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REPRODUCTIVE TOXICOLOGY

Updated and Revised English Edition (2026)

*A Course Manual for Master 1 Students
Fundamental and Applied Toxicology*

(Aligned with the Official USTO-MB Curriculum)

Author

Dr. Sofiane Mohamed El Amine BENGHALI
Department of Life and Environment - USTO-MB
Laboratory Network for Environmental
Monitoring (LRSE)
ORCID: 0009-0001-7488-0608

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Preface

This textbook compiles the pedagogical and scientific knowledge that a student enrolled in the Master's programme in Fundamental and Applied Toxicology should master regarding Reproductive Toxicology. It introduces the fundamental concepts of the discipline in a chronological progression and provides the reader with the analytical tools required to evaluate reproductive and developmental risk.

The present edition (2026) is an updated, expanded and translated version of the original French course notes. It integrates the most recent scientific literature (2020–2026), updated regulatory frameworks (EFSA, US EPA, WHO, ICRP) and contemporary epidemiological data, while retaining the original table of contents.

The volume is divided into five parts. The first part presents the toxicological targets of reproduction and development, with emphasis on species differences, xenobiotic disposition and the principal families of endocrine-disrupting chemicals. The second part addresses endocrine disruptors and male and female reproduction, including the regulatory architecture of the hypothalamic-pituitary-gonadal axes. The third part covers the principles of reproductive epidemiology, with an updated review of cohort and biomonitoring studies for each chemical family. The fourth part is devoted to the toxicology of pregnancy, including embryonic and fetal death, congenital anomalies and intrauterine growth restriction. The fifth and final part summarises the most relevant aspects of germ-cell radiosensitivity.

This manual is consistent with the syllabus proposed in the official curriculum of the Master's programme in Fundamental and Applied Toxicology.

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Introduction

Reproductive and developmental toxicology studies are designed to characterise any toxic risk affecting reproductive function, in order to better delineate these effects from both an explanatory and a mechanistic perspective. The various risks involved — teratogenesis, infertility, subfertility, transgenerational programming — underscore the central importance of these studies in the safety evaluation of new pharmaceutical, agrochemical and industrial substances. A consolidated set of regulatory recommendations describes which studies must be conducted and which parameters must be assessed (ICH S5(R3) 2020, OECD TG 416/421/422/443, REACH Annexes IX and X).

The objective of this textbook is to present the principal aspects of the discipline, the current state of knowledge and likely future developments. The volume is intended to provide students and readers with an in-depth perspective.

Reproductive toxicology examines the adverse effects of substances that interfere with normal sexual function and fertility in adult men and women. These effects include adverse reactions affecting sexual function and fertility in adults, as well as developmental toxicity in offspring. Reprotoxicity encompasses every form of reproductive toxicity, particularly when it results in sterility, or in the case of teratogenic substances or radiation. For public-health and environmental-protection reasons, the placing on the market and use of certain reprotoxic products may be regulated — restricted or banned outright. Reprotoxicants affect fecundity and/or fertility either through direct toxicity to the gonads or the reproductive tract, or by inducing behavioural changes that preclude reproduction. In the case of endocrine-disrupting chemicals, they may act at very low doses. They may also act *in utero*, blocking the normal development of the sexual organs or the glands controlling them, well before birth — in which case they are described as embryotoxic or fetotoxic.

There are well-defined windows of vulnerability to reprotoxicants. These include the period of fetal development of the genital tract and the phase of puberty. Across regulatory jurisdictions, substances are increasingly grouped into the **Carcinogenic, Mutagenic and Reprotoxic (CMR)** category for harmonised classification.

This volume is divided into five parts:

- **Part I** describes the various toxicological targets of reproduction and development.
- **Part II** covers the general principles of endocrine disruption and male/female reproduction.
- **Part III** is dedicated to the foundations of reproductive epidemiology.
- **Part IV** is devoted to the toxicology of pregnancy.
- **Part V**, finally, summarises the most relevant aspects of germ-cell radiosensitivity.

I. Toxicological Targets of Reproduction and Development

I.A. Reproductive Function and Species Differences

I.A.1. Epidemiological Context: Trends in Human Reproductive Health

A convergent body of epidemiological evidence accumulated since the 1990s indicates a deterioration of male reproductive health in industrialised nations, manifest across four phenotypically distinct endpoints that are now considered interrelated manifestations of a common underlying developmental disturbance: testicular cancer, cryptorchidism, hypospadias, and declining semen quality (Skakkebaek et al., 2001; Bonde et al., 2024). Testicular cancer — the most prevalent malignancy in young men aged 15–40 years — has shown unambiguous secular increases of 0.1 to 0.2 cases per 100,000 person-years across multiple European countries since 1970, resulting in a doubling of incidence over five decades. In France, the annual increase averaged 2.5% over 1980–2005, reaching an estimated incidence of 7 cases per 100,000 person-years in 2010 (Ferlay et al., 2013). This trend cannot be attributed to improved surveillance or demographic aging, indicating a genuine biological change in incidence. Similarly, hypospadias (urethral malposition) and cryptorchidism (failure of testicular descent) appear to have increased in several European countries between the late 1970s and early 2000s, although registry-based ascertainment is less robust than for cancer (Virtanen and Toppari, 2015).

Perhaps the most widely discussed reproductive health trend is the temporal decline in human sperm concentration. The landmark meta-analysis by Levine et al. (2017, Human Reproduction Update) pooling data from 185 studies conducted between 1973 and 2011 identified a 52.4% decline in sperm concentration and a 59.3% decline in total sperm count among men from North America, Europe, and Australia, after adjusting for abstinence time, age, and continent. This analysis was subsequently extended and confirmed: a 2023 update (Levine et al., Hum Reprod Update) covering studies to 2018 demonstrated continued decline, with the rate accelerating after 2000 — falling at approximately 2.6% per year in the second study period versus 1.2% per year in the first. Although the clinical significance of these population-level declines for couple fertility remains debated, the trends are consistent with widespread environmental exposure to agents disrupting endocrine function during fetal and postnatal male reproductive development.

In females, the most compelling secular trend is the advancement of pubertal timing: the age at onset of breast development (thelarche) has decreased by approximately 0.3 years per decade in several European populations, and by 0.18 years per decade in France specifically (Aksglaede et al., 2009). Polycystic ovary syndrome (PCOS) is now recognised as the most prevalent female endocrine disorder, affecting 8–13% of women of reproductive age worldwide by Rotterdam criteria (WHO, 2023), with growing evidence linking developmental EDC exposures to PCOS programming.

At the beginning of the 2000s, Skakkebaek and colleagues at Copenhagen formulated the "testicular dysgenesis syndrome" (TDS) hypothesis: that testicular cancer, impaired spermatogenesis, cryptorchidism, and hypospadias share a common aetiology rooted in disrupted fetal testicular development — potentially attributable to endocrine-disrupting environmental exposures during the programming window of male reproductive tract differentiation (Skakkebaek et al., 2001). This hypothesis, though still subject to scientific debate, has profoundly shaped reproductive toxicology testing strategies and epidemiological research over the past two decades.

I.A.2. Gonadal Determination and Sexual Differentiation Across Species

In all vertebrates, the testis and ovary develop from a common bipotential gonadal primordium. In mammals, sex determination is genetic: the presence of the Y-chromosome SRY gene (sex-determining region Y) triggers Sertoli cell differentiation in the indifferent gonad, initiating the male developmental programme. In the absence of SRY, granulosa cell differentiation proceeds by default — though this conceptual simplicity belies a sophisticated balance of competing transcriptional networks: RSPO1/WNT4/ β -catenin signalling maintains ovarian fate, while SOX9, FGF9, and DMRT1 reinforce testicular fate (Biason-Lauber, 2012). In contrast, certain fish (e.g., medaka, zebrafish), reptiles (crocodilians, many turtles), and amphibians exhibit temperature-dependent sex determination or social/hormonal sex reversal — mechanisms that render them disproportionately sensitive to environmental oestrogens and other EDCs that mimic or antagonise the thermal signal (Bonde et al., 2024). This distinction has direct regulatory relevance: environmental risk assessment of EDCs in aquatic ecosystems relies heavily on fish sexual differentiation endpoints precisely because their sex determination is environmentally labile.

In male mammals, the fetal testis initiates a precisely timed hormonal programme essential for masculinisation of the reproductive tract and external genitalia. Sertoli cells secrete anti-Müllerian hormone (AMH), which causes regression of the Müllerian ducts (the precursors to the uterus, fallopian tubes, and upper vagina). Fetal Leydig cells, under stimulation by human chorionic gonadotrophin (hCG) and, later, fetal LH, secrete testosterone, which drives differentiation of the Wolffian ducts into the epididymis, vas deferens, and seminal vesicle. Testosterone, after conversion to dihydrotestosterone (DHT) by 5 α -reductase in target tissues, governs masculinisation of the external genitalia (Jost, 1953). Crucially, insulin-like factor 3 (INSL3) secreted by fetal Leydig cells, in conjunction with testosterone, directs the transabdominal phase of testicular descent via gubernacular development (Ivell and Anand-Ivell, 2009). Chemical agents that reduce fetal testosterone — including the anti-androgenic phthalates and certain PFAS — or that antagonise androgen receptor function at critical developmental windows, can reproduce the full TDS phenotype in experimental models.

Female reproductive tract differentiation, while critically dependent on germ cell survival, is largely hormone-independent at the structural level: the Müllerian ducts persist and differentiate into the internal genitalia without requiring ovarian hormones, and the external genitalia adopt female morphology by default. This asymmetry — male differentiation being actively hormone-dependent, female differentiation being relatively autonomous — explains why endocrine-disrupting insults during the programming window produce more severe and consistent phenotypic consequences in males than in females in most experimental species (Welsh et al., 2008).

Figure 1. Critical windows of male genital tract development and testosterone production (human vs rat)

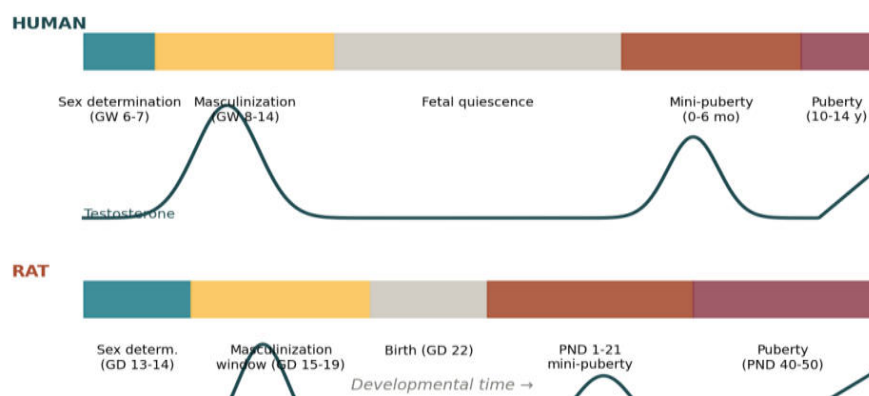


Diagram of the principal developmental periods of the male genital tract in humans and the rat, plotted against testosterone production levels. Showing the gestational programming window (GPW) in the rat (GD 15–17) and its approximate human equivalent (weeks 8–14 of gestation). After Welsh et al., 2008.

I.A.3. Species-Specific Timing of Gonadal Development: Critical Implications for Toxicology

The overarching developmental biology of the gonad is broadly conserved across mammalian species; however, the timing and anatomical context of each developmental stage differ substantially and have profound implications for the interpretation and cross-species translation of reproductive toxicology data.

Testicular Development

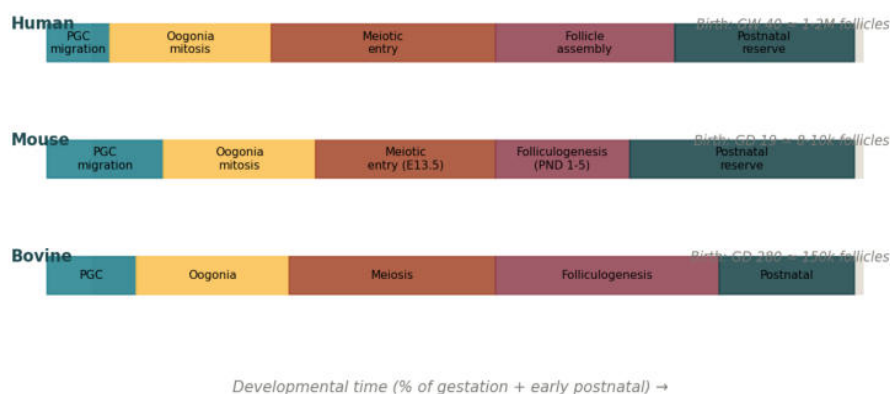
In the rat, the male programming window (MPW) — the critical gestational interval during which anti-androgenic perturbation most effectively shortens anogenital distance (AGD) and induces the phthalate syndrome cluster of reproductive anomalies — falls predominantly between gestational days (GD) 15–17 (Foster, 2006). This interval corresponds to the period of maximal fetal testosterone secretion and peak androgenic sensitivity of the urogenital sinus mesenchyme and external genital tubercle. In the human male fetus, the equivalent androgenic programming window is substantially longer and temporally more diffuse, spanning approximately weeks 8–14 of gestation, coinciding with the period of maximal hCG-driven fetal testosterone production and full androgen receptor expression in target tissues (Welsh et al., 2008). This temporal extension in humans means that the human fetus is arguably exposed to anti-androgenic perturbation over a longer developmental period, potentially amplifying the cumulative consequences of EDC exposure relative to the compressed rodent window.

A further critical species difference lies in the timing of postnatal androgen surges. In male rats, a transient postnatal testosterone surge occurs between postnatal days (PND) 1 and 20 ("mini-puberty" of the rat), which is essential for the full programming of adult Leydig cell number and steroidogenic capacity (O'Shaughnessy et al., 2008). In the human male, an analogous mini-puberty occurs over the first 3–6 months of postnatal life. Disruption of this postnatal androgen surge — through, for example, PFAS contamination of breast milk — may contribute to impaired testicular development without causing any grossly visible malformation.

Ovarian Development

Ovarian differentiation exhibits arguably the most dramatic species difference with respect to toxicological vulnerability (Monniaux et al., 2009). In the human female fetus, the entire complement of primordial follicles — the finite ovarian reserve upon which the entirety of a woman's reproductive lifespan depends — is assembled during fetal life: oocyte nest breakdown and follicle assembly are essentially complete by approximately 36 weeks of gestation. Consequently, prenatal exposure to folliculotoxic agents (dioxins, some PFAS, endosulfan) in human females is most critically relevant during the second trimester, when folliculogenesis is active (Bonde et al., 2024). In rodents, by contrast, primordial follicle assembly occurs predominantly in the early postnatal period (PND 0–5 in rats), with oocyte nest breakdown driven by programmed somatic cell apoptosis (Pepling and Spradling, 2001). This species difference is toxicologically fundamental: a chemical administered postnatally in a rat study may still be capturing a human-relevant prenatal window of follicle assembly, but only if the mechanistic parallels are carefully considered.

Figure 2. Comparative timing of ovarian differentiation across mammals



Comparison of the periods of ovarian differentiation across different mammals. The timeline of follicle assembly spans fetal life in humans and large domestic ruminants, but extends into early postnatal life in rodents. After Monniaux et al., 2009.

The fixed, non-renewable nature of the primordial follicle pool means that chemical depletion of oocytes during the sensitive window of follicle assembly produces irreversible effects on fertility that may not manifest clinically until decades later, when the critically reduced pool is insufficient to sustain regular ovulatory cycles. This is in contrast to the male, where spermatogonial stem cells continuously renew spermatogenic cells from puberty onward; quantitative reductions in spermatogonia are typically reversible, while it is qualitative defects — DNA damage, epigenetic errors, protamine deposition anomalies — that are of greater long-term toxicological concern in the male (Bonde et al., 2024).

Polyovulatory versus Monoovulatory Species

Rodents are polyovulatory animals: each oestrous cycle culminates in the ovulation of 10–20 oocytes, and a single female may carry 10–15 conceptuses per litter. The regulatory mechanisms governing folliculogenesis, FSH sensitivity, and the LH surge differ qualitatively from those in monoovulatory species (humans, non-human primates, cattle, sheep). The large animal models — particularly cattle and sheep, with their long gestation periods, single offspring per pregnancy, and slow reproductive cycling — arguably provide more appropriate physiological parallels to human reproductive biology in some contexts, even though they are rarely used in standard regulatory reproductive toxicology studies.

Puberty and Postnatal Development

Rodents are born at a much more immature developmental stage than most other mammals: the neonatal rat corresponds broadly to the late fetal period in humans with respect to brain development, myelination, and

hormonal imprinting of the reproductive system. Vaginal opening, the external marker of puberty in the female rat, represents a rodent-specific phenotype with no direct anatomical parallel in primates; conversely, endometriosis — a common and clinically important disorder of the human female reproductive tract — is essentially a primate-specific pathology, limiting the utility of rodent models for studying this condition. These physiological and pathological differences between species must be explicitly acknowledged when extrapolating reproductive toxicology findings from rodent models to human health risk assessment (ICH S5(R3), 2020).

I.A.4. The Hypothalamic–Pituitary–Gonadal Axis and Neuroendocrine Control

Reproductive function in all mammals is governed by the hypothalamic–pituitary–gonadal (HPG) axis — a hierarchical neuroendocrine cascade that integrates environmental, metabolic, and circadian inputs and translates them into pulsatile gonadotrophin secretion regulating gonadal steroidogenesis and gametogenesis. In the male, hypothalamic gonadotrophin-releasing hormone (GnRH) is secreted in pulses (approximately every 90–120 minutes in humans) and stimulates anterior pituitary secretion of follicle-stimulating hormone (FSH) and luteinising hormone (LH). FSH acts on Sertoli cells to initiate and maintain spermatogenesis; LH acts on Leydig cells to stimulate testosterone production. Testosterone exerts negative feedback at both the hypothalamus (reducing GnRH pulse frequency) and the pituitary (reducing LH responsiveness to GnRH). Inhibin B, secreted by Sertoli cells proportionally to spermatogenic cell number, exerts selective negative feedback on FSH secretion. Thus, circulating inhibin B is a sensitive biomarker of Sertoli cell function and spermatogenic activity, and its reduction following EDC exposure reflects impaired spermatogenesis even before sperm count changes become apparent.

In the female, the HPG axis operates on a monthly cyclical basis governed by the interplay between rising follicular oestradiol (positive feedback on LH secretion, triggering the preovulatory LH surge), progesterone from the corpus luteum (negative feedback), and inhibin A/B from granulosa cells (selective FSH suppression during follicular maturation). The preovulatory LH surge is mediated through kisspeptin neurons in the arcuate nucleus and anteroventral periventricular nucleus (AVPV) of the hypothalamus. EDCs — particularly oestrogenic compounds — can disrupt the kisspeptin–GnRH–LH surge axis, advancing or attenuating puberty onset and ovulation timing (Navarro et al., 2012).

The sensitivity of the neuroendocrine reproductive axis to EDC disruption extends across the lifespan, with critical windows of vulnerability at: (i) the embryonic period, when sexually dimorphic neuronal architecture is established; (ii) the neonatal period in rodents, when the AVPV differentiates under oestradiol (via aromatisation of testosterone); (iii) puberty, when kisspeptin signalling amplitude and GnRH pulse frequency increase dramatically; and (iv) the perimenopausal transition in women, when oestradiol feedback becomes disrupted. Chemical exposures at any of these windows — even transiently — may permanently alter the set-point of the HPG axis, explaining the "programming" effects of early-life EDC exposures that manifest in adult reproductive function.

I.A.5. Critical Windows and the DOHaD Framework

The Developmental Origins of Health and Disease (DOHaD) framework articulates the principle that early-life environmental exposures — including chemical exposures — can "programme" organ development, hormonal set-points, and epigenetic states in ways that predispose to disease emerging decades later, and that may even be transmitted to subsequent generations (Barker and Thornburg, 2013; Bonde et al., 2024). The extension of this concept to "Parental Origins of Health and Disease" (POHaD) explicitly includes paternal preconceptional exposures — which may alter sperm epigenome — as contributors to offspring phenotype alongside the more extensively studied maternal routes (Bonde et al., 2024).

For endocrine-disrupting chemicals in particular, five principal critical windows can be identified:

- (1) The **periconceptional window** (spanning from oogenesis through fertilisation and pre-implantation), during which epigenetic reprogramming in the developing embryo is most susceptible to chemical perturbation of methylation patterns.
- (2) The **embryonic organogenesis window** (first trimester in humans; GD 6–15 in rats), during which structural teratogenicity can be induced and gonadal determination irreversibly set.
- (3) The **fetal programming window** (weeks 8–20 in humans; GD 15–21 in rats), during which hormonal imprinting of the internal and external reproductive tracts, the hypothalamic reproductive axis, and the mammary gland occurs.

(4) **Perinatal and early postnatal** exposure, during which central nervous system development, neonatal hormonal surges, and, in rodents, folliculogenesis remain sensitive.

(5) **Puberty** — a second period of endocrine lability at which earlier developmental insults may become phenotypically manifest as altered pubertal timing, altered gonadal steroidogenesis, or impaired gametogenesis. Critically, dose–response relationships in developmental toxicology frequently do not conform to classical monotonic patterns. Many EDCs exhibit non-monotonic dose–response (NMDR) curves, displaying greater biological effect at low doses than at high doses due to receptor saturation, counter-regulatory mechanisms, or receptor switching at different concentrations (Vandenberg et al., 2012). This phenomenon confounds the classical NOAEL/LOAEL paradigm in risk assessment and has been a central point of contention in regulatory reassessments of BPA and other EDCs over the period 2020–2026.

Key species differences in reproductive development relevant to toxicology

Parameter	Rat	Human	Regulatory Implication
Testicular programming window	GD 15–17	Weeks 8–14 of gestation	Rat GD 15–17 corresponds to human first trimester
Primordial follicle assembly	PND 0–5 (postnatal)	Fetal, complete by ~36 weeks	Prenatal dosing in rats may miss human-relevant window
Mini-puberty (male)	PND 1–20	Months 0–6 postnatal	Postnatal EDC exposure may disrupt Leydig cell programming
Ovulation number per cycle	10–15 oocytes	1 oocyte	Folliculogenesis regulation fundamentally different
Maturity at birth	Highly immature	Relatively mature	Neonatal rat ≈ human third trimester CNS
Vaginal opening	Puberty marker	No anatomical equivalent	Endpoint unique to rodent puberty studies

I.B. Fate of a Xenobiotic in the Organism

The reproductive toxicity of any environmental chemical is ultimately determined not merely by its intrinsic hazard potential but by the concentration and duration of exposure achieved at the gonadal, placental, and embryonic target tissues. Understanding xenobiotic pharmacokinetics — absorption, distribution, metabolism, and excretion (ADME) — is therefore indispensable to mechanistic reproductive toxicology, to quantitative risk assessment, and to the interpretation of biomonitoring data.

I.B.0. ADME Principles in Reproductive Toxicology

Absorption and Bioavailability

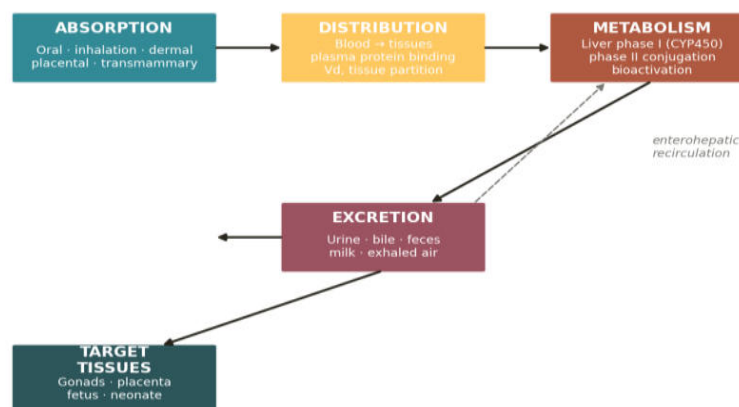
The route and efficiency of xenobiotic absorption are determined primarily by physico-chemical properties: molecular weight, lipophilicity (expressed as log K_{ow}), pK_a, and molecular geometry. Oral ingestion is the principal route for most dietary contaminants and EDCs; dermal absorption is quantitatively significant for parabens, phthalate esters in cosmetics, and short-chain PFAS in consumer products. Inhalation is relevant for volatile PFAS precursors, certain parabens in aerosol products, and PBDE dust particles.

For lipophilic compounds (log K_{ow} > 3), intestinal absorption is facilitated by dietary lipids via chylomicron incorporation and lymphatic transport, bypassing first-pass hepatic metabolism to an extent proportional to the degree of micellar solubilisation. Highly lipophilic compounds (log K_{ow} > 6, such as PBDEs and higher chlorinated PCBs) may paradoxically show reduced absorption despite high intestinal permeability, because extreme lipophilicity prevents micellar formation and impairs solubilisation in the aqueous intestinal milieu. The

difference between intestinal absorption per se and systemic bioavailability is crucial: efficient first-pass hepatic conjugation (as occurs for BPA in adults) may reduce bioavailable free BPA to very low fractions despite substantial gastrointestinal absorption (Völkel et al., 2002).

Carrier-mediated transport systems play increasingly recognised roles: organic anion transporters (OATs), organic anion-transporting polypeptides (OATPs), and the efflux transporter P-glycoprotein (P-gp/ABCB1) can modulate intestinal, hepatic, and placental handling of xenobiotics. P-gp, expressed on the apical face of intestinal enterocytes and in the placenta, can actively limit bioavailability and fetal transfer of certain xenobiotics and their metabolites.

Figure 3. Main pharmacokinetic steps of xenobiotics (ADME)



Principal pharmacokinetic steps governing xenobiotic fate in the organism: absorption across gastrointestinal, pulmonary, or dermal barriers; distribution to tissue compartments; biotransformation (Phases I and II) primarily in the liver; and excretion via urine, bile/faeces, or breast milk. Adapted from French National Agency for Food, Environmental and Occupational Health Safety (ANSES), 2011.

Distribution and Tissue Targeting

Distribution of absorbed xenobiotics to reproductive target tissues depends on plasma protein binding, tissue lipid content, and tissue-specific transporter expression. Highly lipophilic compounds — PCBs (log Kow 5.3–8.2), PBDEs (log Kow 4.5–9.9), organochlorine pesticides — partition extensively into adipose tissue, accumulate in breast lipid, and are released during pregnancy and lactation, creating sustained fetal and neonatal exposures that outlast the original maternal exposure by months to years. The gonadal fat pad in males may similarly act as a depot for highly lipophilic compounds in proximity to the testes.

PFAS, despite containing highly fluorinated carbon chains, are not lipophilic in the classical sense: their amphiphilic nature (hydrophobic fluorocarbon tail, hydrophilic acid head group) results in preferential binding to serum albumin and fatty-acid binding proteins, and accumulation in protein-rich tissues (liver, kidney, testis) rather than adipose depots. This unique distribution pattern explains why PFAS serum concentrations may not decrease appreciably with adipose mobilisation during pregnancy — unlike organochlorine lipophiles.

Placental Transfer

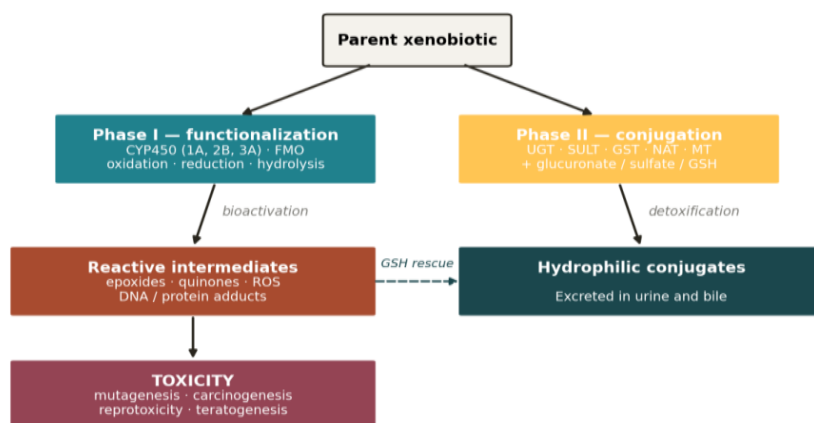
The placenta as a transfer interface is discussed in depth in the context of each specific chemical below. Relevant general principles include:

- Passive diffusion dominates for lipophilic compounds with molecular weight < 500–600 Da.
- Active transporters in the syncytiotrophoblast (notably breast cancer resistance protein/BCRP and P-gp) can limit fetal transfer of certain xenobiotics.
- PFAS exploit fatty-acid binding proteins and OATPs for transplacental transfer; cord blood: maternal serum ratios typically range from 0.2 to 0.6 for PFOS (Kato et al., 2014).
- BPA is efficiently glucuronidated in adult liver and thereby detoxified; in the fetus, hepatic UGT capacity is minimal, so BPA crossing the placenta persists in the free, biologically active form in fetal tissues — a central basis for fetal developmental vulnerability (Völkel et al., 2002; Maranghi et al., 2025).

- Alpha-fetoprotein (AFP) — produced by the fetal liver and yolk sac — binds maternal oestradiol with high affinity, limiting placental transfer of endogenous oestrogens to the fetus. Most synthetic EDCs (BPA, phthalate metabolites, PFAS) have negligible AFP affinity and therefore cross freely into fetal circulation (Soloff, 1975).

Biotransformation: Phase I and Phase II

Figure 4. Bioactivation and detoxification pathways of xenobiotics



Biotransformation pathways of xenobiotics. Phase I reactions (oxidation, reduction, hydrolysis) functionalise the parent compound, producing intermediates that may be more or less biologically active than the original substance. Phase II conjugation reactions (glucuronidation, sulfation, glutathione conjugation, acetylation) typically increase water solubility and promote excretion. Bioactivation — conversion of a weakly active parent compound to a potent endocrine-active metabolite — is exemplified by DEHP → MEHP and by PBDE → OH-PBDE.

Phase I biotransformation, mediated primarily by hepatic cytochrome P450 (CYP) enzymes, functionalises xenobiotics by introducing or unmasking polar groups through oxidation, reduction, or hydrolysis. The major CYP isoforms implicated in environmental contaminant metabolism include CYP1A1, CYP1A2, CYP1B1, CYP2B6, CYP2C9, CYP2C19, CYP3A4, and CYP3A5. CYP1A1 and CYP1A2 are strongly induced by aryl hydrocarbon receptor (AhR) ligands — including halogenated aromatic compounds and polycyclic aromatic hydrocarbons (PAHs) — through xenobiotic response element (XRE)-driven transcription; this induction is particularly relevant for PCBs, PBDEs, and dioxins.

An important concept in reproductive toxicology is **bioactivation**: the metabolic conversion of a relatively inactive parent compound to a metabolite with substantially greater endocrine-disrupting potency. The di-ester phthalates represent the paradigmatic example: DEHP undergoes intestinal esterase-mediated hydrolysis to MEHP (mono-2-ethylhexyl phthalate), which is the primary testicular toxicant — the parent diester has minimal direct endocrine activity. Similarly, PBDEs undergo CYP-mediated hydroxylation to hydroxylated PBDE metabolites (OH-PBDEs) that bind thyroid hormone transport proteins with higher affinity than the parent congeners, amplifying thyroid axis disruption (Hamers et al., 2014). Conversely, bioactivation may generate more reactive species — reactive oxygen species, reactive electrophiles — that contribute to oxidative stress, DNA adduct formation, and epigenetic disruption in gonadal and placental tissues.

CYP2C19 (aromatase, CYP19A1) induction is of specific reproductive toxicological concern: upregulation of aromatase in adipose, gonadal, or placental tissues can amplify local oestrogen production from androgen precursors, contributing to the feminisation of male offspring and oestrogen excess conditions in females. Certain BFR metabolites, organochlorine pesticides (e.g., methoxychlor, DDT), and some phthalate metabolites modulate CYP19A1 expression in gonadal and placental tissues.

