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Unraveling Phthalate-Induced Male Reproductive Toxicity: A Comparative Study of Di-n-butyl phthalate (DBP), Diethyl phthalate (DEHP), and Di-2-Ethylhexyl Phthalate (DEHP)

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ABSTRACT: Phthalates are ubiquitous environmental plasticizers with established endocrine-disrupting properties. Growing evidence implicates di-n-butyl phthalate (DBP), diethyl phthalate (DEP), and di(2-ethylhexyl) phthalate (DEHP) in male reproductive dysfunction; however, their comparative molecular mechanisms require integrated evaluation. This review synthesizes studies published between 2016 and 2026 examining mechanistic pathways of phthalate-induced male reproductive toxicity. Seventy-four studies, including in vivo animal experiments, in vitro cellular models, and human epidemiological investigations, met inclusion criteria. Across models, DBP and DEHP consistently reduced sperm count, motility, and viability, disrupted testicular histoarchitecture, and suppressed testosterone production, while DEP demonstrated comparatively weaker effects. Oxidative stress emerged as a central mechanism, characterized by increased reactive oxygen species, lipid

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peroxidation, antioxidant depletion, and mitochondrial dysfunction. These alterations were closely associated with activation of apoptotic pathways, including caspase signaling and Bax/Bcl-2 imbalance, leading to germ cell loss. Phthalates also downregulated key steroidogenic enzymes such as StAR, CYP11A1, CYP17A1, and 3β-HSD, interfered with androgen receptor and PPAR signaling, and disrupted hypothalamic–pituitary–gonadal axis regulation. Emerging transcriptomic and epigenetic evidence indicates persistent DNA methylation changes and altered gene expression patterns, suggesting potential long-term or transgenerational effects. Comparatively, DEHP demonstrated the highest reproductive toxicity, followed by DBP, with DEP exhibiting lower potency. The convergence of experimental and epidemiological findings supports a unified mechanistic framework in which oxidative stress, endocrine disruption, apoptosis, and epigenetic reprogramming collectively impair male reproductive health. These findings highlight the need for mechanistically informed risk assessment strategies that account for mixture exposure and cumulative biological effects.

Keywords: *Phthalates; Male reproductive toxicity; Oxidative stress; Endocrine disruption; Epigenetics.*

1.0 INTRODUCTION

Phthalates, including di-n-butyl phthalate (DBP), diethyl phthalate (DEP), and di(2-ethylhexyl) phthalate (DEHP), are widely used plasticizers classified as low molecular weight (LMW) or high molecular weight (HMW) compounds, with DBP and DEHP being of significant regulatory concern due to their established male reproductive toxicity. Human exposure occurs mainly through ingestion, inhalation, and dermal contact, with metabolism involving conversion to monoesters that mediate toxic effects. These phthalates have been linked to adverse male reproductive outcomes such as reduced testosterone levels, impaired spermatogenesis, decreased testis and epididymis weights, altered sperm parameters, and disrupted steroid hormone metabolism[1-3]. Mechanistically, oxidative stress, endocrine disruption, inflammation, apoptosis, and altered gene expression in pathways such as focal adhesion and steroidogenesis contribute to toxicity, while combined exposures may exacerbate these effects[4-6]. Antioxidants such as quercetin, lycopene, selenium compounds, zinc, and vitamin E have shown potential in mitigating

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phthalate-induced damage by attenuating oxidative stress and restoring hormonal balance[7-9]. Despite extensive experimental evidence and growing epidemiological data, integrative comparative analyses delineating similarities and differences in the molecular mechanisms underlying DBP-, DEP-, and DEHP-induced male reproductive toxicity remain limited.

Therefore, this structured narrative review aims to synthesize and contrast the molecular pathways through which DBP, DEP, and DEHP impair male reproductive function, integrating evidence from in vivo, in vitro, and human studies to evaluate the relative strength and consistency of mechanistic findings across biological models.

2.0 METHODOLOGY

2.1. Eligibility Criteria

This review included original experimental and epidemiological studies investigating the effects of Di-n-butyl phthalate (DBP), Diethyl phthalate (DEP), and Di(2-ethylhexyl) phthalate (DEHP) on male reproductive function with a clearly defined mechanistic component. Eligible studies were required to (i) involve mammalian models (in vivo rodent studies), human-derived cell lines, primary testicular cell cultures, or human epidemiological cohorts; (ii) assess male reproductive endpoints such as sperm count, motility, morphology, testosterone levels, Leydig or Sertoli cell function, germ cell apoptosis, or testicular histopathology; and (iii) report molecular or biochemical mechanisms including oxidative stress biomarkers, steroidogenic enzyme expression (e.g., StAR, CYP11A1, 3β-HSD), apoptosis signaling pathways (e.g., caspases, Bax/Bcl-2), endocrine receptor modulation, mitochondrial dysfunction, or epigenetic alterations.

Studies included were restricted to peer-reviewed articles published in English. Both acute and chronic exposure models were considered, provided that exposure routes and doses were clearly described. For epidemiological studies, inclusion required quantitative assessment of phthalate metabolites (e.g., urinary monoesters) and correlation with male reproductive parameters.

