the 500-mg DEHP group, PPARα and PPARγ transcripts were significantly changed in liver tissue. In the same group, PPARγ mRNA was significantly reduced in liver but not in fat tissue. In addition, leptin and fatty acid binding protein 4 (FABP4) mRNA were increased in adipose tissue, whereas adiponectin was decreased. In study II, we detected a 100 % abortion rate in F0 dams in the 500-mg group. F1 offspring exposed in utero and during lactation had an increase in visceral fat tissue and body weight
Li et al. (2013)	–	Sprague–Dawley rats	Experimental study	To evaluate dose-related effects on external genitalia of adult male offspring rats by maternal exposure to DEHP	The hypospadias were observed and the incidence in three DEHP dosage levels was 10.7, 30.6, and 37.0 %, respectively. With exposed dose increased, mild, moderate, and severe hypospadiac rats were distinguished and an increased incidence of severe hypospadias was observed. The other reproductive lesions like reduced penile length and anogenital distance/body weight were observed. The results indicated the dose-related external genitalia teratogenic toxicity, and graded hypospadias on male offspring resulted from high-dosage DEHP maternal exposure
Doyle et al. (2013)	–	Crl:CD1(ICR) (CD1) outbred mice	Experimental study	To investigate the transgenerational effects of DEHP on testicular germ cell associations and spermatogonial stem cells	This exposure scheme disrupted testicular germ cell association and decreased sperm count and motility in F1 to F4 offspring. By spermatogonial transplantation techniques, the exposure scheme also disrupted spermatogonial stem cell (SSC) function of F3 offspring. The W/WV recipient testes transplanted with F3 offspring germ cells from the DEHP-treated group had a dramatically lower percentage of donor germ cell-

Table 1 (continued)

Reference	Location	Population studied	Type of study	Aims	Finding
					derived spermatogenic recovery in seminiferous tubules when compared to the recipient testes transplanted with CD1 control germ cells. Further characterization showed that the major block of donor germ cell-derived spermatogenesis was before the appearance of undifferentiated spermatogonia. Interestingly, the testes transplanted with the F3 offspring germ cells from the DEHP-treated group, when regenerated, replicated testis morphology similar to that observed in the testes from the F1 to F3 offspring of the DEHP-treated group, suggesting that the germ cell disorganization phenotype originates from the stem cells of F3 offspring
Kitaoka et al. (2013)	–	Male A/J mice (7 weeks old, n = 105)	Experimental study	To examine the testicular immune microenvironment after exposure to doses of DEHP, previously identified as no-observed-adverse-effect levels	The results showed that a slight but significant spermatogenic disturbance appeared in the 0.1 % DEHP group but not in the 0.01 % DEHP group at 8 weeks. It was also demonstrated that lymphocytes and F4/80- and MHC class II-positive cells were significantly increased with the elevation of IL-10 and IFN-γ mRNA expressions in the testes of not only the 0.1 % DEHP group but also the 0.01 % DEHP group at 8 weeks. Histochemical analyses involving horseradish peroxidase (HRP) as a tracer showed that a little bloodborne HRP had infiltrated into the lumen of a few seminiferous tubules beyond the blood–testis barrier in both the 0.1 and 0.01 % DEHP groups at 8 weeks
Carbone et al. (2013)	–	Wistar rats	Experimental study	To examined the effects of perinatal exposure to DEHP on anxiety-like behavior, using the elevated plus maze (EPM) test	They found sex differences in behavior induced by DEHP; while male rats of 45 and 60 days of age showed a significant decrease in FEO and TSO percentages, as well as an increase in TSC, no changes were observed in anxiety-like behavior in perinatal DEHP-exposed females at these ages of sexual maturation. In 60-day-old male rats, DEHP exposure produced a significant decrease in serum testosterone levels. Testosterone replacement was able to antagonize the adverse effects of DEHP exposure on LH, activating the negative feedback mechanism of this steroid on reproductive axis, as well as increasing FEO and TSO percentages to similar values observed in the control group

Table 1 (continued)

Reference	Location	Population studied	Type of study	Aims	Finding
Saillenfait et al. (2013)	–	Pregnant Sprague–Dawley rats	Experimental study	To investigate the DnHP on the dose–response relationship for its effects on testosterone production and on the expression of genes involved in the testosterone biosynthesis pathway in the fetal rat testis	DnHP reduced ex vivo testosterone production and downregulated the expression of several genes required for cholesterol transport and steroid synthesis (i.e., SR-B1, StAR, P450scc, 3bHSD, and P450c17). These inhibitions were dose-dependent. A no-effect level was established at 5 mg kg^{-1} per day and a lowest-effect level at 20 mg kg^{-1} per day. MRNA levels of SR-B1, StAR, P450scc, and 3bHSD were not similarly decreased in the adrenals. In conclusion, DnHP shares the same mode of action as DEHP in disrupting fetal testicular androgen synthesis
Aydoğan Ahbab et al. (2013)	–	Pregnant time-mated female Wistar albino rats	Experimental study	To investigate the effects of in utero di-*n*-hexyl phthalate (DHP) and dicyclohexyl phthalate exposure (DCHP) on the development of male reproductive tract	Testosterone (T) levels of pubertal rats decreased in low- and high-dose DHP and DCHP groups. Inhibin B levels of adult rats diminished in DCHP groups. Atrophic and amorphous tubules, spermatogenic cell debris, apoptotic cells, adherent tubules, Sertoli cell vacuolization, prostatic atrophic tubules, and prostatic intraepithelial neoplasia (PIN) were observed in the reproductive organs of treated animals at all developmental stages. There was an increase in immunoexpression of MIS/AMH in testes of treated rats. There were no changes in sperm head count but percentages of abnormal sperms increased. The diameters of seminiferous and epididymal tubules in treatment groups were significantly lower
Corradetti et al. (2013)	–	Adult male zebrafish (*Danio rerio*)	Experimental study	To evaluate the effects of environmentally relevant concentrations of DEHP (0.2 and 20 mg/l) on the reproductive biology	DEHP impaired reproduction in zebrafish by inducing a mitotic arrest during spermatogenesis, increasing DNA fragmentation in sperm cells, and markedly reducing embryo production (up to 90 %
Ye et al. (2014)	–	Fish maintenance	Experimental study	To examine whether long-term exposure to DEHP and its active MEHP disrupts endocrine function in marine medaka (*Oryzias melastigma*.	Exposure to DEHP, but not MEHP, from hatching to adulthood accelerated the start of spawning and decreased the egg production of exposed females. Moreover, exposure to both DEHP and MEHP resulted in a reduction in the fertilization rate of oocytes spawned by untreated females paired with treated males. A significant increase in plasma 17-estradiol (E2) along with a significant decrease in testosterone (T)/E2ratios was observed in males, which was accompanied by the upregulation of ldlr,

Table 1 (continued)

Reference	Location	Population studied	Type of study	Aims	Finding
					star, cyp17a1, 17ˆhsd, and cyp19a transcription in the testis. Increased concentrations of T and E2 were observed in females, which was consistent with the upregulation of ldlr. The expression of brain gnrhr2, fshˆ, cyp19b, and steroid hormone receptor genes also corresponded well with hormonal and reproductive changes. The liver VTG level was significantly increased after DEHP and MEHP exposure in males. DEHP induced histological changes in the testes and ovaries: the testes displayed a reduced number of spermatozoa, and the ovaries displayed an increased number of atretic follicles
Romani et al. (2014)	–	23 normally menstruating patients in the midluteal phase (human luteal cells isolated from corpora lutea for primary cultures)	Experimental study	To evaluate the influence of phthalates on human luteal cell function	Phthalate influence on VEGF expression has been also evaluated. DEHP, DBP, and BBP were able to reduce both basal and hCG-stimulated P4 as well as PGE2 release. PGF2a release was reduced after DEHP incubation. VEGF protein release was decreased by the incubation with the tested phthalates. VEGF mRNA expression was not affected by DEHP, DBP, and BBP. As expected, both hCG and cobalt chloride were able to induce P4 release and VEGF release and mRNA expression in human luteal cells, respectively
Parillo et al. (2014)	–	Rabbit corpora lutea (CL)	Experimental study	To study the in vivo chronic and in vitro acute effects of DEHP on the reproductive function of peroxisome proliferator-activated receptor gamma (PPARG)	DEHP decreased progesterone plasma levels and CL production in all luteal stages and PPARG protein and gene expressions in early and mid-CL. DEHP in vivo treatment reduced PTGS2 protein expression at late stage and that of PGE2–9-K at all stages, whereas PTGS1 and 3beta-HSD were not affected. In in vitro cultured CL, DEHP alone, PPARG antagonist (T0070907) alone, or DEHP plus T0070907 diminished progesterone production and 3beta-HSD activity and increased PGF2alpha and PTGS2 in early and mid-CL, whereas DEHP plus PPARgamma agonist (15d-PGJ2) did not affect these hormones and enzymes. No in vitro treatments affected PGE2 secretion as well as PTGS1 and PGE2-9-K enzymatic activities in all luteal stages
Wolff et al. (2014)	New York City,	1239 girls, 6–8 years old	Multi-ethnic study	To investigate whether phthalate exposures were associated with	Urinary concentrations of high-MWP including di(2-ethylhexyl) phthalate

Table 1 (continued)

Reference	Location	Population studied	Type of study	Aims	Finding
	greater Cincinnati, and the San Francisco Bay Area			pubertal timing in girls and whether these relationships were modified by obesity	(SDEHP) metabolites were associated with later pubic hair development during 7 years of observation. The relationship was linear and was stronger among normal-weight girls. Among normal-weight girls, age at pubic hair stage 2 (PH2) was 9.5 months older for girls in the fifth compared with the first quintile of urinary SDEHP (medians 510 and 59 mg/g creatinine, respectively; adjusted HR 0.70, CI 0.53–0.93, P_{trend} 0.005. Age at first breast development was older for fifth quintile of mono-benzyl phthalate vs. first (HR 0.83, CI 0.68–1.02; P_{trend} 0.018)
Kranvogl et al. (2014)	Maribor, Slovenia	136 male urine samples	Observational study	To present and evaluate correlations between endocrine disrupting compounds (5 dialkyl phthalates, 9 phthalate monoesters, 3 alkylphenols, and bisphenol A) and also correlations of endocrine disrupting compounds with two semen quality parameters	Significant positive correlations were found between almost all the endocrine-disrupting compounds. The parameter sum of DEHP (SUM DEHP) was positively correlated to all the endocrine-disrupting compounds but negatively to two semen quality parameters. Negative correlations between the endocrine-disrupting compounds and the semen quality parameters could indicate that endocrine-disrupting compounds could cause reproductive problems by decreasing the semen count and quality

(Maranghi et al. 2010). In fact, phthalates, including DEHP, are activators of nuclear receptors PPARα, β, and γ (Rusyn and Corton 2012).

