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THE OVERVIEW OF CURRENT EVIDENCE ON THE REPRODUCTIVE TOXICITY OF DIBUTYL PHTHALATE

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Abstract
Over the past years, many legitimate concerns have been raised about the effects of dibutyl phthalate (DBP) as an endocrine disruptor, especially on reproduction. The aim of this publication is to critically review the literature related to the developmental and reproductive toxicity of DBP in animals. Several electronic databases were systematically searched until 2019. Studies were qualified for the review if they: linked exposure to DPB with reproduction, were published in English after 1990, and were conducted on animals. In the studies of the testicular effects of DBP on experimental animals, the most common effects of exposure included reduced fertility, atrophic changes in male gonads, degenerative changes in the epididymis, as well as a reduction in sperm count and motility, cryptorchidism, hypospadias, poor sperm quality and other genital defects (decreased testicular weight, delayed spermatogenesis, Leydig cell aggregation, impaired Sertoli cell maturation, and significant inhibitions of testicular enzymes). The embryotoxic effects of DBP on laboratory animals included mainly an increase in fetal resorption and a decrease in live births. The teratogenic effects of DBP also manifest as skeletal malformations in fetuses, malformations of male gonads and other genital effects. On the basis of the literature data, it is clearly demonstrated that DBP shows anti-androgenic effects; however, there are also reports confirming its weak estrogenic effect. Additionally, lower doses cause more adverse effects than the highest dose, which is an important fact because of the widespread environmental exposure to DBP. The studies clearly confirm that DBP is an endocrine disruptor. Int J Occup Med Environ Health. 2021;34(1):15–37

Key words:
reprotoxicity, toxicology, dibutyl phthalate, endocrine disruptor, embryotoxicity, teratogenicity

INTRODUCTION

Dibutyl phthalate (DBP; CAS 84-74-2) occurs as colorless to light yellow oily liquid with a weak odor characteristic of esters. It is used in industry as a plasticizer, in the synthesis of polymers, as a laboratory agent, in analytics, in the production of polyvinyl chloride items, in ceramics and propellants, as a solvent (e.g., in the production of maleic anhydride), and as a metal working fluid [1].

It is worth to mention that phthalates, when used in electrical and electronic equipment, e.g., in cables or capacitors, may have a negative impact on recycling, as well as on human health and the environment, primarily during the processing of this waste equipment [2]. In addition, approx. 8.4 million tons of plasticizers are produced globally every year, of which Europe produces approximately 1.5 million metric tons [3]. Another interesting fact is that

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DBP is manufactured in and/or imported to the European Economic Area in 1000–10 000 tons a year [4].

Dibutyl phthalate traded on the market is recognized by the European Chemicals Agency (ECHA) as a substance which can disrupt hormonal balance, referred to as an endocrine disrupting chemical or an endocrine disruptor [5]. An endocrine disruptor is defined as an "exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations." Such substances can lead, among others, to fertility disorders, genital development disorders, hormone-dependent cancer (e.g., breast, prostate, ovary or testicle cancer), damage to the fetus (including its nervous system), and metabolism disorders [6].

In accordance with Annex XVII of Regulation (EC) No. 1907/2006 of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH Regulation), establishing the European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No. 793/93 and Commission Regulation (EC) No. 1488/94, as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC [7], the possibilities of using phthalates are very limited.

In accordance with Regulation (EC) No. 1272/2008 of the European Parliament and of the Council on classification, labelling and packaging of substances and mixtures, amending and repealing Directive 67/548/EEC and 1999/45/EC, and amending Regulation (EC) 1907/2006 (CLP Regulation) and amending Regulation (EC) No. 1907/2006 [8], DBP has the assigned reproductive toxicity category 1B (Repr. 1B) and aquatic acute category 1 (Aquatic acute 1).

The aim of this study is to summarize and analyze literature data related to DBP and its effect on reproduction and development in animals. As previous reviews which focused on this matter were performed in 1999–2014 [9–12], there is a need to reevaluate and assess the new data on DBP and its effect on reproduction.

METHODS

The literature review was performed on the basis of the Internet databases of peer-reviewed scientific journals, including EBSCO Discovery Service, Science Direct, Scopus, PubMed and MEDLINE, as well as data available on ECHA and European Commission's websites. In the preparation of this study, papers published in 1990–2019, only in English, were considered. Relevant studies were also identified through a review of the references cited in all the published studies. Studies where DBP was used in combination with other phthalates or other substances, or examining other outcomes, were excluded. Only original articles were included.

In the preparation of this study, the following keywords: "dibutyl phthalate application" (75 articles found, 14 excluded), "dibutyl phthalate reprotoxicity" (156 articles found, 53 excluded) "dibutyl phthalate embryotoxicity" (245 articles found, 122 articles excluded), and "dibutyl phthalate teratogenicity" (229 articles found, 115 excluded) were used. In total, 401 articles were initially retrieved. Excluded articles were unrelated (N = 217), inaccessible (N = 23) or duplicate (N = 64). Finally, this review included animal studies published in English, in peer-reviewed journals, since 1990. This period was chosen because a growing body of literature providing data on the reproductive toxicity of DBP and using different methods was published. So, there is a need to reevaluate both older and new data on this topic. Abstracts of the remaining articles were read in search of the adverse effects of DBP on the reproductive system in laboratory animals.

RESULTS

Reprotoxicity – testicular effects

Data on the effects of DBP action on reproduction, which manifest on the testes of treated animals, are presented in

Table 1 (16 studies: 11 on rats, 2 on rabbits, 2 on mice, 1 on monkeys) [13–27]. The table is divided by animal species (rats, mice, rabbits, monkeys) and exposure time (short-time exposure – up to 28 days: 12 studies, and sub-chronic exposure – up to 13 weeks: 4 studies).

Mice treated with DBP orally at the lowest doses (1–10 mg/kg bw) had a reduced anogenital distance and testicular weight with delayed spermatogenesis, which is directly related to impaired and reduced Sertoli cells proliferation/maturation [24]. Delayed spermatogenesis seems to appear in mice in a much lower dose (≥1 mg/kg bw) [24] than in rats (≥250 mg/kg bw) [17]. In addition, the late onset of certain toxic effects in rats may also be related to the conditions under which the experiment was conducted (the route of administration and the duration of exposure). The most sensitive species, in terms of the reprotoxic effects of DBP, are mice. Some authors have concluded that male reproductive toxicity occurs through mechanisms related to these corresponding with oxidative stress [16,20,21].

A higher dose in rats (31 mg/kg bw) caused testicular atrophy and increased sperm abnormalities [19]. The rodents treated with DBP doses of >100 mg/kg bw (rats) or 163 mg/kg bw (mice) had a reduced sperm count and quality, with abnormal morphology of the seminiferous tubules [18,19]. Additionally, an increase in the serum testosterone level was observed in the mice which received DBP via food [23].