Exclusion criteria comprised: (i) studies evaluating mixed chemical exposures without isolating the specific effects of DBP, DEP, or DEHP; (ii) non-mammalian models; (iii) studies lacking reproductive endpoints; (iv) reviews, conference abstracts, editorials, and

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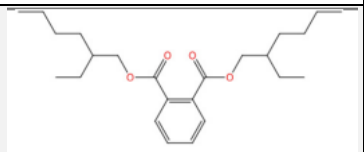
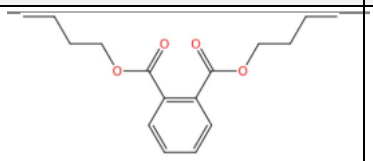
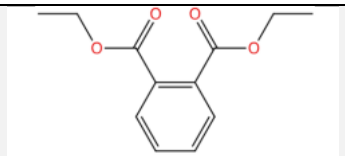
case reports; and (v) articles without accessible full text. Studies focusing solely on female reproductive toxicity or non-reproductive systemic toxicity were also excluded unless male-specific mechanistic data were extractable.

Table 1. Parent Phthalates (DBP, DEP, and DEHP) According to Molecular Weight with Their Primary and Secondary Metabolites

Molecular Class	Parent Phthalate	Primary Metabolite	Secondary Metabolites
High Molecular Weight (HMW) Phthalate	**DEHP** (Di(2-ethylhexyl) phthalate)	MEHP (Mono(2-ethylhexyl) phthalate)	MEHHP (Mono(2-ethyl-5-hydroxyhexyl) phthalate); MEOHP (Mono(2-ethyl-5-oxohexyl) phthalate); MECPP (Mono(2-ethyl-5-carboxypentyl) phthalate)
Low Molecular Weight (LMW) Phthalates	**DBP** (Dibutyl phthalate)	MnBP (Mono-n-butyl phthalate)	MHnBP (Mono(3-hydroxybutyl) phthalate)
	DEP (Diethyl phthalate)	MEP (Monoethyl phthalate)	—

Table 2. Overview of Selected Phthalates (DEHP, DBP, and DEP)

Phthalate Name	Abbreviation	CAS Registry Number	Molecular Formula	Chemical structure	Molecular Weight (g/mol)
Di-(2-ethylhexyl) phthalate	DEHP	117-81-7	$C_{24}H_{38}O_4$		390.56
Di-n-butyl phthalate	DBP	84-74-2	$C_{16}H_{22}O_4$		278.34
Diethyl phthalate	DEP	84-66-2	$C_{12}H_{14}O_4$		222.24

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2.2. Search Strategy

A comprehensive literature search was conducted to identify peer-reviewed studies investigating the molecular mechanisms of male reproductive toxicity induced by di-n-butyl phthalate (DBP), diethyl phthalate (DEP), and di(2-ethylhexyl) phthalate (DEHP). The electronic databases PubMed, Google Scholar, PubChem, Scopus, and Web of Science Core Collection were systematically searched to ensure broad biomedical and toxicological coverage. The search strategy combined controlled vocabulary and free-text keywords using Boolean operators ("AND", "OR"). The primary search terms included:

- ("Di-n-butyl phthalate" OR "DBP")
- ("Diethyl phthalate" OR "DEP")
- ("Di(2-ethylhexyl) phthalate" OR "DEHP")

These were combined with outcome-related and mechanistic terms, including:

- "male reproductive toxicity"
- "testicular toxicity"
- "spermatogenesis"
- "Leydig cells"
- "Sertoli cells"
- "oxidative stress"
- "reactive oxygen species"
- "mitochondrial dysfunction"
- "steroidogenesis"
- "androgen synthesis"
- "hypothalamic–pituitary–gonadal axis"
- "apoptosis"
- "endocrine disruption"

A representative search string used in PubMed was structured as follows:

("DBP" OR "di-n-butyl phthalate" OR "DEP" OR "diethyl phthalate" OR "DEHP" OR "di(2-ethylhexyl) phthalate") AND ("male reproductive toxicity" OR "testicular toxicity" OR "oxidative stress" OR "steroidogenesis" OR "apoptosis" OR "spermatogenesis").