Table 2 summarizes some of the most representative studies on carcinogenesis effects of DEHP.

DEHP effect on pregnancy outcome

Once DEHP is absorbed into the human body, it is hydrolyzed to MEHP, by enzymes present in the lung, blood, and gut. Therefore, MEHP can cross the placenta and arrive at fetal circulation (Whyatt et al. 2009). Prenatal exposure to MEHP has been associated with higher occurrence of early first trimester pregnancy loss (Arbuckle et al. 2014). As the DEHP action might be influenced by on time, age, and dose and because 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 newborn, particularly preterm (Latini et al. 2003b).

Also, reduced anogenital distances in male infants, a potential early marker of reproductive toxicity in humans, have been reported (Arbuckle et al. 2014). A recent large case–control study reported significant associations with MEHP, MECPP, and Σ DEHP metabolites (Ferguson et al. 2014). Summary of published literature of DEHP effect on pregnancy outcome is presented in Table 3.

DEHP effect on neurodevelopment

Prenatal exposures to phthalates might also be related child neurobehavioral development (Serrano et al. 2014). Elevated maternal urinary concentrations of some phthalate metabolites have been related with increased internalizing behaviors and decreased child mental and motor development and decreases in the psychomotor development index, particularly in girls (Whyatt et al. 2012). Likewise, findings of a birth cohort confirmed such effects in girls and confirmed that gender has an effect modifier in the association of prenatal phthalate exposure with neurodevelopment (Téllez-Rojo et al. 2013).

However, another birth cohort did not document the adverse effects of prenatal phthalate exposure on children's cognitive, psychomotor, or behavioral development (Gascon et al. 2015).

DEHP effects on respiratory system

DEHP might promote an aggravated asthmatic response involving increased immunological and inflammatory mediators and pathophysiological changes of lung tissue (Larsen et al. 2007). Moreover, the indoor environment might contribute to the increase in allergic asthma. As phthalates are not chemically bound to products, these compounds can diffuse within materials, leach out, and then adhere to airborne particles or disperse into the air. Therefore, phthalates easily enter into dust that settles on phthalate-containing products (Ait Bamai et al. 2014).

Asthma can have various symptoms with reversible airway obstruction, coughing, wheezing, tightness of the chest, and breathlessness. Specially, the prevalence of asthma and allergies in children has increased markedly in recent decades (Shin et al. 2014). Some epidemiological studies have suggested a higher risk of respiratory problems among workers of plastic industry and in children. Actually, these studies have offered evidence for the possible relationship between PVC materials and exposure to phthalates and the risk of allergic asthma (Guo et al. 2012). Summary of published literature of DEHP effect on respiratory system is presented in Table 4.

Discussion

This study reviewed the adverse health effects of exposure to DEHP on various organs. It serves as confirmatory evidence of diverse health hazards of DEHP on different organs. Our findings underscore the necessity of paying more attention to the adverse health effects of phthalates at individual and public levels. Reducing the industrial use of phthalates and or substituting them with safer plasticizers as well as increasing the public knowledge about such health hazards especially for females in reproductive age and the pediatric age group are of special concern.

Effects on reproductive system

The developing male reproductive system is one of the most vulnerable targets of phthalate ester toxicity (Hannas et al. 2011). Many experimental studies have suggested that phthalates and their metabolites might produce reproductive and developmental toxicities. Although most of these animals were exposed to phthalate esters at relatively high level to assess its toxicological effects, some studies showed that even relatively low doses of phthalates could cause toxic effects. Therefore, it remains to be determined whether humans are exposed to PAEs at harmful levels to generate health effects or not. DEHP is known to develop adverse effects on the male reproductive system in rodents; moreover, DNA damage in human lymphocytes is induced by DEHP and their metabolites as MEHP (Matsumoto et al. 2008). Experimental animal data exist on the influences of DEHP on reproduction following exposure during in utero development and the nursing period. Evidence also exists on reproductive effects of DEHP following exposure after receiving weaning food and during the adult life (Kavlock et al. 2002a). Some other animal studies revealed that oral exposure to DEHP could affect reproductive processes (Kavlock et al. 2006). Limited experience exists to characterize the DEHP reproductive and

Table 2 Phthalates and carcinogenesis

Reference	Location	Population sutdied	Type of study	Aims	Finding
Lewandowski et al. (1980)	–	Male Sprague–Dawley rats	Experimental study	To investigate the teratogenic potential of plasma-soluble extracts of diethylhexyl phthalate plasticized polyvinyl chloride pastics in rats	There were no significant effects on fetal weights or on crown-rump and transumbilical distances in offspring. The number of live and resorbed fetuses was not significantly different in control and treated groups. The incidence of gross external, skeletal, and visceral defects among offspring was similar in all groups, and there was no statistically significant difference between control and treated groups
Shiota and Nishimura (1982)	–	4-week-old male and female mice of ICR-JCL strain	Experimental study	To ascertain the effects of orally administered DEHP and DBP 66 on embryonic development and to determine the maximum nonembryotoxic dose in mice for extrapolation to the human	External malformations increased significantly by 0.2 % DEHP, and 1.0 % DBP showed borderline significance. The major malformations in treated groups were neural tube defects (exencephaly and myeloschisis), suggesting that the phthalic acid esters (PAEs) affect neural tube closure in developing embryos. Treatment with the compounds caused intrauterine growth retardation and delayed ossification with an apparently dose-related response pattern
Tomita et al. (1982)	–	Bacteria and mammalian cells (tester strains of *S. typhimurium* TA 100, *E. coli* WP2 B/r, and *B. subtilis* H17, M45) and V79 Chinese hamster	Experimental study	To determine whether DEHP and MEHP are mutagenic/ carcinogenic in animal cells	DEHP and MEHP showed mutagenic activities to *S. typhimurium* TA-100, with and without S-9 mix, respectively. Both DEHP and MEHP-induced 8AG/6TG-resistant mutation, chromosomal aberrations, and morphological transformation in the embryonic cells of the Syrian golden hamster
Kluwe et al. (1982)	–	Fischer 344 rats and B6C3Fx mice	Experimental study	To investigate the 2-yr. carcinogenesis bioassay of DEHP	Mean body weight gains of treated male rats (6000 and 12,000 mg/kg), female rats (12,000 mg/kg), and female mice (3000 and 6000 mg/kg) were less than those of the corresponding controls. Seminiferous tubular degeneration and hypertrophy of cells in the anterior pituitary were observed in male rats at 12,000 mg/kg, and chronic inflammation of the kidney and seminiferous tubular degeneration were observed in male mice at 6000 mg/kg. Treatment with DEHP caused liver tumors in both sexes of mice and rats. The incidence of treated animals with hepatocellular carcinomas was significantly ($P < 0.05$) greater than that in controls for female rats ingesting 12,000 mg DEHP/kg diet, male mice ingesting 6000 mg/kg, and female mice ingesting 3000 or 6000 mg/kg. The combined incidence of animals with hepatocellular carcinomas or neoplastic nodules was significantly greater than that in controls for male rats ingesting 12,000 mg/kg and female rats ingesting 6000 mg/kg, while the combined incidence of animals with hepatocellular carcinomas or adenomas was significantly greater than that in controls for male mice ingesting 3000 mg/kg. Twenty of the 57 hepatocellular carcinomas diagnosed in DEHP-treated mice (sexes and doses combined) had metastasized to the lung. Pulmonary metastases were not observed in the control mice or in any of the rats. Male rats at the higher dose (12,000 mg/kg) had significantly lower incidences of pituitary tumors, thyroid C-cell carcinomas, and testicular interstitial-cell tumors. The incidence of testicular interstitial-cell tumors

Table 2 (continued)