In the subsequent dose range (200–250 mg/kg bw), in addition to the symptoms listed above, decreased sperm motility, significant changes in testicular enzymes, defective spermatogenesis and shrunken tubules were observed in rats receiving DBP orally [15,17,20].

Treating rats with DBP at a dose of 359 mg/kg bw/day for 13 weeks via food resulted in a lower body weight gain [23].

During short-term exposure (15 days), in the Wistar rats receiving a higher dose of DBP (400 mg/kg bw/day) via gavage, degeneration and even absence of spermatogenesis occurred in most of the seminiferous tubules [16].

In the rabbits treated with DBP at a dose of 400 mg/kg bw/day (given via gavage for 8 weeks) increased liver weight, degeneration and atrophy of the seminiferous tubules, disintegration and shed of the seminiferous epithelial cells, changes in testicular enzyme levels, and in serum testosterone and androgen levels, were observed [26]. A lower serum testosterone level, and decreased prostate and testicular weights, were noticed in the rabbits receiving DBP orally at a dose of 520 mg/kg bw/day [25]. Abnormal sperm count was doubled in the lower dose; however, the rabbits exposed to the higher dose had a decreased sperm count. In the rabbits treated with DBP at a dose of 400 mg/kg bw/day, the epididymal sperm count did not change whereas a lower dose (≥100 mg/kg bw/day) in rodents caused adverse effects on sperm production [15,18,19]. Administering DBP at a dose of 520 mg/kg bw resulted in a decreased progressive and mass sperm motility and live sperm percentage, along with a significant elevation of testicular malondialdehyde, and without changes in other testicular enzymes. Finally, a decreased sperm count was observed. At this dose, the non-reproductive organs weight did not change, as was observed in the rodents receiving a lower dose [19,21].

When given orally at a dose of 500 mg/kg bw/day, DBP caused subsequent toxic effects in rodents, which are described in detail in Table 1 [15,17,21,24]. However, Mitsuhashi et al. [19] found no changes in the serum and testicular testosterone levels despite the use 500 mg/kg bw of DBP in rats.

In the Wistar rats treated with DBP at a dose of 600 mg/kg bw/day (via gavage for 15 days), necrosis of the seminiferous tubules was observed [16].

Increasing the dose to 720 mg/kg bw/day (via food) and extending the exposure time to 13 weeks in F344 rats induced focal atrophy of the seminiferous tubules and degeneration of the germinal epithelium [23].

The mice given 812 mg/kg bw/day of DBP via food for 13 weeks showed increased zinc concentrations in the testes [23].

Table 1. The effects of dibutyl phthalate (DBP) on male animal reproduction – short-term and sub-chronic exposure

Study data	Results	Reference
Rats		
short-term exposure		
Sprague Dawley rats (males – lack of data, 5–14 days old); DBP was given subcutaneously in corn oil; doses: 0, 5, 10, 20 mg/animal (0, 250, 500, 1000 mg/kg bw) for 9 consecutive days (examination after 17 and 28 days of treatment); the animals were killed on day 24 or 31 of their life	– 1000 mg/kg bw/day o significantly reduced body weight (day 15–17) o decreased testicular weight and seminal vesicles (day 28) o mild hyperplasia of Leydig cells in the seminiferous tubules (day 17) o presence of multinuclear germ cells – NOAEL 500 mg/kg bw/day	13
Wistar rats (10 males, 10 weeks old); DBP was given orally via gavage in corn oil; doses: 0, 2000 mg/kg bw for 9 consecutive days; the animals were killed 24 h after the last treatment	– 2000 mg/kg bw/day o decreased body weight gain o decreased spermatozoa motility (66%) o slightly decreased sperm count o increase in abnormal spermatozoa (77%) o decreased relative testicular weight o increased activity of the marker enzymes of oxidative stress o degeneration of the seminiferous tubules o defoliation and necrosis of spermatocytes	14
Sprague-Dawley rats (10 males/group); DBP was given orally via gavage in corn oil; doses: 0, 100, 250, 500 mg/kg bw/day for 2 weeks; the animals were killed at the end of treatment	– ≥250 mg/kg bw/day o decreased sperm and count motility o significant inhibition of the superoxide dismutase, glutathione peroxidase and glutathione in the testes o increased level of malondialdehyde in the testes – 500 mg/kg bw/day o decreased body weight and testicular weight o disintegration and shed of the seminiferous epithelial cells o atrophy of the seminiferous tubules	15
Wistar rats (24 males); DBP was given orally via gavage in corn oil; doses: 200, 400, 600 mg/kg bw/day for 15 days	– ≥200 mg/kg bw/day o decreased testicular weight, sperm and count motility o decreased testosterone and FSH level o decreased testicular lactate dehydrogenase activity o decreased testicular antioxidant enzyme levels and serum total antioxidant capacity o degenerative changes in the testes o absence of sperm in some seminiferous tubules – >400 mg/kg bw/day o degeneration and absence of spermatogenesis in most seminiferous tubules – 600 mg/kg bw/day o necrosis of some seminiferous tubules	16

Table 1. The effects of dibutyl phatalate (DBP) on male animal reproduction – short-term and sub-chronic exposure – cont.

Study data	Results	Reference
Rats – cont.		
short-term exposure – cont.		
Wistar rats (6 males/group, 5 weeks old); DBP was given orally in ground-nut oil; doses: 250, 500 and 1000 mg/kg bw for 15 consecutive days; the animals were killed on day 16	– 250 mg/kg bw/day o shrunken tubules and defective spermatogenesis – ≥500 mg/kg bw/day o decreased testicular weight o marked degeneration of the seminiferous tubules o decreased acid phosphatase and sorbitol dehydrogenase levels o increased lactate dehydrogenase level o increased glucose-6-phosphate dehydrogenase, γ-glutamyl transpeptidase and β-glucuronidase levels	17
Sprague-Dawley rats (20 males/group); DBP was given orally via gavage; doses: 0, 100, 250, 500 mg/kg/day for 21 consecutive days; the animals were killed 24 h after the last treatment	– ≥100 mg/kg/day o abnormal morphology of the seminiferous tubules o reduced sperm quality o heteromorphosis of the mitochondrion and nucleus in the spermatic or spermatogonial cell	18
F344 rats (9 males/group, 10 weeks old); DBP was given orally in corn oil; doses: 0, 31.25, 125, 500 mg/kg bw/day for 4 weeks	– 31.25 mg/kg bw/day o increased sperm abnormalities o testicular atrophy – 125 mg/kg bw/day o increased relative liver and kidneys weight o decreased sperm count – 500 mg/kg bw/day o decreased body weight gain o serum and testicular testosterone level within the normal range – LOAEL 31.25 mg/kg bw/day	19
Sprague-Dawley rats (males); DBP was given orally; dose: 250 mg/kg bw/day for 4, 8 or 12 weeks	– 250 mg/kg bw/day o decreased sperm count o increased production of abnormal sperm o Sertoli cells vacuolization at the longest treatment o changes in the serum testosterone level o irregular arrangements of the seminiferous tubules o decreased glutathione peroxidase and superoxide dismuase levels	20
Sprague-Dawley rats (6 males/group); DBP was given orally via gavage; dose: 500 mg/kg bw/day for 4 weeks	– 500 mg/kg bw/day o increased liver weight o decreased testicular weight o decreased sperm count and motility	21
F344 rats (5 males/group, 6 weeks old); DBP was given via food; doses: 0, 61, 225, 1535 mg/kg bw/day for 28 days	– 1535 mg/kg bw/day o degeneration of the seminiferous tubules o diminished spermatogenesis o prominent vacuolization of spermatogonia o morphological changes in sperm – NOAEL 225 mg/kg bw/day	22