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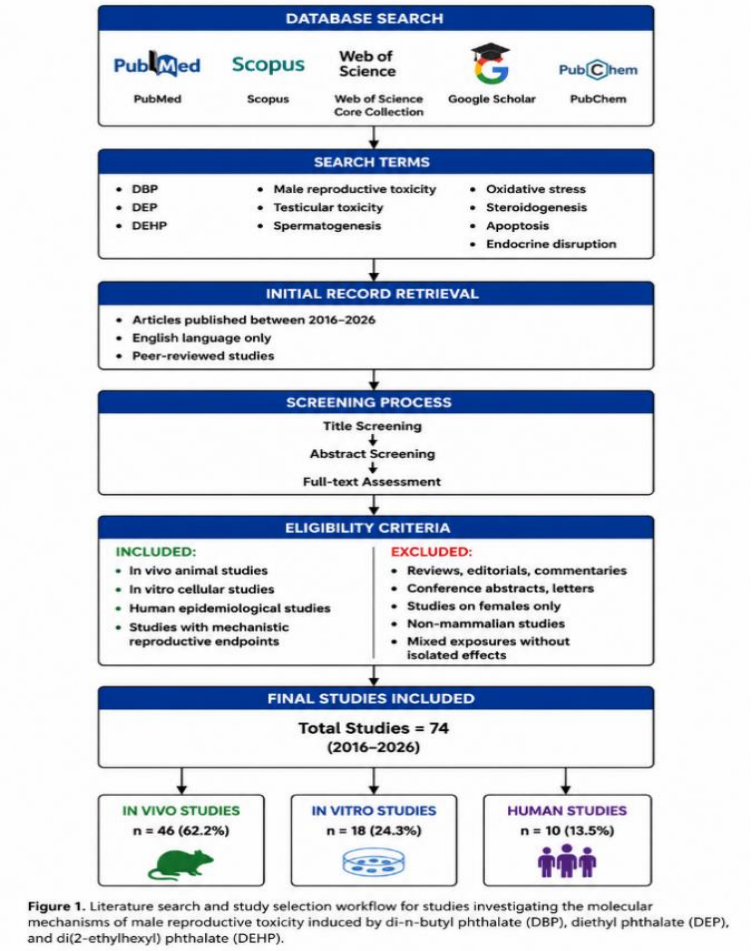

Figure 1. Literature search and study selection workflow for studies investigating the molecular mechanisms of male reproductive toxicity induced by di-n-butyl phthalate (DBP), diethyl phthalate (DEP), and di(2-ethylhexyl) phthalate (DEHP).

Fig 1: Literature search flow identifying peer-reviewed studies investigating the molecular mechanisms of male reproductive toxicity induced by di-n-butyl phthalate (DBP), diethyl phthalate (DEP), and di(2-ethylhexyl) phthalate (DEHP) using electronic databases PubMed, Google Scholar, PubChem, Scopus, and Web of Science.

The search was restricted to articles published between **2016 and 2026**. The year 2016 was selected as the starting point to capture mechanistic studies employing modern molecular, biochemical, and omics-based approaches, which became more prevalent in reproductive toxicology research during this period. Only articles published in English were included. Reference lists of eligible articles were manually screened to identify additional relevant studies not retrieved during the initial database search.

2.3. Study Selection Process

All records identified through database searches were exported into a reference management software (mybib), where duplicate entries were automatically detected and

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removed, followed by manual verification to ensure accuracy. The study selection process was conducted in three sequential screening stages:

Stage 1: Title Screening

Titles were screened to exclude clearly irrelevant studies, including:

- Environmental monitoring studies without biological endpoints
- Studies focused exclusively on female reproductive toxicity
- Non-phthalate toxicants
- Review articles (though these were screened for reference mining)

Stage 2: Abstract Screening

Abstracts of potentially relevant studies were evaluated against predefined inclusion criteria:

- Experimental or epidemiological studies assessing DBP, DEP, or DEHP exposure
- Studies reporting male reproductive endpoints
- Investigations describing molecular, cellular, or biochemical mechanisms (e.g., oxidative stress, steroidogenic disruption, apoptosis pathways)

Studies lacking mechanistic insight or focusing solely on exposure assessment without reproductive outcomes were excluded at this stage.

Stage 3: Full-Text Assessment

Full-text articles were retrieved and assessed for eligibility. Studies were included if they:

- Examined direct effects of DBP, DEP, or DEHP on male reproductive tissues or cells
- Reported mechanistic endpoints such as oxidative stress biomarkers, mitochondrial dysfunction, steroidogenic enzyme expression (e.g., StAR, CYP11A1, 3β-HSD), inflammatory signaling pathways, or apoptotic markers
- Used in vivo, in vitro, or human epidemiological designs relevant to mechanistic interpretation

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Studies were excluded if:

- Full text was unavailable
- Data were insufficient for mechanistic evaluation
- Exposure involved complex mixtures without distinguishable effects of DBP, DEP, or DEHP

3.0 RESULTS

3.1. Overview of Selected Studies

A total of 74 studies met the inclusion criteria and were included in the qualitative synthesis. Of these, 46 (62.2%) were in vivo animal studies, 18 (24.3%) were in vitro cellular investigations, and 10 (13.5%) were human epidemiological studies incorporating mechanistic biomarkers. The predominance of experimental animal models reflects the mechanistic focus of this review, particularly in delineating oxidative stress pathways, steroidogenic disruption, apoptotic signaling, mitochondrial dysfunction, inflammatory responses, and epigenetic alterations induced by DBP, DEP, and DEHP. Rodents were the principal in vivo models, with Wistar and Sprague–Dawley rats representing the majority, followed by C57BL/6 and CD-1 mice. A limited number of studies employed alternative models such as zebrafish for developmental and transgenerational assessments. Exposure windows varied across gestational, lactational, prepubertal, adult, and multigenerational designs, allowing evaluation of both developmental programming and adult-onset reproductive toxicity.