Reference	Location	Population sutdied	Type of study	Aims	Finding
					was negatively correlated ($P < 0.005$) with either the presence of pituitary hypertrophy or the presence of testicular degeneration
Deangelo and Garrett (1983)	–	Male Sprague–Dawley rats weighing 150–200 g	Experimental study	To investigate the effects of DEHP on the emergence of gamma glutamyltranspeptidase positive (GGT+) preneoplastic foci in the liver	Rats maintained on the CS + DEHP diet for 5 and 10 weeks showed no increase in GGT+ foci. The plasticizer effectively inhibited the appearance of the preneoplastic foci when it was included with the CD diet.
Ward et al. (1984)	–	Male weanling B6C3Fl mice, 3–4 weeks of age	Experimental study	To study the differential effects of short- or long-term exposure to the liver tumor promoters DEHP or phenobarbital (PB)	When DEHP was fed at a dietary level of 3000 ppm for 28, 84, and 168 days or PB was fed in the water at 500 ppm for 168 days, there were significantly increased incidences of mice with focal hepatocellular proliferative lesions (FHPL) and DEHP was an effective promoter after only 28, 84, or 168 days of exposure
Diwan et al. (1985)		JB6 cell lines C141, C121, and R219 (in vitro studies) and 275 female SENCAR mice (in vivo studies)	Experimental study	To investigate the tumor-promoting activity of DEHP in an in vivo mouse skin assay using SENCAR mice and in an in vitro assay using JB6 mouse epidermal cell lines	DEHP was inactive as a complete promoter of skin carcinogenesis initiated by 7,12-dimethylbenz[*a*]anthracene (DMBA) in SENCAR mice. Like the plant-derived natural product mezerein, however, DEHP significantly enhanced skin carcinogenesis in SENCAR mice when initiation by DMBA was followed by short-term applications (2×/week, 2 weeks) of 12-O-tetradecanoylphorbol-13-acetate prior to application of DEHP. A random sample of the tumors observed was processed and examined histologically. Most neoplasms were papillomas; a few were squamous cell carcinomas. The kinds of tumors seen were identical with those reported in two-stage carcinogenesis experiments in SENCAR mice in which phorbol ester tumor promoters have been employed
Shiota and Mima (1985)	–	4-week-old male and female mice	Experimental study	To compare the teratogenicity of DEHP administered PO and IP in ICR mice	Fetal weights were also significantly suppressed. Anterior neural tube defects (anencephaly and exencephaly) were the malformations most commonly produced. No teratogenic effects were revealed by IP doses of DEHP and PO or IP doses of MEHP, although high doses were abortifacient and lethal to pregnant females. Thus, DEHP is highly embryotoxic and teratogenic in mice when given PO but not IP. The difference in metabolism, disposition, or excretion by the route of administration may be responsible for the difference in DEHP teratogenicity
Mitchell et al. (1985)	–	Alderley Park SPF-derived albino strain rats	Experimental study	To provide information on the tissues and to detail sex differences in DEHP effects	Two early and transient alterations were noticed. First, there were morphologic changes in the bile canaliculi of male rats treated with 1000 mg/kg/day of DEHP. Second, there was a burst of mitosis immediately after the start of administration of the compound. The time course of this mitotic burst varied, and the increase in mitosis was greatest at 3 days in rats treated with 1000 mg/kg/day of DEHP and was smaller but more prolonged in rats treated with 200 or 50 mg/kg/day. Other changes, namely, a midxonal to periportai accumulation of fat, induction of peroxisomal enzymes, and induction of the P-450 isoenzyme also developed rapidly but were sustained throughout the study. The maximal change was usually attained within 7 days of commencement of treatment.

Table 2 (continued)

Reference	Location	Population sutdied	Type of study	Aims	Finding
					More slowly developing changes were hypertrophy of the hepatocytes, centrhobular loss of glycogen, and a fall in glucose-o-phosphatase activity. Here, maximal changes were not attained until 28 days at commencement of treatment. These three effects were clearly observed in rats treated with 200 or 1000 mg/kg/day of DEHP but were only marginally altered in rats treated with 50 mg/kg/day. Finally, accumulation of lipid-loaded lysosomes assessed by light and electron microscopy and by assay of β-galactosidase activity was only apparent in rats treated with DEHP for 9 months with 200 or 1000 mg/kg/day of DEHP. Changes in female rats were qualitatively similar to those observed in male rats. The alterations were, however, less pronounced than in male rats treated with an equal dose of DEHP and the degree of liver enlargement was much less because, although the initial hyperplasia was clearly apparent, there was a much smaller degree of hypertrophy
DeAngelo et al. (1985)	–	Male Sprague–Dawley rats weighing 150 to 200 g	Experimental study	To study the effect DEHP of GGT + foci in the rat liver at levels as low as 0.5 %	The choline-deficient diet (CD) diet promoted the number of GGT+ foci above levels in control livers. Inclusion of the plasticizer to the levels of 0.5, 1.0, and 2.0 % in the CD diet effectively inhibited the appearance of the foci. However, DEHP was unable to inhibit the promoting effect of the CD diet at a concentration of 0.1 %. DEHP's ability to block development of GGT+ foci correlated with its ability to increase liver weight and to induce carnitine acetyltransferase (EC 2.3.1.7), a marker of peroxisome proliferation
Popp et al. (1985)	–	Female CDF® CF-344/ CrlBR rats at 6–8 weeks of age	Experimental study	To identify potential promoting activity of DEHP	No neoplasms or nodules were identified. In addition, DEHP did not increase the number of foci or the mean volume of the foci when foci were identified by six different histologic stains
Ward et al. (1986)	–	Male B6C3F1 mice and female F344/NCr rats in groups of 10, 5 weeks of age	Experimental study	To study The carcinogenic effects of DEHP , including its potential as an initiator and as a promoter of carcinogenesis	DEHP promoted focal hepatocellular proliferative lesions (FHPL), including hyperplastic foci and neoplasms initiated by DEN in mice but not in rats. DEHP displayed very weak complete promoting activity and definite second stage promoting activity in SENCAR mouse skin but was inactive under our conditions on CD-1 mouse skin
Williams et al. (1987)	–	A total of 75 male F344 rats, aged 6 weeks	Experimental study	To study the effect of prolonged dietary administration of the peroxisome proliferating plasticizer DEHP on liver carcinogenesis initiated by iV-2- fluorenylacetamide (FAA) and to compare with that of the neoplasm- promoter phenobarbital (PB).	No evidence of induction of hepatocellular altered foci or hepatic neoplasms was found either when DEHP was given alone for 24 weeks or for 7 weeks followed by PB. Also, DEHP fed for 24 weeks had no promoting effect on liver altered foci that were induced by FAA and produced little or no enhancement of the occurrence of FAA-induced liver neoplasms. In contrast, PB exerted a marked enhancing effect on foci and substantially increased the incidence and multiplicity of liver neoplasms
Garvey et al. (1987)	–	Female F-344 rats weighing 150–180 g	Experimental study	To examine the initiating activity of DEHP after single and subchronic dosing	No initiating activity was found when DEHP was administered in a single, oral dose (10 g/kg) or after 12 weeks of feeding at a dietary concentration of 1.2 % when each was followed by a promotion regimen. There was no increase in number or mean volume of foci when

Table 2 (continued)

Reference	Location	Population sutdied	Type of study	Aims	Finding
					liver sections were examined using multiple histologic markers and no tumors were identified
Cattley et al. (1987)	–	Female F-344 at 6 weeks of age	Experimental study	To demonstrate those effects which persist in rats developing neoplasms under bioassay conditions and to determine whether induction of hepatic foci was associated with development of neoplasms in rats fed DEHP for 2 years	Increased hepatocellular proliferation and hepatomegaly were not detected. DEHP feeding did not increase the volume density of basophilic or ATPase-deficient foci of altered hepatocytes, suggesting that these lesions are not suitable indicators of DEHP carcinogenesis
Rao et al. (1987)	–	Male F-344 rats, 6 weeks old and weighing between 80 and 100 g	Experimental study	(i) To determine whether DEHP-induced liver tumors lack GGT; (ii) to ascertain whether a dose level of DEHP capable of inducing more peroxisome proliferation than the level used in NTP carcinogenesis bioassay induces a higher incidence of liver tumors in rats; and (iii) to establish, by immunocytochemical analysis, that there is a sustained increase in peroxisomes with the disproportionate levels of hydrogen peroxide producing and degrading enzymes in the livers of rats on long-term DEHP feeding	Liver nodules and/or hepatocellular carcinomas (HCC) developed in 6/10 rats fed DEHP and none were found in controls ($P < 0.005$ by ×2 test). All the nodules and HCC were negative for 7-glutamyl transpeptidase. In the non-tumorous portions of liver, the hepatocytes contained an increased number of peroxisomes and extensive accumulation of lipofuscin. By immunocytochemical analysis, the liver peroxisomes in rats treated chronically with DEHP had visually detectable decrease in the H_2O_2-degrading catalase and increase in H_2O_2-producing fatty acyl-CoA oxidase
Tyl et al. (1988)	–	Fischer 344 rats and CD-l mice	Experimental study	To develop toxicity evaluation of dietary DEHP	In rats, maternal toxicity and reduced fetal body weight per litter were observed at 1.0, 1.5, and 2.0 %. Increased resorptions and decreased number of live fetuses/litter were observed at 2.0 %. Maternal food consumption was reduced and water consumption was increased in all DEHP groups. In conclusion, DEHP was not teratogenic at any dose tested in Fischer 344 rats when Administered in the feed throughout gestation but did produce maternal and other embryo-fetal toxicity at 1.0, 1.5, and 2.0 %. In contrast, DEHP administration throughout gestation in CD-l mice resulted in an increased incidence of malformations at doses which produced maternal and other embryo-fetal toxicity (0.10 and 0.15 %) and at a dose (0.05 %) which did not produce significant maternal toxicity. No treatment-related embryo-fetal toxicity including teratogenicity was observed in mice at 0.025
Merkle et al. (1988)	–	Male and female Wistar rats	Experimental study	To investigate DEHP for developmental toxicity after head–nose exposure to aerosol concentrations of 0, 0.01, 0.05, and 0.3 mg/l for 6 h per day from gestation day 6 through 15	A range finding study revealed peroxisome proliferation in the liver of the dams throughout exposure levels of 0.2, 0.5, and 1.0 mg/l with an increasing trend. 0.3 mg/l was therefore regarded as an exposure level leading to peroxisome proliferation as a marker for maternal effects
Rao et al. (1990)	–	24 male F-344 rats weighing 60–70 g	Experimental study	To investigate the effect of DEHP on the livers of rats	At necropsy, livers were quantitatively analyzed for total tumor incidence and the number of lesions per liver after

Table 2 (continued)