Table 1. The effects of dibutyl phthalate (DBP) on male animal reproduction – short-term and sub-chronic exposure – cont.

Study data	Results	Reference
Rats – cont.		
sub-chronic exposure		
F344 rats (10 males/group, 29–30 weeks old); DBP was given via food: 0%, 0.25%, 0.5%, 1.0%, 2.0%, 4.0% (average: 0, 176, 359, 720, 1540, 2964 mg/kg bw/day) for 13 weeks	– ≥359 mg/kg bw/day o decreased body weight gain – 720 mg/kg bw/day o decreased body weight o degeneration of the germinal epithelium o focal atrophy of the seminiferous tubules – ≥1540 mg/kg bw/day o decreased relative and absolute testicular, cauda epididymal and left epididymal weight o decreased serum testosterone level o decreased zinc concentration in the testes o decreased sperm count, motility and number of spermatid heads per testis and per gram testis o atrophy of Sertoli cells with vacuolated cytoplasm – 2964 mg/kg bw/day o degeneration of the germinal epithelium in all seminiferous tubules with no spermatogenesis – NOAEL 359 mg/kg bw/day	23
Mice		
short-term exposure		
C57BL/6J mice (males, number of animals – no data, 4 days old); DBP was given orally in corn oil; dose range: 1–500 mg/kg bw/day for 10 days	– ≥1 mg/kg bw/day o decreased testicular weight o delayed spermatogenesis o reduced Sertoli cell proliferation o reduced anogenital distance – ≥10 mg/kg bw/day o impaired Sertoli cell maturation – 500 mg/kg bw/day o decreased serum testosterone and testicular o androgen activity – LOAEL ≥1 mg/kg bw/day	24
sub-chronic exposure		
B6C3F1 mice (10 males/group, 29–30 weeks old); DBP was given via food: 0%, 0.125%, 0.25%, 0.5%, 1.0%, 2.0% (average: 0, 163, 353, 812, 1601, 3689 mg/kg bw) for 13 weeks	– ≥163 mg/kg bw/day o increased serum testosterone level – ≥812 mg/kg bw/day o increased zinc concentration in the testes – 3689 mg/kg bw/day o reduced left epididymal weight o reduced body weight o higher number of spermatid heads per gram in the testes – NOAEL 353 mg/kg bw/day	23

Table 1. The effects of dibutyl phthalate (DBP) on male animal reproduction – short-term and sub-chronic exposure – cont.

Study data	Results	Reference
Rabbits		
sub-chronic exposure		
adult male rabbits (5 males/group, 6–6.5 months old); DBP was given orally 3 times a week for 7 weeks; dose: 520 mg/kg bw/day	– 520 mg/kg bw/day o decreased prostate and testicular weight o reduced intratesticular and serum testosterone levels o decreased sperm count o no changes in the total superoxide dismutase, glutathione peroxidase activities, FSH and LH serum concentration	25
Dutch-Belted rabbits (6 males/group, 25 weeks old); DBP was given orally via gavage in a mixture of deionized water with corn syrup; doses: 0, 400 mg/kg bw/day at PNW 4–12 (8 weeks)	– 400 mg/kg bw/day o reduced serum testosterone level o increased abnormal sperm o altered response to gonadotropin release o reduced accessory sex glands weight – LOAEL 400 mg/kg bw/day	26
Monkeys		
short-term exposure		
marmoset monkeys (4 males/group, 4–6 days old); DBP was given orally via gavage in corn oil; doses: 0, 500 mg/kg bw/day for 14 consecutive days; the animals were killed 4 h after the last treatment	– 500 mg/kg bw/day o increased Leydig cell volume in the testes	27

DBP – dibutyl phthalate; FSH – follicle stimulating hormone; LH – luteinizing hormone; LOAEL – lowest observed adverse effect level; NOAEL – no-observed adverse effect level; PNW – postnatal week.

When given in subcutaneous injections for 9 consecutive days at a dose of 1000 mg/kg bw, DBP resulted in the presence of multinuclear germ cells and hyperplasia of Leydig cells in the seminiferous tubules [13]. In the monkeys exposed orally via gavage to 500 mg DBP/kg bw/day, steroidogenesis suppression by fetal type Leydig cells was observed [27]. The same process occurred in rodents but with a higher dose – 1000 mg/kg bw/day given via subcutaneous injections [13,27].

When given via food at a dose of 1540 mg/kg bw/day for 13 weeks, DBP resulted in the atrophy of Sertoli cells with vacuolated cytoplasm, decreased zinc concentrations in the testes, a decreased number of spermatid heads per testis and per gram testis, decreased testicular weight, and a lower serum testosterone level [23]. In another study in which F344 rats received DBP (1535 mg/kg bw/day) via food for 28 days, the animals demonstrated seminiferous degeneration, diminished spermatogenesis with morphological changes in sperm, and vacuolization of spermatogonia [22].

The rats treated with DBP orally at a dose of 2000 mg/kg bw/day (via gavage) for 9 days had most of the previously described adverse effects (Table 1), with an additional effect being an increased activity of the marker enzymes of oxidative stress [14]. Exposing rats to 2964 mg/kg bw/day of DBP, which was given via food for 13 weeks, only resulted in degeneration of the germinal epithelium in all seminiferous tubules [23].

The result of treating mice with the highest dose of DBP (3689 mg/kg bw/day), which was given via food, appears

interesting as it caused an increased number of spermatid heads per gram in the testis [23].

In conclusion, in the studies of DBP reprotoxicity in experimental animals, the most common effects of exposure were reduced fertility [14–26], atrophic changes in male gonads [19,23], as well as a reduction in sperm parameters [14–16,18,21,23,25], and other genital defects [13,20–22,24,26,27]. Laboratory animals were exposed orally (via gavage or food) and via subcutaneous injections. No literature data on the inhalation exposure were found.