In vitro studies primarily utilized Leydig and Sertoli cell lines, including MA-10, TM3, and TM4 cells, as well as primary testicular cell cultures. These models were employed to assess steroidogenic enzyme expression, reactive oxygen species generation, mitochondrial membrane potential disruption, apoptotic markers, and activation of signaling pathways such as Nrf2, NF-κB, and MAPK. Epidemiological studies were predominantly cross-sectional or cohort-based and relied on urinary metabolite quantification to estimate exposure. Outcomes included serum reproductive hormones, semen quality parameters, DNA fragmentation indices, and oxidative stress biomarkers. Oral administration was the most common exposure route in animal studies, reflecting typical human dietary exposure, although intraperitoneal injection, drinking water incorporation, and dietary admixture

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were also reported. Administered doses ranged from environmentally relevant levels below 5 mg/kg/day to high-dose mechanistic exposures up to 1000 mg/kg/day, depending on study objectives and exposure duration. In vitro concentrations generally ranged from submicromolar to several hundred micromolar levels. Human studies reported exposure as urinary metabolite concentrations and commonly evaluated dose–response relationships across exposure strata while adjusting for relevant confounders.

3.2 In Vivo Evidence (Rodent Models)

In vivo rodent studies consistently demonstrate that exposure to phthalates such as DBP, DEP, and DEHP induces significant male reproductive toxicity characterized by testicular morphological changes including seminiferous tubule degeneration, Leydig cell hypoplasia, and disrupted spermatogenesis. These structural alterations are accompanied by impaired sperm quality, evidenced by reduced sperm count, motility, viability, and increased DNA fragmentation[10-12]. Hormonal disruptions are also prominent, with decreased serum testosterone levels and imbalances in luteinizing hormone (LH) and follicle-stimulating hormone (FSH), reflecting Leydig cell dysfunction and hypothalamic-pituitary-gonadal axis perturbations[10,13]. Comparative potency among these phthalates generally shows DEHP and DBP exerting stronger reproductive toxicity than DEP, with DEHP often causing more pronounced steroidogenic enzyme inhibition and oxidative stress[10,14]. The mechanisms underlying these effects involve oxidative stress induction, mitochondrial dysfunction, apoptosis activation, inflammatory responses, and altered gene expression in steroidogenesis pathways[12,14]. Overall, rodent models provide robust evidence that phthalate exposure disrupts testicular structure and function leading to compromised male fertility.

3.3 In Vitro Mechanistic Studies

In vitro mechanistic studies reveal that phthalates and endocrine disruptors inhibit Leydig cell steroidogenesis by downregulating key steroidogenic enzymes and signaling pathways, such as the LH/CAMP/PKA axis, leading to reduced testosterone synthesis[15-17]. Sertoli cell function is compromised through disruption of tight junctions that maintain the blood-testis barrier (BTB), with decreased expression of anchoring junction proteins and connexins like Cx43, which are critical for maintaining BTB integrity and intercellular communication[18].

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Germ cell apoptosis is frequently triggered via mitochondrial dysfunction, oxidative stress, and activation of apoptotic markers including caspase-3 and p53-related pathways, often linked to altered Sertoli and Leydig cell support[16,19]. Differential gene expression profiling highlights distinct transcriptional changes in Leydig and Sertoli cells under toxicant exposure or genetic conditions such as Klinefelter syndrome, affecting steroidogenesis, inflammatory responses, BTB maintenance, and spermatogenesis[20,21]. Additionally, microRNAs transferred from Sertoli to Leydig cells can modulate steroidogenic factor-1 (SF-1) expression, further inhibiting testosterone production[17]. These findings collectively elucidate complex cellular interactions and molecular disruptions underlying male reproductive toxicity in vitro.

Table 3: Overview of multiple studies on the toxicological effects of phthalates (DBP, DEP, DEHP, and mixtures) on male reproductive health, detailing doses, exposure routes, models, and key outcomes

S/N	Phthalate(s)	Dose & Duration	Exposure Route	Model	Main Toxicological Effects
1	DEHP, DBP, DEP	Human epidemiology; varied environmental exposures	Environmental/occupational	Humans	Robust evidence for DEHP and DBP reducing anogenital distance, semen quality, testosterone; moderate/slight evidence for others[1]
2	PAEs-contaminated microplastics (including phthalates)	30 days	Oral (mice)	Mice	Enhanced reproductive toxicity vs. PAEs alone: sperm physiology alterations, spermatogenesis disruption, oxidative stress[4]
3	DEP	Various animal studies; peripubertal/adult exposures; doses vary widely	Oral and others	Rodents	Moderate evidence for sperm effects without androgen-dependent developmental toxicity; some