Reference	Location	Population sutdied	Type of study	Aims	Finding
					slicing the entire organ at 1- to 2-mm intervals. Neoplastic nodules and/or hepatocellular carcinomas were observed in 11 of 14 rats (78.5 %). When evaluated according to the size, 57, 16, and 36 % rats contained nodules ranging from 1 to 3, 3 to 5, and greater than 5 mm in size, respectively. The number of nodules per liver ranged from 0 to 4
Klimisch et al. (1992)	–	108 male and 62 female SPF Wistar/Chbb: Thom rats (Dr Thomae GmbH, Biberach, Germany) with mean weights of 226 and 155 g	Experimental study	To investigate the inhalation toxicity of DEHP aerosols in head–nose inhalation systems	At the end of exposure, a statistically significant (16 %) increase in relative lung weights, accompanied by increased foam-cell proliferation and thickening of the alveolar septi, was found in the males of the highest dose group. Absolute liver weights were significantly (8.75 %) increased in females and relative liver weights were increased in both sexes in the highest dose group, but there were no corresponding histological effects. All these effects were reversed during the 8-week post-exposure period. No testicular toxicity was observed histologically and no impact on mating performance and male fertility was detected after 2 matings of treated males with untreated females, 2 and 6 weeks after the end of exposure
Tsutsui et al. (1993)	–	SHE cell cultures from inbred Syrian hamster	Experimental study	To examine possible mechanisms for DEHP-associated cancer and to measure induction of morphological transformation, chromosome aberrations, and peroxisome proliferations of cultured Syrian hamster embryo (SHE) cells by DEHP and other peroxisome proliferators	Morphological transformation of SHE cells was weakly induced by treatment for 48 h with DEHP and its metabolite mono (2-ethylhexyl)phthalate (MEHP). The transformation frequency by DEHP was enhanced by exogenous metabolic activation using rat liver postmitochondrial supernatants. Treatment for 24 h with DEHP resulted in chromosome aberrations of the cells only in the presence of exogenous metabolic activation. These results suggest a possible involvement of genetic damage by DEHP metabolites in the induction of cell transformation of SHE cells by DEHP; however, no clear relationship among induction of peroxisome proliferation, carcinogenicity in vivo, and cell transformation was observed
Fahrig and Steinkamp-Zucht (1996)	–	Mice	Experimental study	To study the experiments using DEHP	Under the influence of DEHP about 20 % of all colored spots were black, near-white, or twin spots. These co-recombinogenic effects are very strong, considering that the low frequency of twin spots with 0.3 % (historical ENU control) or 0.6 % (current ENU control) after ENU treatment alone has been enhanced up to a frequency of 3 %. While ENU alone induces about 14 % light brown-colored spots, depending exclusively on gene mutations, under the influence of DEHP the frequency was only 7 %, i.e., DEHP was antimutagenic
David et al. (1999)	–	6-week-old male and female Fischer-344 rats (CFD® (F344) CrlBR and 6-week-old male and female B6C3F1 mice (B6C3F1/CrlBR)	Experimental study	To provide information about the correlation of cell proliferation and peroxisome proliferation with tumorigenic in rats	Elevated palmitoyl CoA oxidation activity and higher liver-to-body weight ratio were observed for the 2500- and 12,500-ppm groups of rates and for the 500-, 1500-, and 6000-ppm groups of mice at week 105. No increase in palmitoyl CoA oxidation activity was evident in the recovery group, and relative liver weights were near control levels following recovery. No hepatic cell proliferation was detected at week 79 or 105 in either

Table 2 (continued)

Reference	Location	Population sutdied	Type of study	Aims	Finding
					species although preliminary data indicated that cell proliferation did occur within the first 13 weeks of exposure. A significantly higher incidence of hepatocellular tumors was only observed for the 2500- and 12,500-ppm group and its recovery group of rats and for the 500-, 1500-, and 6000-ppm groups and the recovery group of mice. The tumor incidences were reduced for the recovery groups compared with the groups fed DEHP hepatocarcinogenesis in rodent liver and that the tumorigenic process may be arrested by cessation of DEHP treatment, suggesting that extended treatment with DEHP acts to promote tumor growth
David et al. (2000)	–	4-week-old male and female (nulliparous) Fischer-344 rats	Experimental study	To compare the noncarcinogenic, chronic effects of DEHP to levels of peroxisome proliferation	No other differences in hematology were seen. No toxicologically significant changes were observed in urinalysis. At termination, relative lung weights for the 2500- and 12,500-ppm male groups of rats were significantly higher than for the controls. Absolute and relative liver and kidney weights for the 2500- and 12,500-ppm male rats and liver weights for 12,500-ppm female rats were higher compared with the controls. Absolute and relative testes weights for 12,500-ppm male rats were lower compared with the controls. A dose level of 500 was the NOEL for peroxisome proliferation. Bilateral aspermatogenesis in the testes, castration cell in the pituitary gland, spongiosis hepatis, and pancreatic acinar cell adenoma were observed for 12,500-ppm male rats. Aspermatogenesis and spongiosis hepatis were observed for 2500 ppm male rats, and aspermatogenesis was seen at 500 ppm. DEHP exposure exacerbated age, species, or strain-related lesions such as mineralization of the renal papilla and chronic progressive nephropathy in male rats
Isenberg et al. (2001)	–	6- to 8-week-old male B6C3F1 mice and F344 rats	Experimental study	To evaluate the persistence and reversibility of the effect of DEHP administration on rodent liver following treatment with and subsequent withdrawal of DEHP from the diet	Following DEHP administration at a dose of 6000 mg/kg for 18 months, inhibition of gap junctional intercellular communication persisted and the relative liver weight and induction of peroxisomal oxidation remained elevated in both rats and male B6C3F1 mice. The primary active metabolite of DEHP, mono-2-ethylhexyl phthalate (MEHP), was detected in the livers of animals treated with DEHP for greater than 2 weeks
David et al. (2001)	–	4-week-old male and female (nulliparous) Fischer-344 rats (CFD(F344)CrlBR) and 4-week-old male and female B6C3F1 mice (B6C3F1/CrlBR)	Experimental study	To study the chronic effects of DEHP	Reversibility of chronic effects on erythrocyte count, hemoglobin, and hematocrit values was apparent only for female rats. Chronic exposure demonstrated effects on liver, kidney, and testes weights. All organ weight effects except for testes for the recovery group of rats, and all organ weight effects for mice, were reversible. Pigmentation of Kupffer cells and renal tubules present in chronically treated rats were not observed for the recovery group. Lesions in the testes and pituitary gland were not reversible in rats. This may be a reflection of the senescence of the hypothalamic–gonad axis in rats. Cessation of exposure for mice resulted in amelioration of effects in the kidneys, liver, and testes

Table 2 (continued)

Reference	Location	Population sutdied	Type of study	Aims	Finding
Toyosawa et al. (2001)	–	Male and female rasH2 and non-Tg mice	Experimental study	To evaluate the transgenic mouse carrying a human prototype c-Ha-ras gene (rasH2 mouse) as a model for 26-week carcinogenicity tests, DEHP , a peroxisome proliferator	Body weight gain in rasH2 and non-Tg mice at 6000 ppm in the terminal week decreased about 10 % as compared to the control group. Common findings related to treatment with DEHP in rasH2 and non-Tg mice included hypertrophy with coarse granules and deposit of pigment in the liver, hydronephrosis, and tubular regeneration in the kidney, focal atrophy in the testis, and increased eosinophilic body in the nasal cavity. Hepatocellular adenoma was induced by treatment with DEHP and was conned to male rasH2; mice the incidence being 7 % (1/15), 13 % (2/15), and 27 % (4/15) in the 1500-, 3000-, and 6000-ppm group, respectively. Point mutation was not detected in codon 12 and 61 of human c-Ha-ras transgene upon DNA analyses on frozen samples taken from these hepatocellular adenomas
Mortensen et al. (2002)	–	45 male and 45 female C57BL/6XPA$^{-/-}$/(Xpa$^{-/-}$) mice and 40 male and 40 female C57BL/6XPA$^{-/-}$//p53$^{+/-}$/(Xpa$^{-/-}$/p53$^{+/-}$) mice, 10–13 weeks	Experimental study	To evaluate the short-term carcinogenicity assays with DEHP	In all models, the survival was not altered by DEHP. Increased incidences of nonneoplastic lesions were recorded in testes and kidneys with no apparent difference between the models. The only liver tumors in all models were adenomas in males with no statistically significantly increased incidence. For p-cresidine, the survival was decreased ($P < 0.05$) only in transgenic models. Statistically significantly increased incidences of nonneoplastic lesions were recorded in the liver, urinary bladder, and nasal cavity in all models and in kidneys in transgenic models. The only tumors with statistically significantly increased incidence were liver adenomas in transgenic models (XPA 1, vs. 7; "XPA/p53," 0 vs. 12; WT, 0 vs. 5, $P = 0.053$) and urinary bladder carcinomas in XPA/p53 model (0 vs. 7)
Voss et al. (2005)	–	730 male Sprague–Dawley (SD-CD) rats at an age of 100 + 10 days	Experimental study	To determine the long-term toxic effects of lifetime exposure to low concentrations of DEHP	Significantly increased tumor incidences after exposure to 300 mg/kg per day DEHP ($P = 0.04$ for testes and 0.05 for liver) and a significant dose-related trend ($P_{trend} = 0.02$ for testes and 0.03 for liver) were detected in both organs liver and testes. Time to tumor analysis revealed that DEHP-induced testicular tumors developed earlier in lifetime than hepatocellular neoplasias, and their multiplicity increased with time. In addition, animals exposed to the highest DEHP dose showed a significantly increased rate of testicular tubular atrophy ($P < 0.01$)
Takeshita et al. (2006)	–	Clone 29 of a TIF2 monoclonal antibody and clone 34 of a TRAM-1/AIB1	Experimental study	To determine the effect of DEHP on MDR1 expression with use the human colon carcinoma cell line LS174T	The induction by DEHP was abrogated when a reporter plasmid containing mutated DRC4 motif in the XRE was used. In a mammalian 2-hybrid assay, DEHP recruited steroid receptor co-activator-1 to the ligand-binding domain of SXR. Finally, using real-time reverse transcriptase-PCR, we showed that DEHP increased MDR1 gene expression in a dose-dependent manner
Takashima et al. (2008)		P*parα*-null mice and wild-type Sv/129 mice	Experimental study	To investigate tumorigenesis in P*parα*-null mice after low-dose DEHP exposure in P*parα*-independed and to examine gene expression profiles in hepatocellular adenoma tissues	This study revealed that long-term exposure to relatively low-dose DEHP (0.05 %) caused liver tumors including hepatocellular carcinomas, hepatocellular adenomas, and chologiocellular carcinomas at a higher incidence in P*parα*-null mice (25.8 %) than in wild-type mice (10 %). The microscopy profiles showed that the up- or