Besides the dose, the most important difference, when comparing these data, is the exact exposure time and what comes through it – the cumulative dose in the animal body. Noteworthy is the fact that lower doses cause more adverse effects than the highest dose (3689 mg/kg bw/day) [23].

Embryotoxicity and teratogenicity

Data on the embryotoxic and teratogenic effects of exposure are presented in Table 2 (32 studies conducted on rats) [28–60]. Only rat studies assessed the embryotoxic and teratogenic effects of DBP. The table is divided by exposure time (females exposed at different stages of pregnancy: 22 studies, females exposed at different periods of pregnancy and lactation: 7 studies, and 2-generation groups: 3 studies).

In the pregnant rats treated with the lowest dose of DBP (1.5–3.0 mg/kg bw via food), from gestational day (GD) 15 to postnatal day (PND) 21, changes in the mammary glands in both sexes, with degeneration and atrophy of the mammary gland follicles, were observed along with an increased relative weight of the pituitary in males and reduced spermatocyte development in the F1 generation [55].

A higher dose of DBP (10 mg/kg bw) given to pregnant rats from GD 14 until delivery had a negative impact not only on the dams (longer gestation, a reduced female body weight gain) but also on F1 males (a decreased anogenital distance and a reduction in the testosterone level in adult males) [37].

When the dose was increased to 15–30 mg/kg bw, a reduction in the follicle stimulating hormone was observed when DBP was given at GD 15–PND 21 [55].

Dibutyl phthalate given orally to pregnant rats at a dose of 50 mg/kg bw at GD 12–19 resulted in a reduction of the testicular testosterone level and some enzymes (Table 2) [34]. When the treatment with the same dose of DBP was elevated from GD 14 until delivery, a reduction in body weight, prostate and epididymis in F1 males was observed [37].

In a 2-generation study, F0 females exposed to ≥80 mg/kg bw of DBP via food before gestation resulted in a decrease in the live pup weight and the total number of live pups per litter. In turn, 1 in 20 F1 males, when given ≥52 mg/kg bw of DBP via food, had absent or not fully developed epididymis [23,60].

Treating the dams with 100 mg/kg bw of DBP (via gavage) on GD 1, 7 and 14 resulted in a decreased sperm count, motility, viability with morphological changes in sperm, and a decreased steroidogenic enzyme activity level in F1 males [32].

When pregnant CD rats were treated orally with 100 mg/kg bw/day of DBP at GD 12–21, the following effects were observed: a delay in preputial separation in F1 males [44]; metaplasia of the prostate epithelial cells, an increase in androgen receptor expression, metalloproteinase-9 activity and the proliferation index [52]; a decrease in the testes size and weight, Leydig cell hyperplasia areas, degeneration of the seminiferous tubules and Sertoli cells, an increase in the luteinizing hormone level, a decrease in the testosterone level [43]; Leydig cell clusters, multinucleated germinative cells, and an increase in the interstitial component [53]; testicular Leydig cells [42]; an increase in the epithelial compartment of the prostate gland, an increase in the incidence of metaplasia, inflammation and endothelial prostate cancer [54]; and retained areolas or nipples in F1 males [45,46].

When given at a dose of 100 mg/kg bw/day to the dams at GD 12–19, DBP caused a reduction in testicular mRNA

Table 2. Embryotoxic and teratogenic effects of dibutyl phthalate (DBP) in female rats – exposure at different stages of pregnancy, lactation and a 2-generation study

Study data	Results	Reference
Females exposed at different stages of pregnancy		
Spraque-Dawley rats (pregnant females); DBP was given orally at GD 14; single dose: 0, 500, 1000, 1500 or 2000 mg/kg bw; the dams were killed at GD 21	– ≥1000 mg/kg bw o increased incidence of skeletal malformations – ≥1500 mg/kg bw o statistically significantly decreased female body weight gain o statistically significantly decreased uterine weight o increased resorption frequency o reduced fetal body weight – 2000 mg/kg bw o reduced number of live fetuses	28
Wistar rats (pregnant females); DBP was given via gastric intubation on 1 day, between GD 6 and 16; single dose: 1500 mg DBP/kg bw; at GD 20, the females were killed to assess malformations in the fetuses	– decreased female body weight gain – fetuses with skeletal malformations and/or internal/external malformations were observed only when DBP was given at GD 8, 9 or 15 – after day 8, only deformations of the cervical vertebrae were noted – in the fetuses treated with DBP on day 9, deformations of the cervical and thoracic vertebrae, ribs, and dilatation of the renal pelvis were observed – DBP given at GD 15 resulted in cleft palate and sternebrae fusion	29
Sprague Dawley rats (55 pregnant females); DBP was given orally via gavage at GD 17 (20 females) or at GD 18 (35 females); dose: 500 mg/kg bw; the animals were killed after 2, 4, 6, 24 h, or given the second dose after 24 h and then killed 48 h after the first dose	– 500 mg/kg bw o multinucleated germ cells were observed in the testes of male fetuses 4–6 h after exposure at GD 18 (no changes were observed at GD 17) o elevated seminiferous cord diameter in the testes during the fetal period and cell death	30
Wistar rats (pregnant females); DBP was given orally via gavage at GD 7–9, 10–12 or 13–15; doses: 750, 1000 or 1500 mg/kg bw	– ≥750 mg/kg bw o significantly increased skeletal malformations (deformities of the vertebratal column in the thoracic and cervical regions of the ribs) – in dams exposed at GD 7–9 o significantly increased incidence of skeletal malformations, including those visible to the outside, e.g., cleft palate and fusion of the sternebrae – in the dams exposed at GD 13–15 significantly increased postimplantation loss – ≥1500 mg/kg bw o 100% postimplantation loss (in all females regardless of the GD at which they were exposed) – LOAEL 750 mg/kg bw/day (teratogenicity)	31

Table 2. Embryotoxic and teratogenic effects of dibutyl phthalate (DBP) in female rats – exposure at different stages of pregnancy, lactation and a 2-generation study – cont.