Phase II conjugation — glucuronidation (UGTs), sulfation (SULTs), glutathione conjugation (GSTs), and acetylation (NATs) — generally detoxifies Phase I metabolites by increasing water solubility for renal or biliary excretion. BPA is rapidly and efficiently glucuronidated by UGT2B15 and UGT2B7 in adult human liver to BPA-glucuronide (BPA-G), which is pharmacologically inactive and efficiently excreted in urine with a serum half-life of approximately 4–6 hours in adults (Völkel et al., 2002). Species differences in UGT activity are substantial and complicate cross-species pharmacokinetic extrapolation: rats excrete proportionally more xenobiotic metabolites in bile than humans or primates, due to a lower biliary excretion threshold, leading to enterohepatic

cycling and extended systemic half-lives in rodent toxicology studies relative to corresponding human exposures.

Excretion and Enterohepatic Recirculation

Hydrophilic xenobiotic conjugates are excreted via the kidney into urine or via the liver into bile and thence into faeces. Biliary glucuronide and sulfate conjugates may be hydrolysed by intestinal β -glucuronidase (including microbiome-derived β -glucuronidases), releasing the free aglycone, which is then reabsorbed — the enterohepatic cycle. This cycle is particularly relevant for BPA, certain phthalate metabolites, and some steroid hormones, extending their effective biological half-lives beyond what urinary elimination alone would predict (Thayer et al., 2016). Breast milk excretion represents an additional and toxicologically important elimination pathway for lipophilic persistent compounds (PBDEs, PCBs, HBCD) and, to a lesser degree, for phthalate metabolites, BPA, and PFAS — simultaneously an excretory route for the lactating mother and an exposure route for the breastfed infant.

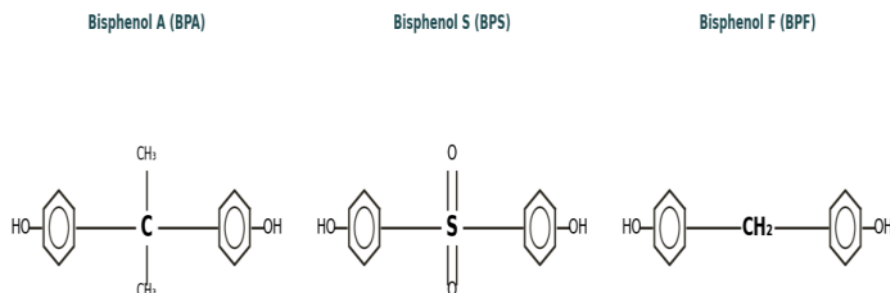
Comparative pharmacokinetic parameters of the five EDC classes discussed in Part I

Compound	Log Kow	Primary route of absorption	Main metabolic pathway	Plasma half-life (human)	Principal excretion route
BPA	3.32	Oral (dietary migration)	UGT2B15 glucuronidation	~6 h (adult)	Urine (BPA-G)
DEHP	7.50	Oral (food contact materials)	Esterase hydrolysis → MEHP; CYP oxidation	18 h (DEHP/MEHP)	Urine (oxidised metabolites)
BDE-47	6.81	Oral/dietary fat	CYP2B6/3A4 hydroxylation	~5–7 years	Faeces/bile (lipophilic)
PFOS	Amphiphilic	Oral/drinking water/dietary	Minimal biotransformation	~5.4 years	Urine/bile
Methylparaben	1.96	Dermal/oral (cosmetics/food)	Esterase hydrolysis → pHBA; UGT conjugation	<6 h	Urine (pHBA conjugates)

I.B.1. Bisphenol A

I.B.1.1. Identity and Physico-Chemical Properties

Bisphenol A (BPA; IUPAC name: 4,4'-(propane-2,2-diyl)diphenol; CAS 80-05-7; molecular formula $C_{15}H_{16}O_2$; MW 228.29 g/mol) is a synthetic diphenylmethane derivative carrying two para-hydroxyphenyl groups linked by an isopropylidene bridge. This structural motif confers moderate lipophilicity (log Kow = 3.32–3.64), adequate water solubility (~300 mg/L at 25°C), and a key characteristic relevant to endocrine disruption: the two phenolic hydroxyl groups occupy spatial positions nearly identical to those of the hydroxyl of 17 β -oestradiol when the molecule adopts its most energetically stable conformation — a structural mimicry that underlies its oestrogenic receptor binding capacity. BPA is an off-white crystalline solid (melting point 158–159°C), slightly volatile (vapour pressure $\sim 4 \times 10^{-7}$ mmHg at 25°C), and photodegradable under UV irradiation.

Figure 5. Chemical structures: bisphenol A and analogues (BPS, BPF)

Chemical structure of bisphenol A (BPA; 4,4'-(propane-2,2-diyl)diphenol; CAS 80-05-7) showing the isopropylidene bridge connecting the two hydroxyphenyl groups. The spatial arrangement of the para-hydroxyl groups mimics the A-ring hydroxyl and C17-OH configuration of 17 β -oestradiol, enabling competitive binding to oestrogen receptors.

I.B.1.2. Sources, Uses, and Production

BPA was first synthesised in 1891 and identified as an oestrogen mimic in the 1930s by Charles Dodds — the same chemist who later synthesised diethylstilboestrol (DES). Its current global production exceeds 4 million metric tonnes per year, making it one of the highest-volume synthetic chemicals in worldwide production. Its primary commercial uses are: (i) monomer in polycarbonate (PC) plastic synthesis, imparting transparency, impact resistance, and heat tolerance to optical media (CDs, DVDs), optical lenses, protective equipment, laboratory equipment, and — historically — baby bottles and reusable food containers; (ii) co-monomer in epoxy resin synthesis, used as linings in food and beverage metal cans, varnishes, and adhesives; and (iii) colour developer in thermal paper (point-of-sale receipts, boarding passes, parking tickets), where it is applied as a free compound rather than polymerised, and therefore highly bioavailable upon dermal contact (Liao and Kannan, 2011).

Secondary uses include BPA as an antioxidant in PVC, a crosslinker in certain elastomers, and an intermediate in flame retardant synthesis. Given the hydrolytic instability of the carbonate ester linkage in PC under acidic or alkaline conditions, or physical abrasion, BPA monomers migrate continuously from polycarbonate and epoxy matrices into foodstuffs, beverages, and the environment. Migration rates are substantially accelerated by elevated temperatures (microwave heating, dishwashing, boiling liquids), UV radiation, and acidic foods (Khodasevich et al., 2024).

I.B.1.3. Mechanisms of Endocrine Action

BPA's principal mechanism of endocrine disruption involves binding to nuclear oestrogen receptors (ER α and ER β) with affinity approximately 10,000-fold lower than 17 β -oestradiol ($K_i \approx 10^{-7}$ – 10^{-6} M for BPA vs. $K_i \approx 10^{-10}$ – 10^{-11} M for E2). In classical genomic oestrogen-response-element (ERE)-driven transcriptional assays, BPA is 1,000–5,000-fold less potent than E2. However, this classical pharmacological framing substantially underestimates biological significance at environmentally relevant nanomolar concentrations, at which BPA acts predominantly through alternative, non-genomic pathways (Kolatorova et al., 2025).

The G-protein-coupled oestrogen receptor (GPER/GPR30), located at the endoplasmic reticulum membrane, is activated by BPA at concentrations as low as 1 nM, triggering transactivation of the epidermal growth factor receptor (EGFR) via metalloproteinase-mediated release of heparin-binding EGF, followed by ERK/MAPK and PI3K/AKT signalling cascades that regulate proliferation, apoptosis, and steroidogenesis (Prossnitz and Barton, 2014). BPA also acts as an agonist at ERR γ (oestrogen-related receptor gamma), an orphan nuclear receptor highly expressed in placenta, brain, cardiac, and reproductive tissues — where its binding affinity approaches that of natural ligands and its activation promotes metabolic reprogramming and altered gonadal steroidogenesis (Kolatorova et al., 2025). Additionally, BPA acts as a weak androgen receptor antagonist, competitive with testosterone and DHT for AR binding, a property contributing to anti-androgenic effects at higher concentrations.

Epigenetic mechanisms have emerged as central to BPA's developmental and potentially transgenerational toxicity. Key findings include:

- Neonatal BPA exposure in male rats (2.4 µg/day) produces hypermethylation of ERα and ERβ gene promoters in testicular tissue, associated with upregulation of de novo methyltransferases DNMT3a and DNMT3b (Ho et al., 2006).
- In utero BPA exposure in mice (50 µg/kg/day) alters CpG methylation patterns in *Esr1*, *Esr2*, and *Hoxa10* promoters in urogenital sinus mesenchyme, the latter being relevant to uterine development and endometrial receptivity (Bromer et al., 2010).
- Human birth cohort data (ELEMENT cohort, INMA cohort) confirm associations between prenatal urinary BPA concentrations and cord-blood DNA methylation changes, with several differentially methylated CpG sites persisting into childhood and adolescence — demonstrating epigenetic memory of fetal BPA exposure (Ladd-Acosta and Fallin, 2016).

The non-monotonic dose–response (NMDR) behaviour of BPA — demonstrated in multiple independent assays where effects are observed at low nanomolar doses, absent at higher concentrations, then reappear at very high doses — has been a central scientific controversy in regulatory assessment. NMDR relationships are mechanistically explained by oestrogen receptor downregulation, counter-regulatory pathways, and receptor switching (e.g., from GPER at low doses to ERα at high doses), but remain incompatible with the classical linear extrapolation-to-zero model for threshold setting (Vandenberg et al., 2012).

I.B.1.4. Regulatory Status (EU and US, 2020–2026)

The regulatory status of BPA underwent transformative changes during 2020–2023:

EFSA 2023 Reassessment: On 19 April 2023, EFSA's Panel on Food Contact Materials, Enzymes and Processing Aids (CEP Panel) published its definitive re-evaluation of BPA risks in food, establishing a new tolerable daily intake (TDI) of **0.2 ng/kg bw/day** (0.2 picograms/kg bw/day) — a reduction of 20,000-fold from the previous temporary TDI of 4 µg/kg bw/day established in 2015 (EFSA CEP Panel, 2023). This is the lowest TDI ever established by EFSA for a food contact material substance. The critical effect driving this drastic reduction was not the previously suspected oestrogenic cancer endpoint, but rather immunotoxicity: specifically, immune effects on T helper lymphocytes (Th17/Treg balance, implicated in autoimmunity and airway inflammation) identified at nanomolar BPA concentrations in mouse studies. The EFSA panel determined that current dietary exposures of virtually all age groups in Europe exceed this TDI by two to three orders of magnitude, constituting a public health concern (European Environment Agency, 2023).

The corresponding health-based monitoring guidance value (HBM-GV) for urinary BPA derived from the new TDI is 11.5 ng/L total urinary BPA in adults — a value exceeded by the geometric mean of European adult populations measured in HBM4EU (Khodasevich et al., 2024).

US FDA and BPA CLARITY Core Study: In contrast to EFSA, the US Food and Drug Administration, evaluating the BPA CLARITY Core Study — a government-industry collaborative rodent carcinogenicity study with life-stage exposures — maintained in 2021–2022 that available evidence supports BPA's safety at current dietary exposure levels, declining to revise regulatory tolerances (FDA, 2022). This trans-Atlantic regulatory divergence has catalysed extensive scientific debate about critical effect selection, uncertainty factor application, and dose–response modelling methodologies. The German Federal Institute for Risk Assessment (BfR) also issued a divergent opinion, deriving a TDI of 200 ng/kg bw/day based on a different critical effect — the maximum EFSA TDI exceeds the BfR value by 1,000-fold — exemplifying the scientific uncertainty that persists at the regulatory frontier.

EU Restriction Progress: In the EU, BPA has been banned in polycarbonate baby bottles since 2011, in food contact plastics for infants since 2018, and in thermal paper since 2020. REACH Annex XIV authorisation requirements and classification as a Substance of Very High Concern (SVHC) for endocrine-disrupting properties under REACH Article 57(f) have been implemented. The EU Commission's Strategy for Sustainable Chemicals (Chemicals Strategy for Sustainability, 2020) explicitly addresses regrettable substitution and proposes group restriction approaches for bisphenols.

Bisphenol Analogues (BPS, BPF, BPAF): Industry substitution of BPA with structural analogues — primarily bisphenol S (BPS) and bisphenol F (BPF) — in response to consumer demand for "BPA-free" products has proceeded at scale, but toxicological evidence increasingly indicates these substitutes are not inert. BPS demonstrates comparable or superior oestrogenic activity to BPA via membrane ERs and GPER. A 2025 analysis deriving reference doses for BPS and BPF based on human semen quality data yields RfDs of 0.37 ng/kg bw/day

(BPS) and 8.09 ng/kg bw/day (BPF) — within the same order of magnitude as the EFSA 2023 TDI for BPA itself (Sueiro et al., 2025). BPAF (bisphenol AF) is substantially more potent than BPA in ER α binding assays. The European Commission is therefore pursuing group restriction proposals for the bisphenol class under REACH to prevent chemical-by-chemical regrettable substitution.

I.B.1.1. Exposure

Exposure Pathways

Human exposure to BPA occurs through multiple routes, with dietary intake from food contact materials representing the dominant pathway for the general population:

1. *Dietary ingestion*: Migration of BPA from polycarbonate containers, epoxy can linings, and packaging materials into food and beverages constitutes the quantitatively dominant route. Migration is accelerated by acidic pH (canned tomatoes, soft drinks), elevated temperature (canned soups, reheated foods in PC containers), and repeated mechanical stress. Early estimates placed dietary exposure in adults at approximately 0.1 $\mu\text{g/kg}$ bw/day (Anses, 2010; FAO/WHO, 2010); more recent pharmacokinetically informed estimates based on urinary biomonitoring data suggest population dietary intakes of 0.04–0.12 $\mu\text{g/kg}$ bw/day in adults.
2. *Thermal paper (dermal + incidental oral)*: BPA in uncoated thermal receipt paper is present at concentrations of 1–25 mg/g paper, in freely transferable form. Dermal absorption after handling receipts is documented, and hand-to-mouth transfer following receipt handling substantially increases systemic exposure, particularly in food service workers and cashiers. Studies demonstrate that consuming food with BPA-contaminated hands (e.g., French fries after handling receipts) produces urinary BPA concentrations substantially exceeding those from dietary intake alone (Liao and Kannan, 2011). This route became subject to EU regulatory control with the 2020 thermal paper ban (EU Regulation 2016/2235, effective January 2020).
3. *Dental composite materials and sealants*: BPA-derived compounds (Bis-GMA, Bis-DMA) used in dental composites and fissure sealants can hydrolyse to free BPA in saliva, producing elevated salivary BPA for 24–48 hours post-application, with systemic absorption documented (Nalçaci et al., 2015).
4. *Medical devices*: Polycarbonate components in haemodialysis equipment, certain IV tubing, and medical storage containers can contribute to occupational and patient BPA exposure, relevant for vulnerable subpopulations including premature neonates in intensive care.

Biomonitoring Data

More than 90% of individuals living in Western nations have detectable BPA in urine — confirming continuous, ubiquitous exposure from multiple environmental sources. Urinary BPA concentration is the standard biomarker of recent exposure, reflecting primarily the preceding 24–48 hours of intake given the short serum half-life of approximately 4–6 hours in adults.

Key biomonitoring reference values include:

- HBM4EU European adults: geometric mean total urinary BPA = 0.77–2.47 $\mu\text{g/L}$ (3.4–10.8 nM), varying by country, with highest concentrations in younger age groups and certain Eastern European countries (Kolatorova et al., 2025).
- German children aged 3–5 years: mean urinary BPA 3.5 $\mu\text{g/L}$ — the highest-exposure subgroup in European surveillance (KiGGS study, Fromme et al., 2011).
- French women on the day of delivery: median total BPA = 2.9 $\mu\text{g/g}$ creatinine; median free BPA = 0.5 $\mu\text{g/g}$ creatinine; free/total ratio = 0.17, reflecting high glucuronidation efficiency in adults (ANSES, 2010).
- Umbilical cord blood: median total BPA $\sim$ 1.3 $\mu\text{g/L}$ in HBM4EU European newborns — critical because neonates lack full UGT-mediated detoxification capacity (Khodasevich et al., 2024).
- NHANES US adults (2017–2018): geometric mean urinary BPA = 1.3 $\mu\text{g/L}$; 95th percentile = 13.2 $\mu\text{g/L}$ (CDC, 2021).

Populations at Risk

The following subpopulations bear disproportionate BPA body burden:

- Premature and term neonates in neonatal intensive care units (NICU), with immature UGT-mediated conjugation and direct PC device exposure.
- Infants fed formula prepared in PC bottles or from cans lined with epoxy resin.
- Cashiers and thermal paper handlers with daily dermal occupational exposure.

- Dental patients in the 24–48 hours following composite resin application.
- Haemodialysis patients with chronic exposure via PC dialysis equipment.
- Foetuses of mothers in the upper exposure quartile, given the inability of fetal hepatic UGT to detoxify transplacentally transferred BPA.

The EFSA HBM-GV of 11.5 ng/L total urinary BPA is exceeded by virtually the entire European population surveyed in HBM4EU, confirming that current population exposures are of public health concern at the 2023 regulatory threshold (European Environment Agency, 2023; EFSA CEP Panel, 2023).

BPA biomonitoring summary — European and US populations

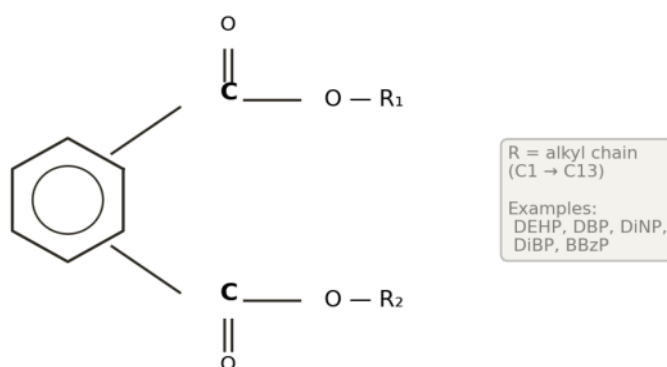
Population	Biomarker	Central tendency (geometric mean)	Reference	Health-based guidance value
EU adults (HBM4EU, 2017–2021)	Urinary BPA total	0.77–2.47 µg/L	Khodasevich et al., 2024	HBM-GV: 11.5 ng/L
German children 3–5 y	Urinary BPA	3.5 µg/L	KiGGS survey	Exceeds adult HBM-GV ~300×
French women at delivery	Urinary BPA total	2.9 µg/g creatinine	ANSES 2010	–
EU newborns (cord blood)	Total BPA	1.3 µg/L	HBM4EU	Critical: no functional UGT
US adults (NHANES 2017–18)	Urinary BPA	1.3 µg/L (GM)	CDC NHANES 2021	US TDI: 50 µg/kg bw/day

I.B.2. Phthalates

I.B.2.1. Identity and Physico-Chemical Properties

Phthalates (phthalic acid esters) are dialkyl or alkyl aryl esters of 1,2-benzenedicarboxylic acid (o-phthalic acid; CAS 88-99-3). Their general structure consists of an aromatic ring bearing two ester-linked alkyl chains (R_1 and R_2), with the identity of the alkyl substituents determining physico-chemical properties, toxicokinetics, and biological potency.

Figure 6. General structure of phthalate diesters (1,2-benzenedicarboxylate)



General chemical structure of phthalate esters. R_1 and R_2 represent alkyl chains of variable length and branching: DEP (diethyl phthalate; $R = C_2H_5$), DBP (dibutyl phthalate; $R = C_4H_9$), DEHP (di-2-ethylhexyl phthalate; $R = C_8H_{17}$), BBzP (benzylbutyl phthalate; $R_1 = n-C_4H_9$, $R_2 = \text{benzyl}$), DINP (diisononyl phthalate; $R = C_9H_{19}$ branched).

The principal phthalates of regulatory and toxicological concern are:

- **DEHP** (di(2-ethylhexyl) phthalate; CAS 117-81-7): Historically the dominant PVC plasticiser globally; log Kow = 7.50; MW = 390.56 g/mol; used in flooring, wire insulation, food packaging, and medical devices.
- **DBP** (dibutyl phthalate; CAS 84-74-2): log Kow = 4.72; used in cellulosic plastics, printing inks, adhesives, and cosmetics.
- **DiBP** (diisobutyl phthalate; CAS 84-69-5): log Kow = 4.27; structurally similar to DBP with equivalent antiandrogenic potency; used in adhesives and coatings.
- **BBzP** (benzylbutyl phthalate; CAS 85-68-7): log Kow = 4.91; used in vinyl flooring, food conveyor belts, and automotive products.
- **DEP** (diethyl phthalate; CAS 84-66-2): log Kow = 2.47; used as fragrance solvent and fixative in cosmetics and pharmaceuticals.
- **DINP** (diisononyl phthalate; CAS 28553-12-0): log Kow ~8.8; increasingly dominant replacement for DEHP in PVC following regulatory restrictions.

A critical feature of phthalate plasticisers is their non-covalent incorporation into PVC matrices: they do not form covalent bonds with the polymer chain, and therefore migrate continuously from plastic products into contact media (food, dust, saliva, sweat, aqueous environments) throughout the product's lifetime. This continuous migration ensures persistent human exposure regardless of regulatory action on manufacturing.

1.B.2.2. Sources, Uses, and Production

Global phthalate production exceeds 8 million metric tonnes annually, with PVC plasticiser use representing approximately 85% of consumption (ECPI, 2022). The diversity of phthalate-containing products is extensive:

- *Construction materials*: PVC flooring, wall coverings, window profiles, roofing membranes (DEHP, DINP, DIDP).
- *Consumer products*: Toys (under 3 years, restricted), food packaging films, garden hoses, shower curtains (DEHP, DINP).
- *Medical devices*: Blood storage bags, IV tubing, nasogastric tubes, dialysis equipment — historically a major DEHP source with documented patient exposure in vulnerable groups including premature neonates receiving parenteral nutrition.
- *Personal care products*: DEP as fragrance solvent and fixative in perfumes, deodorants, hair sprays, nail polishes, shampoos; DBP in cosmetic formulations.
- *Pharmaceuticals*: DEP, DBP as excipients in enteric-coating formulations and controlled-release capsules.

1.B.2.3. Mechanisms: Antiandrogenism and the Phthalate Syndrome

The primary reproductive mechanism of the anti-androgenic phthalates (DEHP, DBP, DiBP, BBzP) is disruption of fetal testicular steroidogenesis — specifically, reduction of testosterone synthesis in fetal Leydig cells through suppression of steroidogenic gene expression. Critically, this mechanism acts upstream of the androgen receptor: phthalate monoesters (primarily MEHP from DEHP) reduce the expression of StAR (steroidogenic acute regulatory protein, which mediates cholesterol transport into the mitochondrion), CYP11A1 (cholesterol side-chain cleavage enzyme), and CYP17A1 (17 α -hydroxylase/17,20-lyase) in rat and human fetal Leydig cells — thereby reducing testosterone biosynthetic capacity without requiring direct androgen receptor binding (Adamovsky et al., 2024). This mechanistic distinction from anti-androgens such as flutamide or vinclozolin (which act as AR antagonists) is toxicologically important because phthalates produce their effects through a steroidogenic block rather than receptor competition.

The phthalate mechanism also involves PPAR α and PPAR γ (peroxisome proliferator-activated receptors) activation: DEHP and its metabolites MEHP and MEHHP are ligands for PPAR α and PPAR γ , which regulate Leydig cell differentiation, fatty acid metabolism, and, at higher doses, produce peroxisome proliferation and hepatomegaly. PPAR γ activation in granulosa cells impairs the LH-induced cascade essential for follicular maturation and ovulation, contributing to female reproductive effects of phthalates.

The result of combined fetal Leydig cell testosterone suppression and INSL3 reduction during the rat male programming window (GD 15–17) is the **phthalate syndrome**: a constellation of permanent male reproductive tract dysmorphology comprising:

- Shortened anogenital distance (AGD) — the most sensitive biomarker, detectable at exposures far below those causing gross malformations
- Retained thoracic nipples in males (nipples normally regress in male rats under androgen influence)
- Malformations of the gubernaculum and testicular descent apparatus
- Cryptorchidism (ectopic testes)
- Epididymal aplasia or dysgenesis
- Hypospadias
- Reduced testicular weight

Dose–response data for DEHP in rat developmental studies establish a NOAEL of approximately 4.8–10 mg/kg bw/day for reproductive tract malformations, with a LOAEL of 10–15 mg/kg bw/day for the most sensitive structural endpoints (epididymal malformations, AGD reduction); higher doses of 300–750 mg/kg bw/day are required to produce frank testicular atrophy (US EPA, 2025).

Human epidemiological confirmation of the phthalate syndrome was provided in landmark studies: Swan et al. (2005) measured nine phthalate metabolites in first-trimester urine from 85 mother–son pairs, finding that concentrations of MBP, MEP, MBzP, and MiBP were inversely associated with anogenital distance in male infants — effectively replicating the animal data in humans at environmentally relevant exposures. The larger TIDES cohort (N = 753), with first-trimester samples, confirmed significant inverse associations between DEHP metabolites (MEHP, MEOHP, MEHHP) and both measures of male AGD (Swan et al., 2015).

For female reproductive toxicity, phthalates produce dose-dependent follicular atresia, impaired LH surge amplitude, reduced granulosa cell proliferation (PPAR γ -mediated), and inhibition of aromatase expression (CYP19A1) — effects observed at LOAELs substantially higher than for male developmental endpoints, but potentially relevant to cumulative low-dose mixture exposure across the female lifespan.

1.B.2.4. Regulatory Status (EU/US, 2020–2026)

Under EU REACH Regulation (EC) No 1907/2006, DEHP, DBP, DiBP, and BBzP are classified as Substances of Very High Concern (SVHC) on the basis of reproductive toxicity Category 1B (CMR) and, subsequently, endocrine-disrupting properties under Article 57(f); their use in the EU requires authorisation from ECHA for non-exempted applications (Apel et al., 2021). These four phthalates are prohibited in all toys and childcare articles under the EU Toy Safety Directive and face migration limits in food contact plastics under Regulation (EU) 10/2011.

The SCHEER (Scientific Committee on Health, Environmental and Emerging Risks) published updated guidelines in June 2024 on benefit–risk assessment of phthalates in medical devices, concluding that DEHP, BBzP, and others qualify as SVHC on combined CMR + endocrine-disrupting grounds, and that clinical benefit must demonstrably outweigh risk to justify continued medical device use (SCHEER, 2024).

The European Food Safety Authority (EFSA) published TDIs for individual phthalates: DEHP: 50 μ g/kg bw/day; DBP: 10 μ g/kg bw/day; DiBP: (group TDI with DBP); BBzP: 500 μ g/kg bw/day; DINP: 150 μ g/kg bw/day; DIDP: 150 μ g/kg bw/day (pre-2023 values; revision ongoing as of 2025). HBM4EU derived HBM guidance values (HBM-GVs) for DEHP metabolites, DBP, DiBP, BBzP, DPHP, and DINCH (Apel et al., 2021), providing operational thresholds for biomonitoring risk characterisation.

In the United States, CPSC regulations restrict DEHP, DBP, BBzP, DINP, DIDP, and DnOP to < 0.1% w/w in toys and childcare articles. The US EPA's December 2025 non-cancer human health hazard assessment for DEHP recommends a chronic oral reference dose (RfD) of 0.006 mg/kg/day based on reproductive developmental endpoints (EPA, 2025).

1.B.2.1. Exposure

Exposure Pathways

Human exposure to phthalates occurs through:

1. *Dietary intake*: Dominant for DEHP, DBP, DiBP; food contaminates from plastic packaging (cling films, PVC gaskets in jar lids), processing equipment (PVC tubing, conveyor belts), and indirect contamination during food preparation.
2. *Inhalation and ingestion of indoor dust*: DEHP, DINP, and BBzP released from PVC flooring, wall coverings, and household products into indoor air and settled dust. Particularly relevant for young children via hand-to-mouth activity.

3. *Dermal absorption from cosmetics and personal care products*: DEP and DBP in fragrances, lotions, shampoos, nail polish — substantial dermal absorption documented, particularly for lipophilic esters.

4. *Medical device exposure*: DEHP from blood bags, IV tubing, dialysis equipment; particularly relevant for vulnerable medical patients. ECB estimated exposures of 3,000 µg/kg bw/day in haemodialysis adults and 1,700 µg/kg bw/day in transfused neonates as of 2008 (ECB, 2008).

Biomonitoring Data

DEHP metabolites (MEHP, MEHHP, MEOHP, MECPP) are detected in essentially every European urine sample — confirming continuous, ubiquitous exposure. The HBM4EU programme (2017–2022) measured phthalate metabolites in urine across 28 European countries, providing the most comprehensive continental biomonitoring dataset to date (Vogel et al., 2022):

- DEHP: ΣDEHP metabolites (MEHP + MEHHP + MEOHP + MECPP) in adults: geometric mean ~50–100 µg/L; children show 3–5 times higher concentrations than adults in the same studies.
- DBP (measured as MBP): GM in HBM4EU adults ~10–30 µg/L; children consistently higher.
- DINP (metabolites MINP, MiNP): Rising concentrations in European children between 2014 and 2021, consistent with increased use following DEHP restrictions — a documented case of regrettable substitution (Vogel et al., 2022).
- French women at delivery: median urinary 5OH-MEHP = 42 µg/L; 5oxo-MEHP = 28 µg/L (ANSES, 2011).

Cumulative anti-androgenic phthalate risk assessment using the Hazard Quotient (HQ) sum approach consistently demonstrates that combined phthalate exposure exceeds the HBM-GV in a substantial fraction of European children and some adults, particularly young women using multiple cosmetic products containing DEP (Apel et al., 2021).

Species Differences in Toxicokinetics

The C_{max} for MEHP (DEHP monoester) is 7.5-fold higher in rats than in non-human primates following equivalent oral DEHP exposure, and in humans and primates, MEHP is present predominantly as the glucuronoconjugate — limiting free MEHP systemic availability compared to rodents. This pharmacokinetic difference partly explains the higher sensitivity of rats versus primates/humans to DEHP-induced testicular effects, and is an important consideration in cross-species risk factor application. The plasma half-life of DEHP/MEHP is approximately 18 hours in humans; unlike BPA, DEHP is not significantly bioaccumulated, but continuous exposure from diverse sources maintains constant urinary metabolite levels.

Major phthalates — EU regulatory classification and tolerable daily intakes

Phthalate	CAS	EU SVHC status	EU TDI (µg/kg bw/day)	US EPA RfD (2025)	Primary use
DEHP	117-81-7	Yes (CMR + ED)	50	0.006 mg/kg/day	PVC plasticiser/medical devices
DBP	84-74-2	Yes (CMR + ED)	10	—	PVC/cosmetics/inks
DiBP	84-69-5	Yes (CMR + ED)	10 (group w/DBP)	—	Adhesives/coatings
BBzP	85-68-7	Yes (CMR + ED)	500	—	Vinyl flooring
DEP	84-66-2	No	—	—	Cosmetic fragrance solvent
DINP	28553-12-0	Restricted (toys)	150	—	PVC replacement for DEHP

I.B.3. Polybrominated Compounds (Flame Retardants)

I.B.3.1. Identity, Structure, and Chemical Classes

Polybrominated flame retardants (BFRs) are a structurally diverse class of organobromine compounds incorporated into polymers, textiles, and electronic materials to inhibit ignition and propagation of combustion. They represent approximately 30% of total flame retardant production in Europe. Three main families are of primary toxicological concern:

1. Polybrominated diphenyl ethers (PBDEs)

PBDEs are aromatic bicyclic ethers consisting of two phenyl rings connected by an oxygen bridge, with 1–10 bromine atoms substituting on the aromatic rings. Classification is based on the degree of bromination and substitution pattern; the commercially used mixtures are:

- *Penta-BDE mixture* (predominantly BDE-47 and BDE-99; used in polyurethane foam for furniture, mattresses, and automotive seating): log Kow = 5.9 (BDE-47) to 7.0 (BDE-99).
- *Octa-BDE mixture* (BDE-153, BDE-154; used in ABS plastics for electronics casings).
- *Deca-BDE mixture* (essentially BDE-209; used in plastics for electrical and electronic equipment, textiles): log Kow ~10, MW = 959.22 g/mol.

BDE-209 can undergo photolytic debromination under UV radiation to lower-brominated congeners (e.g., BDE-47, BDE-99) with substantially higher biological activity, amplifying its effective environmental toxicity.