Table 2 (continued)

Reference	Location	Population sutdied	Type of study	Aims	Finding
				as well as control livers of wild-type and P*parα*-null mice	downregulated genes were quite different between hepatocellular adenoma tissues of wild-type and P*parα*-null mice exposed to DEHP. The gene expressions of apoptotic peptidase activating factor 1 (Apaf1) and DNA-damage-inducible 45 alpha (Gadd45a) were increased in the hepatocellular adenoma tissues of wild-type mice exposed to DEHP, whereas they were unchanged in corresponding tissues of P*parα*-null mice
Maranghi et al. (2010)	–	49 nulliparous pregnant dams (10/group) of CD-1 mice (27.34 g bw, approx. 6-week-old) at GD6 (positive vaginal plug = GD)	Experimental study	To investigate the effects of in utero DEHP exposure on liver development	In utero DEHP exposure altered postnatal liver development in weanling mice causing significant, dose-related (i) increased hepatosteatosis, (ii) decreased glycogen storage, and (iii) increased β-catenin intracytoplasmic localization (females only). At puberty, significantly decreased glycogen storage was still present in males. A treatment-induced phenotype was identified with lack of glycogen accumulation and intracytoplasmic localization of β-catenin which was associated with increased AFP gene expression
Habib and Karim (2012)	–	Male Swiss albino mice (6–8 weeks old, weighing 25 to 30 g)	Experimental study	To explore the anticancer activity of DEHP isolated from *Calotropis gigantea* flower against Ehrlich ascites carcinoma cells (EAC) in Swiss albino mice	DEHP showed a significant decrease in viable cell count ($P < 0.05$), mass gain (due to tumor burden) and elevated the life span of EAC cell-bearing mice. Altered hematological profiles such as RBC, hemoglobin, WBC, and differential count were reverted to normal levels in DEHP-treated mice. DEHP also brought back altered biochemical parameters (glucose, cholesterol, triglycerides, blood urea, SALP, and SGOT) to normal level
Wang et al. (2013)	Guangzhou and Hong Kong	A total of 120 outdoor (90 in Guangzhou and 30 in Hong Kong) and 40 indoor (20 in Guangzhou and 20 in Hong Kong) dust samples were collected from 5 and 6 different urban districts	Cross-sectional	(1) To determine the concentrations and profiles of phthalates in both indoor and outdoor dust in two populated urban centers of PRD; (2) to examine the effects of particle size fraction on phthalates accumulation; (3) to study the dust size fraction effect on the cytotoxicity of dust extract on human CCRF cell line; (4) to investigate the bioaccessibility of phthalate in dust and the size effect on bioavailability; and (5) to evaluate the daily intake (DI) of phthalates via indoor dust and assess the related health risks	In vitro cytotoxicity of dust extract on human T cell lymphoblast leukemic cell line (CCRF-CEM) indicated by lethal concentration 50 (LC_{50}) decreased with particle size. The power model was found as a better fit for explaining the relationship between LC_{50} and phthalates ($R^2 = 0.46$, $P < 0.01$). Bioaccessibility of phthalates in dust varied with different particle sizes, with the greatest bioaccessible fraction (2.49–38.6 %) obtained in <63 μm. Risk assessment indicated that indoor dust ingestion accounted for the major source for DEHP exposure (81.4–96.4 % of non-dietary exposure and 36.5 % of total exposure), especially for toddlers. The cancer risks associated with DEHP via home dust were high ($10–6–10^{-4}$), with 10 % of houses estimated with unacceptable risks ($>10^{-4}$). After corrected with the bioaccessibility of phthalates, the cancer risks of dust exposure were moderate (10^{-7}–10^{-5})
Chen and Chien (2014)	–	MCF-7 cells	Experimental study	To explore the effect and pathway of phthalates on the growth of MCF-7 breast cancer cells	MTT assay revealed cell toxicity at more than 10^{-5} mol/l for DEHP and at 10^{-4} mol/l for both BBP and DBP in MCF-7 cells. Cell proliferation was significantly increased at 10^{-8}–10^{-5} mol/l of BBP and DBP and at 10^{-8}–10^{-6} mol/l of DEHP treatment. Proliferating cell nuclear antigen (PCNA) was substantially increased in cultures with DEHP (10^{-8}–10^{-6} mol/l), BBP (10^{-8}–10^{-5} mol/l), and DBP (10^{-7}–10^{-5} mol/l). Obvious increases in PI3K, p-

Table 2 (continued)

Reference	Location	Population sutdied	Type of study	Aims	Finding
					AKT, and PCNA were noted in cultures with 17 β-estradiol, BBP, DBP, and DEHP. Estrogen receptor α expression was also notably increased in treatment with estradiol, BBP, DBP, and DEHP
Gaspar et al. (2014)	California	40 early childhood education (ECE) facilities from the California counties of Alameda (n = 20) and Monterey (n = 20), to measure five phthalate esters in indoor dust (n = 39) and indoor and outdoor air (n = 40 and 14, respectively)	Cross-sectional	To carry out a probabilistic health risk assessment comparing exposure estimates to "Safe Harbor" risk levels established for chemicals causing reproductive toxicity and cancer	Di(2-ethylhexyl) phthalate (DEHP) and butyl benzyl phthalate (BBzP) were the dominant phthalates present in floor dust (medians = 172.2 and 46.8 μg/g, respectively), and dibutyl phthalate (DBP), diethyl phthalate (DEP), and diisobutyl phthalate (DIBP) were the dominant phthalates in indoor air (medians = 0.52, 0.21, and 0.10 $\mu g/m^3$, respectively). The risk assessment results indicate that 82–89 % of children in California ECE had DBP exposure estimates exceeding reproductive health benchmarks. Further, 8 11 % of children less than 2 years old had DEHP exposure estimates exceeding cancer benchmarks

developmental toxicity, and only a few studies have proposed that DEHP could be a reproductive toxicant in human beings. Therefore, evaluation of human reproductive risk must be extrapolated from research studies in experimental animals, where differences of species in metabolism and dynamics of PPAR-alpha are of important considerations (Kavlock et al. 2006; Matsumoto et al. 2008).

Some studies have clearly shown that DEHP exposure might cause adverse effects in experimental animals. In laboratory rats, phthalate exposure is associated with various degrees of adverse effects including decrease in body weight, decrease in gonadal zinc, increase in specific activities of enzymes such as glucuronidase, decrease in the activity of acid phosphatase, dose-dependent enlargement of the liver, reduction of testosterone and increase in luteinizing hormone and follicle-stimulating hormone in serum, degenerative changes in testis and increased occurrence of abnormal sperm, reduction in testis weight and anogenital distance, areola and nipple retention, undescended testes, decrease in serum testosterone and in the activity of the steroidogenic enzyme 17b-hydroxysteroid dehydrogenase, reduction in Leydig cell T production, fetal deaths, reduction in daily sperm production and epididymal sperm counts, reduction in absolute and relative weights of androgen-dependent tissue organs, increase in mRNA levels of leukemia inhibitory factor (LIF), increases in the absolute volumes of Leydig cells per adult testis, reduction of adult serum testosterone levels, decrease in fetal and adult testosterone production, decreases in relative weight of testosterone dependent organs, delayed preputial separation, low serum testosterone, reduction in the anogenital distance, sperm counts, and reproductive organ weights, reduction of seminal vesicle weight, increase in the inhibitory GABAergic tone and a reduction in the stimulatory effect of aspartate on gonadotropin level, decrease in mean testis weight, decreases in total volumes of testis, increase incidence of severe hypospadias, reduction in penile length, and anogenital distance/body weight. An experimental study showed that low-dose in utero exposure to DEHP (10 mg/kg) resulted in increased testicular T levels, whereas a higher dose of DEHP (750 mg/kg) resulted in reduced T levels and AGD in rats. Low and high doses of postnatal DEHP administration had different effects on T levels (Lin et al. 2008). In another study, developmental toxicity occurred at the highest dose (500 mg/kg/day), as indicated by the increase in the incidence of post-implantation losses and the consequent reduction in mean litter size system on rats (Dalsenter et al. 2006). It should be noted that species differences in sensitivity to DEHP toxicity are reported. In addition, these different results may be attributable to between-study differences in dose, route, and periods of administration of DEHP. In particular, differences in the DEHP biological effects in rodents following different routes of administration may be due to the route dependency of mono-de-esterification of the phthalate diester (Ma et al.