Study data	Results	Reference
Females exposed at different stages of pregnancy – cont.		
Wistar rats (pregnant females); DBP was given via gavage in corn oil at GD 1, 7 and 14; doses: 0, 100, 500 mg/kg bw/day; at PND 100, the F1 male rats were used for mating with normal females	– F1 generation ○ ≥100 mg/kg bw □ decreased fertility (decreased sperm count, motility, viability) □ morphological abnormalities in sperm (hypoosmotic swelling tail coiled sperms) □ decreased serum testosterone level □ decreased steroidogenic enzyme activity level	32
Sprague-Dawley rats (groups of 3–4 pregnant females); DBP was given orally via gavage at GD 12–21; doses: 0, 500 mg/kg bw/day; examination of the male reproductive tract at GD 16–21, and at PND 3, 7, 16, 21, 45 and 70	○ 500 mg/kg bw/day □ multinucleated gonocytes □ increased number of gonocytes and aggregates of Leydig cells in the testes during the fetal period □ decreased number of spermatocytes (at PND 16 and 21) connected with mild to severe degeneration of the seminiferous epithelium (at PND 70) □ ipsilaterally malformed epididymides leading to obstruction of testicular fluid flow	33
Sprague-Dawley rats (groups of 11 pregnant females); DBP was given via gavage in corn oil at GD 12–19; doses: 0, 0.1, 1, 10, 30, 50, 100, 500 mg/kg bw/day	○ ≥50 mg/kg bw □ reduced testicular testosterone level □ dose-dependent reduction in the protein concentration of the scavenger receptor, mRNA, cytochrome P450 side-chain cleavage, P450c17 and 3β-hydroxysteroid dehydrogenase ○ NOAEL 30 mg/kg bw/day (decreased testosterone level)	34
pregnant CD rats; DBP was given via food at GD 12–19; doses: 0, 100, 500 mg/kg/day; the animals were killed 4 h or 24 h after treatment.	○ >100 mg/kg/day □ reduction in testicular mRNA concentration, cytochrome P450 family and P450 family 17, steroidogenic acute regulatory protein □ reduced testicular testosterone level □ significant reduction in the male offspring anogenital distance □ Leydig cell aggregates, multinucleated gonocytes, increased cord diameters	35
Wistar rats (pregnant females); DBP was given orally via gavage in corn oil at GD 13.5–20.5/21.5; doses: 0, 4, 20, 100, 500 mg/kg bw/day	– F1 males ○ ≥100 mg/kg bw/day □ decreased testicular testosterone level □ presence of multinuclear gonocytes □ abnormal Leydig cell aggregation □ 500 mg/kg bw/day □ decreased fertility □ increased incidence of cryptorchidism □ decreased testicular weight	36

Table 2. Embryotoxic and teratogenic effects of dibutyl phthalate (DBP) in female rats – exposure at different stages of pregnancy, lactation and a 2-generation study – cont.

Study data	Results	Reference
Females exposed at different stages of pregnancy – cont.		
rats (pregnant females); DBP was given orally via gavage from GD 14 until delivery; doses: 2, 10 and 50 mg/kg bw	– ≥10 mg/kg bw o significant reduction in female body weight at GD 21 o elevated length of gestation o decreased anogenital distance in F1 males o reduced testosterone level in adult F1 males – 50 mg/kg bw o reduced body weight in F1 males o slightly reduced epididymis and prostate weight in F1 males o slightly decreased daily sperm production and testicular spermatid count	37
rats (pregnant females); DBP was given orally via gastric intubation; doses: 0, 500, 630, 750 or 1000 mg/kg bw at GD 7–15	– ≥500 mg/kg bw/day o significantly decreased maternal body weight gain (a statistically significant change from a dose of 630 mg/kg bw) o resorptions of the implanted embryos o decreased fetal weight (a statistically significant change from a dose of 750 mg/kg bw) – ≥630 mg/kg bw/day o increased number of dead fetuses o increased incidence of malformations (mainly cleft palate; a statistically significant change from a dose of 750 mg/kg bw only in young males) o decreased fetal weight o increased incidence of postimplantation loss – 1000 mg/kg bw/day o complete resorption of the implanted embryos o 2 maternal deaths	38
Wistar rats (pregnant females); DBP was given orally via gavage; doses: 0, 500 mg/kg bw/day at GD 13–21	– 500 mg/kg bw/day o unilateral cryptorchidism, infertility, hypospadias, testis abnormalities in F1 males (similar to those seen in people with TDS) o 60% cryptorchidism, hypospadias, testicular abnormalities (similar to those seen in people with TDS) o immature Sertoli cells	39
Sprague-Dawley rats (pregnant females); DBP was given orally via gavage in corn oil; doses: 0, 250, 500, 700 mg/kg bw/day at GD 10–19; the pups were killed at PND 31 or 42	– ≥250 mg/kg bw o decreased testicular, seminal vesicles, epididymides and Cowper's gland weight o significantly delayed testis descent o decreased serum testosterone level	40
Sprague-Dawley rats (pregnant females); DBP was given orally via gavage; doses: 0, 500 mg/kg bw/day at GD 12–20	– 500 mg/kg bw/day o testicular atrophy in males (decreased sperm production, degeneration of seminiferous epithelium) o malformations of the male reproductive system o Leydig cell aggregation o reduced testosterone level o multinucleated gonocytes	41

Table 2. Embryotoxic and teratogenic effects of dibutyl phthalate (DBP) in female rats – exposure at different stages of pregnancy, lactation and a 2-generation study – cont.

Study data	Results	Reference
Females exposed at different stages of pregnancy – cont.		
Sprague Dawley rats (20 pregnant females); DBP was given orally at GD 12–21; doses: 10, 30, 50 or 100 mg/kg bw/day	– 100 mg/kg bw/day o reduced testicular weight o testicular Leydig cells o decreased serum testosterone level o reduced LH level at PNW 5–7 and its increase at PNW 9–17 (compared to the controls)	42
Sprague Dawley rats (4 pregnant females); DBP was given orally in corn oil at GD 12–21; dose: 100 mg/kg bw/day; the young males were killed at PNW 20	– 100 mg/kg bw/day o decreased testes size and testicular weight at PNW 20 o Leydig cell hyperplasia areas o degeneration of the seminiferous tubules and Sertoli cells (disturbed spermatogenesis in several seminiferous tubules) o increased LH level o decreased testosterone level	43
CD rats (pregnant females); DBP was given orally via gavage at GD 12–21; doses: 0, 100, 250 or 500 mg/kg bw/day	– ≥100 mg/kg bw o delayed preputial separation – ≥250 mg/kg bw o malformations of the reproductive organs in F1 males (decreased anogenital distance and retained thoracic nipples) – 500 mg/kg bw o in male offspring: hypospadias, epididymal agenesis, prostate and vas deferens, cryptorchidism, degeneration of the seminiferous epithelium, intersticial cell hyperplasia of the testis o intersticial cell adenoma (in 2 F1 males) o reduced body weight of 1 female after GD 18 and birth of dead or exhausted pups – in F1 females, no abnormalities related to the development of reproductive organs and kidneys were observed	44
CD rats (pregnant females); DBP was given orally via gavage at GD 12–21; doses: 0, 0.5, 5, 50, 100, 500 mg/kg/day	– 100 mg/kg/day o retained areolas or nipples in the male offspring – 500 mg/kg/day o decreased anogenital distance, hypospadias o partially developed or absent epididymis, seminal vesicles, ventral prostate and vas deferens o decreased weight of the epididymis, testes, ventral and dorsolateral prostates, levator anti-bulbocavernosus and seminal vesicles at PND 110 o prevalent seminiferous tubule degeneration, intersticial cell adenoma, focal intersticial cell hyperplasia – NOAEL 50 mg/kg bw/day – LOAEL 100 mg/kg bw/day	45, 46

Table 2. Embryotoxic and teratogenic effects of dibutyl phthalate (DBP) in female rats – exposure at different stages of pregnancy, lactation and a 2-generation study – cont.