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					hepatic effects noted[22]
4	DEHP and other phthalates	Varied animal studies	Mainly oral	Rats and mice	DEHP most potent; adverse effects on male reproductive tract development and semen quality; mice less sensitive than rats[8]
5	Mixture: DEHP, DBP (MPEs)	16 mg/kg/day & 450 mg/kg/day for 90 days	Oral gavage	Rats	Decreased testis/epididymis weights; lowered serum hormones; abnormal sperm; testicular histopathology; transcriptomic changes in focal adhesion and signaling pathways[5]
6	DBP	Various animal/human studies	Multiple	Rodents/humans	Declined fertility and testis weight with transitional phthalates like DBP and BBP; minor effects from DINP/DIBP[2]
7	Mixture of six phthalates (DMP, DEP, DBP, BBP, DEHP, DNOP)	Low doses for 15 weeks	Oral gavage	Rats	Decreased serum/testicular testosterone; testicular damage; downregulation of steroidogenic proteins; apoptosis promotion[3]

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8	Various phthalate esters	In vivo/in vitro models	Multiple	Rodents/humans	Sperm toxicity linked to oxidative stress imbalance causing structural/functional sperm damage[23]
9	DEHP, DBP metabolites (MEHP/MBP)	Various antioxidant intervention studies	Animal models	Rodents	Antioxidants mitigate oxidative stress-induced reproductive toxicity by restoring hormonal balance and reducing apoptosis[7]
10	Mixture of DEHP, DBP & BBP (MPEs) 16 mg/kg/day for 91 days + quercetin treatment	Oral gavage	Rats	MPEs caused decreased sex hormones and abnormal sperm/testis pathology reversed by quercetin via improved testosterone biosynthesis[9]	

This compilation shows that oral exposure in rodent models is the predominant experimental approach. Doses range from environmentally relevant low levels (~16 mg/kg/day) to high mechanistic doses (>400 mg/kg/day). DEHP consistently emerges as the most potent phthalate affecting male reproductive parameters including hormone disruption (notably testosterone), sperm quality decline, testicular histopathology changes such as atrophy or Leydig cell hyperplasia, and altered gene expression related to steroidogenesis and cell adhesion pathways. Mixtures often exacerbate toxicity compared to single compounds. Oxidative stress is a common mechanistic pathway underlying these toxicities. Antioxidants like quercetin show promise in mitigating these adverse effects[1,4,22,23].

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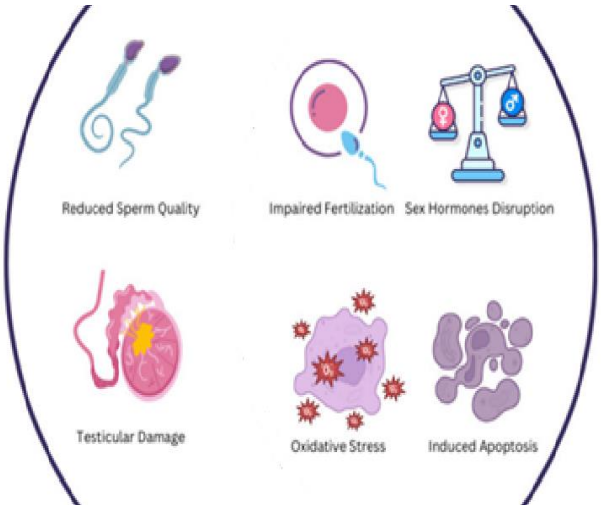

Fig. 2. Main reproductive toxicity outcomes of phthalates in males as described in the various studies. Image adapted from Moghazy *et al*. (2025).

3.4. Epidemiological Evidence in Males

Epidemiological evidence links phthalate exposure, particularly to DEHP and DBP, with adverse male reproductive outcomes including reduced semen quality, altered hormone levels, and shorter anogenital distance (AGD)[1,24]. Urinary metabolites such as mono-n-butyl phthalate (MnBP), monoethyl phthalate (MEP), and mono-(2-ethylhexyl) phthalate (MEHP) serve as biomarkers of exposure and have been associated with decreased serum testosterone and impaired sperm parameters in multiple human studies[1,25]. Dose–response relationships have been observed, for example, higher urinary MEHP correlates with lower total and free testosterone and increased sperm DNA damage and apoptosis[25]. Prenatal exposure studies show that maternal phthalate metabolites correlate negatively with adult testicular volume and reproductive hormone levels in male offspring, suggesting long-term effects from early-life exposure[26]. While the overall epidemiological evidence is strongest for DEHP and DBP, moderate associations exist for DINP and BBP, with weaker or limited data for DIBP and DEP[1,27]. Confounding factors such as diet, lifestyle, and co-exposures complicate interpretation, highlighting the need for further well-designed longitudinal studies to clarify causal pathways[28].