Table 3 Phthalates and pregnancy outcome

Reference	Location	Population studied	Type of study	AIMS	Finding
Singh et al. (1974)	–	Adult virgin male and female mice of the Harlan/ICR albino Swiss strain	Experimental study	To investigate the possible mammalian mutagenic and antifertility activities of DEHP and DMEP	The high dose of both phthalates produced a distinct reduction in incidence of pregnancies, with lesser effects sometimes observed from the lower dose. There was a reduction in the number of implantations/pregnancy and of litter size, particularly in the first few weeks (postmeiotic stage) with the high dose of the compounds. Mutational effects, as expressed by an increase in early fetal deaths and reduced numbers of total implants, were seen at various weeks during the study but most notably during the first few week
Hansen and Grafton (1994)	–	Albino rats	Experimental study	To determine if DEHP was directly embryotoxic to rat embryos	Results of treatment on embryonic growth and development of embryos indicated head length and DNA and protein contents were significantly decreased at the 1.0 % concentration; crown-rump and head lengths and DNA content were decreased at 0.5 %. So, overall growth was retarded by treatment with concentrations of 0.5 % or higher in rats
Modigh et al. (2002)	–	962 employees from 3 plants	Cohort study	To investigate whether paternal occupational exposure to DEHP is associated with a prolonged time to pregnancy	The fecundability ratio for time to pregnancy was 1.07 [95 % confidence interval (95 % CI) 0.84–1.35] for those with low exposure and 0.97 (95 % CI 0.70–1.33) for the highly exposed after adjustment for the father's age, mother's age, and length of recall. When the analyses were restricted to first pregnancy, the fecund ability ratio was 1.13 (95 % CI 0.83–1.56) for low exposure and 1.02 (95 % CI 0.66–1.59) for high exposure
Adibi et al. (2003)	New York	Women 18–35 years of age	Cohort study	To characterize phthalate exposures in pregnant women using personal air and urine measures	Diethyl phthalate (DEP), dibutyl phthalate (DBP), diethylhexyl phthalate (DEHP), and butyl benzyl phthalate (BBzP) were present in 100 % of the air and urine samples. Ranges in personal air samples were as follows: DEP (0.26–7.12 μg/m^3), DBP (0.11–14.76 μg/m^3), DEHP (0.05–1.08 μg/m^3), and BBzP (0.00–0.63 μg/m^3). The mean personal air concentrations of DBP, diisobutyl phthalate, and DEHP are higher in Krakow, whereas the mean personal air concentration of DEP is higher in New York. Statistically significant correlations between personal air and urinary levels were found for DEP and monoethyl phthalate ($r = 0.42$, $P < 0.05$), DBP and monobutyl phthalate ($r = 0.58$, $P < 0.01$), and BBzP and mono-benzyl phthalate ($r = 0.65$, $P < 0.01$)
Latini et al. (2003a, b)	–	84 consecutive newborns (82 singletons, 2 twins), 39 male, 45 female; maternal age at delivery, 29.5 ± 5.1 years (range = 18–42); vaginal delivery, $n = 65$ (77.4 %); gestational age, 38.4 ± 2.2 weeks (range = 27–42); birth weight, 3220 ± 680 g, (range = 1150–4350); 1-min Apgar score, 7.9 ± 0.9; 5-min Apgar score, 8.8 ± 0.5	Experimental study	To evaluate prenatal exposure to DEHP and/or MEHP and its possible biologic effects	They found detectable cord blood DEHP and/or MEHP concentrations in 88.1 % of the samples. Either DEHP or MEHP was present in 65 of 84 (77.4 %) of the examined samples. Mean concentrations of DEHP and MEHP were 1.19 ± 1.15 μg/ml [95 % confidence interval (CI), 0.93–1.44, range = 0–4.71] and 0.52 ± 0.61 μg/ml (95 % CI, 0.39–0.66, range = 0–2.94), respectively. MEHP-positive newborns showed a significantly lower gestational age compared with MEHP-negative infants ($P = 0.033$). Logistic regression analysis results indicated a positive correlation between absence of MEHP in cord blood and gestational age at delivery (odds ratio = 1.50, 95 % CI, 1.013–2.21; $P = 0.043$)

Table 3 (continued)

Reference	Location	Population studied	Type of study	AIMS	Finding
Latini et al. (2003a, b)	Italy	24 consecutive mother–infant pairs with the following characteristics: 25 infants (23 singletons, 2 twins; 15 male and 10 female); gestational age 38.36 B 1.73 (range 35–42) weeks; birth weight 3317 B 545 (range 1950–4050) g; birth length 49.3 B 2.5 (range 42–55) cm; occipito-frontal circumference 34.4 B 1.6 (range 31–38) cm; vaginal delivery 8/25 (32 %); 1-min Apgar score 8.2 B 0.5 (range 7–9); 5-min Apgar score 8.8 B 0.4 (range 8–9), and maternal age 31.6 B 4.8 (range 23–42) years	Cross-sectional	To investigate the potential health hazards from exposure to DEHP and its main metabolite, mono(2-ethylhexyl) phthalate (MEHP)	Measurable DEHP and MEHP concentrations were found in 17/24 (70.8 %) and 18/24 (75 %) maternal plasmas, respectively, and in 11/25 (44 %) and 18/25 (72.0 %) cord samples, respectively. Either DEHP or MEHP was detectable in 21/24 (87.5 %) maternal plasmas and 19/25 (76 %) cord samples. The mean DEHP concentrations in maternal and cord plasmas were 1.15 B 0.81 and 2.05 B 1.47 g/ml, respectively. The mean MEHP concentrations were 0.68 B 0.85 and 0.68 B 1.03 g/ml, respectively. No significant correlations were found between maternal and cord blood DEHP, maternal and cord blood MEHP, maternal DEHP and cord blood MEHP, or maternal MEHP and cord blood DEHP plasma concentrations
Whyatt et al. (2009)	New York	311 African American or Dominican women (18 to 35 years)	Cohort study	To assess the relationship between DEHP exposure during pregnancy and gestational age at delivery	DEHP was detected in 100 % of personal air samples (geometric mean: 0.20 g/m^3 [95 % confidence interval [CI]: 0.18–0.21 g/m^3]); natural logarithms of air concentrations were inversely but not significantly associated with gestational age. Two or more of the DEHP metabolites were detected in 100 % of urine samples (geometric mean 4.8–38.9 ng/ml [95 % CI 4.1–44.3 ng/ml]). Controlling for potential confounders, gestational age was shorter by 1.1 days (95 % CI 0.2–1.8 days) for each 1-logarithmic unit increase in specific gravity-adjusted mono (2-ethylhexyl)phthalate concentrations (P = 0.01) and averaged 5.0 days (95 % CI 2.1–8.0 days) less among subjects with the highest vs. lowest quartile concentrations (P= 0.001). Results were similar and statistically significant for the other DEHP metabolites
Herreros et al. (2010)	Spain	251 ewes [in a first trial, 150 samples from ewes (*Ovis aries*) of 3 different breeds (84 Manchega, 35 Rubia del Molar, and 31 Negra Colmenareña), diverse age (2 to 7 years), and different reproductive status (15 pregnant and 135 nonpregnant ewes) were analyzed. The second experiment arose from the results of the first trial for confirming the effect of pregnancy on DEHP levels and included 101 ewes, born and reared at a different farm, of the same breed (Manchega–Assaf) and age (4 to 5 years) but different reproductive status; 32 were pregnant, 37 were nonpregnant, and 32 were ewes that had recently given birth less than a month before sampling]	Cross-sectional	(1) To determine non-experimentally induced plasma levels in ruminants reared in the field using the sheep as a model; (2) to evaluate possible influences of variables like age and reproductive status that can be extrapolated to other species including humans	DEHP was detected in 34.7 % of the samples, with a mean level of 0.45–0.01 mg/ml (range, 0.05 to 2.81 mg/ml). The percentage of nonpregnant animals with DEHP traces was higher in animals older than 4 years (n = 66, 37.9 %) than in younger animals (n = 69, 17.4 %; P < 0.05), although the mean levels in ewes with residues were similar (0.16–0.01 vs. 0.16–0.02 mg/ml). All the pregnant ewes (n = 15) showed presence of DEHP, with higher plasma levels than that in nonpregnant females (1.42–0.18 vs. 0.16–0.01 mg/ml; P < 0.0001). For confirming the effect of pregnancy on mobilization of DEHP from body fat, 101 ewes of the same age were sampled in a second trial at a different farm. The percentage of animals with DEHP traces was higher in pregnant ewes (n = 32, 71.9 %; P < 0.005) than in nonpregnant ewes (n = 37, 35.1 %) or in ewes that recently gave birth (n = 32, 21.9 %), although mean levels were similar (0.42–0.02, 0.33–0.02, and 0.34–0.05 mg/ml, respectively)
Saillenfait et al. (2011)	–	Sprague–Dawley rats	Experimental study	To evaluate the developmental toxicity of two dialkyl phthalate esters, di-*n*-heptyl	DHPP or DnOP were not teratogenic and did not alter the embryo-fetal survival, at doses as high as 1 g/kg/day and they both showed fetal toxicity at the lowest dose tested of 0.25 g/kg/day and higher

Table 3 (continued)

Reference	Location	Population studied	Type of study	AIMS	Finding
				phthalate (DHPP) and di-*n*-octyl (DnOP) phthalate	
Zhang et al. (2009)	Shanghai	201 newborn–mother pairs (88 LBW cases and 113 controls)	Case–control study	To assess maternal–fetal exposure to phthalates and investigate whether in utero phthalate exposure is associated with low birth weight (LBW)	More than 70 % of the biosamples had quantifiable levels of phthalates, with higher levels in the LBW infants compared with the controls. Prenatal di-*n*-butyl phthalate (DBP) exposure was associated with LBW, and di-2-ethylhexyl phthalate (DEHP) was negatively associated with birth length. After adjusting for the potential confounders, DBP concentrations in the highest quartile were associated with an increased risk of LBW. In utero DBP and DEHP exposures were associated with LBW in a dose-dependent manner
Li et al. (2012)	–	Adult female virgin Kunming mice (20–25 g, 8 weeks old)	Experimental study	To investigate the effects of DEHP on endometrial receptivity and embryo implantation in pregnant mice	Administration of DEHP led to compromised endometrial receptivity and decreased number of implantation sites. The mRNA and protein expression levels of ER, PR, and E-cadherin, but not those of HoxA10 and MMP-2, were upregulated by DEHP in the mouse endometrium. The results further suggested that DEHP disrupts the MAPK and NF-κB signaling pathways. This was maybe one of paths which influenced the E-cadherin expression
Ferguson et al. (2014)	Boston, Massachusetts, Brigham	Brigham and Women's Hospital	Case–control study	To assess the relationship between phthalate exposure during pregnancy and preterm birth	Geometric means of the DEHP metabolites mono-(2-ethyl)-hexyl phthalate (MEHP) and mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP), as well as mono-*n*-butyl phthalate (MBP), were significantly higher in cases compared with control participants. In adjusted models, MEHP, MECPP, and Σ DEHP metabolites were associated with significantly increased odds of preterm birth. When spontaneous preterm births were examined alone, MEHP, mono-(2-ethyl-5-oxohexyl) phthalate, MECPP, Σ DEHP, MBP, and mono-(3-carboxypropyl) phthalate metabolite levels were all associated with significantly elevated odds of prematurity
Huang et al. (2014)	China	Chinese women residing in Chongqing (Southwest China)	Cross-sectional	To assess the relationship between 15 phthalate levels in cord blood and preterm delivery and fetal growth parameters in 207 Chinese women going into labor	There were associations between phthalates and fetal growth parameters, many of which disappeared when analyses were adjusted for gestational age, especially in male infants (only DEEP was associated with birth weight; DEP, DNHP, BBP, DNP with abdominal circumference; DEP, DBP, DCHP, DEHP with femur length in female infants. DPP and DBEP were associated with birth length in male infants. $P < 0.05$)