Study data	Results	Reference
Females exposed at different stages of pregnancy – cont.		
Sprague-Dawley rats (pregnant females); DBP was given orally via gavage at GD 12–21; dose: 0, 500 mg/kg bw/day	– 500 mg/kg bw/day o decreased androstenedione and testicular testosterone levels o reduced steroidogenesis	47
Sprague-Dawley rats (pregnant females – groups of 3–4 dams); DBP was given via gavage in corn oil at GD 12–21; doses: 0, 500 mg/kg bw/day, testes examination in the offspring	– 500 mg/kg bw/day o enlarged seminiferous cords in fetuses o Leydig cell hyperplasia o atrophic changes in the testes o decreased serum testicular testosterone level o decreased sperm production o reproductive tract malformations o adenomas	48
Sprague-Dawley rats (pregnant females – 4 dams/group); DBP was given orally via gavage at GD 8–18; doses: 0, 33, 50, 100, 300, 600 mg/kg bw/day	– ≥300 mg/kg bw o reduced testosterone production – NOAEL 100 mg/kg bw/day	49
Wistar rats (pregnant females); DBP was given via food at GD 11–21; doses: ~0, 331, 555 or 661 mg/kg bw	– ≥555 mg/kg bw o significantly reduced feed intake and body weight gain o increased incidence of cryptorchidism o decreased anogenital distance of male fetuses – 661 mg/kg bw o decreased weight of female and male fetuses o increased incidence of fetal fusion of the sternebrae and cleft palate – the number of live fetuses, the incidence of postimplantation loss, dead fetuses or resorptions did not differ in comparison to the controls – NOAEL 331 mg/kg bw/day	50
Females exposed at different periods of pregnancy and lactation		
Sprague-Dawley rats (9 pregnant females); DBP was given orally at GD 14.5–PND 6; dose: 500 mg/kg bw/day	– 500 mg/kg bw/day o gonadal dysgenesis (unilateral abdominal cryptorchidism and unilateral anorchism at PND 24, unilateral testicular dysgenesis at PND 90) o slightly reduced anogenital distance at PND 24 o Leydig cell proliferation	51
rats (10 pregnant females); DBP was given orally via gavage at GD 12–PND 21; dose: 100 mg/kg bw/day; the pups were killed at PND 90	– o 100 mg/kg bw/day o metaplasia of the prostate epithelial cells o increased androgen receptor expression, metalloproteinase-9 activity and proliferation index o no change in the serum and testicular testosterone levels o no change in prostate weight	52

Table 2. Embryotoxic and teratogenic effects of dibutyl phthalate (DBP) in female rats – exposure at different stages of pregnancy, lactation and a 2-generation study – cont.

Study data	Results	Reference
Females exposed at different periods of pregnancy and lactation – cont.		
Wistar rats (10 pregnant females); DBP was given orally via gavage at GD 12–PND 21; dose: 100 mg/kg bw/day; 5 dams were killed at GD 20; 5 young males were killed at PND 90	– 100 mg/kg bw/day ○ Leydig cell clusters ○ presence of multinucleated germinative cells ○ increased interstitial component ○ decreased anogenital distance (statistically insignificant)	53
Wistar rats (pregnant females); DBP was given orally via gavage at GD 15–PND 21; doses: 100, 500 mg/kg of DBP; the pups were killed at PND 220	– ≥100 mg/kg ○ decreased anogenital distance in newborn males ○ increased epithelial compartment of the prostate gland ○ increased incidence of metaplasia and inflammation ○ increased incidence of endothelial prostate cancer in males	54
rats (females); DBP was given via food; doses: 0, 20, 200, 2000 and 10 000 ppm (0, 1.5–3.0, 15–30, 150–3000, 750 mg/kg bw at GD 15–PND 21)	– at PND 21 ○ 1.5–3.0 mg/kg bw (20 ppm) □ reduced testicular spermatocyte development in the male offspring □ histopathological changes in the mammary gland in both sexes □ degeneration and atrophy of the mammary gland follicles □ increased relative weight of the pituitary in males ○ >15–30 mg/kg bw (200 ppm) □ reduced FSH ○ >150–3000 mg/kg bw (2000 ppm) □ changes in the pituitary immunoreactive hormones with a similar increase in the percentage of LH ○ 10 000 ppm □ reduced prolactin producing cells in both sexes ○ 200 and 2000 ppm □ increased relative weight of the pituitary in males at PNW 11 ○ ~750 mg/kg bw (10 000 ppm) □ increased relative weight of the pituitary in females at PNW 11 ○ 10 000 ppm □ the male offspring showed reduced anogenital distance and hypoplasia of the nipples (at PND 14) ○ LOAEL 1.5–3 mg/kg bw/day (developmental toxicity)	55

Table 2. Embryotoxic and teratogenic effects of dibutyl phthalate (DBP) in female rats – exposure at different stages of pregnancy, lactation and a 2-generation study – cont.

Study data	Results	Reference
Females exposed at different periods of pregnancy and lactation – cont.		
CD rats (pregnant females (groups of 10 dams); DBP was given orally via gavage from GD 3 through lactation to PND 20; doses: 0, 250, 500 or 750 mg/kg bw; the dams were killed after lactation (at PND 21) and the offspring after reaching puberty (on day 100–105 of their life)	– ≥250 mg/kg bw/day ○ at all doses, the epididymis was underdeveloped or absent at PND 100, and it was connected with germ cell loss and testicular atrophy; hypospadias; and absent or ectopic testes – ≥500 mg/kg bw/day ○ reduced uterine weight ○ reduced anogenital distance in F1 males at birth, as well as small testes, seminal vesicles and the absence of the prostate gland – 750 mg/kg bw/day ○ maternal effects on pregnancy ○ postimplantation loss ○ decreased number of live pups per litter at birth ○ reduced prostate weight and average kidney weight – the effects of DBP on the reproductive system in F1 females were insignificant – the dam's body weight and the amount of feed consumed remained unchanged	56
Sprague-Dawley rats (pregnant females); DBP was given orally via gavage at GD 1–PND 21; doses: 0, 50, 250, 500 mg/kg bw/day	– ≥250 mg/kg bw ○ reduced birth weight ○ reduced body weight gain ○ reduced number of live pups per litter ○ reduced anogenital distance in males ○ reduced epididymis weight (at PND 70) ○ significantly reduced prostate weight ○ absent or underdeveloped epididymis ○ undescended testes, testicular atrophy ○ reduced sperm count and motility – 500 mg/kg bw ○ increased number of dead fetuses	57
2-generation study		
Long Evans rats (groups of 12–13 pregnant females); 2-generation study; exposure from PND 20; DBP was given via food; doses: 0, 250, 500, 1000 mg/kg bw/day	– ≥500 mg/kg bw ○ reduced fertility in F1 females ○ reduced number of litters in F0 females ○ reduced serum progesterone level in F0 females ○ increased estradiol production in F0 females ○ increased weight of the liver and kidneys – NOAEL 250 mg/kg bw/day (F0 female fertility)	58
Sprague-Dawley rats (17 males and 17 females/group); 2-generation study; DBP was given via food; doses: 1, 4, 10, 30, 100, 1000, 10 000 ppm (approx. 0.1, 0.4, 1, 3, 10, 100, 1000 mg/kg bw)	– F1 males ○ 1000 mg/kg bw/day □ atrophy of vas deferens □ reduced anogenital distance □ delayed testis descent □ delayed preputial separation ○ NOAEL 1000 mg/kg bw/day (systemic toxicity, effect on sperm) ○ NOAEL 100 mg/kg bw/day	59