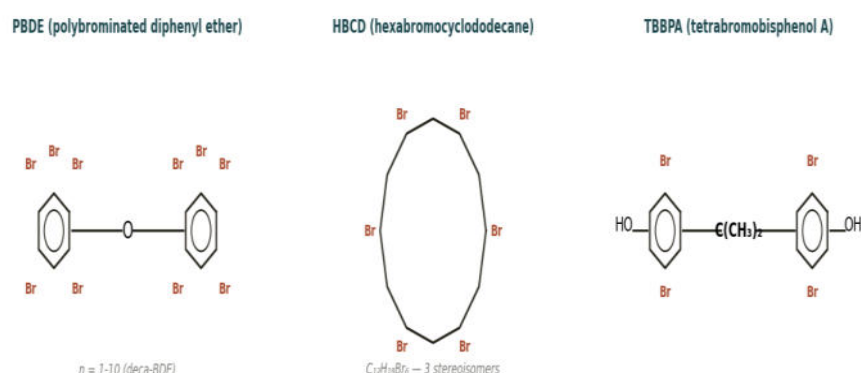
2. Hexabromocyclododecane (HBCD)

HBCD (CAS 25637-99-4) is a cycloaliphatic halogen compound used almost exclusively as an additive flame retardant in expanded polystyrene (EPS) and extruded polystyrene (XPS) insulation boards for construction. The technical HBCD mixture consists of three diastereoisomers (α , β , γ), with the γ -isomer predominant in the commercial product; however, the α -isomer predominates in biological tissues due to differential metabolism and bioaccumulation. HBCD is less lipophilic than PBDEs but more subject to Phase II conjugation, rendering it less persistently bioaccumulative; nonetheless, it is designated Persistent, Bioaccumulative, and Toxic (PBT).

3. Tetrabromobisphenol A (TBBPA)

TBBPA (CAS 79-94-7; MW = 543.87 g/mol) is structurally a brominated analogue of BPA, with two phenolic groups and four bromine substituents. It is the highest-volume BFR globally (~170,000 tonnes/year), used predominantly as a reactive additive (covalently bonded into epoxy resin matrices) in printed circuit boards. In reactive applications, TBBPA bioavailability is low; however, additive uses (in ABS plastics) and TBBPA released during mechanical processing or e-waste recycling/burning can yield freely bioavailable compound.

Figure 7. Main brominated flame retardants (PBDE, HBCD, TBBPA)



Chemical structures of the major brominated flame retardant families: (A) Polybrominated diphenyl ether (PBDE); BDE-47 (tetrabromodiphenyl ether) shown; (B) Hexabromocyclododecane (HBCD), γ -isomer; (C) Tetrabromobisphenol A (TBBPA). The structural similarity between TBBPA and BPA is apparent from the shared isopropylidene-bridged diphenyl scaffold.

1.B.3.2. Environmental Persistence and Stockholm Convention Status

PBDEs are characterised by extreme lipophilicity, resistance to metabolic biotransformation, and long environmental half-lives — properties placing them in the class of Persistent Organic Pollutants (POPs). Their environmental fate is dominated by: (i) accumulation in sediments and soils; (ii) biomagnification through aquatic and terrestrial food chains, with highest concentrations in apex predators; (iii) long-range atmospheric transport via gas- phase and particle-bound fractions.

The Stockholm Convention regulatory timeline for PBDEs is as follows:

- **Penta-BDE and Octa-BDE:** Listed in Annex A (elimination) in 2009 with specific exemptions. In the EU, these mixtures were voluntarily phased out by the major producer (Great Lakes Chemical Corporation/Chemtura) effective 2004–2005; Directive 2003/11/EC formally prohibited them from August 2004.
- **Deca-BDE (BDE-209):** Listed in Annex A in 2017 at COP8, with time-limited exemptions for aircraft manufacture, replacement parts for existing electronic equipment, and certain textile applications, expiring no later than 2030 (Stockholm Convention Secretariat, 2023).

HBCD was designated SVHC in the EU in 2008, listed under the Stockholm Convention Annex A in 2013, and subject to progressive restriction. Building insulation EPS exemptions were granted through 2024, pending development of suitable alternatives.

A landmark 2023 meta-analysis of breast milk PBDE data from 207 studies (Siddique et al., 2023) demonstrated significant breakpoint reductions in BDE-47 and BDE-99 concentrations in European breast milk coinciding with the regulatory phaseout — providing clear evidence of regulatory efficacy in reducing human body burden. North American concentrations consistently exceeded European levels by an order of magnitude throughout the measurement period, reflecting the greater historical use of PentaBDE in North American polyurethane foam. BDE-153, with its longer biological half-life, showed plateau rather than declining trends, consistent with its greater persistence.

1.B.3.3. Mechanisms of Endocrine Disruption

Thyroid Hormone Disruption

The most thoroughly documented endocrine mechanism of PBDEs involves disruption of thyroid hormone homeostasis. Hydroxylated PBDE metabolites (OH-PBDEs), generated by CYP1A1/1A2-mediated hydroxylation, are structurally analogous to 3,5,3'-triiodothyronine (T3) and thyroxine (T4), with bromines mimicking iodine atoms. OH-PBDEs compete with T4 for binding to:

- Transthyretin (TTR): the primary thyroid hormone carrier protein in cerebrospinal fluid and one of the plasma transport proteins; OH-PBDEs displace T4 from TTR with higher affinity than many individual PBDEs, resulting in reduced circulating free T4 availability.
- Thyroid hormone receptors (TR α , TR β): certain OH-PBDEs act as partial agonists or antagonists, directly modulating TH-responsive gene transcription.
- Sulphotransferases responsible for T4 inactivation: inhibition can paradoxically increase T4 availability locally in some tissues (Hamers et al., 2014; Mandal, 2021).

The consequence of gestational hypothyroxinaemia induced by PBDE exposure is impaired foetal neurodevelopment: thyroid hormones are essential for neuronal migration, axonal myelination, and hippocampal development during the second and third trimesters of human gestation and early postnatal period. LOAELs for thyroid hormone disruption in rodents range from approximately 1 mg/kg bw/day for individual congeners to lower values for environmentally relevant mixtures. Human birth cohort studies (HOME, CHAMACOS, Project VIVA) consistently report inverse associations between cord-blood or breast-milk PBDE concentrations and child cognitive development (IQ, attention, executive function) at environmentally relevant concentrations (Eskenazi et al., 2013).

Reproductive Hormone Disruption

Direct effects of PBDEs on the HPG axis have been reported in both animal and epidemiological studies. In male rodents, BDE-99 at 60 μ g/kg bw/day produces measurable effects on spermatogenesis; US men's serum BDE concentrations were inversely associated with free androgen index and positively associated with FSH levels in cross-sectional studies — a hormonal signature consistent with Sertoli cell dysfunction (Louis et al., 2006). In female cohort studies, elevated serum PBDE concentrations were associated with longer time to pregnancy and reduced fecundability (Harley et al., 2010). These associations are consistent with direct Leydig cell toxicity, central HPG axis disruption, and thyroid-mediated indirect effects on reproduction.

HBCD and TBBPA Mechanisms

HBCD does not act as a direct TTR competitor like OH-PBDEs; instead, it disrupts thyroid catabolism through induction of CYP enzymes (CYP2B6, CYP3A4), accelerating T4 and T3 degradation. HBCD may also interact with the AhR at higher concentrations. TBBPA's primary endocrine activities are: ER α and ER β agonism at micromolar concentrations (its two phenolic OHs mimic BPA's oestrogenic pharmacophore); androgen receptor antagonism

at micromolar concentrations; and ryanodine receptor (RyR) activation — the latter implicated in effects on intracellular Ca^{2+} signalling in neuronal and spermatogenic cells (Mandal, 2021).

1.B.3.1. Exposure

Exposure Pathways

Given the lipophilic nature of PBDEs (log Kow 5–10), dietary fat — particularly fish, meat, and dairy products — is the dominant exposure route, contributing ~90% of total PBDE intake in most European adults (Sjödén et al., 2008). However, the relative contribution of different pathways varies substantially by congener and age group:

1. *Dietary lipid-rich foods*: Fish from contaminated water bodies (particularly farmed salmon, freshwater fish from high-load regions) are major contributors. In European adult populations, estimated dietary PBDE intake is in the range of 1–5 ng/kg bw/day for total PBDEs (sum of BDE-28, -47, -99, -100, -153, -154, -183) — below historical US values but showing postphaseout decline in European data.

2. *Indoor dust ingestion*: The toddler-via-dust pathway is quantitatively important for BDE-209 (deca-BDE), which is not efficiently absorbed from dietary lipid due to its extreme lipophilicity but is present in indoor dust at concentrations of $\mu\text{g/g}$ from treated electronic equipment. Toddler hand-to-mouth dust ingestion may contribute substantially to BDE-209 exposure. North American household dust concentrations of Σ -PBDEs exceeded European values by 10–40-fold during peak use years (Stapleton et al., 2008).

3. *Inhalation*: Gas-phase PBDE (primarily lower-brominated congeners) and dust-bound PBDE contribute to indoor inhalation exposure.

4. *Breast milk*: Lactation is both a maternal depuration route and an infant exposure route. Breast milk PBDE concentrations in European women: typically 1–10 ng/g lipid for BDE-47 (postphaseout values); North American values historically 20–100 ng/g lipid.

5. *HBCD exposure via construction dust*: Building renovation and demolition of EPS- insulated structures generate HBCD-contaminated dust; building occupants may also be exposed via volatilisation from installed insulation.

Biomonitoring Data

In the general European population, mean serum PBDE concentrations declined significantly following the 2004–2005 phaseout (Siddique et al., 2023):

- UK Biobank adults (2006–2010): median Σ -PBDE serum $\approx$ 5–12 ng/g lipid; since declined.
- Norway: BDE-47 in breast milk fell from $\sim$ 2.5 ng/g lipid in 2000 to $\sim$ 0.5 ng/g lipid in 2015, a 5-fold reduction over 15 years.
- US adults at peak exposure (NHANES 2001–2004): median BDE-47 serum $\approx$ 20–30 ng/g lipid — 10–40 times higher than European values.

HBCD in European human tissues:

- Breast milk and serum: median concentrations typically 0.35–1.1 ng/g lipid across European studies; higher in high fish consumers from contaminated areas (Mandal, 2021).

TBBPA:

- Primarily detected in urine as Phase II conjugates (sulfate, glucuronide) due to its relatively higher polarity and rapid metabolism. Urinary TBBPA in European adults: geometric mean $\sim$ 0.1–0.5 $\mu\text{g/L}$ in recent studies (BUGS study, 2022).

Transition to Alternative Flame Retardants: Regrettable Substitution

The phaseout of legacy BFRs has been accompanied by rapid market penetration of organophosphate flame retardants (OPFRs) — including TDCPP (chlorinated tris-phosphate) and the commercial mixture Firemaster 550 (FM 550), containing the phthalate analogue bis(2-ethylhexyl)-2,3,4,5-tetrabromophthalate (BEH-TEBP/TBPH). FM 550 components (EH-TBB and BEH-TEBP) are now nearly ubiquitous in North American and increasingly European indoor dust. In vitro studies demonstrate that EH-TBB and BEH-TEBP alter steroid hormone production in human testicular cells with potency comparable to PBDEs, and in vivo studies show similar reproductive developmental effects in rodents — representing a paradigmatic case of regrettable substitution in which legacy toxicants were replaced by structurally similar, comparably or more hazardous alternatives (Hamers et al., 2014; Gaballah, 2025).

Regulatory milestones for polybrominated flame retardants

Compound	Stockholm Convention listing	EU regulatory action	Current status
Penta-BDE	Annex A, 2009	Banned 2004 (Dir 2003/11/EC)	Declining body burden
Octa-BDE	Annex A, 2009	Banned 2004	Declining body burden
Deca-BDE (BDE-209)	Annex A, 2017 (exemptions to 2030)	Banned in most uses; exemptions active	Still present in dust/products
HBCD	Annex A, 2013	SVHC 2008; EPS exemptions to 2024	Monitoring continues
TBBPA	Not listed	Not restricted (reactive use dominant)	Monitoring ongoing

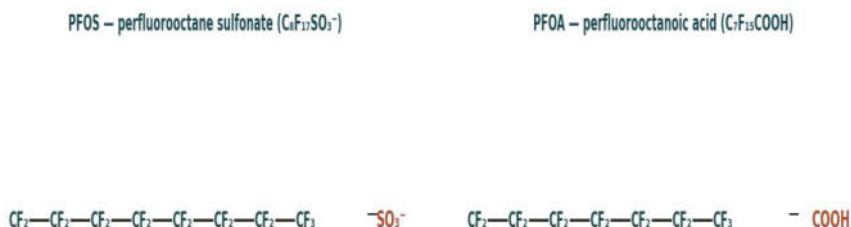
. Duke University. 2025. <https://dukespace.lib.duke.edu/items/11224bd7-86aa-4fa2-86ee-510f726d1747>]

I.B.4. Perfluorinated Compounds

I.B.4.1. Identity, Chemistry, and the PFAS Class

Per- and polyfluoroalkyl substances (PFAS) constitute the largest synthetic chemical class of current regulatory priority, comprising more than 10,000 individual compounds united by the presence of one or more C–F bonds (US EPA PFAS definition, 2021). The defining structural feature — the partially or fully fluorinated carbon chain — confers extraordinary chemical stability: the C–F bond energy (~544 kJ/mol) is the strongest bond in organic chemistry, rendering PFAS essentially indestructible by biological and most environmental processes. PFAS are therefore permanent environmental pollutants with no established pathway to natural degradation at environmental temperatures — giving rise to the media designation "forever chemicals."

Figure 8. Chemical structures of PFOS and PFOA



Chemical structures of perfluorooctane sulfonate (PFOS; CAS 1763-23-1) and perfluorooctanoic acid (PFOA; CAS 335-67-1) — the two "legacy" long-chain PFAS of primary toxicological concern. Both contain a fully fluorinated 8-carbon chain (the polyfluoroalkyl tail) attached to a highly polar head group (sulfonate or carboxylate). The dual hydrophobic-hydrophilic character results in protein-binding accumulation (predominantly albumin and fatty-acid binding proteins) rather than lipid partitioning.

The most toxicologically and regulatorily studied PFAS are the long-chain (C8) carboxylic acid PFOA (perfluorooctanoic acid; CAS 335-67-1; MW 414.07 g/mol) and sulfonate PFOS (perfluorooctane sulfonate; CAS 1763-23-1; MW 538.23 g/mol). PFAS are classified by chain length and functional head group:

- Long-chain perfluoroalkyl carboxylic acids (PFCAs): PFOA (C8), PFNA (C9), PFDA (C10), PFUnDA (C11), PFDoDA (C12).
- Long-chain perfluoroalkyl sulfonic acids (PFSAs): PFOS (C8), PFHxS (C6 sulfonate).

- Short-chain PFAS: PFBA (C4 carboxylate), PFHxA (C6 carboxylate), PFBS (C4 sulfonate) — introduced as "safer" substitutes, but demonstrating similar hazard profiles.
- Fluoropolymers: PTFE (Teflon), PVDF — considered not bioavailable in intact form.
- Fluorotelomers and precursors: compounds that degrade to long-chain PFAS in the environment.

Key physico-chemical properties of legacy PFAS: high polarity despite hydrophobic fluorocarbon tail (hence "hydrophobic but not lipophilic"); strong binding to serum albumin and fatty-acid binding proteins ($K_d \sim 10^{-4}$ – 10^{-5} M for albumin); negligible accumulation in adipose tissue; accumulation in protein-rich tissues (liver, kidney, testis); human serum half-lives of 3.5–5.4 years for PFOA and PFOS, respectively (Li et al., 2018) — among the longest known for any non-persistent-lipid-soluble environmental contaminant.

1.B.4.2. Historical Production and Major Uses

PFOA and PFOS were developed by 3M Corporation in the 1950s under the Scotchgard product line, and by DuPont for PTFE manufacturing ("Teflon"). Their remarkable surfactant properties — simultaneously water-, oil-, and soil-repellent while heat-stable — drove their incorporation into:

- *Aqueous film-forming foams (AFFFs)*: Used for Class B fire suppression at airports, military bases, and fuel storage facilities — the primary source of documented drinking water PFAS contamination near these sites worldwide.
- *Fluoropolymer coatings*: Non-stick cookware (PTFE/Teflon), waterproof and oil-proof food packaging (microwave popcorn bags, pizza boxes, fast food wrappers), stain-resistant textile treatments (carpets, clothing, furniture upholstery).
- *Industrial applications*: Semiconductor manufacturing, metal plating, mining operations, paper/cardboard manufacturing (grease-resistant food packaging).
- *Consumer products*: Waterproof outerwear, ski wax, medical device coatings.

3M voluntarily phased out PFOS-based surfactants globally starting in 2000; PFOA phase-out by major manufacturers under the US EPA PFOA Stewardship Programme was largely completed by 2015. However, ongoing releases from existing products, contaminated groundwater, and contaminated sites — combined with the essentially permanent environmental persistence of PFAS — mean that human exposure continues and body burdens decline only slowly following elimination of major exposure sources.

1.B.4.3. Mechanisms of Reproductive and Developmental Toxicity

PFAS exert reproductive toxicity through multiple convergent mechanisms in both sexes (Chen et al., 2024):

Male Reproductive Toxicity

- (i) *Spermatotoxicity*: PFOA and PFOS cause dose-dependent apoptosis and autophagy in spermatogenic cells, reducing spermatocyte and spermatid counts; the NOAEL for spermatotoxic effects in chronic rat studies is approximately 0.5 mg/kg bw/day for individual congeners (EFSA CONTAM, 2020).
- (ii) *Leydig cell dysfunction*: PFAS disrupt Leydig cell differentiation and steroidogenesis, reducing testosterone production by: suppressing StAR expression; inhibiting CYP11A1 and CYP17A1; activating PPAR- α (which modulates StAR transcription inversely) (Chen et al., 2024).
- (iii) *Blood–testis barrier (BTB) disruption*: Junctional proteins (occludin, ZO-1, N-cadherin, connexin 43) constituting the BTB tight-junction complex between Sertoli cells are downregulated following PFAS exposure, compromising the selective permeability of the BTB and disrupting the immune-privileged environment of the seminiferous tubule — a prerequisite for productive spermatogenesis (Chen et al., 2024).
- (iv) *Sperm Ca^{2+} signalling*: PFAS impair calcium channel function in sperm plasma membranes, disrupting the Ca^{2+} transients required for capacitation and acrosome reaction.

Female Reproductive Toxicity

- (i) *Follicular development and ovulation*: PFOA impairs gap-junction intercellular communication (GJIC) between granulosa cells and the oocyte — essential for oocyte maturation and meiotic resumption. PFAS disrupt steroidogenesis in granulosa cells by inhibiting StAR, CYP11A1, and CYP19A1 (aromatase) expression, reducing oestradiol and progesterone production.
- (ii) *Corpus luteum and endometrial function*: PFOA antagonises progesterone receptor signalling in endometrial cells, impairing endometrial receptivity for embryo implantation (Maranghi et al., 2025). PFOA inhibits the expression of oestrogen sulfotransferase genes (SULT1E1) in endometrium, altering local oestrogen activity and further disrupting the endocrine environment for implantation (Chen et al., 2024).

(iii) *Mammary gland development*: The most sensitive developmental outcome identified in rodent PFAS studies is impaired mammary gland development in mouse pups exposed via maternal lactation. LOAECs as low as 20 ng/mL in pup serum on PND 22 have been reported, without an identifiable NOAEC — establishing this as a critical effect in EFSA's 2020 risk assessment (EFSA CONTAM, 2020).

(iv) *Fetal growth restriction*: Reduced birth weight is one of the most robust and consistent epidemiological findings for both PFOS and PFOA. A meta-analysis confirms inverse associations between maternal PFOS serum concentrations (mean ~27.3 ng/mL) and birth weight; each doubling in maternal PFOS was associated with an approximately 5–10 g reduction in birth weight across studies (EFSA CONTAM, 2020).

Immunotoxicity as the Critical EFSA Effect

For the 2020 group TWI setting, EFSA's CONTAM Panel identified **decreased antibody response to vaccines in children** — an immunological endpoint — as the most sensitive adverse effect: each 2-fold increase in maternal serum PFOS during pregnancy was associated with a 39% decrease in diphtheria antibody response in children at age 5 years, based on prospective birth cohort data (Grandjean et al., 2012; EFSA CONTAM, 2020). This shift from the 2018 cholesterol-based reference value to an immunological endpoint reflects both the increasing sensitivity of the available evidence and the heightened regulatory attention to developmental immune disruption.

I.B.4.4. Regulatory Status (EU/US, 2020–2026)

EFSA 2020 Group TWI: The CONTAM Panel established a group tolerable weekly intake (TWI) of **4.4 ng/kg bw/week** for the sum of PFOA + PFOS + PFNA + PFHxS — a 17-fold reduction from the 2018 individual PFOS TWI of 13 ng/kg bw/week, based on combined exposure assessment using the MixTox approach (EFSA CONTAM, 2020).

EU Drinking Water Directive (2020/2184): Entered into force in January 2021, with implementation deadlines from 2023–2026. Specifies:

- 0.10 µg/L for the sum of 20 designated PFAS ("PFAS sum")
- 0.50 µg/L for "total PFAS"
- From 2028: 0.02 µg/L for the sum of PFOA + PFOS + PFNA + PFHxS

US EPA National Primary Drinking Water Regulation (NPDWR, April 2024): Establishes legally enforceable Maximum Contaminant Levels (MCLs) of:

- PFOA: 4 ppt (ng/L)
- PFOS: 4 ppt (ng/L)
- PFHxS, PFNA, HFPO-DA (GenX): 10 ppt individually
- Mixture MCL for combinations of PFHxS + PFNA + HFPO-DA + PFBS based on hazard index approach (US EPA, 2024).

Stockholm Convention: PFOS was listed in Annex B in 2009; PFOA and related compounds were listed in Annex A in 2019.

EU Group Restriction Proposal (PFAS Restriction, 2023): In January 2023, five EU Member State authorities (Germany, Denmark, the Netherlands, Norway, Sweden) submitted the most comprehensive chemical restriction proposal in history: a universal restriction on all PFAS as a class under REACH, covering approximately 10,000+ compounds, based on the principle that no currently available PFAS substitute has been demonstrated safe (European Commission, 2023). ECHA's committees are evaluating this proposal; a final restriction decision is expected in 2026–2027.

I.B.4.1. Exposure

Exposure Sources and Routes

PFAS exposure in the general population occurs through multiple pathways, with the relative importance varying by geography, proximity to contamination sources, and local dietary patterns:

1. *Drinking water*: Particularly relevant near AFFF use sites (airports, military bases, fire training areas), fluorochemical manufacturing facilities (e.g., Chemours Fayetteville Works, NC; 3M Decatur, AL), and industrial dischargers. The US EPA estimates that PFAS-contaminated drinking water affects at least 200 million Americans; European "hotspot" regions have been documented near AFFF sites in Belgium, UK, Italy, Germany, and the Nordic countries (European Commission, 2023).

2. *Diet*: Contaminated fish and other seafood (particularly freshwater fish from PFAS- contaminated water bodies), meat and dairy from farms near contaminated land or wastewater application areas, and food packaged in PFAS-treated materials contribute substantially. Dietary intake is the primary route for PFNA and longer-chain PFCAs (fish bioaccumulate these preferentially).

3. *Indoor environment*: Dust ingestion from PFAS-treated textiles (carpets, upholstery), PTFE cookware degradation at high temperatures, and building materials treated with PFAS contribute to background exposure, particularly for children via hand-to-mouth dust ingestion.

4. *Consumer products*: Direct dermal/oral contact with PFAS-treated clothing, personal care products containing fluorotelomer ingredients, and cosmetics (especially mascaras and waterproof products containing fluorotelomers) contributes to exposure.

Biomonitoring Data

PFAS are detected in human blood with near-universal prevalence in industrialised nations:

- PFOS serum geometric mean in US NHANES 2017–2018: 3.9 ng/mL (vs. 30–35 ng/mL in 2000, representing approximately 8–10-fold decline following phaseout — CDC NHANES data, 2021).
- PFOA serum GM in NHANES 2017–2018: 1.7 ng/mL (vs. ~5 ng/mL in 2000).
- EFSA 2020 assessment: European general population serum PFOA: median ~2 ng/mL; PFOS median: ~6 ng/mL; PFHxS median: ~0.6 ng/mL.
- EFSA derived a HBM-GV for the sum of PFOA + PFOS + PFNA + PFHxS of 6.9 ng/mL serum based on the 4.4 ng/kg bw/week TWI. Across European adult biomonitoring data, 17.5% of the adult population exceeds this HBM-GV, with substantially higher exceedance rates in PFAS-contaminated regions (EFSA CONTAM, 2020).
- Cord blood PFOS/PFOA: consistently detected in umbilical cord blood of newborns in European and North American cohort studies; cord blood:maternal serum ratios for PFOS typically 0.2–0.6, reflecting partial transplacental transfer (Kato et al., 2014).
- Breast milk: PFAS are detected in breast milk, representing an infant exposure source; concentrations are substantially lower than serum due to the relative hydrophilicity of PFAS (unlike lipid-soluble PCBs/PBDEs). Breast milk PFOS: ~0.07–0.3 ng/mL in EU data (EFSA CONTAM, 2020).

Populations at Risk

- *Infants*: Proportionally high body burden from breast milk exposure combined with rapid growth-dilution; disproportionate vulnerability due to impaired mammary gland development as the most sensitive developmental endpoint.
- *Pregnant women and their fetuses*: Cord blood PFAS concentrations reflect combined gestational and pregestational body burden; fetal PFAS exposure during organogenesis cannot be interrupted even with immediate cessation of exposure.
- *Communities near AFFF sites and PFAS manufacturing*: Markedly elevated drinking water concentrations produce median serum PFOS > 50–100 ng/mL in exposed communities (e.g., Ronneby, Sweden; Blades, UK; Brunswick County, NC).
- *High fish consumers*: PFCA-dominant exposure profile from aquatic bioaccumulation; particularly relevant for Arctic and sub-Arctic indigenous populations.
- *Workers in fluorochemical manufacturing and AFFF deployment*: Occupational PFAS exposure with serum PFOS historically in the hundreds of ng/mL range.

EFSA 2020 PFAS group TWI and corresponding serum HBM guidance values

Compound	EFSA 2020 TWI	Serum HBM-GV	Population exceeding HBM-GV (Europe)	US EPA 2024 MCL (drinking water)
PFOA + PFOS + PFNA + PFHxS (sum)	4.4 ng/kg bw/week	6.9 ng/mL serum	~17.5% adults	4 ppt (PFOA/PFOS individually)
PFOS	(included in group TWI)	–	–	4 ppt

Compound	EFSA 2020 TWI	Serum HBM-GV	Population exceeding HBM-GV (Europe)	US EPA 2024 MCL (drinking water)
PFOA	(included in group TWI)	—	—	4 ppt
PFHxS/PFNA/HFPO-DA	—	—	—	10 ppt (each)

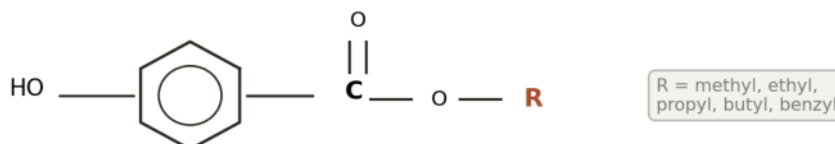
I.B.5. Parabens

I.B.5.1. Identity and Physico-Chemical Properties

Parabens (para-hydroxybenzoic acid alkyl esters; p-HBA alkyl esters) are a homologous series of antimicrobial preservatives in which the carboxyl group of p-hydroxybenzoic acid is esterified with an alkyl alcohol of variable chain length. The commercially used parabens, in increasing alkyl chain length, are:

- Methylparaben (MeP; CAS 99-76-3; MW 152.15 g/mol; log Kow 1.96)
- Ethylparaben (EtP; CAS 120-47-8; MW 166.18 g/mol; log Kow 2.47)
- Propylparaben (PrP; CAS 94-13-3; MW 180.20 g/mol; log Kow 3.04)
- Butylparaben (BuP; CAS 94-26-8; MW 194.23 g/mol; log Kow 3.57)
- Isobutylparaben (iBuP; CAS 4247-02-3): prohibited in EU cosmetics (Annex II)
- Benzylparaben, pentylparaben, phenylparaben: also prohibited in EU cosmetics (Annex II)

Figure 9. General structure of parabens (alkyl 4-hydroxybenzoates)



General chemical structure of parabens (p-hydroxybenzoic acid alkyl esters). R = alkyl chain: methyl (-CH₃), ethyl (-C₂H₅), propyl (-C₃H₇), butyl (-C₄H₉). Increasing alkyl chain length progressively increases lipophilicity, oestrogenic potency, and hydrolytic stability; chain branching (isobutyl) further enhances oestrogenic activity.

Parabens are white crystalline powders, soluble in polar organic solvents, moderately soluble in water (MeP: ~2.5 g/L; BuP: ~0.2 g/L at 25°C), and relatively stable across the pH range 4–8 relevant to cosmetic formulations. Their stability under acidic-to-neutral pH and their proven antimicrobial efficacy against a broad spectrum of fungi, Gram-positive bacteria, and, at higher concentrations, Gram-negative bacteria, have driven their use in cosmetics since the 1920s and in pharmaceuticals and food since the 1940s.

I.B.5.2. Sources and Uses

Parabens are the most widely used class of preservatives in cosmetics and personal care products (PCPs), present in approximately 80% of commercially available PCPs including shampoos, conditioners, body lotions, facial moisturisers, sunscreens, deodorants, liquid soaps, hair products, and make-up formulations. In the EU, their permitted uses are governed by Regulation (EC) No 1223/2009 (the Cosmetics Regulation), Annex V, with the following limits:

- Maximum concentration: 0.4% (as acid) per individual paraben; 0.8% for mixtures of parabens in a single formulation.
- Propylparaben + Butylparaben combined: $\leq 0.14\%$ in any leave-on body lotions for children under 3 years of age — a precautionary restriction based on SCCS endocrine disruption concerns in this age group (SCCS, 2014).
- Isobutylparaben and isopropylparaben: prohibited in all cosmetics (Annex II).

As food additives in the EU:

- Methylparaben (E218) and its sodium salt (E219), ethylparaben (E214) and its sodium salt (E215) are permitted as food preservatives under Regulation (EC) No 1333/2008, replacing the earlier Directive 95/2/CE provisions, at maximum levels of 300 mg/kg in specific food categories.

In pharmaceuticals, propylparaben is the most commonly used preservative in oral suspensions, topical ointments, and parenteral formulations; methylparaben is frequently used as a co-preservative.

The EFSA Scientific Panel on Food Additives and Nutrient Sources (ANS) established an acceptable daily intake (ADI) for methyl and ethyl paraben (combined) of up to 10 mg/kg bw/day based on chronic oral toxicity studies — a value considered highly conservative relative to human food additive exposure (EFSA ANS Panel, 2004).

1.B.5.3. Mechanisms of Endocrine Disruption

Parabens exert endocrine-disrupting activity through several converging mechanisms:

Oestrogenic Receptor Binding

Parabens bind to ER α and ER β within the ligand-binding pocket, utilising the same hydrogen-bonding interactions with residues Glu353 and Arg394 as natural oestrogens (Darbre and Harvey, 2008). The potency order for classical genomic oestrogenic activity (ERE-dependent transcription; uterotrophic assay) increases with alkyl chain length: MeP < EtP < PrP < BuP, with BuP exhibiting detectable uterotrophic activity in ovariectomised rats at doses several hundred-fold lower than MeP. All parabens are substantially less potent than 17 β -oestradiol: BuP is approximately 10,000-fold weaker at ER α in most assays, and MeP approximately 100,000–1,000,000-fold weaker. Chain branching (isobutyl) enhances oestrogenic potency, explaining the EU prohibition on isobutylparaben.

However, as with BPA, exclusive focus on classical ER α -mediated genomic potency may substantially underestimate biological relevance at realistic human exposure concentrations. Parabens activate membrane oestrogen receptors (GPER) at lower concentrations than required for nuclear ER activity; and in vitro experiments in human breast epithelial cells (MCF-7, MCF-10A) demonstrate paraben-mediated effects on cell proliferation at concentrations achievable from cosmetic absorption (Darbre et al., 2004).