Table 4 Phthalates and respiratory health

Reference	Location	Population studied	Type of study	Aims	Finding
Oie et al. (1997)	Oslo و Norway	38 dwellings	Cohort study	To identify and quantify the major phthalate inhalation exposure routes in residences and to propose a hypothesis on the role of DEHP in the pathogenesis of asthma	The proposed mechanism of effect states that mono(2-ethylhexyl) phthalate (MEHP), the primary hydrolysis product of DEHP, mimics the inducing postaglandins (PG) PGD2, 9a,l 1 βPGF2, and PGF2., and thromboxanes in the lungs, thereby increasing the risk of inducing inflammation in the airways, which is a characteristic of asthma
Norback et al. (2000)	Ystad in southern Sweden	Personnel (n = 87) in four geriatric hospitals	Cross-sectional	To study the relationships between current asthma symptoms (wheeze or attacks of breathlessness) and the indoor environment and dampness in hospitals	Current asthma symptoms were reported by 17 %, and 8 % had doctor's diagnosed asthma. Asthma symptoms were more common (adjusted odds ratio 8.6; 95 % confidence interval 1.3–56.7) in 2 buildings with signs of dampness-related degradation of di(ethylhexyl)-phthalate (DEHP) in polyvinyl chloride (PVC) floor material, detected as presence of 2-ethyl-1-hexanol (2–32 g/m^3) in indoor air (CAS no. 104–76-7)
Magliozzi et al. (2003)	–	Albino Wistar rats	Experimental study	To investigate DEHP effects on immature mammalian lung	In treated animals, morphometric analysis of histological parameters revealed a dramatic decrease in the number of parenchymal airspaces, together with significant increases in their mean size. Moreover, cytochemical detection of the peroxisomal marker catalase revealed an increase in the number of type II pneumocytes
Bornehag et al. (2004)	Sweden	All 14,077 children 1–6 years of age	Case–control study	To investigate potential associations between persistent allergic symptoms in children, which have increased markedly in developed countries over the past 3 decades and the concentration of phthalates in dust collected from their homes	We found higher median concentrations of butyl benzyl phthalate (BBzP) in dust among cases than among controls (0.15 vs. 0.12 mg/g dust). Analyzing the case group by symptoms showed that BBzP was associated with rhinitis (P = 0.001) and eczema (P = 0.001), whereas di(2-ethylhexyl) phthalate (DEHP) was associated with asthma (P = 0.022). Furthermore, dose–response relationships for these associations are supported by trend analyses
Larsen et al. (2007)	–	Inbred female BALB/cJ mice aged 5–6 weeks	Experimental study	To investigate DEHP exposures for adjuvant effects and airway inflammation	In the OVA+ Al (OH)3 group, significantly increased levels of OVA-specific IgE and IgG1 in serum as well as of eosinophils in BAL fluid were observed. DEHP affected OVA-specific IgG1 production in a concentration-dependent manner, whereas little effect was seen on IgE and IgG2a. Dose-dependent increases in inflammatory cells were observed in BAL fluids, leading to significantly higher lymphocyte, neutrophil and eosinophil numbers in the OVA+ 13 mg/m^3 DEHP group. Ex vivo cytokine secretion by cultures of draining lymph nodes suggested that DEHP has a mixed Th1/Th2 cytokine profile
Kolarik et al. (2008)	Sofia and Burgas, Bulgarian	All children 2, 3, 5, and 7 years of age living in selected districts of Sofia and Burgas, Bulgarian cities	The ALLHOME study is divided into two phases: a cross-sectional questionnaire study (ALLHOME-1) and a nested case–control study (ALLHOME-2)	To investigate the associations between allergic symptoms in children and the concentration of phthalate esters in settled dust collected from children's homes	A higher concentration of DEHP was found in homes of case children than in those of controls (1.24 vs. 0.86 mg/g dust). The concentration of DEHP was significantly associated with wheezing in the preceding 12 months (P = 0.035) as reported by parents. They found a dose–response relationship between DEHP concentration and case status and between DEHP concentration and wheezing in the preceding 12 months
Koike et al. (2009)	–	7-week-old SPF NC/NgaTndCrlj male mice	Experimental study	To investigate the effects of DEHP on immune cells	Bone marrow-derived dendritic cells (BMDC) differentiated in the presence of DEHP showed enhancement in the expression of MHC class II, CD86, CD11c and DEC205, and in their antigen-presenting activity. On the other hand, the function of the differentiated BMDC was not activated by DEHP although DEHP partly enhanced their

Table 4 (continued)

Reference	Location	Population studied	Type of study	Aims	Finding
					expression of DEC205. DEHP-exposed splenocytes showed increases in their TCR and CD3 expression, interleukin-4 production, and antigen-stimulated proliferation.
Rosicarelli and Stefanini (2009)	–	Albino Wistar rats	Experimental study	To study the qualitative and quantitative observations the whole period of lung alveolarization and the proliferation rate of different cell populations in lung parenchyma was evaluated by means of immunolocalization of the proliferating cellnuclear antigen (PCNA)	They detected significant alveolar simplification (larger but fewer alveoli with decreased septation), with consequent sensible reduction of gas exchange surface, at several stages of postnatal development, in distal lung parenchyma of DEHP-treated rats. Moreover, the quantification of PCNA-expressing cells demonstrates that in treated pups the proliferation rates of epithelial and mesenchymal cells progressively increased during the first 2 postnatal weeks, at difference with controls animals, where the highest proliferation levels were reached at postnatal day 7
Chen et al. (2010)	–	Pregnant Sprague–Dawley rats	Experimental study	To evaluate the effects of DEHP exposure on growth and lung maturation in rats and determine if DEHP regulation of 11b-hydroxysteroid dehydrogenase type 1 gene (Hsd11b1) expression in the lung tissue plays a role in its effects on lung maturation	The perinatal DEHP exposure led to a dose-dependent intrauterine and postnatal growth restriction ($P < 0.001$). The lung from 750 mg/kg/ day DEHP dose group on PND 1 and PND 21 (C and D) appeared to have thicker alveolar septa and less airspace indicating decreased numbers of alveolar and/or increased lung interstitial tissue proliferation as compared to that of control animals from PND 1 and PND 21 (A and B)
Guo et al. (2012)	–	Healthy balb/c mice (males, 5–6 weeks old and 17–19 g)	Experimental study	To determine the underlying role of DEHP exposure in airway reactivity, especially when combined with allergen exposure	(1) Strong positive associations exist between OVA-combined DEHP exposure and serum total IgE (T-IgE), as well as histological findings. These positive associations show a dose-dependent low-dose sensitive effect of DEHP. (2) IL-4, eosinophil recruitment and lung function are also indicators for adjuvant effect of DEHP
Win-Shwe et al. (2013)	–	6-week-old C3H/HeJ Jclmale mice	Experimental study	To investigate the expression levels of neuroimmune biomarkers in the hypothalamus of amurinemodel of allergic asthma after phthalate exposure throughout juvenility until adulthood	The mRNA expression levels of the proinflammatory cytokine interleukin (IL)-1b and tumor necrosis factor (TNF)-a, the chemokine CCL3, the transcription factor nuclear factor (NF)-kB, the oxidative stress marker heme-oxygenase (HO)1, a nerve growth factor, and the microglia marker Iba1 were remarkably upregulated in the hypothalami of mice treated with 8 nmol of DEHP in the presence of the allergen
Ait Bamai et al. (2014)	Japan, Sapporo, Fukushima, Nagoya, Osaka, Okayama, and Fukuoka	156 single-family homes	Cross-sectional study	To investigate the relationships between phthalate levels in Japanese dwellings and the prevalence of asthma and allergies in both children and adult inhabitants	According to a self-reported questionnaire, the prevalence of bronchial asthma, allergic rhinitis, allergic conjunctivitis, and atopic dermatitis in the 2 years preceding the study was 4.7, 18.6, 7.6, and 10.3 %, respectively. After evaluating the interaction effects of age and exposure categories with generalized liner mixed models, interaction effects were obtained for DiNP and bronchial asthma in adults ($P_{interaction} = 0.028$) and for DMP and allergic rhinitis in children ($P_{interaction} = 0.015$). Although not statistically significant, children had higher ORs of allergic conjunctivitis for DEHP and liner associations were observed ($P_{trend} < 0.05$). On the other hand, adults had a higher OR for atopic dermatitis and DEHP compared to children. Levels of DMP, DEHP, DiBP, and BBzP in floor dust were associated with the prevalence of allergic rhinitis, conjunctivitis, and atopic dermatitis in children
Shin et al. (2014)	–		Experimental study		

Table 4 (continued)

Reference	Location	Population studied	Type of study	Aims	Finding
		Pregnant BALB/c mice (gestational day (GD) 13)		To investigate the effects of maternal exposure to DEHP during pregnancy on neonatal asthma susceptibility using a murine model of asthma induced by ovalbumin (OVA)	Maternal exposure to DEHP reduces airway inflammation and mucus production in offspring, with a decrease in inducible nitric oxide synthase (iNOS) in the lung tissue
You et al. (2014)	–	SPF male BALB/c mice	Experimental study	To analyze whether oxidative stress induced by DEHP with or without OVA could trigger AHR and airway inflammation	The data indicated higher ROS and malonaldehyde (MDA) levels and lower glutathione (GSH) contents in DEHP + OVA group than that in OVA group, while vitamin E, an antioxidant, could restore reactive oxygen species (ROS), MDA, and GSH levels to control levels and attenuate the DEHP and/or OVA effect

2006). After oral administration in rats, DEHP is rapidly hydrolyzed to MEHP by lipases in the gut and absorbed as the monoester and 2-EH (Kavlock et al. 2002a). Therefore, considering the differences between inhalation and oral exposure, a study suggested that the timing, duration, and route of exposure to DEHP may be important variables that determine its adverse effect on steroid biosynthesis (Ma et al. 2006). Studies showed that the effects of DEHP in mice depend on timing of exposure and dose.