Table 2. Embryotoxic and teratogenic effects of dibutyl phthalate (DBP) in female rats – exposure at different stages of pregnancy, lactation and a 2-generation study – cont.

Study data	Results	Reference
2-generation study – cont.		
Sprague-Dawley rats (groups of 20 males and 20 females); 2-generation study (controls: 40 females and 40 males); DBP was given via food; doses: 0, 52, 256 or 509 mg/kg bw/day (males) and 0, 80, 385 or 794 mg/kg bw/day (females); the F1 males and females exposed to the highest dose of DBP were involved in the study on fertility, pregnancy and mating to deliver F2 offspring	– F0 females ○ ≥80 mg/kg bw/day □ decreased live pup weight □ decreased number of live pups per litter ○ 794 mg/kg bw/day □ increased relative weight of the liver and kidneys (both males and females) □ decreased body weight □ lower birth weight of F1 pups – F0 males ○ 509 mg/kg bw/day □ increased relative weight of liver and kidneys □ increased relative right cauda epididymal weight ○ parameters related to the sperm and estrous cycles in the F0 generation remained unchanged – F1 males ○ ≥52 mg/kg bw/day □ absence or not fully developed epididymis (1 in 20 animals) ○ ≥256 mg/kg bw/day □ significantly increased relative kidney weight □ absence or incompletely developed epididymis (1 in 20 animals) □ atrophic changes in the testes (1 in 20 animals) □ degeneration of the seminiferous tubules (3 in 10 animals) ○ 509 mg/kg bw/day □ decreased body weight and relative weight of all reproductive organs, and increased relative weight of the kidneys and liver □ absence or not fully developed epididymis (12 in 20 animals) □ atrophic changes in the testes (4 in 20 animals) □ degeneration of the seminiferous tubules (8 in 10 animals) □ statistically significantly reduced sperm count □ cryptorchidism (3 in 20 animals), underdeveloped seminal vesicles (4 in 20 animals) and underdeveloped penis or foreskin (4 in 20 animals) – F1 females ○ 794 mg/kg bw/day □ statistically significantly decreased body weight and decreased absolute right ovary weight, liver and kidney weight ○ in F1 females, there were no changes in the frequency and length of the estrous cycle – F2 live-born young males and females ○ lower birth weight in all dose groups (a statistically significant difference) – LOAEL 52/80 mg/kg bw/day (embryotoxicity) – NOAEL 385 mg/kg bw/day (maternal toxicity)	23, 60

GD – gestational day; PND – postnatal day; TDS – testicular dysgenesis syndrome.
Other abbreviations as in Table 1.

concentration, cytochrome P450, and steroidogenic acute regulatory protein, along with an increased cord diameter [35]. When given at a dose of 100 mg/kg bw/day (via gavage) at GD 13.5–20.5/21.5, in F1 males besides the altered testosterone level (which was observed at lower doses – beginning from 10 mg/kg bw [34,37]), multinucleated gonocytes and abnormal Leydig cell aggregation were also noted [36], and when the exposure time was GD 1–PND 21, a reduction in sperm count, motility and birth weight was observed [57]. Noteworthy is the fact that Scarano et al. [52] did not observe changes in the serum and testosterone levels or in prostate weight at a dose of 100 mg/kg bw, but these effects were noted at lower doses (≥10 mg/kg bw) [37].

In the subsequent dose range of 250–300 mg/kg bw, DBP given orally to pregnant rats, in addition to the symptoms listed above, also resulted in significant delayed testis descent, a decrease in the seminal vesicles, epididymides and Cowper's gland weight (exposure time: GD 10–19) [40], germ cell loss, testicular atrophy, hypospadias, absent or ectopic testes (exposure time: GD 3–PND 20) [56], retained thoracic nipples (exposure time: GD 12–21) [44], and a reduced testosterone production (exposure time: GD 8–18) [49].

In a 2-generation study where DBP was given via food at a dose of 256 mg/kg bw/day, degeneration of the seminiferous tubules, incompletely developed or absent epididymis, and atrophic changes in the testes were observed [23,60].

When given orally to pregnant females at GD 7–15, at a dose of 500 mg/kg bw, DBP resulted in increased resorptions of the implanted embryos [38], and at GD 12–21 decreased body weight was observed in 1 female after 18 days of pregnancy, and a birth of dead/exhausted pups was noted [44]. When the exposure time changed (GD 3–PND 20), a reduction in uterine weight was observed [56]. A significant increase in the number of dead fetuses was observed when exposure to DBP covered a period of GD 1–PND 21 [57].

In a 2-generation study conducted on females treated orally with 500 mg/kg bw, the following changes were observed in F0 females: a decrease in the number of litters and the serum progesterone level, coupled with an increased estradiol production; and in F1 females: decreased fertility [58]. When the dams were exposed to 500 mg/kg bw of DBP orally, F1 males had additional symptoms which were not described above, such as a decreased sperm production, degeneration of the seminiferous epithelium (exposure time: GD 10–20) [41], obstruction of testicular fluid flow due to malformed epididymides (exposure time: GD 12–21) [33], cryptorchidism (exposure time: GD 13.5–20.5/21.5) [36], hypospadias, immature Sertoli cells (exposure time: GD 13–21) [39], the absence of the prostate gland (exposure time: GD 3–PND 20) [56], partially developed or even absent epididymides, seminal vesicles, ventral prostate, and vas deferens, a decreased weight of the ventral and dorsolateral prostates, and the levator ani-bulbocavernosus muscle, intersticial cell adenoma, focal intersticial cell hyperplasia (exposure time: GD 12–21) [44–46], Leydig cell proliferation (exposure time: GD 14.5–PND 6) [51], a decreased androstenedione level (exposure time: GD 12–21) [47], and adenomas (exposure time: GD 12–21) [48].