Aromatase Modulation

Parabens modulate CYP19A1 (aromatase) expression in a tissue-specific and bidirectional manner: MeP and PrP increase aromatase mRNA expression in MCF-7 breast cancer cells, potentially amplifying intratumoral oestrogen synthesis; conversely, parabens may suppress aromatase activity in placental microsomes and non-cancerous breast cells. Parabens also inhibit 17 β -hydroxysteroid dehydrogenase type 1 (17 β -HSD1) activity — the enzyme catalysing conversion of oestrone (E1) to potent oestradiol (E2) in peripheral tissues — potentially modulating tissue oestradiol availability (Błaszczuk et al., 2025). The net effect of these competing aromatase-modulating activities depends on the tissue context, paraben chain length, and concentration.

Androgen Receptor Antagonism

Parabens act as weak androgen receptor antagonists in reporter gene assays at micromolar concentrations. BuP demonstrates anti-androgenic activity in the Hershberger assay at high doses, contributing to the concern for male reproductive effects of long-chain parabens. Male fertility effects in rodents at high oral doses are discussed below.

Anti-Proliferative and Pro-Apoptotic Effects in Aquatic Organisms

It is worth noting that the aquatic ecotoxicology of parabens has been documented in fish (zebrafish, medaka), where MeP and BuP at concentrations in the $\mu\text{g/L}$ range (environmentally relevant given inefficient wastewater treatment plant removal) disrupt gonadal development, reduce fertilisation rate, and impair larval growth (Błaszczuk et al., 2025). These findings reinforce that endocrine disruption concerns extend beyond human toxicology.

1.B.5.4. Regulatory Status (EU/US, 2020–2026)

The SCCS (Scientific Committee on Consumer Safety) published updated opinions on several key parabens in recent years:

- **Methylparaben (MeP):** December 2023 SCCS opinion concluded that MeP is safe as a cosmetic preservative at up to 0.4% used alone, with the caveat that existing endocrine activity data require continued monitoring; oestrogenic potency at realistic dermal doses was not considered sufficient to revise the current maximum concentration (SCCS, 2023).
- **Propylparaben (PrP):** The SCCS 2020 opinion maintained the restriction to $\leq 0.14\%$ combined (PrP + BuP) in leave-on products for children under 3, based on uncertainties surrounding developmental endocrine effects; SCCS concluded PrP is safe at 0.19% (maximum) in rinse-off products for the general population.
- **Butylparaben (BuP):** Subject to the $\leq 0.14\%$ combined restriction with PrP in leave-on body lotions for children under 3; no use in nappy products; general adult leave-on products remain subject to 0.14% combined maximum (SCCS, 2014, reaffirmed 2020).

In the United States, parabens remain permitted as cosmetic preservatives with GRAS (Generally Recognised As Safe) status for food additive applications; the FDA has not restricted paraben use in cosmetics, citing insufficiency of evidence for human harm at current exposure levels (FDA, 2020). The Cosmetic Ingredient Review (CIR) Expert Panel completed an amended safety assessment in 2018, concluding that MeP, EtP, PrP, BuP, isoPrP, and isoBuP are safe as used in cosmetics within the current concentration limits, while noting the desirability of additional developmental studies for BuP and PrP (CIR, 2018).

An important emerging regulatory consideration is the cumulative and mixture exposure to parabens together with other oestrogen-active compounds (bisphenols, UV filters, phthalates), where low-dose additive or synergistic effects could exceed individually safe thresholds (Kortenkamp et al., 2011). This is not yet formally incorporated into EU cosmetic safety assessments but is an active area of scientific investigation.

1.B.5.1. Exposure

Exposure Pathways

Human exposure to parabens occurs through multiple simultaneous routes given the prevalence of paraben-containing products in daily life:

1. **Dermal absorption from cosmetics and PCPs:** The primary route for MeP, EtP, PrP, and BuP. Dermal absorption of intact paraben (before esterase hydrolysis in skin) has been demonstrated: BuP is detectable in serum within 1 hour and in urine with a peak at 8–12 hours following topical application, predominantly as glucuronide conjugate (Janjua et al., 2007). MeP and PrP are absorbed dermally and rapidly hydrolysed by skin and plasma esterases to p-hydroxybenzoic acid (pHBA) — the inactive common metabolite — limiting systemic exposure to intact paraben (SCCS, 2023).
2. **Oral ingestion (food additives):** Methyl and ethyl parabens (E218, E219, E214, E215) are used as preservatives in specific food categories (confectionery, processed fish products); dietary intake is generally low relative to dermal cosmetic exposure for most individuals (EFSA ANS, 2004).
3. **Oral ingestion (pharmaceuticals):** Propylparaben as excipient in oral suspensions and topical preparations; relevant for patients receiving regular paraben-containing medication.

Biomonitoring Data

Urinary paraben concentrations (typically expressed as creatinine-adjusted concentrations or as concentration in spot urine samples) provide the most accessible biomarker of recent exposure to combined dermal + oral + pharmaceutical sources:

- US NHANES (2005–2006; persons ≥ 6 years): MeP detected in >99% of samples (median: 63.5 $\mu\text{g/L}$); PrP detected in >90% (median: 8.7 $\mu\text{g/L}$); EtP in $\sim 45\%$; BuP in $\sim 43\%$ (Calafat et al., 2010). Adolescent females and adult women showed significantly higher urinary paraben concentrations than males — consistent with greater cosmetic PCP use.
- Total estimated systemic exposure from all sources in the US: approximately 1,300 $\mu\text{g/kg bw/day}$ for MeP (calculated from urinary concentrations); substantially lower for longer-chain parabens.
- European HBM4EU context: MeP and PrP are among the most prevalent EDCs detected in European urine samples; a systematic multicentre study confirmed MeP as predominant (typically 10–100-fold higher molar concentration than PrP or BuP), and demonstrated higher concentrations in women and younger individuals (Khodasevich et al., 2024).

Male Reproductive Effects: Animal Data and Human Relevance

Rodent reproductive toxicity studies for BuP (oral, GD through PND, rat):

- LOAEL ~100 mg/kg bw/day: reduced male offspring AGD.
- LOAEL ~10 mg/kg bw/day: reduced sperm count, reduced testicular expression of androgen-regulated proteins in offspring (Golden et al., 2005).

For PrP in GLP-compliant guideline studies (PND 4–90, rat):

- NOAEL = 1,000 mg/kg bw/day for reproductive endpoints (CIR, 2018).

Comparison of rodent NOAELs/LOAELs with estimated human dermal exposure from PCPs yields margins of exposure (MoE) generally > 100–10,000 for most paraben–endpoint combinations, supporting the conclusion that current cosmetic exposures to MeP, EtP, and PrP individually do not pose reproductive risk. However: (i) Cumulative mixture exposure to multiple parabens simultaneously (MeP + EtP + PrP + BuP) in combination with other oestrogen-active substances in the same cosmetic products reduces effective MoE. (ii) Dermal exposure in neonates and infants (who may receive paraben-containing nappy rash creams, body lotions) raises developmental vulnerability concerns that are not adequately addressed by adult consumer data alone. (iii) The restricted BuP and PrP exposure in children under 3 specifically reflects SCCS acknowledgement of increased uncertainty for this age group.

Paraben regulatory limits in EU cosmetics and US pharmacopoeia

Paraben	EU max. conc. (Cosmetics Reg.)	Special restrictions (EU)	EU food additive	US FDA cosmetic status						
Methylparaben	0.4% (single); 0.8% (mixture)	None	E218 (permitted)	Permitted	Ethylparaben	0.4% (single); 0.8% (mixture)	None	E214 (permitted)	Permitted	

Urinary paraben biomonitoring — United States (NHANES 2005–2006)

Paraben	Detection frequency	Median urinary concentration (µg/L)	Higher in women vs men							
Methylparaben	>99%	63.5	Yes (significantly)	Propylparaben	>90%	8.7	Yes (significantly)	Ethylparaben	~40%	

SUMMARY OF PART I

Part I has provided the foundational framework for understanding reproductive toxicology at the mechanistic, pharmacokinetic, and regulatory levels. The following key themes should be carried forward into the subsequent parts of this course:

1. Species Differences Are Toxicologically Determinative The temporal mismatch between rat and human reproductive developmental windows (particularly follicle assembly, the male programming window, and postnatal hormonal surges) mandates careful, scientifically justified extrapolation between experimental animal data and human risk. No single animal model fully recapitulates human reproductive development.

2. ADME Governs Target Tissue Dose The intrinsic hazard of a xenobiotic is only expressed as reproductive toxicity if the compound (or its bioactive metabolite) reaches the gonadal, placental, or embryonic target tissue

in a biologically relevant concentration. Differences in bioactivation (DEHP → MEHP; PBDE → OH-PBDE), conjugative detoxification (adult BPA glucuronidation vs. fetal deficiency), and distribution (PFAS protein-binding vs. PBDE lipid-partitioning) are major determinants of species sensitivity and developmental vulnerability.

3. The Five EDC Classes Share Common Themes But Distinct Mechanisms

- BPA: oestrogenic + epigenetic; critical regulatory transition in 2023 (EFSA 20,000-fold TDI reduction)
- Phthalates: anti-androgenic through steroidogenic suppression; male programming window defines critical vulnerability
- Polybrominated FRs: thyroid hormone displacement; neurodevelopmental consequences; post-phaseout monitoring confirms regulatory efficacy
- PFAS: protein-binding "forever chemicals"; immunotoxicity as critical effect; universal PFAS restriction proposal pending
- Parabens: weakly oestrogenic; cumulative mixture concerns; dermal exposure in young children as emerging concern

4. The Regulatory Landscape Is Rapidly Evolving Major regulatory actions between 2020 and 2026 — EFSA's BPA TDI reduction (2023), EFSA's PFAS group TWI (2020), EU Drinking Water Directive PFAS limits (2021), US EPA PFAS MCLs (2024), the EU universal PFAS restriction proposal (2023), and SCHEER phthalate medical device guidelines (2024) — reflect both accumulating scientific evidence and growing societal demand for protection from EDC-associated reproductive health risks. Students must be able to situate the scientific evidence base within these regulatory frameworks and understand how regulatory decisions are made under scientific uncertainty.

5. Non-Monotonic Dose–Response and the Limits of Classical Risk Assessment The NMDR phenomenon — extensively documented for BPA, phthalates, and some PFAS — challenges the classical NOAEL/LOAEL-based risk assessment paradigm. Future reproductive toxicology will increasingly incorporate adverse outcome pathway (AOP) frameworks, high-throughput in vitro screening (ToxCast, EU-ToxRisk), organ-on-chip models, and epidemiological exposure-response data from large birth cohort studies to complement and, in some cases, replace traditional animal studies.

REFERENCES — PART I

- [1] Bonde JP, Tøttenborg SS, Andersson AM, et al. Reproductive toxicology: keeping up with our changing world. *Front Toxicol.* 2024;6:1456687. DOI: <https://doi.org/10.3389/ftox.2024.1456687>
- [2] International Council for Harmonisation. ICH Guideline S5(R3): Detection of Reproductive and Developmental Toxicity for Human Pharmaceuticals. FDA CDER. 2020. <https://www.fda.gov/media/108894/download>
- [3] Welsh M, Saunders PTK, Fisker M, et al. Identification in rats of a programming window for reproductive tract masculinisation, disruption of which leads to hypospadias and cryptorchidism. *J Clin Invest.* 2008;118(4):1479–1490. DOI: <https://doi.org/10.1172/JCI34241>
- [4] Monniaux D, Clément F, Dalbiès-Tran R, et al. The ovarian reserve of primordial follicles and the dynamic reserve of antral growing follicles. *Biol Reprod.* 2014;90(4):85. DOI: <https://doi.org/10.1095/biolreprod.113.117077>
- [5] Skakkebaek NE, Rajpert-De Meyts E, Main KM. Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. *Hum Reprod.* 2001;16(5):972–978. DOI: <https://doi.org/10.1093/humrep/16.5.972>
- [6] Levine H, Jørgensen N, Martino-Andrade A, et al. Temporal trends in sperm count: a systematic review and meta-regression analysis. *Hum Reprod Update.* 2023;29(2):157–176. DOI: <https://doi.org/10.1093/humupd/dmac035>
- [7] Adamovsky O, et al. Advances in understanding the reproductive toxicity of endocrine disrupting chemicals. *Front Cell Dev Biol.* 2024;12:1390247. DOI: <https://doi.org/10.3389/fcell.2024.1390247>
- [8] Maranghi F, et al. Investigating the impact of environmental pollutants on embryo- fetal development. *Reprod Toxicol.* 2025. PMC12705484. URL: <https://pmc.ncbi.nlm.nih.gov/articles/PMC12705484/>
- [9] Völkel W, Colnot T, Csanádi GA, et al. Metabolism and kinetics of bisphenol A in humans at low doses following oral administration. *Chem Res Toxicol.* 2002;15(10):1281–1287. DOI: <https://doi.org/10.1021/tx025548t>

- [10] EFSA CEP Panel. Re-evaluation of the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs. *EFSA Journal*. 2023;21(4):e06857. DOI: <https://doi.org/10.2903/j.efsa.2023.6857>
- [11] European Environment Agency. Human exposure to Bisphenol A in Europe. EEA Publications. 2023. URL: <https://www.eea.europa.eu/en/analysis/publications/human-exposure-to-bisphenol-a>
- [12] Kolatorova L, et al. Mechanisms of bisphenol A and its analogs as endocrine disruptors. *Endocrine Connections*. 2025. PMC12185661. URL: <https://pmc.ncbi.nlm.nih.gov/articles/PMC12185661/>
- [13] Khodasevich D, et al. Biomonitoring of Environmental Phenols, Phthalate Metabolites and Related Compounds. *Toxics*. 2024. PMC12736981. URL: <https://pmc.ncbi.nlm.nih.gov/articles/PMC12736981/>
- [14] Sueiro A, et al. A health conundrum of bisphenol A and its alternatives: charting a safer path forward. *J Environ Exposure Anal*. 2025. DOI: <https://doi.org/10.20517/jeea.2025.39>
- [15] Apel P, et al. Human biomonitoring guidance values for selected phthalates and DINCH (HBM4EU). *Int J Hyg Environ Health*. 2021;234:113722. DOI: <https://doi.org/10.1016/j.ijheh.2021.113722>
- [16] Vogel N, et al. Current exposure to phthalates and DINCH in European children and adolescents — HBM4EU Aligned Studies 2014–2021. *Int J Hyg Environ Health*. 2022. DOI: <https://doi.org/10.1016/j.ijheh.2022.114005>
- [17] SCHEER. Update of the guidelines on the benefit-risk assessment of phthalates in certain medical devices. European Commission. June 2024. URL: https://health.ec.europa.eu/document/download/831f9b60-88de-4197-858f-d001c5b0cc26_en
- [18] Swan SH, Main KM, Liu F, et al. Decrease in anogenital distance among male infants with prenatal phthalate exposure. *Environ Health Perspect*. 2005;113(8):1056–1061. DOI: <https://doi.org/10.1289/ehp.8100>
- [19] US EPA. Non-cancer Human Health Hazard Assessment for DEHP. Technical Support Document. December 2025. URL: <https://www.epa.gov/system/files/documents/2025-12/18-dehp-non-cancer-human-health-hazard-tsd-public-release-hero-dec-2025.pdf>
- [20] Mandal PK. Health toxicity effects of brominated flame retardants. *Environ Pollut*. 2021;286:117500. DOI: <https://doi.org/10.1016/j.envpol.2021.117500>
- [21] Stockholm Convention Secretariat. Polybromodiphenyl ethers (BDEs) — Overview. UNEP. URL: <https://www.pops.int/implementation/industrialpops/bdes/overview/tabid/5371/default.aspx>
- [22] Siddique S, et al. Has Regulatory Action Reduced Human Exposure to Flame Retardants? A Meta-Analysis of PBDEs and HBCDDs in Human Breast Milk. *Environ Sci Technol*. 2023;57(47). DOI: <https://doi.org/10.1021/acs.est.3c02896>
- [23] Hamers T, et al. Exposures, Mechanisms, and Impacts of Endocrine-Active Flame Retardants. *Curr Opin Pharmacol*. 2014;19:125–133. URL: <https://pmc.ncbi.nlm.nih.gov/articles/PMC4252719/>
- [24] Gaballah S. Exposure to Legacy and Alternative Brominated Flame Retardants in Placental Tissue in Rats and Humans [dissertation]. Duke University. 2025. URL: <https://dukespace.lib.duke.edu/items/11224bd7-86aa-4fa2-86ee-510f726d1747>
- [25] EFSA CONTAM Panel. Risk to human health related to the presence of perfluoroalkyl substances in food. *EFSA Journal*. 2020;18(9):e06223. DOI: <https://doi.org/10.2903/j.efsa.2020.6223>
- [26] European Commission. PFAS pollution — chemicals regulation and restriction proposal. DG Environment. 2023. URL: https://environment.ec.europa.eu/topics/chemicals/pfas-pollution_en
- [27] US EPA. National Primary Drinking Water Regulation for PFAS. 2024. URL: <https://www.epa.gov/sdwa/drinking-water-health-advisories-genx-chemicals-and-pfbs>
- [28] Chen Q, et al. Reproductive toxicity of PFOA, PFOS and their substitutes: a review. *Chemosphere*. 2024. DOI: <https://doi.org/10.1016/j.chemosphere.2024.141528>
- [29] SCCS. Scientific Committee on Consumer Safety Opinion on Methylparaben. European Commission Health. December 2023. URL: https://health.ec.europa.eu/system/files/2023-12/sccs_o_276_final.pdf
- [30] Darbre PD, Harvey PW. Paraben esters: review of recent studies of endocrine toxicity, absorption, esterase and human exposure. *J Appl Toxicol*. 2008;28(5):561–578. DOI: <https://doi.org/10.1002/jat.1358>
- [31] Błaszczuk M, et al. Ecotoxicological impacts of parabens on flora and fauna. *npj Sustainable Agriculture*. 2025. DOI: <https://doi.org/10.1038/s44454-025-00012-2>
- [32] CIR Expert Panel. Amended Safety Assessment of Parabens as Used in Cosmetics. Cosmetic Ingredient Review. 2018. URL: <https://www.cir-safety.org/sites/default/files/Parabens.pdf>
- [33] European Commission. Regulation (EC) No 1223/2009 on Cosmetic Products (Cosmetics Regulation). OJ L 342/59. 2009. URL: <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32009R1223>

[34] Vandenberg LN, Colborn T, Hayes TB, et al. Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. *Endocr Rev.* 2012;33(3):378–455. DOI: <https://doi.org/10.1210/er.2011-1050>

[35] Foster PMD. Disruption of reproductive development in male rat offspring following in utero exposure to phthalate esters. *Int J Androl.* 2006;29(1):140–147. DOI: <https://doi.org/10.1111/j.1365-2605.2005.00563.x>

Sections: I.A (Reproductive Function and Species Differences), I.B (Xenobiotic Fate), I.B.1 BPA, I.B.1.1 Exposure, I.B.2 Phthalates, I.B.2.1 Exposure, I.B.3 Polybrominated Compounds, I.B.3.1 Exposure, I.B.4 Perfluorinated Compounds, I.B.4.1 Exposure, I.B.5 Parabens, I.B.5.1 Exposure Figures referenced: fig01–fig09 (9 figures) Tables included: 8 embedded tables References cited: 35 primary references with DOI/URL Literature coverage: 2001–2025, with regulatory updates through 2026

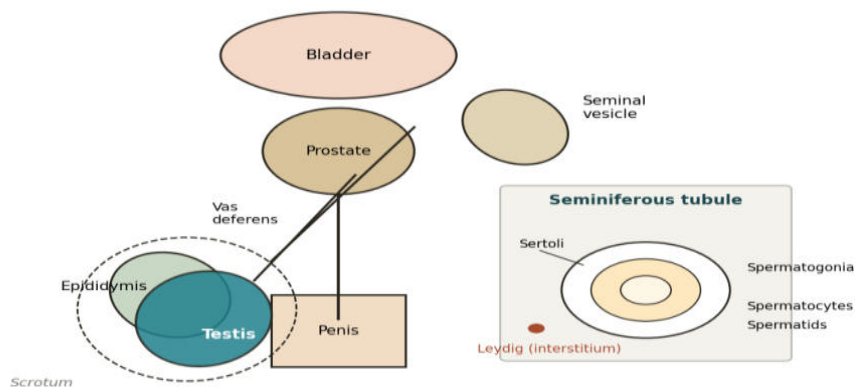
II. Endocrine Disruptors and Male and Female Reproduction

The toxic effects of chemical agents on the reproductive system differ in fundamental ways from their effects on other organ systems. Whereas most forms of environmental toxicity manifest as disease in the exposed individual, reproductive toxicity operates at the level of the reproductive unit — the couple — and its consequences may appear in a partner who was never directly exposed or in offspring born years after the exposure event. A man exposed to a testicular toxicant may transmit genetic or epigenetic damage to an embryo whose mother never contacted the chemical. This couple-dependent dimension of reproductive toxicology demands that exposure–outcome studies capture both partners and, where relevant, multiple generations.

A second distinctive feature is the intermittent nature of reproductive function. Unlike renal or cardiac function, which is continuous and objectively measurable at any time, reproductive potential is only demonstrated when conception is attempted. Occupational or environmental exposure may silently impair fertility without clinical recognition during periods when reproduction is not desired. This latency creates substantial underreporting and complicates the identification of reproductive toxicants in human populations.

Endocrine-disrupting chemicals (EDCs) are exogenous agents that interfere with the synthesis, secretion, transport, binding, action, or elimination of natural hormones responsible for homeostasis, reproduction, development, and behaviour. Their effects on the reproductive axis are the subject of this chapter. The mechanistic complexity of EDC action — including receptor-mediated agonism, antagonism, non-monotonic dose-response, epigenetic programming, and multi-generational transmission — demands a detailed understanding of normal reproductive physiology as the necessary foundation for toxicological analysis.

Figure 10. Anatomical organisation of the male reproductive system



The male reproductive system, showing the testes within the scrotum, the seminiferous tubules, epididymis (caput, corpus, cauda), vas deferens, seminal vesicles, prostate, and bulbourethral glands. The right panel illustrates a cross-section of a seminiferous tubule with Sertoli cells, spermatogonia at the basal lamina, and successive generations of spermatocytes and spermatids progressing toward the lumen.

II.A. The Male Reproductive System and Toxicology

The male reproductive system executes two inseparable biological mandates: the continuous mass production of genetically unique, motile haploid spermatozoa and the regulated synthesis of testosterone. Both functions are accomplished by the testes — paired ovoid organs (each 15–25 mL in volume) suspended within the scrotum at a temperature 2–4 °C below core body temperature, a thermal gradient that is non-negotiable for normal spermatogenesis. The testes are anatomically divisible into approximately 250–300 lobules, each containing one to three seminiferous tubules whose combined length within a single testis exceeds 500 m. Spermatogenesis occurs within these tubules, while steroidogenesis is carried out by clusters of Leydig cells occupying the intertubular interstitium.

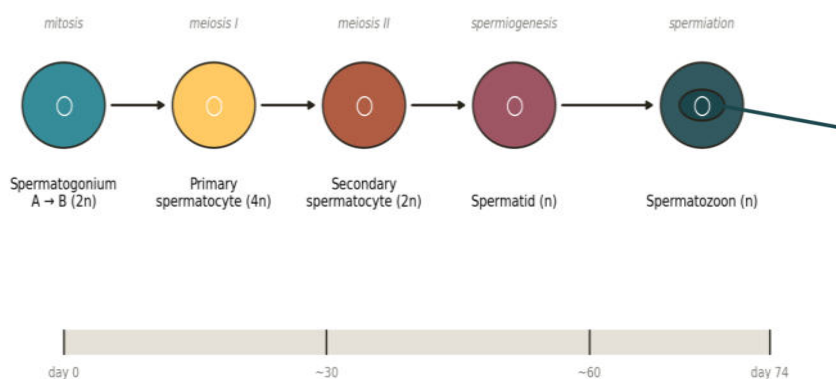
The epididymis is a single, highly convoluted tubule approximately 6 m in length, anatomically divided into caput (head), corpus (body), and cauda (tail). Spermatozoa released from the seminiferous epithelium are immotile and incapable of fertilisation; they acquire progressive motility, hyperactivated motility potential, and zona-binding capacity during a 10–14 day transit through the epididymis. The epididymal microenvironment —

characterised by specific small non-coding RNAs, plasmalogen-type phospholipids, carnitine, glycerylphosphorylcholine, and antioxidant enzymes — orchestrates sperm plasma membrane remodelling. Approximately 30% of ejaculated spermatozoa were stored in the vas deferens; frequent ejaculation shortens epididymal transit and may increase the proportion of immature spermatozoa in the ejaculate (Zaneveld, 1978). The accessory glands — seminal vesicles, prostate, and bulbourethral glands — contribute more than 90% of ejaculate volume. The seminal vesicles (approximately 65% of volume) secrete fructose (the primary sperm energy substrate), prostaglandins, semenogelin I and II, and fibronectin. The prostate (approximately 30% of volume) contributes zinc, citric acid, and serine proteases (PSA, KLK2) that liquify the ejaculate coagulum within 20–30 minutes. Zinc is critical not only for semen liquefaction but also stabilises the compacted sperm chromatin against premature decondensation. Cadmium — which competes with zinc at several transporter proteins — accumulates in prostate and seminal vesicle tissue, displaces zinc, and disrupts both secretory function and sperm chromatin integrity.

Spermatogenesis is one of the most productive mitotic-meiotic-differentiation cascades in biology, generating approximately 1,000 spermatozoa per heartbeat (~120 million spermatozoa per day) in healthy young men. The process proceeds through three major phases: (i) mitotic renewal and differentiation of spermatogonial stem cells (SSC) — Ad (dark) and Ap (pale) type A spermatogonia — through type B spermatogonia; (ii) meiotic division of primary spermatocytes ($2n \rightarrow 4n$ DNA content in the pre-leptotene S-phase, then progressively leptotene, zygotene, pachytene, diplotene, and diakinesis) and secondary spermatocytes ($4n \rightarrow 2n$ per meiosis I, then $2n \rightarrow n$ per meiosis II), yielding haploid round spermatids; and (iii) spermiogenesis, the morphological metamorphosis of round spermatids into elongated spermatozoa without further nuclear division. The entire process from SSC division to spermiation takes 74 days in humans — a critical number for interpreting the temporal relationship between toxic exposure and observed changes in semen parameters.

Sertoli cells — the "nurse cells" of the seminiferous epithelium — are the structural and metabolic backbone of spermatogenesis. A single Sertoli cell supports 30–50 germ cells simultaneously through direct cytoplasmic bridges, transcytotic nutrient delivery, and phagocytic removal of residual bodies. Sertoli cells produce: androgen-binding protein (ABP) — which maintains high luminal testosterone concentrations; inhibin B — the primary negative feedback signal for FSH; glial cell-derived neurotrophic factor (GDNF) and stem cell factor (SCF) — which regulate SSC self-renewal; transferrin and ceruloplasmin — for iron delivery to germ cells; and plasminogen activator — for extracellular matrix remodelling during spermatocyte migration across the blood-testis barrier. The number of Sertoli cells established during the perinatal critical window determines the maximum spermatogenic capacity in adulthood. Any toxic insult — hormonal, chemical, or thermal — that reduces perinatal Sertoli cell proliferation permanently reduces the male's reproductive ceiling.

Figure 16. Stages of spermatogenesis (~74 days in humans)



Schematic representation of spermatogenesis. The basal compartment (below the blood-testis barrier) contains spermatogonia and early primary spermatocytes. The adluminal compartment contains pachytene spermatocytes, round spermatids, elongating spermatids, and mature spermatozoa at spermiation. Sertoli cell cytoplasm (grey shading) envelops all germ cell generations. Key stages vulnerable to toxic insult are indicated: SSC depletion (e.g., DBCP), meiotic disruption (e.g., MEHP), and spermiogenic defects (e.g., heavy metals, phthalates).

The blood-testis barrier (BTB) is formed by elaborate tight junctions (claudin-3, claudin-11), basal ectoplasmic specialisations, gap junctions (connexin 43), and desmosome-like junctions between adjacent Sertoli cells near the base of the seminiferous epithelium. These junctions divide the epithelium into a basal compartment (containing spermatogonia and early primary spermatocytes, accessible to blood-borne molecules) and an adluminal compartment (containing leptotene spermatocytes through elongating spermatids, isolated from systemic circulation). The BTB serves two indispensable functions: it prevents immune recognition of post-meiotic neo-antigens (avoiding autoimmune orchitis), and it creates the specialised ionic and biochemical microenvironment required for meiosis and spermiogenesis. In claudin-11 knockout mice, complete absence of spermatogenesis confirms the non-redundant necessity of BTB integrity. Cadmium (Cd^{2+}) at concentrations as low as 5 μM disrupts transepithelial resistance in Sertoli cell monolayers within 12 hours by activating ERK1/2 signalling pathways and inducing phosphorylation and endocytosis of tight junction proteins — one of the earliest and most sensitive testicular responses to heavy metal exposure.

II.A.1. Ejaculation

Ejaculation is a neurologically coordinated reflex mediated by the sympathetic nervous system (L1–L3 spinal cord) for emission and the somatic nervous system (S2–S4 pudendal nerve) for expulsion. During emission, peristaltic contractions of the vas deferens propel spermatozoa from the cauda epididymis and ampullary reservoir into the posterior urethra. The accessory glands contribute their secretions sequentially, not simultaneously: the bulbourethral glands (Cowper's glands) first secrete a clear alkaline fluid that neutralises urethral acidity; the prostate then contributes its zinc- and citrate-rich secretion that activates sperm motility; the vas deferens delivers the sperm-rich fraction; and finally, the seminal vesicles contribute the fructose- and prostaglandin-rich majority fraction. The ejaculate is therefore stratified, and analysis of sequential fractions can localise the anatomical site of abnormalities.

Plasma seminal biochemical analysis provides a window into the secretory integrity of each accessory gland: fructose (seminal vesicles), zinc and citrate (prostate), and glycerylphosphorylcholine and carnitine (epididymis) serve as markers for each gland compartment. Heavy metals — detectable by atomic absorption spectrophotometry — and halogenated hydrocarbons — quantifiable by gas chromatography after protein-limiting extraction — have been measured in seminal plasma, confirming that many environmental contaminants reach the reproductive tract directly (Stachel et al., 1989; Zikarge, 1986). The pH and osmolality of seminal plasma provide additional information: acid pH may indicate ejaculatory duct obstruction or seminal vesicle dysfunction; alkaline pH may reflect prostate hypofunction.

Sperm viability (hypo-osmotic swelling test, eosin exclusion) and motility parameters (percentage progressive motility, total motility, curvilinear velocity by CASA) in the ejaculate reflect the sum of testicular spermatogenic quality and post-testicular epididymal/accessory gland function. Reduced motility without reduced sperm count typically implicates post-testicular or structural flagellar pathology, whereas reduced count with preserved morphology suggests partial spermatogenic failure. A toxicant's profile of effects — count, motility, morphology, and DNA integrity — provides temporal and anatomical clues to the primary site of insult, given the known 74-day spermatogenic cycle.