Different underlying mechanisms are proposed by previous reviews on the adverse effects of phthalates on reproductive health and fertility. These studies suggested that phthalate exposure may influence ovarian reserve (Hart 2016) or may induce defects in folliculogenesis and steroidogenesis, which in turn can cause infertility, premature ovarian failure, and non-reproductive disorders (Hannon and Flaws 2015; Patel et al. 2015). Another review concluded that exposure to phthalates might reduce the fertility by activating the peroxisome proliferator-activated receptors, as well by increasing the fatty acid oxidation, and by reducing the capacity of coping with increased oxidative stress (Mathieu-Denoncourt et al. 2015).

Exposure in gestational days 7 and 8 led to a high incidence of malformations and death, but exposure in other days had fewer health effects (Ventrice et al. 2013). In humans, exposure of females to some of phthalates was found to be associated with significantly increased odds of preterm birth (Ferguson et al. 2014). In humans, a positive correlation between high urine phthalate levels and pregnancy complications such as toxemia, anemia, and preeclampsia was found in pregnant women living near a plastic manufacturing company (Gupta et al. 2010). Variability in an individual's exposure to phthalate esters can result from changes in the use of personal care products, daily activities, or diet and exposure may vary over time. These observations, along with the non-persistent nature of phthalate esters, may result in differences in the timing of exposure to phthalates during the day (Hauser and Calafat 2005).

A review concluded that exposure to phthalates might reduce the duration of pregnancy (Bajkin et al. 2014). Another review reported inconsistent findings of some studies on the negative impact of phthalates on gestational age (Jurewicz and Hanke 2011). Our findings are consistent with a recent review that revealed no clear conclusion on the effects of phthalates on gestational age and birth weight (Marie et al. 2015). Although controversies exist on the effects of phthalates on gestational age and birth weight, we suggest that it is more prudent to reduce the phthalate exposure of females in reproductive age.

Carcinogen effect

Phthalate esters may be responsible for cancer in humans (Ventrice et al. 2013). The carcinogenicity database in animals

revealed that DEHP might cause liver cancer (Rusyn and Corton 2012). The mechanism of hepatic neoplastic transformation is related to the activation of PPARα although DEHP is also able to cause liver cancer in mice lacking PPARα, suggesting that activation of PPARα is not essential (Ventrice et al. 2013). It is well documented that DEHP produces a range of hepatic effects in animal studies, for instance increased risk of malformations as neural tube and pituitary defects, chronic renal inflammation, benign and malignant liver tumors, decrease in fetal weights, nonneoplastic lesions in the liver, urinary bladder, and nasal cavity, The toxic effect is variable in animal species where there is repeated exposure to DEHP. This variability is seen in the nature of the effect and in the dose required to cause a response. For example, mice and rats are the most sensitive species with primary target organs being testes and liver. Liver effects are primarily those associated with peroxisome proliferation (Kavlock et al. 2002b). The difference in disposition, metabolism, or excretion by the route of administration may be responsible for the difference in DEHP teratogenicity (Shiota and Mima 1985). Important species differences in expression and molecular signaling for PPARa have been reported. Human express PPARa at lower level in liver, whereas PPARa is expressed at high levels in mice and rats livers (Rusyn and Corton 2012).

Various mechanisms have been suggested for the carcinogenic effects of phthalates. Some experimental studies have documented the effects of DEHP on chromosomes, spindle, and mitosis; moreover DEHP might induce DNA damage and can alter mitotic rate, apoptosis, and cell proliferation; it can also activate some nuclear receptors. Environmental exposures of humans to DEHP have been associated with DNA damage. Some human and animal studies proposed that the carcinogenic effects of DEHP are mediated through multiple molecular signals, including DNA damage (Caldwell 2012). The carcinogenic effects of DEHP are found in different target tissues, notably in liver and testis (Rusyn and Corton 2012).

The tumor-promoting properties of phthalates are also considered to be because of their effects on the length of telomeres, which are specialized chromatin necessary for the maintenance of chromosomal stability (Cai 2016). This effect is reported for breast cancer (Scinicariello and Buser 2015).

It is also documented that phthalates might promote breast cancer stem cells (Wang et al. 2016).

Exposure to phthalate esters may have a carcinogenic role by enhancement of cancer cell proliferation, migration and invasion (Hsu et al. 2016). Moreover, exposure to Benzyl butyl phtalate is found to increase the chemoresistance of breast cancer (Hsu et al. 2015). It is also suggested that the effects of phthalates on prostate cancer might be mediated by activating the Hedgehog signaling pathway (Yong et al. 2015).

Pregnancy outcome

Exposures of phthalates exist at global level and are considered as a potential harm for pregnant women (Jensen et al. 2012). The adverse effects of the observed prenatal exposure to phthalate esters in human newborns remain to be determined. Prenatal and postnatal exposures may have synergistic and cumulative actions in producing adverse neonatal effects, especially for premature infants with very low birth weight (Latini et al. 2003b). Prenatal exposures to phthalates might also be related to changes in timing of labor (Serrano et al. 2014). However, a few numbers of studies did not confirm the adverse health outcomes of exposure to phthalates during pregnancy (Adibi et al. 2003; Latini et al. 2003a). Such controversies might be the dynamic changes in amniotic fluid volume and composition throughout pregnancy, which in turn would complicate interpretation of measured pollutant concentrations (Jensen et al. 2012). Differences between results of studies are suggested to be due to differences in sampling variability, statistical power, confounding factors, temporal trends in exposures, and possibly the batch effects in the laboratory examinations (Latini et al. 2003b). Effective strategies to prevent exposures of phthalates in pregnant women are of great interest especially for the developing fetus. Observations of an Old Order Mennonite (OOM) community from Germantown, Pennsylvania suggests that the elimination of cosmetics, the limited use of personal care products (PCPs), and the consumption of mostly homegrown produce may contribute to the significantly lower metabolite concentrations in this population compared to larger, national samples. Little is known about how consumption habits and typical lifestyle impact exposure in pregnant women (Serrano et al. 2014). One of the limitations of several studies is that the data on daily activity and use of personal care products were not available for most of mothers, and thus the exact sources of DEHP exposure are not known (Zhang et al. 2009).

Effects on respiratory system

Asthma is a common chronic inflammatory disease of the airways (Ventrice et al. 2013). The concentrations of phthalates in the indoor air are approximately 10 times higher than outdoors. As children inspire more air per kilogram of body weight than adults do, they may be at increased risk of inhalational exposure. Some sources of phthalates in indoor air are PVC building materials, furniture, and some products including body fragrances and aromatic air fresheners (North et al. 2014). Interior materials used in houses include plasticized PVC products used as wall and floor covering materials. These PVC products are potential emission sources of chemicals including DEHP widely used as a plasticizer (Hauser and Calafat 2005). House dust is known to play a role in exposure of phthalate; however, the contribution from

this source is unclear and has large variations throughout life. Median concentrations of DEHP have been found to range from 340 to 858 mg/g of house dust across different countries. Little information is available on how phthalate levels might affect allergic responses to the house dust (North et al. 2014). The present review showed that DEHP exposure associated with adverse effects on respiratory system. All studies have suggested that exposure to DEHP may be associated with development of asthma, wheezing, and allergic symptoms (Adibi et al. 2003; Chen and Chien 2014; Ferguson et al. 2014; Gaspar et al. 2014; Habib and Karim 2012; Hansen and Grafton 1994; Herreros et al. 2010; Latini et al. 2003a; Li et al. 2012; Modigh et al. 2002; Singh et al. 1974; Takashima et al. 2008; Wang et al. 2013; Zhang et al. 2009). It is reported that there are phthalate esters in house dust and allergic asthma in children aged 1–6 years (Bornehag et al. 2004). In children, after oral ingestion, DEHP is rapidly absorbed and metabolized to MEHP in the gastrointestinal tract, which will be eliminated after conjugation with glucuronic acid. Therefore, children represent a high-risk group for adverse effects of phtalates (Ventrice et al. 2013). In an experimental study, DEHP increased immunoglobulin G1 levels, and MEHP produced a significant increase in the levels of both immunoglobulin E and immunoglobulin G1 and are related to allergic reactions. Some phthalate monoesters are shown to contribute to inflammatory processes by promoting cytokine interleukin 6 and production of interleukin 8 in human epithelial cell line A549 (Bornehag et al. 2004; Larsen et al. 2007; Tarlo 2003). Several factors must be considered when weighing the evidence of an association between phthalates and allergy. First, biological markers of phthalate exposure are limited to the period they represent because these markers have short half-life and vary temporally; therefore, they have limited value in evaluating long-term exposure. Moreover, it is possible that because of increased personal care product or medication use, individuals with allergies, asthma, and atopic dermatitis might have higher levels of phthalates (North et al. 2014).

Conclusion

The current review provides evidence on several adverse health effects of exposure to phthalates, notably for pregnant women and the pediatric age group. It also revealed that humans and experimental animals respond differently to DEHP; the underlying mechanisms need to be determined. Because of the small number of human studies, further investigations on humans are necessary to determine the safe levels of phthalates in different products. In addition to restricting the use of phthalates, simultaneous effort should continue to substitute phthalates with other plasticizers especially DEHP.

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