3.5. Comparative Molecular Mechanisms

3.5.1 Oxidative Stress and Mitochondrial Dysfunction

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DEHP consistently induces oxidative stress and mitochondrial dysfunction across multiple organs, primarily by increasing reactive oxygen species (ROS) and lipid peroxidation (measured as malondialdehyde, MDA), while depleting key antioxidants such as superoxide dismutase (SOD) and glutathione (GSH). Studies show DEHP exposure leads to impaired mitochondrial function characterized by reduced ATP production, disrupted mitochondrial membrane potential, swelling, and structural damage in liver, kidney, heart, brain, and pancreatic cells[29-33]. Mechanistically, DEHP activates oxidative stress signaling pathways including NF-κB, MAPK, and Nrf2-mediated antioxidant defenses; however, prolonged or high-dose exposure overwhelms these defenses causing inflammation and cell damage[34-37]. DEHP also downregulates mitochondrial biogenesis regulators such as PGC-1α, NRF1, and TFAM, further impairing mitochondrial homeostasis[36-38]. Compared to DEHP, data on DBP and DEP's oxidative stress effects are less detailed in these studies; however, DBP shares antiandrogenic effects but with less emphasis on mitochondrial dysfunction in the available research. DEP shows comparatively weaker oxidative stress induction and mitochondrial impairment than DEHP[30]. Overall, DEHP exhibits the strongest capacity among these phthalates to induce oxidative stress and mitochondrial dysfunction through multiple molecular pathways involving antioxidant depletion, lipid peroxidation elevation, and disruption of mitochondrial biogenesis and dynamics.

Table 4. Overview of the main toxicological effects of phthalates on reproductive health with different doses, exposure routes, and in vivo models among studies included.

Cell Line(s)/Sample(s)	Human Tissue Type	Phthalate(s) Used	Concentration and Time of Exposure	Main Effects	Reference
Rat testicular tissue	Testes	DEHP	850–950 mg/kg/day for 30 days	Decreased sperm count, motility, daily sperm production; increased oxidative stress markers; altered enzyme activities; decreased testosterone; increased	39

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				FSH and LH; inflammation and hematological changes indicating impaired spermatogenesis	
Murine spermatozoa	Sperm cells	DEHP	Acute exposure, not specified	Impaired sperm capacitation, acrosome reaction; increased reactive oxygen species (ROS); altered ion channel activity leading to reduced fertilization ability	40
Postnatal male mouse testicular cells	Testicular cells	DEHP	40 and 80 µg/kg/day	Inhibited early meiotic progression via Trp53-mediated p38-MAPK pathway; disrupted Sertoli-germ cell communication; downregulated genes critical for spindle organization	41
Male rat testicular cells	Testes	Mixture including DEHP and DBP	Low-dose mixture for 15 weeks	Decreased serum and testicular testosterone; testis structural damage; downregulation of	3

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				steroidogenic proteins; cell cycle arrest and apoptosis promotion	
Mouse testis and epididymis	Testis, epididymis	DEHP	35 days oral administration	Reduced sperm quality and histological abnormalities in testes and epididymides; transcriptomic changes related to oxidative stress, immune response, and cell death pathways	42
Rat Leydig cells	Testicular cells	DBP	Not specified	Inhibited androgen production and proliferation via antagonism of androgen receptor signaling	1
Rat testicular tissue	Testes	DBP	Included in mixture exposures	Reduced sex hormones, abnormal testicular structure, increased abnormal sperm rate	43, 44
Animal models (various)	Sperm cells	DEP	Peripubertal and adult exposures (varied doses)	Moderate evidence of sperm effects without strong antiandrogenic action; developmental toxicity at higher doses	22
Leydig cells	Testicular cells	Bis(2-ethylhexyl)tetrabromopht	Not specified	Inhibited androgen	45

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		halate (TBPH)		production and proliferation; antagonistic activity on androgen receptor signaling pathway	
Mouse spermatozoa	Sperm cells	Mixture of DEHP, DINP, DIBP, DBP, DEP, BBP	1–500 µg/mL for 90 minutes	Decreased sperm motility and hyperactivity; abnormal capacitation and acrosome reaction; reduced fertilization rates	46
Rat testicular tissue	Testes	Mixture of DEHP, DBP, BBP	16 mg/kg/day for 91 days	Decreased sex hormones; abnormal testicular structure; increased abnormal sperm rate; altered steroidogenic proteins	5, 9
Mouse spermatozoa	Sperm cells	Various phthalate esters	Not specified	Oxidative imbalance leading to compromised sperm parameters	23
Male rat reproductive organs	Testes, epididymis	Mixture of phthalates (MPEs)	160 mg/kg/day for 70 days	Decreased organ weights and sex hormones; increased sperm malformation; testicular tissue injury	8
Male mice (in vivo exposure)	Testis	Phthalate-contaminated microplastics	30 days exposure	Enhanced reproductive toxicity with	4

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				altered sperm physiology and oxidative stress	

This table summarizes key in vitro and some ex vivo findings on phthalate-induced male reproductive toxicity. Phthalates commonly impair androgen production, sperm motility, capacitation, and induce oxidative stress leading to structural and functional damage in male reproductive cells. Concentrations and exposure durations vary widely but effects are observed even at low micromolar levels in vitro. Mechanisms include antagonism of androgen receptor signaling, disruption of steroidogenesis, oxidative imbalance, and altered gene expression related to cell adhesion and hormone pathways[4,5,44,45]