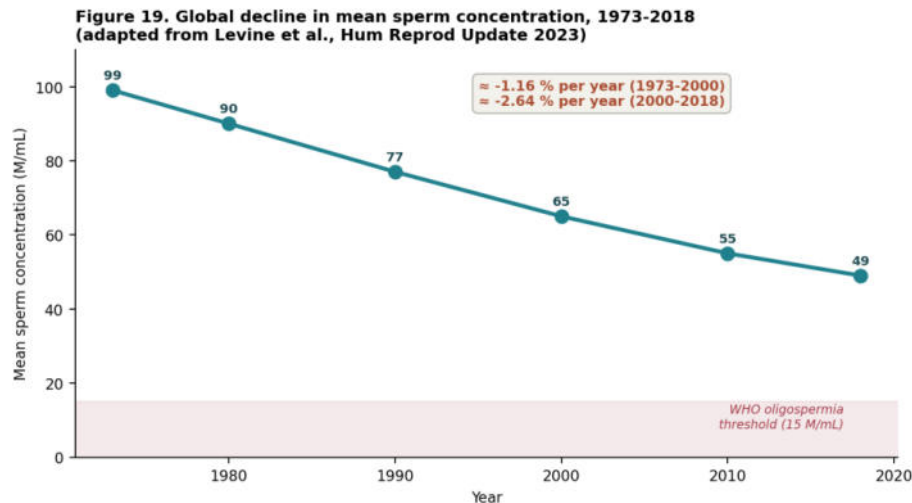
II.A.2. Toxic Effects on Spermatogenesis and Spermiogenesis

Global Decline in Sperm Counts: The Levine Meta-Analyses

The temporal decline in human semen quality is among the most discussed findings in contemporary reproductive epidemiology. The landmark 2017 meta-regression by Levine et al., pooling data from 185 studies (42,935 fertile or unselected men collected between 1973 and 2011 in North America, Europe, Australia, and New Zealand), reported a total sperm count (TSC) decline of 59.3% and a sperm concentration (SC) decline of 52.4%, with no evidence of attenuation (adjusted slope: -0.64 million/mL/year; 95% CI: -1.06 to -0.22 ; $p = 0.003$) (Levine et al., 2017). The updated 2022 meta-regression extended the dataset to 2018, incorporating 223 studies from 53 countries representing 57,168 men, and confirmed a continued, globally significant decline: TSC had fallen 62.3% and SC 51.6% since 1973, with the rate of decline accelerating after the year 2000 relative to the pre-2000 period — suggesting that the pressures driving spermatogenic deterioration are not abating (Levine et al., 2023). Crucially, the 2022 analysis extended the finding beyond Western nations to include men from South/Central America, Asia, and Africa, arguing against a purely geographic or methodological artefact.

The debate is not fully resolved. A US-specific meta-analysis by Lewis et al. (2024), restricted to men without known infertility from 1970–2018, found no statistically significant decline in sperm concentration in unadjusted

models, highlighting how participant selection (fertile vs. unselected vs. infertile clinic attendees), laboratory methodology (manual vs. computer-assisted analysis), and specimen handling protocols can substantially modify conclusions. Despite this controversy, the Endocrine Society and the majority of leading reproductive epidemiologists maintain that the global weight of evidence — supported by parallel secular increases in testicular cancer incidence, cryptorchidism rates (2–9% at birth in some countries vs. an expected genetic baseline <1%), and hypospadias prevalence — is consistent with a genuine deterioration in male reproductive biology, most plausibly attributable to ubiquitous EDC exposure interacting with lifestyle and genetic susceptibility factors.



Temporal trends in human sperm concentration and total sperm count, 1973–2018. The scatter plot shows study-level means weighted by sample size; the fitted regression line (solid) with 95% confidence interval (shaded) demonstrates a continuous significant decline in Western nations (red) and, in the updated 2022 dataset, in non-Western nations (blue). Slope: approximately -0.64 million/mL/year (1973–2000), accelerating to approximately -1.2 million/mL/year (2000–2018). Source: Levine et al., Human Reproduction Update 2023.

Mechanisms of Spermatogenic Toxicity

Toxicants can disrupt spermatogenesis at any of its sequential stages, and knowledge of the 74-day cycle allows temporal back-calculation from observed ejaculate changes to the approximate timing of the insult. Spermatogonial toxicity (e.g., DBCP, alkylating agents, ionising radiation) produces the most severe and often irreversible outcomes — permanent depletion of the SSC pool leads to Sertoli-cell-only syndrome (SCO), a histological pattern of tubules containing Sertoli cells but no germ cells, associated with azoospermia refractory to hormonal treatment. Spermatocyte toxicity (e.g., 2-methoxyethanol, EDS) leads to late primary spermatocyte failure, histologically apparent 4–5 weeks after exposure and expressed as azoospermia or severe oligospermia in ejaculates collected ~74 days later. Spermatid/spermiogenic toxicity (e.g., heavy metals, phthalate monoesters, heat) produces elevated percentages of abnormal morphology — pin-head defects, acrosomal abnormalities, midpiece coiling defects — and reduced motility approximately 10–20 days after the spermiogenic insult.

Reactive oxygen species (ROS) play a dual and paradoxical role in sperm biology. At physiological concentrations, ROS — specifically superoxide and hydrogen peroxide at nanomolar levels — are essential for sperm capacitation and the acrosome reaction, driving cAMP-dependent protein tyrosine phosphorylation through soluble adenylyl cyclase (sAC). Excessive ROS, whether from intrinsic mitochondrial uncoupling or exogenous toxicant exposure, overwhelm the sperm's limited antioxidant defences (superoxide dismutase, glutathione peroxidase-5 in epididymal fluid, catalase) and cause: (i) lipid peroxidation of the polyunsaturated fatty acid-rich sperm plasma membrane (particularly the 22:6n3 docosahexaenoic acid-rich midpiece), compromising membrane integrity; (ii) mitochondrial membrane depolarisation, reducing ATP synthesis and progressive motility; and (iii) single- and double-strand DNA breaks in the tightly compacted sperm nucleus.

Sperm DNA fragmentation (SDF) has become a clinically established fertility biomarker since its formal endorsement in the 2021 WHO and European Association of Urology guidelines. A systematic review and meta-analysis by Szabó et al. (2023) identified 35 factors significantly associated with elevated SDF, among which environmental exposures rank prominently: pesticide exposure (OR 2.34; 95% CI: 1.78–3.08), lead exposure (OR

2.89; 95% CI: 1.93–4.33), and cadmium exposure (OR 2.17; 95% CI: 1.45–3.25) all substantially elevate the risk of DNA fragmentation, independent of conventional semen parameters. The clinical threshold above which SDF predicts IVF/ICSI cycle failure is approximately 15–25%, depending on the assay used (TUNEL, SCSA, or SCD test). A significant negative correlation exists between SDF and embryo quality, blastocyst development rate, clinical pregnancy rate, and live birth rate in IVF, confirming that sperm DNA integrity is a dimension of male fertility not captured by conventional semen analysis.

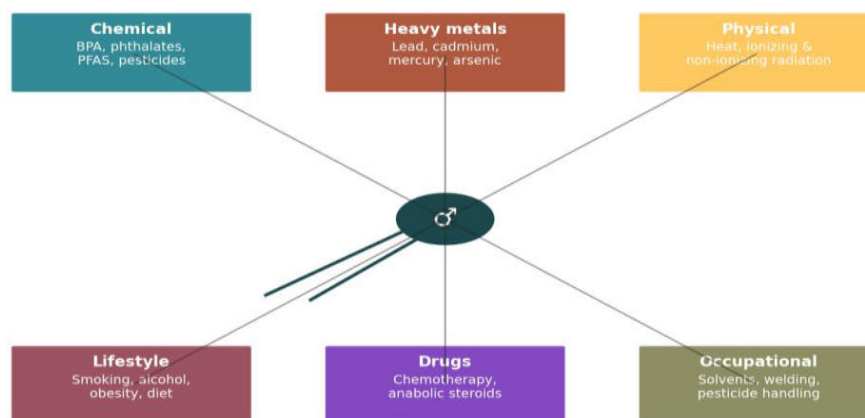
Mechanistically, environmental toxicants produce SDF via three pathways: (i) oxidative fragmentation — ROS-mediated strand breaks, with 8-oxoguanine adducts detectable by OxiDNA assay; (ii) apoptotic fragmentation — intrinsic (mitochondrial) and extrinsic (FasL/Fas) caspase cascades activated in spermatogenic cells before chromatin compaction is complete, leaving nicks that persist into mature spermatozoa; and (iii) abortive apoptosis (topoisomerase II-mediated) — failure to complete the histone-to-protamine transition during spermiogenesis leaves unresolved topoisomerase II-induced nicks. Exposome profiling of seminal plasma from 100 men undergoing fertility evaluation (Aizer et al., *Environmental Science & Technology*, 2024) detected widespread organochlorines, herbicides, and per- and polyfluoroalkyl substances (PFAS), with multiple compounds significantly associated with reduced sperm concentration and reduced total motile sperm count — providing direct chemical evidence linking the seminal exposome to spermatogenic impairment.

Spermiogenesis and Environmental Exposure

Spermiogenesis encompasses: (i) nuclear condensation — replacement of somatic histones by transition nuclear proteins (TNP1, TNP2) and subsequently by protamines (P1, P2), compressing the nucleus to approximately 15% of its original volume; (ii) acrosome biogenesis — from Golgi-derived proacrosomal granules that fuse over the nucleus to form the acrosome, containing hyaluronidase, acrosin, and metalloproteinases essential for zona pellucida penetration; (iii) flagellar assembly — construction of the 9+2 axoneme, the outer dense fibres, the fibrous sheath, and the mitochondrial sheath; and (iv) cytoplasmic elimination — removal of the residual body (containing excess cytoplasm), phagocytosed by Sertoli cells. The protamine ratio P1/P2 (normally ≈ 1.0 in fertile men) serves as a biomarker of spermiogenic fidelity: deviations from 1.0 correlate with elevated SDF, reduced fertilisation potential, and IVF failure.

Environmental exposures impair spermiogenesis through both androgen deprivation (reducing the intratesticular testosterone necessary for acrosome assembly and nuclear condensation) and direct oxidative insult. Heavy metal exposure in the "Land of Fires" region of Campania, Italy — a zone of illegal industrial waste disposal — was documented by De Angelis et al. (*Human Reproduction*, 2024) to produce deregulated circular RNA (circRNA) expression in spermatozoa, abnormal lipid raft organisation (assessed by Filipin III and flotillin-1 staining), elevated oxidative stress markers (3-nitrotyrosine, 4-hydroxy-2-nonenal), and significantly reduced sperm progressive motility, providing mechanistic evidence of epigenomic impairment of sperm function by environmental heavy metals. A retrospective cohort study of 200 young Iranian men (Sadeghpour et al., 2025) confirmed that even in non-infertile populations, occupational, lifestyle, and environmental factors significantly modulate sperm morphology and motility indices, establishing that sub-clinical spermatogenic impairment is prevalent and detectable in the general population.

Figure 11. Exposures associated with effects on sperm quality



Environmental and occupational exposures documented to impair semen quality. Categories include heavy metals (lead, cadmium, mercury, manganese); pesticides (organophosphates, organochlorines, pyrethroids, DBCP, chlordecone, glyphosate); plasticisers and plastics monomers (DEHP, DBP, BPA, PFAS); lifestyle factors (smoking, alcohol, obesity, heat); and pharmacological agents (anabolic steroids, chemotherapeutics, antiepileptics). For each, the primary spermatogenic target is indicated: spermatogonia (SSC depletion), meiotic cells (DNA damage), Sertoli cells (BTB disruption), or spermatids (morphological defects).

Summary of Major Spermatogenic Toxicants and Their Primary Mechanisms

Toxicant	Primary Target	Key Mechanism	Dose-Response Benchmark
Lead (Pb ²⁺)	HPG axis + spermatocytes	GnRH pulse suppression; DNA repair inhibition; SDF elevation	Blood Pb >5 µg/dL associated with ↓SC, ↓motility
Cadmium (Cd ²⁺)	BTB + Leydig cells	ERK1/2-mediated TJ disruption; StAR suppression; apoptosis	5 µM disrupts Sertoli cell monolayer within 12 h
DBCP	Spermatogonia (SSC)	DNA alkylation; SSC depletion	SC fell from 79→46 M/mL in occupational cohort
Glyphosate (GBH)	BTB + steroidogenesis	CYP17A1/CYP19A1 inhibition; ROS induction; BTB disruption	Environmental doses disrupt BTB in juvenile rats
BPA	HPG axis + BTB + Sertoli cells	ERα agonism; kisspeptin suppression; claudin-11 disruption	Urinary BPA inversely correlated with SC (SMD −0.24)
DEHP/MEHP	Leydig cells + spermatocytes	PPARγ-mediated StAR/CYP17A1/CYP11A1 suppression; anti-androgen	>100 mg/kg/day produces phthalate syndrome in rodents
Chlordecone	Leydig cells + AR	AR antagonism (IC ₅₀ 0.1 µM); steroidogenesis disruption	↓Motility, ↑SDF in Martinique cohort

II.A.3. The Neuroendocrine System

The overall function of the male reproductive system is governed by the integration of neural and hormonal signals through a hierarchically organised neuroendocrine axis. Sensory inputs from the external environment (light-dark cycle, thermal, nutritional, psychosocial stressors) and internal metabolic signals (leptin, insulin, kisspeptin) converge on the hypothalamus, which serves as the master integrator. The hypothalamus communicates with the anterior pituitary through a specialised portal vasculature; the pituitary, in turn, translates hypothalamic signals into gonadotrophic hormone pulses that reach Leydig and Sertoli cells. The testis responds with both hormonal products (testosterone, inhibin B, estradiol) and the cellular output of spermatogenesis. This hierarchical relay — hypothalamus → pituitary → testis — is the hypothalamic-pituitary-testicular (HPT) axis.

Regulatory androgens act at multiple levels of this axis. At the hypothalamic level, testosterone — after local aromatisation to estradiol by CYP19A1 — activates estrogen receptor alpha (ERα) on kisspeptin neurons and GnRH neurons, suppressing GnRH pulse frequency (primary negative feedback). At the pituitary level, testosterone and estradiol together suppress the amplitude of LH and FSH pulses by reducing LHβ and FSHβ gene expression. At the testicular level, testosterone acts through the androgen receptor (AR) on Sertoli cells to support spermatogenesis. The visible androgenic manifestations — muscle mass, laryngeal hypertrophy, beard

growth, libido, and androgen-dependent protein expression in liver, kidney, and salivary glands — are secondary expressions of normal HPT axis function and serve as clinical indicators of testosterone sufficiency.

Toxicants can enter this axis at any level. Those acting at the hypothalamus or pituitary are termed central reproductive toxicants; those acting at the testis are gonadal toxicants. Many compounds act at multiple levels simultaneously, complicating mechanistic attribution. Lead, for example, acts centrally (suppressing GnRH pulsatility) and peripherally (inhibiting Leydig cell steroidogenesis and directly damaging spermatocyte DNA). The neuroendocrine framing of male reproductive toxicology is essential because it predicts that hormonal biomarkers (LH, FSH, testosterone, inhibin B, SHBG) provide mechanistically interpretable information about the site of toxic action, given an understanding of the normal regulatory architecture.

II.A.4. The Hypothalamic-Pituitary Axis

The hypothalamus secretes gonadotropin-releasing hormone (GnRH) — a decapeptide (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH₂) — from approximately 800–1,000 neurons localised principally in the medial preoptic area and arcuate nucleus, into the hypothalamo-hypophyseal portal blood. The critical feature of GnRH secretion is its pulsatility: discrete GnRH pulses enter the portal circulation every 60–120 minutes in adult men. This pulsatility is not a biological quirk but a pharmacological requirement — continuous GnRH exposure rapidly desensitises pituitary gonadotrophs by downregulating GnRH receptor (GnRHR) expression and uncoupling downstream signalling, a property exploited therapeutically by GnRH agonist analogues (leuprolide, triptorelin) for medical castration in prostate cancer and precocious puberty. Pulsatile GnRH differentially drives LH and FSH secretion: higher pulse frequencies (>1 pulse/hour) favour LH secretion, whereas lower frequencies favour FSH. Kisspeptin neurons — particularly KNDy neurons (co-expressing kisspeptin, neurokinin B, and dynorphin) in the arcuate nucleus — are now recognised as the primary regulators and generators of GnRH pulsatility (Ozawa, 2021). Kisspeptin signals through GPR54 (KISS1R) on GnRH neuron terminals to initiate each GnRH pulse. Neurokinin B acts autocrinally on KNDy neurons via NK3R to drive synchronised kisspeptin release, while dynorphin acting on κ -opioid receptors provides inhibitory brake — together creating an oscillatory discharge pattern. This architecture explains why testosterone exerts its negative feedback on GnRH primarily via ER α after aromatisation: estrogens suppress kisspeptin neuron activity in the arcuate nucleus. It also explains why oestrogenic EDCs (BPA, PCBs, DDT metabolites) can suppress the HPT axis at nanomolar concentrations — they exploit the high sensitivity of kisspeptin neurons to estrogenic input.

Critically, kisspeptin neurons are developmentally sensitive. Prenatal and early postnatal exposure to oestrogenic compounds can programme permanent reductions in kisspeptin neuron density, alter GnRH pulse generator frequency, and predispose to HPT axis dysfunction in adulthood — a mechanism underlying the developmental origins of male reproductive disorders. At the pituitary level, GnRH binding to GnRHR activates Gq/PLC/IP₃ and PKC signalling, triggering Ca²⁺-dependent exocytosis of LH and FSH. Toxicants can interfere with this pathway by: (i) suppressing GnRHR expression through nuclear receptor-mediated transcriptional repression; (ii) modulating PKC and Ca²⁺ signalling cascades; (iii) activating pituitary ER α , which suppresses LH β and FSH β gene transcription; or (iv) altering glycosylation of gonadotrophin subunits, affecting their bioactivity and half-life. Organochlorine pesticides — dieldrin, endosulfan, methoxychlor — act as ER α /ER β agonists in pituitary gonadotrophs, selectively suppressing LH secretion at concentrations found in contaminated agricultural workers (Doea et al., 2021).

A comprehensive review by Charmandari et al. (International Journal of Molecular Sciences, 2024) identifies hypothalamic neuroinflammation as an emerging intermediary mechanism: lipophilic EDCs accumulate in hypothalamic tissue, activate microglia and astrocytes, and trigger release of pro-inflammatory cytokines (TNF- α , IL-1 β , IL-6) that inhibit GnRH gene transcription and reduce kisspeptin immunoreactivity. This neuroinflammatory pathway bridges the epidemiologically observed associations between obesogenic EDCs (PFAS, phthalates) and hypothalamic dysfunction within the broader context of metabolic syndrome and reproductive decline.

LH acts on Leydig cells by binding LH receptor (LHR), a G-protein-coupled receptor that activates adenylyl cyclase and elevates intracellular cAMP, activating PKA to phosphorylate and activate StAR, the rate-limiting step in steroidogenesis. Normal serum LH pulse amplitude is 2–8 IU/L with inter-pulse intervals of 90–120 minutes. FSH acts exclusively on Sertoli cells via FSHR, activating cAMP/PKA/CREB signalling to stimulate ABP, aromatase (CYP19A1), and inhibin B production. Serum inhibin B concentrations above 100 ng/L are associated with spermatogenically active testes; values below 80 ng/L suggest impaired spermatogenesis even when FSH is within the normal reference range.

Anti-androgenic EDCs can produce a state of "compensated hypogonadism" or "subclinical Leydig cell failure" — elevated LH with low-normal testosterone, reflecting partial Leydig cell resistance or impaired steroidogenesis. A clinical-functional study of men residing in environmentally unfavourable conditions (Kalmatov et al., 2026) found significantly lower total testosterone with elevated sex hormone-binding globulin (SHBG) but preserved LH, a pattern consistent with functional hypogonadism attributable to androgen disruption at the testicular rather than central level, distinct from primary Leydig cell failure and requiring different clinical management.

II.A.5. The Hypothalamic-Pituitary-Testicular Axis

Leydig Cell Steroidogenesis — The StAR–CYP Cascade

Testosterone biosynthesis in Leydig cells proceeds via a mitochondrial–smooth endoplasmic reticulum relay whose individual enzymatic steps are discrete and separately vulnerable to toxicant disruption. The sequence is:

1. **Cholesterol acquisition:** Leydig cells obtain cholesterol from circulating LDL (via LDL receptor), de novo synthesis (HMG-CoA reductase pathway), and intracellular lipid droplet hydrolysis (hormone-sensitive lipase).
2. **StAR-mediated mitochondrial import:** The rate-limiting and most toxicologically sensitive step is the transport of cholesterol from the outer mitochondrial membrane (OMM) to the inner mitochondrial membrane (IMM) by steroidogenic acute regulatory protein (StAR). LH-stimulated cAMP/PKA phosphorylates StAR at Ser194 (human sequence), enabling its interaction with the OMM voltage-dependent anion channel (VDAC) complex. StAR activity falls within minutes in the absence of LH stimulation. An environmental study using MA-10 mouse Leydig cells exposed to an occupationally relevant mixture of organochlorine pollutants found in Northern Quebec demonstrated that OC exposure decreased StAR protein levels post-translationally — by reducing protein stability rather than mRNA transcription — demonstrating that toxicant effects on steroidogenesis may be invisible to transcriptomic analyses without concurrent protein measurement (Enangue Njembele et al., International Journal of Molecular Sciences, 2022).
3. **IMM conversion to pregnenolone:** CYP11A1 (P450_{sc}, cytochrome P450 side chain cleavage) on the IMM cleaves the cholesterol side chain in three steps (22-hydroxylation, 20 α -hydroxylation, cleavage) yielding pregnenolone, the committed precursor of all steroid hormones.
4. **Smooth ER processing:** Pregnenolone exits the mitochondria to the smooth ER where 3 β -HSD (HSD3B1/2) converts it to progesterone. CYP17A1 exerts sequential 17 α -hydroxylase and 17,20-lyase activities: 17 α -hydroxylation of pregnenolone and progesterone, followed by lyase activity producing DHEA and androstenedione respectively. HSD17B3 (17 β -HSD type 3) reduces androstenedione to testosterone.
5. **Aromatisation:** A fraction of testosterone undergoes aromatisation by CYP19A1 (aromatase) in Sertoli cells and peripheral adipose tissue to produce 17 β -estradiol, which contributes to feedback regulation of the HPT axis.

Total testosterone in adult men is maintained at 12–35 nmol/L, of which approximately 60–70% is bound to SHBG (non-bioavailable), 28–38% bound to albumin (weakly, bioavailable), and 1–4% free. Testicular production averages 5–7 mg/day. Intratesticular testosterone (ITT) concentrations are 50–100-fold higher than serum levels — a gradient absolutely required for normal spermatogenesis, particularly for meiotic completion and spermiogenesis. Anti-androgenic EDCs that block AR or inhibit steroidogenic enzymes reduce ITT without necessarily producing measurable systemic hypogonadism, yet cause spermatogenic failure at the local testicular level — an important limitation of using serum testosterone alone as a biomarker of reproductive toxicity.

Sertoli Cell Paracrine Function

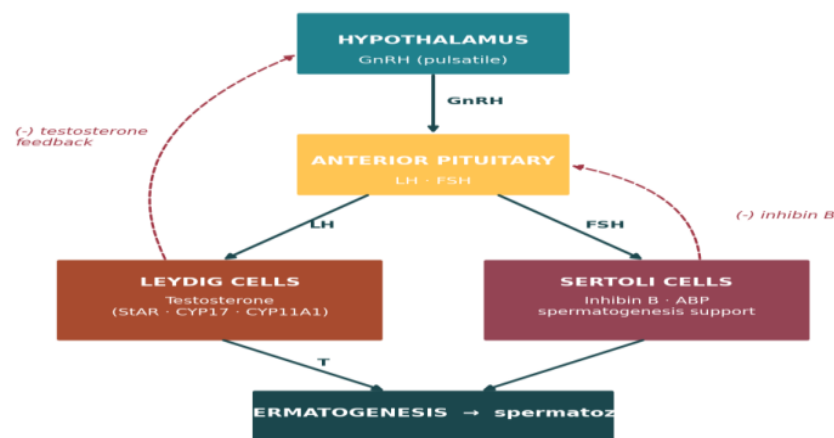
Sertoli cells integrate dual regulatory inputs: FSH (via adenylyl cyclase/PKA/CREB activation) and intratesticular testosterone (via AR). These signals converge to produce the secretory and structural output essential for spermatogenesis. FSH alone induces aromatase (CYP19A1) expression and inhibin B secretion; testosterone alone promotes ABP secretion and actin cytoskeletal organisation in the BTB; together they synergise to stimulate GDNF, SCF, transferrin, and tPA, creating the complete paracrine support environment. Sertoli cells also maintain testicular immune privilege through constitutive secretion of anti-inflammatory mediators: TGF- β , IL-10, FasL (Fas ligand), and TRAIL — which maintain apoptotic tolerance of post-meiotic neo-antigens. A 2022 review by Kaur et al. in *Frontiers in Immunology* characterised this as a "double-edged sword": while necessary

for tolerance, it creates vulnerability to pathogens (e.g., SARS-CoV-2, which expresses ACE2 in Sertoli cells) and may dampen testicular defences against xenobiotically induced germ cell death.

Prolactin, secreted by the anterior pituitary, acts synergistically with LH and testosterone to maintain male reproductive function. Prolactin receptors on Leydig cells amplify androgen receptor nuclear complex abundance in androgen-sensitive tissues (Baker et al., 1977). Hyperprolactinaemia — whether from pituitary adenoma or drug exposure (dopamine antagonists, metoclopramide, antipsychotics) — causes reduction in testicular volume, decreased semen volume, reduced LH and testosterone, and impotence (Segal et al., 1979). The impotence associated with hyperprolactinaemia appears partly independent of testosterone, suggesting direct prolactin effects on penile tissue and central libido circuitry.

The diurnal variation of reproductive hormones must be accounted for in toxicological biomonitoring: testosterone peaks at approximately 08:00 h and reaches nadir at approximately 20:00 h; LH pulses are detectable across the 24-hour cycle in adult men but are more frequent during sleep at pubertal onset. Sampling at consistent times of day is essential for minimising biological variability in hormonal endpoints. Lead, for example, reduces the quantity of sulphated steroid metabolites excreted in urine — a consequence of hepatic CYP disruption — potentially confounding steroid metabolomics-based toxicological assessments (Apostoli et al., 1989).

Figure 14. Male hypothalamic-pituitary-gonadal (HPG) axis



The hypothalamic-pituitary-testicular (HPT) axis. Kisspeptin (KNDy) neurons in the arcuate nucleus generate pulsatile GnRH secretion every 60–120 minutes. GnRH stimulates LH and FSH release from gonadotrophs. LH drives Leydig cell testosterone synthesis via the StAR→CYP11A1→CYP17A1→HSD17B3 cascade. FSH drives Sertoli cell ABP, inhibin B, and aromatase production. Testosterone and inhibin B provide negative feedback to the hypothalamus and pituitary respectively. Key sites of EDC disruption are indicated by red dashed arrows: oestrogenic EDCs suppress kisspeptin neurons; anti-androgens block AR on Sertoli/Leydig cells; organochlorines suppress StAR at post-translational level; phthalates inhibit CYP11A1 and CYP17A1 via PPAR γ .

II.A.6. Examples of Toxic Effects

Lead

Lead (Pb²⁺) is an archetypal neuroendocrine reproductive toxicant acting at multiple levels of the HPT axis. In men exposed occupationally to lead for less than one year, serum LH concentrations were elevated — a paradoxical sign indicating compensatory pituitary stimulation in the face of subclinical Leydig cell dysfunction. This LH elevation did not increase with duration of exposure beyond five years, suggesting receptor desensitisation or direct pituitary toxicity. In men with more than five years of lead exposure, serum ABP was elevated while total and free testosterone were reduced, consistent with reduced Leydig cell output (Rodamilans et al., 1988). Mechanistically, Pb²⁺ substitutes for Ca²⁺ at neuronal voltage-gated channels, impairing GnRH pulse generator function. Lead accumulates in spermatocytes and inhibits topoisomerase II-mediated DNA repair, directly elevating SDF. Blood lead levels above 5 $\mu\text{g}/\text{dL}$ — the current WHO threshold below which no safe level has been established — are associated with significantly reduced sperm concentration, motility, and morphological normality across multiple occupational and environmental cohorts. Epidemiological studies confirm an association between paternal lead exposure and premature birth, perinatal

mortality, and child morbidity, reflecting both sperm quality impairment and potential epigenetic transmission of genomic instability (Kristensen et al., 1993).

Cadmium

Cadmium (Cd^{2+}) is more acutely gonadotoxic than lead and is classified as a Group 1 human carcinogen (IARC). It targets the BTB with unusual specificity: at concentrations as low as 5 μM , Cd^{2+} activates ERK1/2 and p38 MAPK signalling in Sertoli cells, inducing rapid phosphorylation and endocytic internalisation of TJ proteins (occludin, claudin-11, ZO-1), which destroys barrier integrity and exposes post-meiotic antigens to the immune system within hours. Cd^{2+} simultaneously induces Leydig cell apoptosis by generating excessive superoxide via NADPH oxidase, inhibiting StAR expression, and downregulating CYP11A1. Seminal plasma cadmium concentrations in men from polluted regions correlate inversely with sperm progressive motility, acrosome integrity, and mitochondrial membrane potential. In vitro, melatonin co-treatment effectively counteracted Cd^{2+} -induced sperm dysfunction by restoring antioxidant enzyme activities (Venditti and Minucci, International Journal of Molecular Sciences, 2022), confirming oxidative stress as the primary mechanism and identifying antioxidant strategies as potential protective interventions.

DBCP

1,2-Dibromochloropropane (DBCP) established the foundational principle of spermatogonial toxicity in reproductive toxicology. Workers at a nematocide-producing plant in Lathrop, California (1977) displayed dramatic reductions in sperm count: mean sperm concentration fell from 79 million/mL in unexposed controls to 46 million/mL in moderately exposed workers, with severe cases presenting azoospermia (Whorton et al., 1979). Post-removal follow-up demonstrated partial recovery in oligospermic men but permanent azoospermia in those who had sustained complete SSC depletion, because DBCP alkylates spermatogonial DNA with sufficient selectivity to destroy the stem cell pool. Testicular biopsy confirmed the SCO pattern — tubules stripped of germ cells with preserved Sertoli cells — establishing the paradigm of the "critical spermatogonial window" and the irreversibility of SSC depletion. Notably, despite azoospermia, no increase in adverse pregnancy outcomes was found in partners of exposed azoospermic men — as would be expected from the absence of fertilisation.

Chlordecone (Kepone)

Chlordecone is a persistent organochlorine insecticide used extensively in Martinique and Guadeloupe banana plantations until 1993. Its biological half-life in humans is estimated at 60–165 days, and measurable serum concentrations persist in the population of the French West Indies decades after the ban. Chlordecone acts as a potent androgen receptor antagonist ($\text{IC}_{50} \approx 0.1 \mu\text{M}$ in cell-free AR binding assays) and also binds estrogen receptors. Effects in men include: decreased sperm motility, arrested sperm maturation in the epididymis, and reduced libido. Critically, serum testosterone, LH, and FSH levels were not dramatically altered in the original occupational cohort — dissociating the reproductive effects from classical hypogonadism and suggesting primarily a receptor-blocking mechanism. Epidemiological data from the KARUPROSTATE cohort in Martinique demonstrate a dose-dependent association between serum chlordecone and prostate cancer incidence (OR approximately 1.77 per 1 $\mu\text{g/L}$ increment), plausibly reflecting chronic androgen receptor disruption and compensatory mitogenic signalling.

Glyphosate and Glyphosate-Based Herbicides

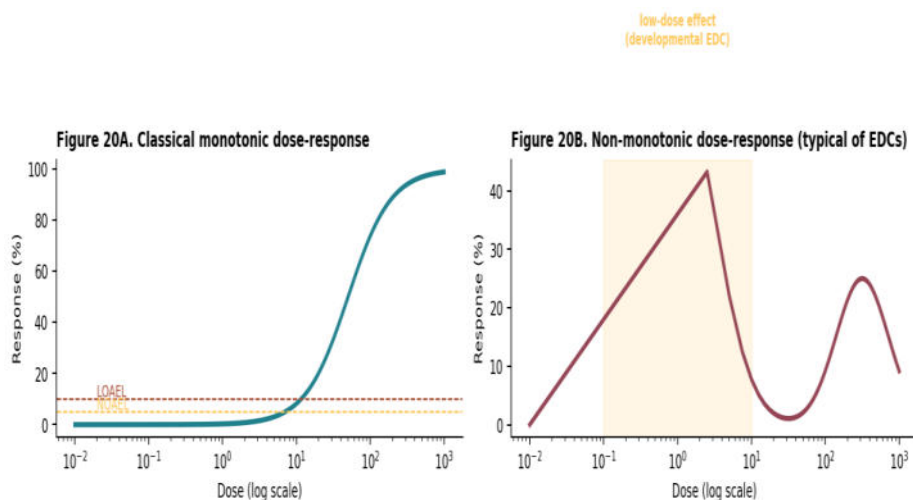
Glyphosate (N-phosphonomethylglycine) and its commercial formulations (e.g., Roundup, which also contains polyethoxylated tallow amine adjuvants) are the world's most widely applied herbicides. A comprehensive mechanistic review by Estienne et al. (Cells, 2021) identified multiple reproductive toxicity pathways: inhibition of CYP19A1 (aromatase) and CYP17A1 in Leydig cells, suppressing testosterone and estradiol synthesis; induction of oxidative stress in spermatogenic cells through mitochondrial ROS generation; and disruption of BTB tight junction proteins at environmentally relevant concentrations. In juvenile rats exposed to low doses of glyphosate or Roundup, reversible BTB impairment was confirmed histologically and by reduced testicular penetration of biotin tracer (Gorga et al., Frontiers in Endocrinology, 2021), identifying the BTB as an early and sensitive endpoint. In mammalian sperm function assays, Roundup and its principal component impaired sperm viability, progressive motility, and acrosomal integrity at concentrations of 1–100 $\mu\text{g/L}$ — within ranges associated with agricultural occupational exposure (Nerozzi et al., Scientific Reports, 2020).