In a 2-generation study, the F1 males exposed to 509 mg/kg bw/day of DBP via food also developed some atrophic changes in the testes, and had an underdeveloped penis or foreskin, besides the adverse effects which were also observed in lower doses [23,60]. Detailed symptoms which were noted during these studies are presented in Table 2.

The dams treated orally with 630 mg/kg bw had also a decreased fetal weight and an increased incidence of implantation loss (exposure time: GD 7–15) [38]. A slightly higher dose (661 mg/kg bw) of DBP given at GD 11–21 caused a higher incidence of fetuses malformations (cleft palate, fusion of the sternebrae) [50].

When the dose of DBP given orally to pregnant rats on GD 7–9 increased to 750 mg/kg bw, an additional defor-

mity of the vertebratal column in the thoracic and cervical regions of the ribs was observed [31]. When the exposure time changed from 15 GD to 21 PND, a reduction in the prolactin cells in both sexes, an increased relative weight of the pituitary in both sexes, and hypoplasia of the nipples in males were noted [55].

Treating pregnant Sprague-Dawley rats with 1000 mg/kg bw of DBP orally at GD 12–20 resulted in the complete resorption of implanted embryos and 2 maternal deaths [38].

When given orally to pregnant Sprague-Dawley rats at a dose of 1500 mg/kg bw after GD 8, DBP resulted in deformations of the cervical vertebrae. In the fetuses treated *in utero* on GD 9, deformations of the ribs and dilatation of the renal pelvis were also noted [29].

Treating pregnant females with DBP at a dose 2000 mg/kg bw orally on GD 14 resulted in a reduced number of live fetuses besides the previously mentioned adverse toxic effects [28].

A decreased anogenital distance and a lower testosterone level occurred at the lowest dose of 10 mg/kg bw given from GD 14 until delivery [37]. These effects were observed in practically all the studies related to the observation of utero exposure to DBP [32,34–36,40,42–48,50,51,54,56,57,59]. There is only one alluring report where the authors did not notice changes in the serum and testicular testosterone levels, in which pregnant rats were treated orally with 100 mg DBP/kg bw at GD 12–PND 21 [52]. One of the most common symptoms in F1 males was reduced testicular weight which appeared when DBP was given at a dose of 100 mg/kg bw at GD 12–21 [42]. Leydig cells abnormalities were evident (clusters, aggregation, hyperplasia), starting from a dose of 100 mg/kg bw/day, and the same applied to multinucleated gonocytes and multinucleated germinative cells, regardless of the exposure time (GD 12–21, GD 13.5–20.5/21.5, GD 12–PND 21, and GD 12–19) [35,36,43,53].

In addition to changes related to the testes, epididymides and sperm parameters, DBP was found to cause increased liver and kidney weight, starting from a dose of 500 mg/kg bw [23,58,60]. However, in a study by Mylchreest et al. [56], the authors treated pregnant CD rats with DBP at a dose of 750 mg/kg bw/day at GD 3–PND 20, and noted a reduction in the average kidney weight.

It is also worth paying attention to the maternal effects caused by DBP which started to occur from a dose of ≥80 mg/kg bw given before gestation, and resulted in a decreased number of live pups per litter and a lower live pup weight [23,60]. However, DBP treatment at a dose of 500 mg/kg bw (exposure time: GD 1–PND 21) resulted in an increased number of dead fetuses [57], and exposure to DBP at GD 7–15 at a dose of 630 mg/kg bw caused the same effect [38]. The fact that there were no changes in the frequency and length of the estrous cycle in F1 females even at the highest dose (794 mg/kg bw/day) seems quite interesting [23,60]. The observation made by Ema et al. [50] who treated pregnant rats with DBP at a dose of 661 mg/kg bw at GD 11–21, and noted that the number of live fetuses, and the incidence of postimplantation loss, dead fetuses and resorptions, did not differ from controls, also appears alluring.

Fetal malformations started to appear when DBP was used at a dose of 661 mg/kg bw/day (exposure time: GD 11–21) and mainly cleft palate and fusion of the sternebrae were observed [50]. The same malformations were noted at a dose of 750 mg/kg bw given at GD 13–15 [32], while at a dose of 750 mg/kg bw used at GD 7–15, only cleft palate was found to occur [38]. A single treatment with DBP at a dose of 1000 mg/kg bw on GD 14 resulted in the increased incidence of skeletal malformations [28].

A 2-generation study conducted on Sprague-Dawley rats which were given DBP via food resulted in adverse effects only at the highest dose (1000 mg/kg bw/day) [59], which are described in detail in Table 2.

The embryotoxic effects of DBP on laboratory animals include mainly an increase in fetal resorption and a decrease in live births [23,28,38,56,57,60]. The teratogenic effects

of DBP also manifest as skeletal malformations in fetuses (e.g., cleft palate, deformations of the cervical vertebrae, ribs, thoracic vertebrae, and sternebrae fusion) [28,29,31,50], malformations of male gonads (e.g., cryptorchidism or hypospadias) [23,36,39,44–46,50,51,56,57,60], and other genital effects [23,33,35,37,40–46,48,51,55–57,59,60].

CONCLUSIONS

The review of the literature was prepared due to increasing reports concerning the reproductive and developmental toxicity of DBP on laboratory animals.

The results of the presented studies suggest that the most common testicular effects of oral exposure to DBP in laboratory animals were reduced fertility, atrophic changes in male gonads, degenerative changes in the epididymis, as well as a reduction in sperm count and motility, reduced sperm quality, decreased testicular weight, delayed spermatogenesis, Leydig cell aggregation, impaired Sertoli cell maturation, and significant inhibitions of testicular enzymes.

On the basis of the literature data, it is clearly demonstrated that DBP shows the anti-androgenic effects while there are also reports confirming its weak estrogenic effect.

Based on the collected data, it can be assumed that DBP has a non-linear dose-response relationship which is typical for endocrine disruptors where stronger physiological responses can be observed at lower doses than at higher ones. Such a scenario is contrary to the typical toxicological concept assuming a linear relationship between the chemical dose and the effect, better known as "the dose makes the poison" principle. In the case of endocrine disruptors, there probably exist many physiological explanations of this phenomenon, but additional studies are needed for DBP to fully understand the mechanism.

Based on the presented results, the embryotoxic effects of DBP on laboratory animals include an increase in fetal resorption and a decrease in live births. The teratogenic effects of DBP also manifest as skeletal malformations in fetuses which include, e.g., cleft palate, deformations of the cervical vertebrae, ribs, thoracic vertebrae and the sternebrae fusion, dilatation of the renal pelvis, deformations of the vertebratal column, changes in male gonads, such as cryptorchidism or hypospadias, and other testicular abnormalities.

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