3.5.2 Disruption of Steroidogenesis

DEHP and DBP disrupt steroidogenesis primarily by downregulating key enzymes such as StAR, CYP11A1, CYP17A1, and 3β-HSD, leading to impaired testosterone synthesis and Leydig cell dysfunction. In fetal male rats, combined exposure to DEHP and DBP decreases testosterone production and expression of steroidogenic genes including cyp11a, resulting in reproductive malformations and reduced androgen-dependent organ weights in a dose-additive manner. Mechanistic studies in human adrenocortical cells show that DBP reduces CYP11A1 and HSD3β2 protein levels, while its metabolite MBP decreases CYP17A1, collectively impairing early steps of steroid hormone biosynthesis; DBP also induces oxidative stress contributing to this disruption[47]. DEHP exposure increases aldosterone secretion by upregulating enzymes like CYP11B2 in human adrenal cells, indicating complex effects on adrenal steroidogenesis beyond androgen pathways[48]. Hydrolysis of phthalate diesters influences their endocrine effects: short-chain diesters like DBP and DEP are more readily hydrolyzed than DEHP, with DBP showing biphasic androgenic and anti-androgenic effects depending on metabolite presence[49]. Overall, anti-androgenic mechanisms involve inhibition of steroidogenic enzyme expression and function, oxidative stress induction, and Leydig cell impairment, with DEHP generally exerting stronger disruption than DBP or DEP but all contributing to altered steroid hormone homeostasis[50-52].

3.5.3 Apoptosis and Germ Cell Loss

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DEHP and DBP induce germ cell apoptosis through activation of caspase-dependent pathways, including increased cleaved caspase-3 and caspase-9, often linked to mitochondrial damage and reactive oxygen species (ROS) generation. Both phthalates trigger endoplasmic reticulum (ER) stress markers such as GRP78, CHOP, and p-IRE1α, which contribute to apoptosis in spermatogenic cells; however, ER stress can also activate protective autophagy that partially mitigates cell death[53-55]. DEHP exposure elevates the pro-apoptotic Bax/Bcl-2 ratio and reduces anti-apoptotic Bcl-2 expression, promoting spermatogenic arrest and testicular atrophy[54,56]. DBP induces apoptosis via epigenetic modulation of the PTEN/AKT pathway, increasing PTEN expression and inhibiting AKT signaling, which reduces germ cell proliferation and survival[57]. Additionally, DEHP disrupts early meiotic progression by altering p53-mediated pathways and spindle organization genes, further contributing to germline dysfunction[41,58]. Overexpression of nuclear glutathione peroxidase 4 (nGPx4) can protect against DEHP-induced DNA damage and caspase-independent cell death by enhancing chromatin condensation and downregulating apoptotic factors like Bax[59]. Overall, apoptosis and germ cell loss from DEHP and DBP involve complex interactions between oxidative stress, ER stress, mitochondrial pathways, epigenetic regulation, and disruption of key apoptotic regulators.

3.5.4 Endocrine and Receptor-Mediated Effects

DEHP, DBP, and DEP exhibit endocrine-disrupting effects through multiple receptor-mediated pathways. DEHP does not activate estrogen receptor alpha (ERα) but can enhance ERα-mediated transcription under hypoxic conditions, while DBP and DEP show variable estrogenic activity with some phthalates like DBP having limited ER binding affinity[60-62]. DEHP and DINP synergistically activate the glucocorticoid receptor (GR), enhancing dexamethasone-induced GR transactivity, suggesting phthalates can modulate glucocorticoid signaling[63]. Phthalates also interact with peroxisome proliferator-activated receptors (PPARs), particularly PPARγ and PPARδ, affecting lipid metabolism and endocrine function; DEHP shows strong binding affinity to several nuclear receptors including PPARs and androgen receptor (AR), potentially disrupting androgen signaling[64]. Disruption of the hypothalamic-pituitary-thyroid (HPT) axis and hypothalamic-pituitary-gonadal (HPG) axis has been linked to altered steroid hormone levels and receptor dysregulation by DEHP, contributing to reproductive and metabolic dysfunction[65,66].

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Overall, these phthalates interfere with androgen receptor signaling, activate PPAR pathways especially DEHP, and dysregulate endocrine axes such as HPT and HPG, leading to complex endocrine disruption[61,63,64]

3.5.5 Epigenetic and Transcriptomic Alterations

Phthalates such as DEHP and DBP induce significant epigenetic and transcriptomic alterations, including widespread changes in DNA methylation patterns across multiple tissues, with effects often being sex- and tissue-specific. Developmental exposure to DEHP alters DNA methylation at imprinting control regions and gene promoters, impacting genes involved in development and disease susceptibility, with some changes persisting into adulthood[67-69]. These phthalates also dysregulate microRNA expression, which can influence gene networks related to DNA damage response, cell cycle regulation, and chromatin remodeling, potentially contributing to reproductive impairments[70]. Transgenerational inheritance of epigenetic modifications has been demonstrated in animal models exposed ancestrally to mixtures of plastic-derived endocrine disruptors including DEHP and DBP, leading to increased incidence of diseases such as obesity and reproductive disorders in subsequent generations[70]. Transcriptomic analyses reveal that phthalate exposure affects gene clusters associated with reproductive function and chromatin modifiers, suggesting a mechanistic link between environmental exposure and epigenetic reprogramming[70,71]. Overall, these findings highlight that phthalates cause complex epigenetic reprogramming involving DNA methylation changes, miRNA dysregulation, and transgenerational effects that may underlie long-term health risks.