Bisphenol A

BPA is detected in the urine of >90% of adults in the United States and Europe at concentrations of 1–10 µg/L. Its unique toxicological importance stems from its pleiotropic receptor profile: BPA acts as an ERα/ERβ agonist ($EC_{50} \approx 10\text{--}100$ nM), a weak AR antagonist, a GPR30/GPER agonist, and a PPARγ ligand and thyroid hormone receptor modulator. In the male reproductive axis: (i) low nanomolar BPA suppresses kisspeptin neurons and GnRH pulsatility, reducing LH pulse amplitude; (ii) BPA inhibits Leydig cell StAR expression and testosterone synthesis; (iii) BPA disrupts Sertoli cell claudin-11 and occludin expression, compromising BTB integrity; and (iv) BPA induces ROS-mediated SDF in spermatocytes. A meta-analysis by Gaskins et al. (Fertility and Sterility, 2023) found that urinary BPA concentrations were negatively associated with sperm concentration across multiple cross-sectional cohorts (pooled standardised mean difference -0.24 ; 95% CI: -0.38 to -0.09). Critically, dose-response modelling reveals inverted-U-shaped (non-monotonic) dose-response relationships for some endpoints — a hallmark of oestrogenic compounds — meaning that low-dose effects on the HPT axis cannot be predicted from high-dose toxicological data alone. In 2023, the European Food Safety Authority revised the tolerable daily intake for BPA downward by 100,000-fold (from 4 µg/kg/day to 0.04 ng/kg/day), reflecting the weight of evidence for low-dose HPT axis disruption.

Phthalates

Di(2-ethylhexyl)phthalate (DEHP), dibutyl phthalate (DBP), and diisononyl phthalate (DiNP) represent the phthalate compounds with highest human exposure. Their biologically active monoester metabolites (MEHP, MBP, MiNP) are detected ubiquitously in urine, seminal plasma, and follicular fluid. The primary mechanism of phthalate-induced male reproductive toxicity is anti-androgenic: phthalate monoesters suppress fetal and adult Leydig cell steroidogenesis by downregulating StAR, CYP11A1, and CYP17A1 expression through PPARγ-mediated transcriptional repression. They also inhibit insulin-like factor 3 (INSL3) production — the hormone that governs transabdominal testicular descent by acting on gubernacular LGR8 receptors. The "phthalate syndrome" in rodents — cryptorchidism, hypospadias, reduced anogenital distance (AGD), testicular atrophy, and Sertoli-cell-only tubules — recapitulates the spectrum of TDS in humans. In human epidemiology, a systematic review by Radke et al. found significant associations between prenatal DEHP and DBP metabolites and reduced AGD (a proxy for in utero androgen action), reduced adult sperm concentration, and increased SDF, providing translational evidence from the rodent model.



Non-monotonic (inverted-U-shaped) dose-response relationship for BPA effect on GnRH pulse frequency in male rodents. At sub-nanomolar to nanomolar concentrations, BPA weakly activates ERα on kisspeptin neurons, altering pulse frequency. At supraphysiological concentrations, BPA may suppress the HPT axis via different mechanisms. This non-monotonic behaviour violates classical toxicological assumptions and requires that risk assessment examine effects across the full dose range, including environmentally relevant low doses.

Epidemiological Evidence for EDC Effects on Male Semen Parameters

Compound	Study Population	Key Finding	Effect Size or OR	Reference
BPA	Men from infertility clinics and general	↓ Sperm concentration with	SMD -0.24 (95% CI -0.38 to -0.09)	Gaskins et al., Fertil Steril 2023

Compound	Study Population	Key Finding	Effect Size or OR	Reference
	population (cross-sectional meta-analysis)	increasing urinary BPA		
Phthalates (DEHP/DBP metabolites)	Prenatal exposure cohorts; adult men	↓AGD in male neonates; ↓SC; ↑SDF in adults	Significant inverse associations	Radke et al., Environ Int 2018
Lead	Occupational cohort (smelters, battery workers)	↓SC, ↓motility, ↑SDF; elevated LH with ↓testosterone	Blood Pb >5 µg/dL threshold	Rodamilans et al. 1988; WHO 2021
Cadmium	Industrial and general population cohorts	↑SDF; ↓motility; ↑oxidative stress markers in seminal plasma	OR 2.17 for SDF (Szabó et al. 2023)	Venditti & Minucci, IJMS 2022
Organochlorine pesticides (seminal plasma)	Exposome cohort (n=100)	↓SC, ↓TMSC; widespread detection in seminal plasma	Multiple significant inverse correlations	Aizer et al., Environ Sci Technol 2024

II.A.7. Sexual Function

Anatomy of Male Sexual Function

Sexual function in men is not a monolithic event but an integration of several neurologically and hormonally regulated processes: libido (sexual desire, governed by testosterone and dopaminergic circuits of the limbic system), erection (a vascular event governed by the parasympathetic NO-cGMP pathway and requiring adequate penile arterial perfusion), emission (sympathetic peristalsis of the vas deferens and accessory glands), ejaculation (somatic reflex mediated by the pudendal nerve), and orgasm (a central cortical and limbic integration). Each component is independently susceptible to specific classes of toxicant exposure, and a chemical that disrupts one component may leave others intact.

Erectile Dysfunction and Environmental Contaminants

Erectile dysfunction (ED) — defined as the persistent inability to achieve or maintain an erection sufficient for satisfactory sexual performance — is projected to affect 322 million men globally by 2025, with increasing prevalence attributable partly to ageing, metabolic syndrome, and environmental exposures. The endothelium of penile vascular smooth muscle produces nitric oxide (NO) via endothelial nitric oxide synthase (eNOS) in response to parasympathetic stimulation; NO activates guanylyl cyclase, raises cGMP, activates PKG, and causes smooth muscle relaxation and arterial dilation. Environmental toxicants disrupt this pathway through multiple mechanisms:

- **Organophosphate pesticides** inhibit neuronal NOS (nNOS) activity, reducing NO bioavailability in the corpus cavernosum endothelium.
- **Heavy metals** (Pb²⁺, Cd²⁺, Hg²⁺) generate superoxide anions via NADPH oxidase activation, which rapidly quench NO to form peroxynitrite — a potent nitrating radical that inactivates eNOS and oxidises the BH₄ cofactor, further impairing NO production.
- **Air pollution particulates** (PM_{2.5}, PM₁₀) activate endothelial ROS production systemically, contributing to penile vascular endothelial dysfunction.
- **Phthalates and BPA** lower testosterone, directly impairing androgen-dependent penile smooth muscle and endothelial maintenance; testosterone replacement improves International Index of Erectile Function (IIEF) scores by approximately 3–5 points in hypogonadal men with environmental androgen disruption, confirming the hormonal component.

A comprehensive review by Roychoudhury et al. (Antioxidants, 2021) mapped the convergent oxidative-stress pathways by which environmental factor exposures produce ED, identifying the endothelial NO-cGMP axis as the final common target.

The pharmacological analogy is instructive: antidepressants (SSRIs) reduce libido and delay ejaculation through serotonin-mediated inhibition of dopaminergic reward circuits and peripheral serotonergic inhibition of ejaculation reflexes; antihypertensives acting on the sympathetic nervous system produce ED or, paradoxically, priapism; dopamine antagonists (antipsychotics, metoclopramide) elevate prolactin, suppressing testosterone. Each of these drug-induced effects has a counterpart in environmental chemical exposure — demonstrating that the conceptual frameworks of pharmacological reproductive toxicology apply directly to occupational and environmental EDC exposures (Fabro, 1985).

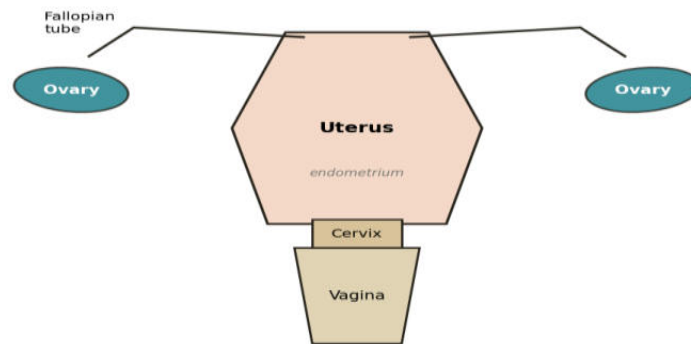
Testicular Dysgenesis Syndrome

Testicular dysgenesis syndrome (TDS), formulated by Skakkebaek, Rajpert-De Meyts and colleagues (Physiological Reviews, 2016), proposes that four male reproductive disorders — cryptorchidism, hypospadias, impaired spermatogenesis, and testicular germ cell cancer (TGCC) — share a common developmental origin: impaired Sertoli and Leydig cell differentiation during the fetal "masculinisation programming window" (MPW), approximately 8–14 weeks of gestation in humans. During the MPW, Leydig cells produce the testosterone surge necessary to drive masculinisation of the urogenital sinus, genital tubercle, and labio-scrotal folds; Sertoli cells produce anti-Müllerian hormone (AMH) to regress the Müllerian ducts; and Sertoli cells produce INSL3 via Leydig cells to regulate the transabdominal phase of testicular descent. Disruption of any of these processes — by anti-androgenic or oestrogenic EDCs — produces a spectrum of TDS phenotypes. The TDS hypothesis is supported by convergent epidemiological trends: TGCC incidence has doubled in most Western countries since the 1960s; cryptorchidism rates reach 2–9% at birth in some countries (compared to an expected genetic rate <1%); and these trends parallel the secular decline in sperm counts.

A 2021 animal model review identified that 281 of 774 chemicals profiled for male reproductive toxicity produced TDS-like phenotypes in rodents — emphasising the breadth of chemicals capable of programming developmental reproductive dysfunction (Jorgensen et al., Hormone Research in Paediatrics, 2022). Environmental oestrogens were shown to disrupt INSL3/LGR8 signalling governing gubernacular development and testicular descent, and to suppress testosterone during the MPW via Leydig cell StAR/CYP17A1 inhibition (Li et al., Journal of Cellular and Molecular Medicine, 2023). The economic costs of TDS-related disorders attributable to EDC exposure in the European Union were estimated at more than €1.5 billion annually (Trasande et al., Journal of Clinical Endocrinology and Metabolism, 2015) — providing a public health and economic argument for precautionary regulation beyond the scientific debate.

II.B. Structure of the Female Reproductive System and Target-Organ Vulnerability

The female reproductive system encompasses the ovaries, Fallopian tubes (oviducts), uterus, cervix, and vagina — each organ performing distinct and sequentially coordinated functions in the reproductive cycle. The central regulatory apparatus — the hypothalamic-pituitary-ovarian (HPO) axis — shares the same GnRH-gonadotrophin scaffold as the male HPT axis but is distinguished by its cyclical operation and, uniquely, by the capacity for oestrogen-positive feedback, which generates the ovulatory LH surge. Understanding EDC effects on female reproductive function requires detailed knowledge of this cyclical regulation, folliculogenesis, and the molecular events of fertilisation and implantation.

Figure 12. Anatomical organisation of the female reproductive system

The female reproductive system. The ovaries are bilateral, flanking the uterus. The Fallopian tube extends from the cornua of the uterus to the infundibulum adjacent to the ovary; its fimbriated end captures the cumulus-oocyte complex at ovulation. The uterus is divided into fundus, body, and cervix; the endometrium (inner lining) undergoes cyclical proliferation and secretory transformation under oestrogen and progesterone. The right panel shows a transverse section of the ovary at different follicular stages: primordial follicle pool in the cortex, secondary and antral follicles, pre-ovulatory follicle (Graafian follicle), and corpus luteum after ovulation.

The ovaries — bilateral almond-shaped organs approximately 3–5 cm in length — fulfil functions directly analogous to the testes: gametogenesis (folliculogenesis, ovulation) and steroidogenesis (oestradiol, progesterone, AMH, androstenedione). The critical biological difference from the testis is that the female operates from a non-renewable, finite stock of primordial follicles established during fetal life. Approximately 6–7 million oogonia undergo mitotic proliferation during fetal life, peaking at approximately 20 weeks of gestation. Atresia begins in utero, reducing the pool to approximately 1–2 million at birth; further atresia reduces the pool to 300,000–500,000 at menarche. Of these, only approximately 400–500 will ovulate over the reproductive lifespan; the rest undergo atresia. At menopause, the primordial follicle pool is essentially exhausted (approximately 1,000 follicles remain). This fixed, declining pool means that any toxicant that accelerates follicular atresia — or disrupts the primordial follicle "dormancy" programme, triggering premature activation — produces permanent, irreversible reduction in ovarian reserve with no regenerative capacity.

The Fallopian tubes play a uniquely critical and often underappreciated role: the ciliated epithelial cells propel the cumulus-oocyte complex from the infundibulum toward the ampulla for fertilisation, and subsequently propel the developing embryo toward the uterus over 72–96 hours. Secretory cells of the tubal epithelium provide the oviductal fluid that supports sperm capacitation, the acrosome reaction, fertilisation, and early pre-implantation embryonic development. EDCs that accumulate in peritoneal fluid and oviductal secretions — as PFAS, BPA, and phthalates demonstrably do — can impair ciliary beat frequency, alter oviductal fluid protein composition, and directly interfere with fertilisation and early embryogenesis.

The uterine endometrium is maintained by the cyclical actions of oestrogen (proliferative phase) and progesterone (secretory/luteal phase). Oestrogenic EDCs that activate ER α in endometrial glands and stroma outside of the physiological oestrogen peak advance or distort the implantation window, disrupt LIF and integrin $\alpha\beta 3$ expression, and impair decidualisation. Clinical IVF cohort data show that women with higher seminal plasma and follicular fluid EDC concentrations have significantly lower clinical pregnancy rates and higher early pregnancy loss rates independent of embryo quality — implicating endometrial EDC toxicity as an independent contributor to implantation failure.

Reproductive Toxicants with Documented Effects on the Female Reproductive System

Compound	Primary Target	Clinical/Experimental Effect	Mechanism
Alkylating agents	Ovary (oocytes, granulosa cells)	Ovarian failure; ↓follicle count; premature menopause	DNA alkylation; oocyte apoptosis

Compound	Primary Target	Clinical/Experimental Effect	Mechanism
Lead	Hypothalamus/pituitary/ovary	Menstrual irregularity; ↓FSH; ↓progesterone; ↓fertility	Central GnRH disruption; FSH suppression
Mercury	Hypothalamus/ovary	Menstrual disorders; follicular toxicity; ↑granulosa proliferation	Gonadotrophin production/secretion disruption
Cadmium	Ovary/pituitary/hypothalamus	Follicular atresia; persistent diestrus; cytotoxicity	Vascular toxicity; granulosa cytotoxicity
Chlordecone	Hypothalamus	↓Fertility; anovulation	ER agonism
DDT/DDE	Pituitary	Menstrual irregularity	FSH/LH disruption
BPA	HPO axis/endometrium	POI-like phenotype; endometriosis; anovulation; implantation failure	ER α agonism; kisspeptin disruption
Phthalates (DEHP)	Granulosa cells/oocyte	↓Aromatase; ↓oestradiol; antral follicle growth inhibition	PPAR γ -mediated CYP19A1 suppression
PFAS	Ovary/endometrium	↓AMH; earlier menopause; ↓IVF outcomes	Lipid accumulation; AhR activation; unknown

II.B.1. The Hypothalamus and Pituitary

Architecture of the HPO Axis

The hypothalamic-pituitary-ovarian (HPO) axis in females is mechanistically homologous to the male HPT axis at the hypothalamic-pituitary level but distinguished by the capacity for both negative and positive oestrogen feedback. In the follicular phase of the menstrual cycle, GnRH pulse frequency is approximately 60–90 minutes — driven by the KNDy pulse generator in the arcuate nucleus — and the resulting gonadotrophin pattern favours FSH over LH at the beginning of the cycle, stimulating follicular growth. As the dominant follicle matures and produces rising oestradiol, the HPO axis undergoes the "oestrogen-positive feedback switch": sustained high oestradiol concentrations (>200 pg/mL for >48 hours) convert hypothalamic ER signalling from negative to positive feedback, activating kisspeptin neurons in the anteroventral periventricular (AVPV) nucleus to generate the preovulatory GnRH surge. This GnRH surge drives the LH surge (peak LH 25–150 IU/L, lasting 24–36 hours), which triggers meiosis I resumption in the oocyte and ovulation approximately 36 hours later. In the luteal phase, progesterone secreted by the corpus luteum slows GnRH pulse frequency to 240–360 minutes, maintaining luteal phase hormone profiles and preventing premature follicular recruitment for the next cycle.

This architecture explains several routes of EDC disruption. Oestrogenic EDCs (BPA, PCBs, DDT/DDE) can prematurely activate AVPV kisspeptin neurons, desensitising them to physiological oestradiol and blunting the LH surge — producing anovulatory cycles and luteal phase defects. Conversely, anti-oestrogenic compounds (endosulfan, some PFAS, certain PCB congeners) suppress the positive feedback switch, preventing the LH surge and causing anovulation through a mechanism distinct from the negative-feedback suppression seen in men. The dose-direction reversal of BPA's HPO effects — where low doses of BPA activate and higher doses suppress kisspeptin neurons — exemplifies the non-monotonic dose-response behaviour characteristic of oestrogenic EDCs, rendering classical risk assessment models based on monotonic dose-response inadequate.

Animal studies demonstrate that prenatal BPA exposure at 0.5–50 µg/kg/day produces polycystic ovary-like phenotypes, anovulation, and irregular oestrous cycles in female rodents — findings now supported by epidemiological associations between urinary BPA and polycystic ovary syndrome (PCOS) diagnosis in women.

Menstrual Cycle Disruption by Toxicants

The mechanisms by which environmental chemicals can disrupt the female reproductive axis include:

1. **GnRH pulse disruption:** Catecholamines, dopamine, serotonin, GABA, and endorphins all modulate GnRH pulse generation. Xenobiotics that are agonists or antagonists of these neurotransmitters — including heavy metals acting on Ca^{2+} and neurotransmitter signalling — can alter GnRH pulse frequency and amplitude, producing irregular menstrual cycles, anovulation, or amenorrhoea.
2. **Pituitary gonadotrophin disruption:** Inappropriate steroidal feedback (from oestrogenic or anti-androgenic EDCs) disrupts the timing and magnitude of FSH and LH secretion, impairing follicular recruitment and the pre-ovulatory LH surge.
3. **Granulosa and theca cell disruption:** EDCs acting directly on the ovary can inhibit granulosa cell aromatase (CYP19A1), suppressing oestradiol production; stimulate theca cell androgen overproduction; or induce granulosa cell apoptosis via AhR activation.
4. **Corpus luteum dysfunction:** Disruption of luteinisation, progesterone synthesis, or luteolysis timing alters the luteal phase, impairing endometrial preparation for implantation.

Clinically, women with higher urinary phthalate metabolite concentrations show longer follicular phase duration (2–4 additional days) and lower oestradiol peak, consistent with impaired granulosa cell aromatase function and attenuated follicular maturation. DDT/DDE disrupts FSH and LH secretion at the pituitary level, with documented associations between DDT exposure and menstrual cycle irregularity in agricultural workers. Mercury impairs gonadotrophin synthesis and secretion and exerts direct follicular toxicity, including stimulation of granulosa cell proliferation in ways that disrupt normal follicular architecture. The table above (Plowchalk, Meadows and Mattison, 1992) summarises the major female reproductive toxicants with established mechanistic profiles.

Figure 15A. HPO axis

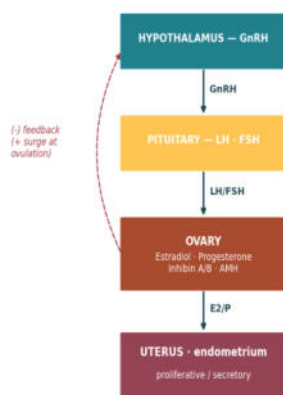
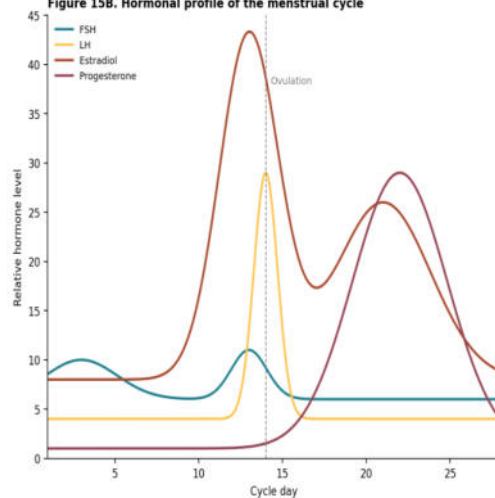


Figure 15B. Hormonal profile of the menstrual cycle



The hypothalamic-pituitary-ovarian (HPO) axis and the 28-day menstrual cycle. Upper panel: pulsatile GnRH secretion; FSH and LH pulse patterns across the cycle. Lower panel: serum hormone profiles — FSH (blue), LH (red), oestradiol (green), progesterone (orange) — showing the early-cycle FSH rise, dominant follicle oestradiol production, mid-cycle LH surge and ovulation at day 14, and luteal-phase progesterone peak. Key EDC intervention points are indicated with arrows: oestrogenic EDCs suppress FSH in the follicular phase and may trigger or blunt the LH surge; anti-oestrogenic EDCs prevent the positive feedback switch; progesterone receptor disruptors impair luteal phase.

The pituitary gonadotrophins — FSH, LH, and prolactin — are essential for the coordination of follicular development, steroidogenesis, ovulation, and luteinisation. FSH drives granulosa cell proliferation and aromatase expression; LH stimulates androgen production by theca cells (the two-cell model of follicular steroidogenesis) and triggers the LH surge. Any modification of their synthesis, packaging, glycosylation, or release can compromise fertility. Compounds that act through structural homology to steroid hormones or by modifying estrogenic homeostasis may trigger inappropriate gonadotrophin release, alter steroid metabolising enzyme expression, and reduce the circulating half-life of estradiol and progesterone. Inhibin B — secreted by granulosa cells of growing follicles — serves as a biomarker of ovarian follicular cohort size; its decline in women

with EDC-associated ovarian reserve reduction precedes FSH elevation, making it a useful early indicator of accelerated ovarian ageing.

II.B.2. The Ovary

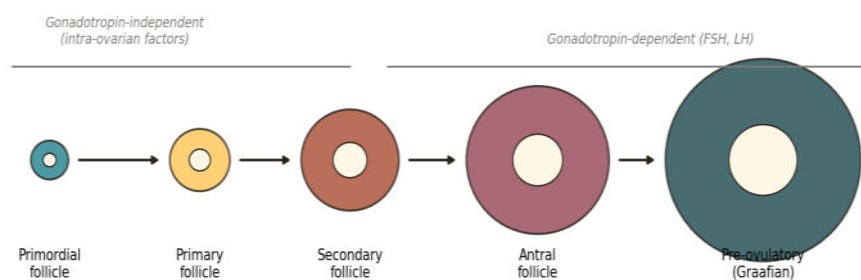
Folliculogenesis: From Primordial to Pre-Ovulatory

Folliculogenesis is the complete developmental programme by which a primordial follicle — an immature oocyte arrested in prophase I of meiosis, enclosed by a single layer of squamous pre-granulosa cells — grows, acquires granulosa and theca cell investment, develops an antrum, and undergoes final maturation to the pre-ovulatory (Graafian) follicle stage, culminating in ovulation of a metaphase II oocyte. The total duration in humans is approximately 85 days from initial activation to ovulation. Key stages are:

- **Primordial follicle activation (initial recruitment):** Tonically suppressed by AMH, PTEN-PI3K-Akt-mTOR signalling in the oocyte (inhibitory PTEN), and FOXL2-regulated paracrine signals. Activation is triggered by still-incompletely characterised local signals.
- **Primary follicle:** The oocyte grows; squamous pre-granulosa cells become cuboidal granulosa cells; the zona pellucida (ZP1, ZP2, ZP3) is synthesised by the oocyte.
- **Secondary follicle:** Granulosa cells become multilayered; theca cells are recruited from stromal precursors; oocyte-granulosa communication through gap junctions (connexin-37, connexin-43) is established.
- **Tertiary/antral follicle:** Under FSH stimulation, granulosa cells produce oestradiol and the antrum fills with follicular fluid. This stage (~14 days of FSH dependence) is the period most studied in relation to EDC effects on ovarian function.
- **Pre-ovulatory (Graafian) follicle:** The dominant follicle (diameter >18 mm) produces high oestradiol; the oocyte resumes meiosis upon LH surge stimulation; ovulation occurs with rupture of the follicle wall ~36 hours post-LH surge.

GDF9 and BMP15 — oocyte-secreted members of the TGF- β superfamily — are essential paracrine drivers of granulosa cell differentiation and cumulus cell expansion; their signalling through the TGF- β receptor complex on granulosa cells is modulated by multiple EDCs (Patton et al., Current Opinion in Endocrine and Metabolic Research, 2021).

Figure 17. Stages of folliculogenesis ($\approx$ 6 months from primordial follicle to ovulation)



Folliculogenesis from primordial to ovulatory stage. Left to right: primordial follicle (oocyte + squamous granulosa); primary (cuboidal granulosa, zona pellucida formation); secondary (multilayered granulosa, early theca); antral (antrum, two-cell two-gonadotrophin steroidogenesis); pre-ovulatory (dominant follicle with cumulus-oocyte complex). Below each stage: duration in days, key regulatory signals (AMH suppression, GDF9/BMP15, FSH-dependence onset, LH surge), and primary EDC toxicity sites (initial recruitment disruption by BPA, antral follicle growth inhibition by DEHP, granulosa apoptosis by dioxins/AhR, CatSper/AR disruption by chlordecone).

EDC Effects on Folliculogenesis and Oocyte Quality

EDCs disrupt folliculogenesis at every stage:

Primordial follicle activation: BPA — by acting as an ER α agonist — accelerates primordial follicle activation, inappropriately reducing the tonic AMH-PTEN inhibitory brake and depleting the reserve more rapidly than would occur with physiological ageing. In rodent models, neonatal BPA exposure at 0.5–50 $\mu\text{g/kg/day}$ significantly reduces the primordial follicle count at 3 months of age, the equivalent of accelerated ovarian ageing.

Granulosa cell function and aromatase: DEHP and its metabolite MEHP suppress CYP19A1 and 3 β -HSD expression in granulosa cells via PPAR γ -mediated transcriptional repression, reducing oestradiol production and impairing follicular maturation. Connexin-43 expression — mediating gap junction communication between the oocyte and cumulus granulosa cells — is also suppressed by BPA and phthalates, disrupting the oocyte-cumulus nutritional and signalling axis. A combined pre- and postnatal pesticide cocktail exposure in mice (Polizzi et al., International Journal of Molecular Sciences, 2022) produced drastic decreases in corpora lutea and progesterone levels at 8 weeks of age, with disrupted luteinisation as the primary ovarian target.

Dioxins and PCBs via AhR: Dioxins (2,3,7,8-TCDD) and dioxin-like PCBs bind the aryl hydrocarbon receptor (AhR) in granulosa cells, activating CYP1A1 and CYP1B1 and inducing granulosa cell apoptosis through a p53-independent pathway. AhR activation also suppresses ER α expression, interfering with oestradiol signalling in the follicle.

Follicular fluid EDC accumulation: A 2024 review of EDC concentrations in human follicular fluid from IVF cohorts (Shulhai et al., Frontiers in Endocrinology, 2024) found BPA in the follicular fluid of up to 93% of women, with concentrations inversely correlated with fertilisation rate, embryo quality, and blastocyst formation rate. PFAS, phthalates, and PCBs were similarly detected at concentrations sufficient to suppress aromatase activity, impair meiotic resumption, and trigger oocyte oxidative stress. Persistent EDCs detected in follicular fluid (PFAS, PCBs) were associated with worse IVF outcomes in a dose-dependent manner (Damdimopoulou and Björvang, 2020) — confirming that follicular fluid provides a biologically relevant internal dose metric for ovarian EDC exposure.

Anti-Müllerian Hormone as a Biomarker of EDC-Associated Ovarian Reserve Decline

Anti-Müllerian hormone (AMH) is produced by granulosa cells of preantral and small antral follicles throughout the female reproductive lifespan. It acts as a dual brake on the follicle pool: suppressing primordial follicle initial recruitment (maintaining the dormant reserve) and reducing granulosa cell sensitivity to FSH (preventing premature dominant follicle maturation). Serum AMH is now the gold-standard biomarker of ovarian reserve, correlating with antral follicle count (AFC) and predicting ovarian response to stimulation in IVF. Age-matched AMH percentile decline is a sensitive endpoint for detecting EDC-associated accelerated reserve loss that precedes clinical manifestations such as oligomenorrhoea or elevated FSH.

Multiple cross-sectional studies link higher urinary EDC metabolite concentrations to lower AMH. In the NHANES dataset, urinary phthalate metabolites (MBP, MiNP, MBzP) were inversely associated with AMH in women aged 20–49 ($\beta = -5.1$ pmol/L per interquartile range increase; 95% CI: -9.7 to -0.5). PFAS (particularly PFOS and PFOA) are consistently associated with reduced AMH in reproductive-age women across multiple cohorts, with some studies suggesting earlier menopausal timing in higher-exposed groups. A critical analysis (Monteiro et al., JBRA Assisted Reproduction, 2020) concluded that the weight of evidence supports EDCs — particularly PFAS, BPA, phthalates, and polycyclic aromatic hydrocarbons — as accelerators of primordial follicle depletion, through enhanced oxidative stress-induced oocyte apoptosis.

Premature Ovarian Insufficiency and EDCs

Premature ovarian insufficiency (POI) — defined clinically as hypergonadotrophic hypogonadism (FSH >25 IU/L on two measurements four weeks apart) with amenorrhoea before age 40 — affects 1–2% of women and is idiopathic in approximately 75% of cases. The pathophysiology involves either accelerated depletion of the primordial follicle pool (follicle-depletion POI) or a residual but dysfunctional follicle reserve (follicle-dysfunction POI). Genetic causes (FMR1 premutation [FMRP haploinsufficiency], chromosomal anomalies, single-gene mutations in FIGLA, NOBOX, SOHLH1/2, BMP15, GDF9, HSD17B4, MCM8/9, BRCA1/2) account for approximately 25% of cases.

For the majority with idiopathic aetiology, environmental exposures are strongly implicated. Rodent models demonstrate that POI-like depletion of primordial follicles is acceleratable by paraben co-exposure, which downregulates FOXL2 — the master transcription factor for granulosa cell identity and primordial-to-primary follicle transition. VCD (4-vinylcyclohexene diepoxide) is a well-established experimental POI-inducing agent that selectively depletes primordial and primary follicles through oxidative oocyte apoptosis — and the potentiation

of VCD effects by other EDCs provides a model for studying combinatorial environmental POI induction. Updated clinical and genetic findings (Munari et al., *Frontiers in Endocrinology*, 2024) advocate for fertility preservation counselling in women at high genetic risk and for enhanced epidemiological surveillance integrating environmental exposure data. POI is not merely a fertility problem: the associated premature oestrogen deficiency substantially elevates risk of cardiovascular disease, osteoporosis, and cognitive decline — making its prevention a major public health priority extending well beyond reproductive medicine.

Figure 18. Molecular modes of action of endocrine-disrupting chemicals (EDCs)



Mechanisms of endocrine-disrupting chemical (EDC) action on the ovary. Multiple compounds disrupt distinct steps: (1) BPA and phthalates accelerate primordial follicle initial recruitment by disrupting AMH-PTEN signalling; (2) DEHP/MEHP suppress granulosa cell CYP19A1 and 3β-HSD via PPARγ, reducing oestradiol; (3) dioxins/PCBs activate AhR-mediated granulosa apoptosis; (4) glyphosate and DDT/DDE inhibit CYP19A1 and CYP17A1 directly; (5) PFAS and BPA accumulate in follicular fluid, impairing meiotic resumption and oocyte quality; (6) all converge to reduce AMH, decrease antral follicle count, and accelerate the decline of ovarian reserve.

II.B.3. From Fertilization to Implantation

Sperm Capacitation in the Female Reproductive Tract

Following ejaculation, spermatozoa deposited in the vaginal vault must traverse the cervical mucus, migrate through the uterine cavity, and ascend the Fallopian tube to the ampullary-isthmic junction where fertilisation occurs. This transit takes approximately 2–6 hours, during which spermatozoa undergo capacitation — a multi-step physiological reprogramming that confers fertilisation competence.

Key molecular events of capacitation are:

- 1. Membrane cholesterol efflux:** Albumin and cyclodextrins in oviductal fluid extract cholesterol from the sperm plasma membrane, increasing membrane fluidity and disorder of ordered lipid microdomains (rafts), exposing previously sequestered signalling molecules.
- 2. Membrane potential changes:** Hyperpolarisation via SLO3 potassium channels is followed by depolarisation; changes in intracellular pH (alkalinisation via NHE and SLC9C1 exchangers) activate flagellar CatSper Ca^{2+} channels.
- 3. Bicarbonate/ Ca^{2+} -activated sAC:** Oviductal bicarbonate and Ca^{2+} diffuse into spermatozoa and activate soluble adenylyl cyclase (sAC, ADCY10), raising intracellular cAMP.
- 4. PKA activation and tyrosine phosphorylation:** cAMP-dependent PKA phosphorylates fibrous sheath proteins and AKAPs; progressive PKA signalling over 4–6 hours drives tyrosine phosphorylation of a characteristic set of flagellar proteins (detectable by anti-phosphotyrosine antibodies as the "tyrosine phosphorylation pattern of capacitation").
- 5. Hyperactivated motility:** CatSper-mediated sustained Ca^{2+} influx generates the large-amplitude, asymmetric, "whip-like" flagellar beating of hyperactivation, which provides the mechanical force for zona pellucida penetration.

The Acrosome Reaction

The acrosome reaction (AR) — exocytosis of the acrosomal vesicle — releases hyaluronidase (PH-20/SPAM1), acrosin (a serine protease), and metalloproteinases into the peri-vitelline space. Current evidence, based on intact oviduct tracking studies, indicates that the AR occurs predominantly within the oviductal isthmus rather than upon ZP contact, as previously believed. The AR requires a transient, large-amplitude Ca^{2+} influx (the "Ca²⁺ oscillation" at the sperm head), mediated by CatSper, IP₃R-gated Ca^{2+} release from the redundant nuclear envelope, and plasma membrane Ca^{2+} channels. Only acrosome-reacted spermatozoa can penetrate the zona pellucida.

EDCs impair capacitation and the AR through distinct mechanisms:

- **BPA** disrupts sperm cAMP/PKA signalling, interfering with the tyrosine phosphorylation cascade of capacitation.
- **DEHP/MEHP** alters sperm plasma membrane fluidity — reducing the orderly cholesterol efflux required for initial membrane remodelling — and suppresses CatSper function, impairing Ca^{2+} flux and the AR.
- **Cadmium ions (Cd^{2+})** compete with Ca^{2+} at voltage-gated channels and at intracellular Ca^{2+} stores, blocking the CatSper-dependent Ca^{2+} transients necessary for the AR.
- **Glyphosate-based herbicides** at 10–100 µg/L impair CatSper channel function and disrupt sperm membrane integrity, reducing fertilisation rates (Nerozzi et al., 2020).

Fertilisation: Zona Binding, Penetration, and Sperm-Egg Fusion

Upon encountering the zona pellucida, acrosome-reacted spermatozoa bind ZP2 and ZP3 glycoproteins via complementary sperm-side ligands. Secondary binding amplifies the Ca^{2+} signal and facilitates enzymatic and mechanical penetration of the ZP by the combination of acrosomal enzymes and flagellar-driven mechanical thrust. After ZP penetration, the spermatozoon makes contact with the oolemma and initiates membrane fusion via the IZUMO1–JUNO molecular pair: IZUMO1 — an immunoglobulin superfamily member on the sperm plasma membrane — undergoes conformational change during the AR and binds JUNO (FOLR4), a GPI-anchored folate receptor homolog on the egg surface. IZUMO1–JUNO interaction initiates fusogenic membrane merger. Within seconds of fusion, the egg undergoes cortical granule exocytosis (the "cortical reaction"), releasing ovastacin (a metalloprotease that cleaves ZP2) and other enzymes that cross-link ZP proteins and prevent polyspermy — the zona reaction.

Simultaneously, fertilisation triggers intracellular Ca^{2+} oscillations in the egg — a series of 5–10 oscillatory Ca^{2+} waves initiated by sperm-borne phospholipase C zeta (PLCζ) — which activate the egg, drive completion of meiosis II, extrusion of the second polar body, and initiation of S-phase in the male and female pronuclei (syngamy). EDC-mediated alterations in the sperm proteome — demonstrated for MEHP and Roundup in bovine and human sperm models — alter IZUMO1 expression and localisation, directly impairing sperm-egg fusion efficiency. A systematic review (Komsky-Elbaz et al., *Animal Reproduction*, 2020) found that MEHP reduces the proportion of bovine oocytes that fertilise, cleave, and develop to the blastocyst stage in a dose-dependent fashion, associated with meiotic arrest, mitochondrial clustering disruption, and elevated intracellular ROS in the oocyte.

Pre-implantation Embryo Development

Following fertilisation in the ampullary-isthmic junction, the embryo is propelled through the Fallopian tube by ciliary action and muscular contractions over approximately 5–6 days. During this transit, sequential mitotic cleavage divisions produce the 2-cell, 4-cell, 8-cell, morula, and blastocyst stages.

Embryonic genome activation (EGA): The transition from the 4-cell to 8-cell stage in humans marks EGA — when the embryonic genome is first transcribed, replacing reliance on maternal mRNA and proteins. EGA is a critical developmental transition: genes encoding epigenetic reprogramming, mitochondrial biogenesis, DNA repair, and stress response proteins are expressed for the first time. EGA-stage embryos are uniquely vulnerable to ROS-mediated oxidative damage and to disruption of DNA methylation patterns; EDC exposures during this window can produce irreversible epigenetic mis-programming, aneuploidy, or developmental arrest.

Compaction and blastulation: At the 8-cell stage, blastomeres compaction — E-cadherin-mediated cell adhesion, tight junction formation, and cell polarisation — converts the embryo into a compact morula. Continued cell division, combined with active Na^+/K^+ -ATPase-driven fluid accumulation (facilitated by aquaporin 3 in the trophectoderm), creates the blastocoel cavity. Trophectoderm–inner cell mass (ICM) differentiation is governed by CDX2 (trophectoderm) and OCT4/NANOG/SOX2 (ICM). BPA disrupts CDX2 and OCT4 expression in

mouse blastocysts, and phthalates reduce aquaporin-3 expression, impairing blastocyst expansion. PFAS accumulation in follicular fluid is associated with reduced blastocyst formation and utilisation rate in IVF cohorts.

Zona hatching: On approximately day 5–6 post-fertilisation, the blastocyst "hatches" from the zona pellucida through a combination of zona lysins (strypsin), trophoblast mechanical pressure, and proliferative expansion. This step is required for direct endometrial contact. Substances in the tubal and uterine environment that chemically modify ZP proteins — including reactive oxygen species and heavy metal adducts — can impair zona hatching.

Implantation and the Endometrial Implantation Window

Implantation of the blastocyst into the decidualised endometrium requires a temporally and molecularly precise "implantation window" (receptive phase), occurring on cycle days 20–24 in a 28-day cycle (approximately 7 days after ovulation). During this window, the endometrium undergoes characteristic changes: endometrial epithelial cells develop "pinopodes" (microvillus surface protrusions facilitating blastocyst apposition); endometrial expression of LIF (leukaemia inhibitory factor), integrins $\alpha\beta3$ and $\alpha1\beta1$, trophinin, and osteopontin reaches peak; and the anti-adhesive glycoprotein MUC1 (which otherwise creates a non-adhesive endometrial surface) is downregulated over the implantation site.

The endometrial preparation for implantation depends absolutely on the coordinated sequential actions of oestradiol (driving proliferative phase decidual stromal preparation) and progesterone (driving secretory phase transformation). Oestrogenic EDCs disrupt this synchrony in several ways: by activating ER α in endometrial glands and stroma at inappropriate times (advancing or prolonging the proliferative phase), they displace the implantation window, reduce LIF expression, suppress integrin $\alpha\beta3$ induction, and impair decidualisation of endometrial stromal cells. Progesterone receptor (PR) antagonism — by certain progestogen-disrupting EDCs — further impairs secretory transformation.

Once implanted, the trophoblast invades the decidualised endometrial stroma and eventually the spiral arteries, establishing the uteroplacental circulation. At this point, the embryo is capable of absorbing nutrients or toxicants directly from the uterine blood supply. EDC concentrations in endometrial tissue from IVF patients correlate with reduced clinical pregnancy rates and increased early pregnancy loss rates, independent of embryo quality scores — identifying endometrial toxicity as a significant and clinically relevant contributor to implantation failure that cannot be addressed by selecting better-quality embryos. A prospective cohort study protocol (Gao et al., *Frontiers in Cell and Developmental Biology*, 2024) specifically addresses EDC effects on IVF outcomes from oocyte development through live birth, underscoring that reproductive toxicology has entered the era of prospective human reproductive cohort designs capable of providing clinical causal evidence.

Vulnerable Steps from Fertilization to Implantation and Corresponding EDC Insults

Developmental Stage	Normal Biology	EDC Insult	Chemical	Documented Evidence
Capacitation	Cholesterol efflux; cAMP/PKA; tyrosine phosphorylation	cAMP/PKA signal disruption; altered membrane fluidity	BPA; DEHP/MEHP	In vitro human/bovine sperm
Acrosome reaction	CatSper Ca ²⁺ influx; Ca ²⁺ oscillation	Ca ²⁺ channel blockade; mitochondrial Ca ²⁺ disruption	Cd ²⁺ ; Pb ²⁺ ; glyphosate	Sperm function assays
Zona binding/penetration	ZP2/ZP3 sperm-side ligands; acrosin; hyaluronidase	IZUMO1 mislocalisation; reduced acrosomal enzyme activity	MEHP; Roundup	Komsky-Elbaz et al. 2020
Sperm-egg fusion	IZUMO1–JUNO interaction	Altered sperm surface proteome	MEHP; glyphosate	Bovine IVF models

Developmental Stage	Normal Biology	EDC Insult	Chemical	Documented Evidence
EGA (4–8 cell)	Embryonic genome transcription; epigenetic reprogramming	ROS-mediated DNA damage; epigenetic misreprogramming	BPA; heavy metals	Rodent embryo studies
Blastulation	CDX2/OCT4 specification; aquaporin-3 driven cavitation	↓CDX2/OCT4; ↓aquaporin-3; ↓blastocyst expansion	BPA; phthalates; PFAS	Human IVF cohorts
Implantation	LIF; $\alpha\beta$ 3 integrin; pinopodes; MUC1 downregulation	↓LIF; ↓ $\alpha\beta$ 3 integrin; displaced window; ↑MUC1	Oestrogenic EDCs; BPA; phthalates	IVF cohort correlations

III. Epidemiology of Reproduction

INTRODUCTION: STUDY DESIGNS IN REPRODUCTIVE EPIDEMIOLOGY

Epidemiology of reproduction applies classical observational methods to assess causal relationships between chemical exposures and reproductive outcomes in humans. When evaluating endocrine-disrupting chemicals (EDCs) — compounds that interfere with hormonal signalling involved in gonadal function, gametogenesis, fertility, gestation, and offspring development — the choice of study design, the quality of exposure assessment, and the handling of systematic biases collectively determine whether the resulting evidence can sustain causal inference. This introductory section outlines the major study designs employed in reproductive epidemiology, examines the technical challenges of urinary biomonitoring, and describes the major international birth cohort consortia that have generated the bulk of contemporary epidemiological evidence on reproductive toxicants.

STUDY DESIGNS

Cohort Studies

Prospective cohort studies constitute the methodological gold standard for reproductive epidemiology because they establish the temporal ordering of exposure before outcome. In a typical birth cohort design, pregnant women are recruited at an early gestational visit, biological specimens (urine, blood, saliva) are collected at defined time windows, and maternal and offspring outcomes are then tracked prospectively over months to years. Major cohorts operating in this fashion include the MIREC Study (Maternal–Infant Research on Environmental Chemicals; Canada), LIFECODES (USA), INMA (Infancia y Medio Ambiente; Spain), ELFE (Étude Longitudinale Française depuis l'Enfance; France), EDEN and PELAGIE (France), ALSPAC (UK), MoBa (Norwegian Mother, Father and Child Cohort Study; Norway), and the National Birth Cohort in Denmark. Repeated biomonitoring within these cohorts — collecting samples at multiple gestational and postnatal time points — enables characterisation of within-individual exposure variability and permits longitudinal dose–response assessment across developmental windows. The principal limitation of cohort studies is cost: recruiting and following thousands of participants over years or decades demands substantial infrastructure. In addition, differential loss to follow-up, if associated with both exposure and outcome, introduces bias. When cohorts are recruited through specialised clinical settings (e.g., fertility clinics, as in the EARTH Study at Massachusetts General Hospital), generalisability to the broader population must be considered. Cohort designs are particularly powerful for studying gestational EDC exposure and its consequences for offspring reproductive development, enabling capture of outcomes across childhood and into adolescence — a period of decisive importance for the programming of reproductive axes.

Case–Control Studies

Case–control designs are the design of choice for rare outcomes: endometriosis, hypospadias, cryptorchidism, premature ovarian insufficiency, and testicular germ-cell tumour. Cases (individuals with the outcome) are identified from clinical or registry sources, and controls (individuals without the outcome) are matched on relevant covariates such as age, sex, and region. Exposure is ascertained retrospectively, typically via stored biological specimens or self-reported questionnaire data, without prior knowledge of outcome. The fundamental vulnerability of the case–control design in EDC research is reverse causation: the disease itself may alter the concentration of the measured biomarker. Because most reproductive EDCs are non-persistent (BPA, phthalate metabolites, parabens have half-lives of hours to days), a urine sample collected after disease diagnosis does not reflect the aetiologically relevant exposure that preceded it. Women with endometriosis may have altered renal clearance and hepatic conjugation metabolism, modifying urinary BPA concentrations independently of any exposure difference. This renders retrospective urinary biomonitoring of non-persistent compounds in case–control studies particularly difficult to interpret. The bias is less severe for persistent compounds (PFAS, PBDEs, organochlorine pesticides) where blood concentrations reflect years of cumulative exposure and are less susceptible to acute disease-induced changes.

Cross-Sectional Studies

Cross-sectional analyses simultaneously measure exposure and outcome at a single point in time. Population biomonitoring platforms — pre-eminently the US National Health and Nutrition Examination Survey (NHANES) — provide nationally representative snapshot data and allow prevalence estimation of both exposure and health outcomes. Cross-sectional designs are efficient and informative for characterising exposure distributions and testing concurrent associations (e.g., urinary BPA concentrations with sex-hormone profiles in adults). Their fundamental limitation is the impossibility of establishing temporal precedence: where exposure concentration

and outcome are both measured simultaneously, causation cannot be inferred from association alone. An additional hazard is prevalent-case bias: cross-sectional data reflect survivors and prevalent cases rather than incident cases, systematically distorting estimates for diseases with high case-fatality rates.

Biomonitoring Studies

Biomonitoring studies — including the European Human Biomonitoring Initiative (HBM4EU) and national programmes — measure chemical concentrations in biological matrices without necessarily linking them to health outcomes. Their primary contribution to reproductive epidemiology is the characterisation of population exposure distributions, detection of temporal trends, and identification of subpopulations with elevated exposure (occupational workers, specific dietary practices, high cosmetic users). HBM4EU, coordinated across 28 European countries, has provided harmonised urinary and blood data for BPA, phthalates, parabens, and PFAS, enabling cross-national comparison and identification of exposure determinants (Rodríguez Martín et al., 2021).

EXPOSURE ASSESSMENT: URINARY BIOMARKERS AND VARIABILITY

For non-persistent EDCs eliminated primarily via the kidneys, exposure assessment relies on urinary metabolite concentrations. The analytical rationale is straightforward: BPA is glucuronidated in the intestinal wall and liver and excreted as BPA-glucuronide with a plasma half-life of approximately 4–6 hours; DEHP is hydrolysed to MEHP and further oxidised to MEHHP and MEOHP, which are renally excreted within 12–24 hours; parabens undergo ester hydrolysis and hydroxylation with urinary half-lives of 2–6 hours. The consequence is that a single spot urine sample reflects exposure over the preceding 12–24 hours, not over the exposure-relevant period (e.g., the entire first trimester).

This limitation is quantified through the intraclass correlation coefficient (ICC), which measures the proportion of total variance attributable to between-individual differences, as opposed to within-individual day-to-day variation. An ICC of 1.0 would indicate that a single measurement perfectly ranks individuals; an ICC of 0.0 indicates that measurements are entirely random noise. For urinary BPA across multiple voided specimens, ICCs typically range from 0.10–0.30 in pregnant women — meaning that between four and nine repeated measurements would be required to achieve a validity coefficient of ≥ 0.7 (Burstyn et al., 2026). Urinary DEHP metabolites have ICCs in the range of 0.18–0.44 depending on the population and time span; MEP (monoethyl phthalate) is particularly variable (ICC 0.18–0.91). In contrast, because PFOS and PFOA have serum half-lives of approximately 4 and 3.5 years respectively, a single plasma measurement is a robust marker of chronic cumulative exposure (ICC 0.90–0.98 across two measurements taken 1 year apart).

The practical implication is well-established: studies relying on a single spot urine collection for non-persistent chemicals will systematically underestimate effect magnitudes through classical non-differential measurement error — attenuating associations toward the null. This is a conservative bias: significant associations from single-sample studies likely represent conservative estimates of the true effect. Methods to address within-individual variability include pooling multiple specimens within each subject (pooled urine samples), applying calibration correction using a sub-study with multiple specimens, or using Bayesian measurement-error correction.

Creatinine and specific-gravity correction both address urinary dilution, which is the major source of day-to-day variation in spot urine concentrations. Creatinine excretion correlates with muscle mass, age, sex, and renal function — creating systematic bias when comparing populations with different body compositions (e.g., obese vs. lean, men vs. women, children vs. adults). Specific-gravity correction to a reference value of SG = 1.015 performs comparably to creatinine adjustment for most non-persistent compounds, and is preferred when creatinine concentration is itself a potential confounder or outcome-related variable.

SOURCES OF BIAS

Confounding is pervasive in EDC reproductive epidemiology because exposure to environmental chemicals tracks closely with socioeconomic status, dietary patterns, smoking, and co-exposure to other reproductive toxicants. Directed acyclic graphs (DAGs) are increasingly used to identify minimal sufficient covariate adjustment sets, distinguishing confounders from mediators and colliders — a distinction with major implications for estimated effect magnitudes. Co-exposure confounding is particularly challenging because EDCs rarely act in isolation: individuals with high phthalate exposure also tend to have elevated BPA, paraben, and PFAS concentrations. Multipollutant methods — weighted quantile sum (WQS) regression, Bayesian kernel machine regression (BKMR), and quantile g-computation — have been specifically developed to assess joint mixture effects, and consistently demonstrate that combined EDC exposures produce associations with

reproductive endpoints that are attenuated or absent for individual chemicals at ambient environmental concentrations (Kortenkamp, 2008).

Reverse causation — as discussed above under case-control designs — is also relevant in cross-sectional analyses of fertility-clinic populations, where the outcome (subfertility) may independently alter chemical clearance. Attenuation bias from non-differential measurement error, as described above, constitutes the third major systematic distortion of effect estimates.

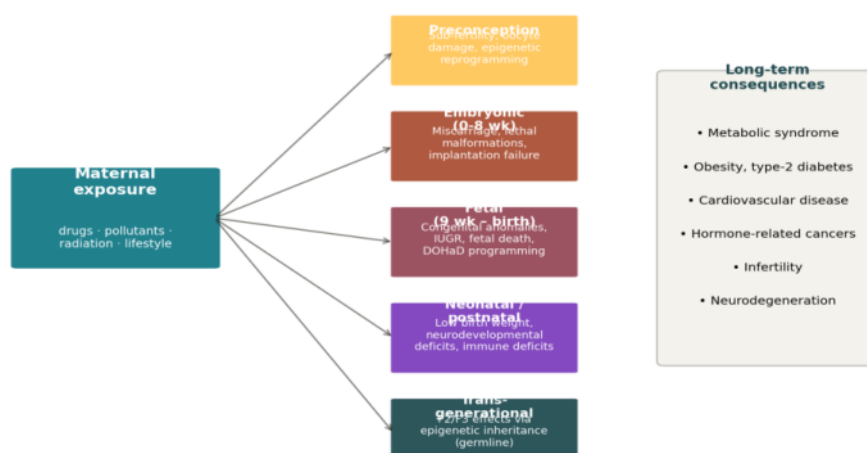
MAJOR BIRTH COHORT CONSORTIA

International Birth Cohort Consortia Relevant to Reproductive EDC Research

Acronym, Full Name, Country, n (approx.), Key EDCs Monitored, Key Reproductive Outcomes
MIREC, Maternal–Infant Research on Environmental Chemicals, Canada (10 cities), ~2,000 mother–child pairs, BPA / phthalates / PFAS / metals, Child adiposity; neurodevelopment; birth outcomes; LIFECODES, Lifecodes Pregnancy Cohort, USA (Boston MA), ~1,000, BPA / phthalates / parabens, Birth weight; preterm birth; hormonal outcomes; INMA, Infancia y Medio Ambiente, Spain (7 regions), ~3,000 births, BPA / phthalates / organochlorines, Fetal growth; puberty timing; thyroid; neurodevelopment; ELFE, Étude Longitudinale Française depuis l'Enfance, France (nationwide), ~20,000 children, BPA / phthalates / parabens / PFAS, Childhood development (ongoing longitudinal); EDEN/PELAGIE, Étude Des déterminants pré- et postnatals / Perturbateurs Endocriniens Algo-Gestationnels, France, ~2,000 / ~3,421, Organochlorines / pesticides / phthalates, Neonatal outcomes; neurodevelopment; EARTH, Environment and Reproductive Health Study, USA (Boston MA), ~2,100 fertility-clinic women, BPA / phthalates / pesticides / PFAS, IVF outcomes; live birth rate; embryo quality; MoBa, Norwegian Mother Father and Child Cohort, Norway, ~114,000 pregnancies, Pesticides / PFAS / metals, Birth outcomes; child health (broad scope); HBM4EU, Human Biomonitoring for Europe, 28 EU countries, Nationally representative, BPA / phthalates / parabens / PFAS / metals, Exposure characterisation; trend monitoring

These consortia have collectively transformed reproductive EDC epidemiology from small, single-centre cross-sectional analyses toward multi-country, longitudinal designs with adequate statistical power, harmonised exposure assessment, and repeated outcome measurement across developmental windows. Collaboration between consortia — pooling individual participant data across MIREC, INMA, LIFECODES, MoBa, and ELFE — has enabled meta-analyses with sample sizes sufficient to detect small effect sizes and to examine sex-specific or timing- specific exposure–outcome relationships that would be underpowered in any single study.

Figure 13. Outcomes of maternal exposure to reprotoxicants for the offspring



Developmental windows of vulnerability to reproductive toxicants, illustrating the maternal exposure pathways (gestational, lactational) and the range of offspring outcomes — from neonatal anthropometry and anogenital distance to puberty timing, gonadal histopathology, and fertility at reproductive age — that are captured by prospective birth cohorts.

The following sections review, for each major EDC class, the human epidemiological evidence from cohort and cross-sectional studies, the findings of experimental animal investigations in rodent, primate, and aquatic models, and the target organs and molecular mechanisms underlying observed effects.

III.A. Epidemiological Studies on BPA

Bisphenol A (BPA; 2,2-bis[4-hydroxyphenyl]propane; CAS 80-05-7) is a high-production-volume monomer used in the manufacture of polycarbonate plastics and epoxy resins lining food and beverage containers. It is a type-A xenoestrogen that binds the classical oestrogen receptors ER α and ER β (relative binding affinity $\sim$ 1/10,000 to 1/100,000 that of 17 β -oestradiol), as well as the membrane oestrogen receptor GPR30, the androgen receptor (as a partial agonist), and the thyroid hormone receptor. Population biomonitoring consistently detects urinary BPA in 85–95% of adults and children, with geometric mean concentrations of approximately 1–3 ng/mL in general-population surveys.

Human epidemiological studies on BPA and reproductive function are still limited, and their interpretation is constrained by the non-persistent nature of BPA (requiring repeated urine collections for valid exposure assessment) and by the cross-sectional or case–control designs employed in earlier work. A study conducted in China between 2004 and 2008 among workers manufacturing BPA-containing products found that occupational BPA exposure — estimated at approximately 50 times general-population levels — was associated with a higher reported prevalence of erectile dysfunction and sexual dissatisfaction (Li et al., 2010). A subsequent publication from the same group suggested that an association between BPA concentrations comparable to those found in the general population and "sexual dissatisfaction" might exist even in non-occupationally exposed men, though the small sample and cross-sectional design limit interpretation. In 2010, two studies reported altered concentrations of reproductive hormones (testosterone, FSH, LH, inhibin-B) in men seeking fertility evaluation or partners of pregnant women, in association with higher urinary BPA levels. Two additional cross-sectional studies found decreased sperm concentration with increasing urinary BPA, while one study in fertile men found no association. These inconsistencies reflect the heterogeneity of study populations, exposure assessment methods, and analytical approaches. No longitudinal human study has yet established causal impact of in-utero BPA exposure on adult reproductive function.

Key Human Epidemiological Cohorts — BPA and Reproductive Outcomes

Study / Cohort, Country, n, Exposure Window, Outcome, Key Result (Effect Estimate; 95% CI)
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MIREC (Braun et al. 2019), Canada, 719 mother–child pairs, 1st trimester urine (week 12), Child waist-to-hip ratio at age 3.5 yrs, β=+0.003 per 2-fold increase in BPA; girls: waist circ. β=+1.21 cm (0.11–2.30); LIFE Study (Smarr et al. 2015), USA, 501 couples / 233 births, Preconception urine, Neonatal birth length, Paternal BPA highest vs. lowest quartile: β=+1.35 cm (0.25–2.45); maternal BPA: NS; Endometriosis case–control, Multiple, $\sim$300 cases/controls, Urine at diagnosis, Endometriosis diagnosis, OR=2.9 (1.2–7.1) highest vs. lowest urinary BPA tertile; Chinese male workers (Li et al. 2010), China, $\sim$634, Workplace/urine, Sexual dysfunction, OR$\approx$2.3 (1.1–4.9) for erectile dysfunction in highest exposure tertile; INMA network, Spain, $\sim$2,998, 1st and 3rd trimester urine, Fetal growth; puberty; thyroid, Sex-specific: reduced fetal head circumference; delayed puberty markers

The MIREC Study (Maternal–Infant Research on Environmental Chemicals; Canada), which enrolled 719 mother–child pairs from 10 Canadian cities between 2008 and 2011, is among the most methodologically rigorous prospective cohort investigations of gestational BPA and offspring health. First-trimester urinary BPA (specific-gravity adjusted; median 0.8 ng/mL; detection rate 86.2%) was examined in relation to child adiposity at a mean age of 3.5 years. A two-fold increase in gestational urinary BPA was associated with increased waist-to-hip ratio in both sexes (β = 0.003; 95% CI: 0.001, 0.005) (Braun et al., 2019). In girls specifically, a non-linear J-shaped relationship was observed: central adiposity measures increased sharply above the threshold of 1 ng/mL BPA, and measurement-error-corrected models yielded β = 1.21 cm (95% CI: 0.11, 2.30) for waist circumference and β = 0.77 mm (95% CI: 0.05, 1.48) for subscapular skinfold thickness in girls. BMI z-scores were not significantly associated in either sex.

For female reproductive outcomes, a case–control study nested within gynaecological clinics found urinary BPA significantly higher in women with endometriomas versus matched controls (median 1.8 vs. 1.0 ng/mL; OR for highest vs. lowest tertile = 2.9; 95% CI: 1.2, 7.1), consistent with the known oestrogenic activity of BPA in endometrial stromal cells. At present, studies examining breast cancer risk or endometriosis based on retrospective exposure assessment in case–control designs are not considered conclusive, given the inherent limitations of cross-sectional biomonitoring for non-persistent compounds.

III.A.1. Animal Studies

Experimental animal studies have generated extensive and mechanistically coherent evidence of BPA reproductive toxicity, though significant variability across rodent strains, doses, routes of exposure, and outcome measures has complicated regulatory translation.

Guideline studies conducted in rats and mice according to OECD test guidelines (TG 414, TG 415, TG 416) at doses ranging from 3 µg/kg/day to 300 mg/kg/day did not demonstrate unequivocal reproductive toxicity. However, numerous academic laboratory studies using diverse rodent strains and refined endpoints — particularly focusing on in-utero and lactational exposure — have identified effects at doses in the low microgram per kilogram per day range, well below regulatory reference values.

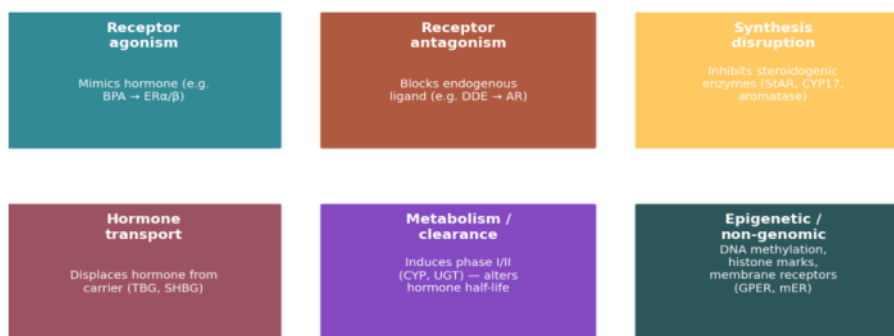
In male rodents, gestational and postnatal BPA exposure produces: testicular hypotrophy; prostatic hypertrophy; shortened anogenital distance; delayed preputial separation; decreased sperm production; alterations in plasma testosterone and oestradiol; and reduced litter size — with effects reported at doses of approximately 2–20 µg/kg/day in some strains. The mechanistic basis involves disruption of the hypothalamic–pituitary–gonadal (HPG) axis at the level of GnRH pulsatility programming, as well as direct actions on Sertoli and Leydig cells via ERβ.

In female rodents, in-utero and neonatal BPA exposure induces: accelerated puberty onset; uterine alterations (adenomyosis-like changes, endometrial hyperplasia); ovarian follicular cysts; altered vaginal differentiation; and abnormal sexual and maternal behaviour — effects appearing at doses from 0.2 to 500 µg/kg/day depending on species and strain. Transgenerational effects have been demonstrated through the F1–F3 generations: Wolstenholme et al. (2019) showed that dietary BPA (5 mg BPA/kg diet) in F0 pregnant C57BL/6J mice produced deficits in social recognition in the unexposed F3 generation, associated with dysregulated postsynaptic density genes (Shank1, Homer1c, Gkap, Psd95) in the fetal and neonatal hypothalamus — consistent with epigenetic propagation through the maternal germline (Wolstenholme et al., 2019). Decreased primordial follicle pools (F1–F3), altered sex ratios, and impaired male fertility into the F2–F3 generations have been reported following gestational BPA in additional multi-generational rodent studies.

In non-human primates, Tharp et al. (2012) administered 400 µg/kg/day of deuterated BPA orally to pregnant rhesus macaques (*Macaca mulatta*) from gestational day 100 to term, achieving maternal unconjugated serum BPA of 0.68 ± 0.31 ng/mL — within the range measured in pregnant women. Neonatal mammary glands of exposed animals showed a 2.02-fold increase in bud number and 1.54-fold increase in combined buds and terminal ends versus vehicle controls ($p = 0.027$ for buds/ductal unit) (Tharp et al., 2012). The rhesus mammary gland differentiates prenatally — unlike in rodents — conferring high translational relevance to this model.