4.0 DISCUSSION

This review integrates evidence from animal experiments, mechanistic in vitro studies, and human epidemiological investigations to provide a comparative understanding of male reproductive toxicity induced by DBP, DEP, and DEHP. Across study designs, a coherent pattern emerges: phthalate exposure compromises spermatogenesis, suppresses testosterone synthesis, disrupts testicular architecture, and impairs sperm function through interconnected molecular pathways.

Rodent studies consistently demonstrate reductions in sperm count, motility, and viability accompanied by seminiferous tubule degeneration, Leydig cell dysfunction, and altered

reproductive hormone levels following exposure to DEHP and DBP[5,9,39]. Long-term and mixture exposures amplify these effects, producing more pronounced suppression of testosterone and greater structural damage compared to single-compound exposure[3,5]. DEP generally exhibits weaker reproductive toxicity, although moderate sperm-related impairments have been reported under certain exposure conditions[22]. Human studies parallel these findings, linking urinary metabolites such as MEHP and MnBP to reduced testosterone levels, impaired semen quality, shortened anogenital distance, and increased sperm DNA damage[1,24,25]. Although observational designs limit causal inference, the concordance between experimental and epidemiological data strengthens biological plausibility.

Mechanistically, oxidative stress and mitochondrial dysfunction appear central to phthalate-induced reproductive injury. DEHP robustly increases reactive oxygen species and lipid peroxidation while depleting antioxidant defenses, leading to disruption of mitochondrial membrane potential, impaired ATP production, and dysregulation of mitochondrial biogenesis pathways[29,31,35]. Activation of stress-responsive signaling pathways such as Nrf2 and MAPK may initially represent compensatory responses but becomes insufficient with sustained exposure[36,37]. Elevated oxidative stress directly compromises sperm capacitation and fertilization capacity[40,46], while antioxidant interventions partially restore hormonal balance and reduce cellular injury, supporting a causal link between ROS generation and reproductive dysfunction[7-9].

Disruption of steroidogenesis is another consistent finding. DBP and DEHP downregulate key enzymes including StAR, CYP11A1, CYP17A1, and 3β-HSD, thereby suppressing testosterone biosynthesis and impairing Leydig cell function[47,50]. Phthalates also interfere with nuclear receptor signaling, including androgen receptor and PPAR pathways, and can modulate glucocorticoid receptor activity[63,64]. These endocrine-disrupting properties are reflected in altered LH and FSH concentrations observed in vivo[5,39], indicating perturbation of hypothalamic–pituitary–gonadal axis regulation.

Apoptotic signaling further contributes to germ cell loss and spermatogenic arrest. DEHP and DBP increase caspase activation, elevate Bax/Bcl-2 ratios, and induce mitochondrial and endoplasmic reticulum stress–mediated apoptosis in germ cells[53,54]. Epigenetic modulation of the PTEN/AKT pathway by DBP suppresses cell survival and

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proliferation[57], while disruption of early meiotic progression through p53-mediated pathways exacerbates germline instability[41]. Emerging transcriptomic and methylation analyses indicate that developmental exposure to phthalates can induce persistent epigenetic reprogramming, with sex- and tissue-specific DNA methylation alterations that may underlie long-term or transgenerational effects[67,69,70,72].

Collectively, the data indicate that DEHP exerts the strongest reproductive toxicity, followed by DBP, with DEP demonstrating comparatively lower potency[1,22,73,74]. Importantly, mixture exposures frequently produce additive or synergistic effects, reflecting real-world exposure scenarios and underscoring limitations of single-compound risk assessment[3-5]. The convergence of oxidative stress, steroidogenic inhibition, receptor-mediated endocrine disruption, apoptosis, and epigenetic modification provides a unified mechanistic framework explaining how phthalates impair male reproductive health across biological systems.

5.0 CONCLUSION

The accumulated evidence demonstrates that DBP and DEHP are potent disruptors of male reproductive function, while DEP exhibits comparatively weaker but measurable effects. Central mechanisms involve oxidative stress–driven mitochondrial dysfunction, suppression of steroidogenic enzymes, endocrine receptor interference, activation of apoptotic pathways, and epigenetic reprogramming. These processes converge to impair testosterone production, disrupt spermatogenesis, and reduce sperm quality. The consistency across experimental and human studies, coupled with evidence of mixture toxicity and potential transgenerational effects, highlights the need for refined regulatory strategies and continued mechanistic investigation to safeguard male reproductive health.

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