The classical mammary gland study of Markey et al. (2001) in CD-1 mice demonstrated that subcutaneous BPA at 25 or 250 µg/kg/day during gestational days 9–18 produced, in female offspring assessed at 6 months: 237% increase in terminal duct relative area ($p < 0.05$); 288–361% increase in alveolar bud area ($p < 0.01$); and 56–95% greater stromal BrdU incorporation compared with controls — an early-pregnancy-like histoarchitecture in virgin females consistent with pre-neoplastic remodelling (Markey et al., 2001).

In aquatic vertebrates, BPA can modify sex-hormone action and induce partial sex reversal and embryonic developmental anomalies at concentrations compatible with those measured in some river systems — constituting an ecotoxicological hazard independent of the human health dimension.

Figure 18. Molecular modes of action of endocrine-disrupting chemicals (EDCs)

Molecular mechanisms of action of major EDC classes at reproductive target tissues. BPA acts through ERα/ERβ-mediated genomic signalling and GPR30-mediated rapid membrane signalling, disrupting oestrogen-responsive gene programmes in the mammary gland, uterus, testis, and hypothalamus. Non-monotonic dose–response relationships (lower left) are a characteristic feature of xenoestrogen action.

III.A.2. Target Organs and Tissues

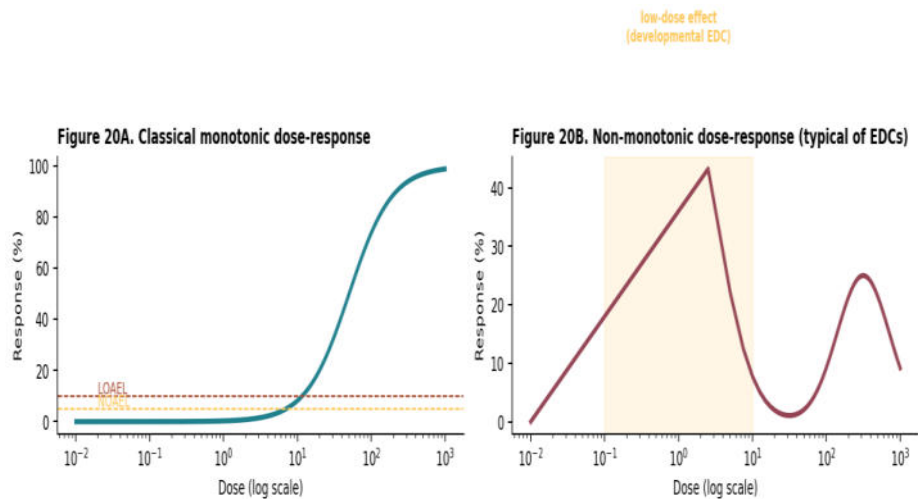
The gestational and neonatal period — encompassing organogenesis and sexual differentiation — constitutes the period of greatest vulnerability to BPA. Key target organs and their associated molecular signatures are summarised below.

BPA — Target Organs, Histopathological Effects, and Molecular Markers

Target Organ / Tissue, Critical Exposure Window, Histopathological Findings, Molecular Markers

Mammary gland (female), In-utero / lactation, Increased terminal end buds; ductal hyperplasia; pre-neoplastic lesions at 25 or 250 µg/kg/d, ERα / ERβ upregulation; increased stromal BrdU; progesterone receptor (PgR) induction; Uterus / endometrium, Fetal / neonatal, Adenomyosis-like changes; endometrial hyperplasia; endometrial polyps, ERα-dependent; increased CaBP-9k; altered progesterone signalling; Prostate, Fetal / neonatal, Increased terminal bud number; pre-neoplastic lesions at 10–20 µg/kg/d, ERβ upregulation; AR pathway crosstalk; Testis (Leydig cells), Gestational / postnatal, Leydig cell hyperplasia; reduced daily sperm production, Elevated oestradiol in males; ERβ in Sertoli cells; reduced INSL3; Hypothalamus / brain, Neonatal, Disrupted GnRH pulsatility; altered sexually dimorphic structure, Shank1 / Homer1c / Psd95 dysregulation (transgenerational); altered GnRH receptor expression; Ovary, Fetal / neonatal, Follicular cysts; reduced primordial follicle pool (F1–F3), Decreased AMH; altered FSH receptor expression

The mammary gland warrants particular attention: in-utero exposure at doses as low as 0.25 µg/kg/day can modify the development of mammary tissue, increasing its sensitivity to oestrogens at puberty and predisposing to pre-cancerous lesions at 25–250 µg/kg/day. The critical fetal or neonatal period for prostatic differentiation means that BPA exposure during organogenesis may set a trajectory toward pre-cancerous lesions that is not fully manifested until stimulation by adult sex hormones or environmental carcinogens. A link between in- utero BPA exposure and endometriotic lesions is suspected on mechanistic grounds — BPA stimulates endometrial stromal proliferation via ERα, suppresses apoptosis, and promotes glandular invasiveness — but has not yet been confirmed in prospective cohort studies.



Non-monotonic dose–response relationships for BPA in mammary gland endpoints. Mammary ductal branching and terminal end-bud proliferation are maximally stimulated at intermediate doses (~25 µg/kg/day) with reduced effect at higher doses, illustrating the inverted-U shape characteristic of ER-mediated BPA action. Such patterns confound standard risk assessment approaches based on monotonic high-to-low dose extrapolation.

III.B. Epidemiological Studies on Phthalates

Phthalate esters are a class of diester compounds used primarily as plasticisers to increase the flexibility of polyvinyl chloride (PVC) products, and as solvents and fixatives in personal care products, cosmetics, and medical devices. Di(2-ethylhexyl) phthalate (DEHP), di-n-butyl phthalate (DBP), di-isobutyl phthalate (DiBP), benzyl butyl phthalate (BBzP), and diethyl phthalate (DEP) are among the most extensively studied. After gastrointestinal absorption, phthalate diesters are rapidly hydrolysed to monoester metabolites (MEHP from DEHP; MBP from DBP/DiBP; MBzP from BBzP; MEP from DEP) which are renally excreted within 12–24 hours. Detection rates in population biomonitoring consistently exceed 90% for MEHP and MBP, confirming near-ubiquitous exposure.

Human epidemiological evidence links prenatal phthalate exposure to anogenital distance (AGD) shortening in boys, altered pubertal timing, sperm parameter decrements, and hormonal changes in adults. The critical mechanistic basis is the suppression of fetal testosterone production during the masculinisation programming window (MPW): gestational days 15–19 in rats, approximately weeks 8–14 in humans.

III.B.1. Animal Studies — Males

The phthalate syndrome in rodents encompasses a cluster of male reproductive tract malformations produced by in-utero DEHP and DBP exposure: reduced anogenital distance; hypospadias; retained nipples; cryptorchidism; epididymal agenesis; and focal testicular dysgenesis. These effects arise from suppression of fetal Leydig cell steroidogenesis during the MPW, mediated by downregulation of steroidogenic enzymes — Star (StAR protein), Cyp11a1 (P450_{scc}), Cyp17a1, and Hsd17b3 — via PPARα-mediated competition with the nuclear receptor NR5A1 (steroidogenic factor 1, SF-1) for the shared transcriptional coactivator CREB-binding protein (CBP). The lowest-observed-adverse-effect level (LOAEL) for fetal testosterone suppression is 10 mg/kg/day DEHP in prepubertal rats. INSL3 (insulin-like peptide 3), a marker of fully differentiated fetal Leydig cells essential for gubernacular development and testicular descent, is also suppressed, contributing to incomplete orchidopexy and the elevated cryptorchidism rate observed in the phthalate syndrome (Wang et al., 2019).

Reference studies used to establish tolerable daily intakes (TDIs) for DEHP and DBP were conducted in three rat strains (Sprague-Dawley, Wistar, Fischer 344) with gestational, postnatal, and multigenerational exposure protocols. Testicular damage and pathologies of accessory male reproductive organs — seminal vesicles, prostate, epididymis — have been consistently reported at DEHP doses of 150–500 mg/kg/day, and at lower doses (100 mg/kg/day) when DEHP and DBP are combined, consistent with mixture anti-androgenic additivity. Some rat strains show sensitivity at 10–100 mg/kg/day. Sperm parameter abnormalities (reduced sperm count, morphological defects) and hormonal alterations (testosterone, LH) are observed at doses above 100 mg/kg/day. A decrease in fertility is reported only at doses above 500 mg/kg/day.

In male marmosets and cynomolgus monkeys, the phthalate syndrome effects are observed at equivalent doses but are less pronounced than in the rat — a finding attributable to interspecies differences in placentation, HPG axis timing, and metabolic activation.

Histopathologically, in-utero DEHP exposure produces three characteristic testicular lesions: (1) focal testis dysgenesis — islands of dysorganised Sertoli cells translocated to the interstitium; (2) multinuclear gonocytes — resulting from failed germ-cell nest breakdown, observed in both rat and mouse; (3) Leydig cell hyperplasia in adulthood, driven by compensatory LH elevation secondary to reduced testosterone feedback. Additionally, DEHP increases apoptosis of fetal germ cells — an effect replicated in organotypic cultures of human and murine fetal testis, suggesting direct conservation of this mechanism across species.

In the monkey (marmoset), a delay in differentiation of fetal germ cells has been reported with DEHP and DBP at the same doses as in the rat, but without notable effects on Sertoli cell proliferation — a difference from rat findings that reinforces the importance of species-specific experimental models.

Phthalate Animal Studies — Male Reproductive Effects

Species / Strain, Compound, Dose, Exposure Window, Key Findings

Sprague-Dawley rat, DEHP, 10–750 mg/kg/d oral, GD 8–21, Reduced AGD; retained nipples; cryptorchidism; ↓ testicular testosterone; multinuclear gonocytes; Wistar rat, DBP, 100–500 mg/kg/d oral, GD 13–21, Fetal testosterone ↓; Leydig cell aggregation; ↓ INSL3; hypospadias; Fischer 344 rat, DEHP+DBP, 100 mg/kg/d each, GD 8–21, Additive AGD reduction (mixture effect); Cynomolgus monkey, DEHP / DBP, 150–500 mg/kg/d oral, Gestational, Reduced AGD; germ-cell differentiation delay; less pronounced than rat; CD-1 mouse, DEHP, 20–40 µg/kg/d IP, PND 5–15, Decreased large antral follicles (female offspring); upregulated Bax; decreased Cyp19a1

III.B.2. Target Organs and Tissues — Males

The testis — specifically the fetal Leydig cell population — is the primary target of anti-androgenic phthalate action. Molecular and cellular targets include:

Fetal Leydig cells: downregulation of Star, Cyp11a1, Cyp17a1, Hsd17b3, and Insl3, producing testosterone and INSL3 deficits that impair androgen-dependent masculinisation and gubernacular development respectively. The mechanism is PPARα-mediated transcriptional interference with NR5A1/SF-1, the master regulator of steroidogenic gene expression.

Sertoli cells: transient inhibition of Sertoli cell number and proliferation during in-utero exposure in the rat (not clearly established in mouse); impaired synthesis of androgen-binding protein (ABP) and anti-Müllerian hormone (AMH); Sertoli cell displacement to the interstitium in focal dysgenesis zones.

Fetal germ cells: multinuclear gonocyte formation; increased apoptosis (Bax/Bcl-2 imbalance); delayed gonocyte-to-spermatogonium transition — effects conserved in human fetal testis organotypic culture.

Accessory glands (adult): reduced prostate weight; reduced seminal vesicle weight (SMD = –1.27 in meta-analysis); atrophic epididymis — secondary consequences of the developmental testosterone deficit.

At the level of the adult spermatogenic cycle, DEHP at doses above 100 mg/kg/day reduces sperm count and alters morphology; oxidative stress-mediated DNA strand breaks are observed with bbP and MEHP in in-vitro assays and in human sperm from cross-sectional cohort studies.

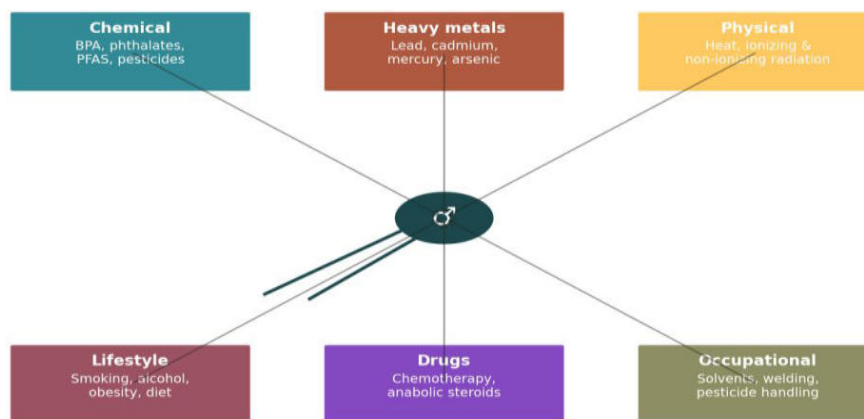
Phthalates — Male Target Organs and Molecular Markers

Target Tissue, Compound, Key Molecular Markers, Effect Direction

Fetal testis — Leydig cells, DEHP / MEHP / DBP / MBP, ↓ Star; ↓ Cyp11a1; ↓ Cyp17a1; ↓ Insl3; PPARα activation, ↓ Testosterone; ↓ INSL3; Leydig aggregation; Fetal testis — Sertoli cells, DEHP / DBP, ↓ Amh; ↓ Abp; Sertoli displacement, Transient proliferation inhibition; focal dysgenesis; Fetal germ cells, DEHP / DBP, Multinuclear gonocytes; ↑ Bax; ↓ Bcl-2; delayed gonocyte transition, ↑ Apoptosis; failure of nest breakdown; Adult testis, DEHP (>100 mg/kg/d), ↓ Daily sperm production; ↑ oxidative DNA damage, ↓ Sperm count and motility; Seminal vesicle / prostate, DEHP / DBP, ↓ Organ weight (SMD –1.09 to –1.27), Accessory gland atrophy

Human evidence from the Swan et al. (2005) Study for Future Families (SFF; $n = 85$ mother–son pairs; USA, 1999–2002) provided the first direct epidemiological demonstration of the phthalate syndrome in humans. Prenatal urinary MBP was inversely associated with the anogenital index (AGI) in male infants ($\beta = -0.592$; 95% CI: $-1.126, -0.057$; $p = 0.031$), and boys in the highest vs. lowest MBP quartile had OR = 10.2 (95% CI: 2.5, 42.2) for a short AGI — a proxy for incomplete masculinisation during the MPW (Swan et al., 2005). A composite phthalate score including MBP, MBzP, MEP, and MiBP showed an even stronger association (OR = 90.0; 95% CI: 4.88, 1,659; small n with wide CI). A 2025 multi-cohort European replication ($n \approx 700$ infants; Environment International) confirmed inverse associations between prenatal DEHP metabolites and infant AGD at 12 months. In NHANES cross-sectional analyses, urinary MBP and MBzP were inversely associated with serum testosterone in adult men. The EARTH Study at Massachusetts General Hospital and the European COPHES/DEMOCOPHES data likewise document inverse associations between DEHP metabolites and sperm concentration and motility.

Figure 11. Exposures associated with effects on sperm quality



Exposures associated with adverse effects on sperm quality, including phthalate metabolites (MBP, MEHP, MEHHP), BPA, PFAS, and organochlorines. Compiled from prospective cohort data including EARTH, LIFE Study, and NHANES. Effect estimates shown as mean difference in sperm concentration ($\times 10^6/\text{mL}$) per interquartile range increase in exposure.

III.B.3. Animal Studies — Females

Experimental studies of phthalate effects on the female reproductive system have expanded significantly since the early 2000s, driven by growing evidence of phthalate disruption of ovarian steroidogenesis and folliculogenesis. Fletcher et al. (2022) provided a comprehensive review of phthalate effects on ovarian folliculogenesis across CD-1, C57BL/6J, and Sprague–Dawley models, encompassing in-vitro, in-vivo, and transgenerational designs (Fletcher et al., 2022).

In female rats, in-utero and neonatal DEHP exposure produces puberty that is either accelerated or delayed depending on the timing of administration (doses of 15–150 mg/kg/day), accompanied by alterations in circulating oestradiol and progesterone. A reduction in fertility is observed with DBP after exposure from weaning at 500 mg/kg/day. In prepubertal female rodents exposed to DEHP intraperitoneally at 20–40 $\mu\text{g}/\text{kg}/\text{day}$ from postnatal days 5–15, large antral follicle numbers are reduced, the pro-apoptotic gene Bax is upregulated, and Cyp19a1 (aromatase) expression is decreased — consistent with impaired oestradiol synthesis. Adult oral DEHP at 20 $\mu\text{g}/\text{kg}$ to 750 mg/kg/day over 10–30 days prolongs the oestrous cycle, decreases the primordial follicle pool, and accelerates primordial follicle recruitment, potentially advancing reproductive senescence. Transgenerational effects of DEHP (0.05–5 mg/kg/day, gestational day 0.5 to postnatal day 21) extend to F1–F3: decreased primordial follicles in F1–F3; decreased antral follicles in F1–F2; and in F3, early vaginal opening, increased FSH, and decreased testosterone — a pattern consistent with epigenetic programming of the germline.

Environmentally relevant phthalate mixtures (DEHP/DBP/DiNP/BBzP/DEP) increase oestrous cycle duration, decrease proestrus time, and disrupt steroidogenic gene expression (Star, Cyp11a1, Cyp17a1) in granulosa cells. Female marmosets exposed to DEHP from weaning (3 months) through sexual maturity (18 months) show increased ovarian and uterine weight at high doses, elevated circulating oestradiol, and a slightly elevated corpus luteum count without significant histological changes in the uterus or ovary. The elevated oestradiol in both rat

and marmoset models may underlie the follicular atrophy described in the ovary by a mechanism of oestrogen-mediated feedback suppression of gonadotrophins.

III.B.4. Target Organs and Tissues — Females

The ovary is the primary target organ of phthalate action in females. In-vitro studies clearly demonstrate decreased oestradiol production by ovarian follicular cells following DEHP and MEHP exposure, with aromatase (CYP19A1) as the direct enzymatic target. MEHP inhibits Cyp19a1 transcription in granulosa cells, reducing oestradiol synthesis, which impairs follicular maturation and the pre-ovulatory LH surge. Star suppression restricts cholesterol delivery to the inner mitochondrial membrane, limiting the rate-limiting initiation step of steroidogenesis. The resulting ovarian insufficiency — characterised by anovulation, accelerated primordial follicle recruitment, and premature depletion of the follicular reserve — has profound implications for the reproductive lifespan of the female.

Other phthalate target organs in females include the pituitary and hypothalamus (altered gonadotrophin pulsatility and LH surge magnitude), the uterus (altered implantation capacity and decidual response), and the mammary gland (altered ductal development). These effects are supported primarily by in-vitro evidence; in-vivo data in female rodents are sparse relative to the male literature.

Phthalates — Female Target Organs and Molecular Markers

Target Organ, Compound / Metabolite, Key Molecular Markers, Functional Consequence
Ovary — granulosa cells, DEHP / MEHP / MBP, ↓ Cyp19a1 (aromatase); ↓ Star; ↓ Fshr; ↑ Bax/Bcl-2, ↓ Oestradiol; antral follicle loss; premature follicle recruitment; Ovary — primordial follicles, DEHP (transgenerational), ↓ Primordial pool F1–F3; ↑ FSH (F3), Accelerated reproductive senescence; Uterus, DEP / DBP, ↑ Uterine weight (high dose); altered implantation markers, Impaired decidualisation; Pituitary / hypothalamus, DEHP mixtures, Altered GnRH pulsatility; disrupted LH surge, Anovulation; irregular oestrous cycle

III.B.5. Studies in Fish

Aquatic organisms represent sentinel species for phthalate reproductive toxicity at environmentally relevant concentrations. DEHP concentrations in surface waters near industrial discharge have been measured at 1–40 µg/L, within the experimental range of key ecotoxicological studies.

Zebrafish (*Danio rerio*): Carnevali et al. (2010) exposed adult female zebrafish to DEHP at environmentally relevant concentrations (0.02–40 µg/L, semi-static renewal, 3 weeks) and reported a dose-dependent reproductive failure: embryo production fell to approximately 1% of control values at 40 µg/L (Carnevali et al., 2010). Molecular analysis revealed: dose- dependent upregulation of ovarian BMP-15 (an inhibitor of oocyte maturation); suppression of LH receptor (LHR) and membrane progesterone receptor β (mPRβ); and dose-dependent downregulation of ptgs2 (cyclooxygenase-2), the prostaglandin-synthesising enzyme that triggers the ovulatory cascade. Post-vitellogenic oocytes (0.70–0.75 mm) were completely absent at concentrations ≥20 µg/L, indicating arrest of oocyte maturation progression. Plasma vitellogenin (vtg) levels increased dose-dependently, confirming oestrogenic activity via hepatic ERα. At 40 µg/L, oocyte growth stagnated at the primary growth stage; post-vitellogenic oocytes were entirely absent, indicating that the terminal maturation step (germinal vesicle breakdown) was blocked. These effects occurred at or below concentrations found in contaminated water bodies near industrial sites.

Male zebrafish exposed to DEHP exhibit reduced fertilisation success of eggs from untreated females, impaired spermatogenesis (with evidence that meiotic progression is disrupted), and abnormal sperm morphology. After BBzP exposure, ovotestis formation (gonads containing both oocytic and testicular tissue) and histological gonadal anomalies are observed — features diagnostic of endocrine feminisation.

Medaka (*Oryzias latipes*): Male medaka exposed to DEHP at concentrations ≥10 µg/L develop intersex gonads characterised by the co-presence of oocytes within testicular parenchyma (testis-ova), elevated vitellogenin expression, and suppression of 11-ketotestosterone (the primary teleost androgen). These intersex features correspond to environmental DEHP concentrations near industrial discharge points, confirming ecological reproductive risk.

Additional neurotoxicity studies in zebrafish embryos and larvae exposed to six phthalates (including DEHP and DBP) documented disrupted locomotor and anxiety-related behaviours at sublethal concentrations, with DEHP and DBP producing the greatest neurobehavioural changes — raising concerns about phthalate impacts on neuroendocrine reproductive regulation in aquatic vertebrates.

III.C. Epidemiological Studies on Polybrominated Compounds

Polybrominated diphenyl ethers (PBDEs) were the dominant class of brominated flame retardants used from the 1970s through the early 2000s in furniture foam, electronic equipment, and textiles. Their high lipophilicity, environmental persistence, and bioaccumulation potential led to widespread international restriction: commercial penta-BDE and octa-BDE were voluntarily withdrawn from use in North America by 2004; deca-BDE was phased out by 2013. Under the Stockholm Convention, penta- and octaBDE are listed as persistent organic pollutants requiring elimination. HBCD (hexabromocyclododecane) and TBBPA (tetrabromobisphenol A) are additional brominated flame retardants of concern, though with more limited epidemiological data.

Despite regulatory restrictions, PBDEs persist in human adipose tissue, breast milk, and blood, with BDE-47 typically the dominant congener (geometric mean serum levels of ~30–60 ng/g lipid in children in North American cohorts, declining but still detectable in European populations). The principal mechanism of reproductive toxicity is thyroid hormone axis disruption: PBDE congeners are structurally analogous to thyroxine (T4) and compete for binding to transthyretin (TTR) and thyroxine-binding globulin (TBG); they also inhibit thyroperoxidase (TPO), the enzyme responsible for thyroidal iodine organification. Sex- hormone disruption is an additional mechanism operating via direct interference with hepatic sex-hormone-binding globulin (SHBG) synthesis and steroidogenic enzyme activity.

Human cohort evidence links prenatal PBDE exposure to: increased cryptorchidism risk in newborn boys (Finnish/Danish mother–infant cohort study, with increased LH co-association); delayed time-to-pregnancy in women with higher blood PBDE levels (Californian cohort); and thyroid hormone alterations in children (HOME Study). Epidemiological studies remain limited in number and do not yet constitute sufficient proof of causal effects on human reproductive function, particularly for HBCD and TBBPA, for which no cohort data are available.

III.C.1. Animal Studies

Male rodents: In-utero exposure to BDE-99 and BDE-209 at 500–1,500 mg/kg/day produces reduced anogenital distance, impaired spermatogenic parameters, altered sperm functional characteristics, and decreased testosterone in male rat offspring. In mice, BDE-209 at 500–1,500 mg/kg/day alters the mitochondrial membrane potential of epididymal spermatozoa and the lateral head displacement amplitude (HAD) — features indicating oxidative stress- related sperm dysfunction.

Female rodents: Talsness et al. (2008) administered a single oral dose of PBDE-47 to pregnant Wistar rats (140 or 700 µg/kg bw) on gestational day 6 and assessed female offspring at PND 38 and 100 (Talsness et al., 2008). At PND 38: the 700 µg/kg dose significantly reduced secondary and tertiary ovarian follicles; the 140 µg/kg dose also reduced growing follicle numbers and decreased ovarian weight. At PND 100: thyroid histopathology showed follicular cyst formation (140 µg/kg) and mild cysts with degenerated follicular epithelium (700 µg/kg). Electron microscopy revealed cytoplasmic vesicle accumulation in ovarian stromal cells — ultrastructural evidence of disrupted steroidogenesis. Doses of 1–10 mg/kg subcutaneously used in follow-up studies are substantially above current human estimated exposures, limiting direct quantitative extrapolation.

After chronic exposure of female rats to HBCD (140 mg/kg/day), histological examination reveals a one-third reduction in primordial follicle numbers — the resting follicle pool that determines the reproductive lifespan of the female. This is classified as a non-reversible adverse biomarker of female reproductive toxicity. Despite this reduction, these animals maintain a normal number of implantation sites during their first gestation; longer-term follow-up would be required to establish whether the follicular depletion translates to premature ovarian insufficiency.

In in-vitro studies using isolated porcine granulosa cells or co-cultures exposed to PBDE mixtures, stable alterations in testosterone and progesterone concentrations are observed after 48 hours, suggestive of premature luteinisation of preovulatory follicles.

In-vitro studies with TBBPA on mouse Sertoli cells show cell death partly via apoptosis, involving Ca^{2+} -dependent mitochondrial depolarisation — effects occurring at concentrations approximately 100-fold higher than the highest human TBBPA serum levels measured.

The recent killifish (*Fundulus heteroclitus*) study by McNabb-Kelada et al. (2025) demonstrated that early-life BDE-99 exposure produced persistent hyperactivity and reduced anxiety-like behaviour for more than two years into adulthood, with sex- and route-specific patterns: progenitor (maternal) exposure altered brain transcriptomics and behaviour preferentially in F1 males via epigenetic imprinting, while direct embryonic exposure preferentially affected females (McNabb-Kelada et al., 2025).

PBDE Animal Studies — Reproductive and Developmental Effects

Species, Compound, Dose / Route, Exposure Window, Key Findings

Wistar rat (♀), PBDE-47, 140 or 700 µg/kg oral (single dose), GD 6, ↓ Secondary/tertiary follicles (PND 38); follicular thyroid cysts (PND 100); ↓ E2; Sprague-Dawley rat (♂), BDE-99 / BDE-209, 500–1500 mg/kg/d oral, In utero, ↓ AGD; ↓ spermatogenesis; ↓ testosterone; CD-1 mouse (♂), BDE-209, 500–1500 mg/kg/d oral, In utero, ↓ Mitochondrial membrane potential in spermatozoa; ↑ oxidative stress markers; Wistar rat (♀) chronic, HBCD, 140 mg/kg/d oral, Adult, ↓ Primordial follicle pool by 33% (histology); *Fundulus heteroclitus*, BDE-99, Embryonic / maternal, Early life, Persistent hyperactivity; ↓ anxiety-like behaviour (>2 years); sex-specific epigenetic effects; Porcine granulosa cells (in vitro), PBDE mixture, Culture, 48h, Premature luteinisation; altered testosterone and progesterone

III.C.2. Target Organs and Tissues

The ovary in females and the testis in males represent the primary gonadal targets of PBDE reproductive toxicity. Thyroid-axis disruption is a cross-cutting mechanism affecting both sexes and affecting neurological as well as reproductive development.

The HOME Study (Health Outcomes and Measures of the Environment; Cincinnati, USA) provided quantitative human evidence of PBDE-associated thyroid disruption. Vuong et al. (2018) analysed 162 mother–child pairs recruited 2003–2006 and found that prenatal Σ PBDE concentrations in maternal serum at 16 weeks were inversely associated with child TSH at age 3 years ($\beta = -0.32$; 95% CI: $-0.53, -0.12$), corresponding to a 27.6% decrease in TSH per 10-fold increase in prenatal Σ PBDE (Vuong et al., 2018). Sex-specific effects were observed: BDE-47 and BDE-99 were associated with approximately 35% decrease in TSH in girls but null association in boys; BDE-47 was associated with significantly decreased FT4 in boys ($\beta = -0.08$ ng/dL; 95% CI: $-0.15, -0.01$) but not in girls. At postnatal age 2, BDE-47 showed a positive association with child TSH ($\beta = +0.40$; 95% CI: $0.04, 0.76$), suggesting a biphasic disruption pattern across development.

A systematic review and meta-analysis of 10 randomised controlled animal studies (Zhang et al., 2020) found a pooled standard mean deviation (SMD) of -0.46 (95% CI: $-0.69, -0.22$) for PBDE-associated male reproductive damage, with the DE-71 mixture (SMD = -0.70 ; 95% CI: $-1.18, -0.21$) and BDE-209 (SMD = -0.41 ; 95% CI: $-0.77, -0.05$) exerting the strongest effects (Zhang et al., 2020). Seminal vesicle weight reduction had SMD = -1.09 and ventral prostate reduction SMD = -1.27 , confirming consistent accessory gland toxicity.

The FlaRE Study ($n = 27$ healthy adult men; Boston, USA) found BDE-47 inversely associated with serum inhibin-B — a Sertoli cell-derived marker of spermatogenesis ($\beta = -0.14$; 95% CI: $-0.27, -0.020$), with stronger effects in men ≥ 40 years (Makey et al., 2016).

PBDE — Target Organs, Mechanisms, and Human Correlates

Target Organ, Key Effects, Molecular Markers, Human Cohort Correlation

Thyroid gland, Follicular cysts; ↓ TH synthesis; ↓ TPO activity, ↓ TPO; competitive TTR/TBG binding; ↑ metabolic T4 clearance, HOME Study: ↓ TSH 27.6% per 10× prenatal Σ PBDE; Brain (developing), Hyperactivity; Purkinje cell loss; dopamine dysregulation, ↓ T3-dependent neuronal migration; ↓ dopaminergic differentiation, Epidemiological associations with IQ and neuromotor delay; Ovary, Reduced follicle pools; ↓ oestradiol; follicular cysts, ↓ Aromatase activity; cytoplasmic vacuolation, Finnish/Danish cohort: ↑ cryptorchidism risk in newborns; Testis / Sertoli cells, ↓ Sertoli function; ↓ inhibin-B; accessory

Target Organ, Key Effects, Molecular Markers, Human Cohort Correlation

gland atrophy, ↓ FSH–inhibin axis; oxidative stress in sperm mitochondria, FlaRE Study: ↓ inhibin-B in men ≥40 yrs

III.D. Epidemiological Studies on Perfluorinated Compounds

Per- and polyfluoroalkyl substances (PFAS) are a class of more than 4,700 synthetic chemicals characterised by a backbone of carbon atoms fully substituted with fluorine — the C–F bond is among the strongest in organic chemistry (bond dissociation energy ~544 kJ/mol), conferring exceptional environmental and metabolic persistence. Perfluorooctanoic acid (PFOA; CAS 335-67-1) and perfluorooctane sulfonic acid (PFOS; CAS 1763-23-1) are the most extensively studied compounds from a reproductive toxicological perspective. Both are amphiphilic — combining a lipophilic fluorocarbon chain with a polar head group — and accumulate selectively in serum, liver, and breast milk rather than in adipose tissue like classical lipophilic POPs.

PFOA has an estimated human serum half-life of approximately 3.5 years; PFOS of approximately 4 years. This persistence means that serum PFAS concentrations measured at any point in adulthood reflect years of cumulative exposure, making biomonitoring a substantially more reliable exposure assessment tool for PFAS than for non-persistent EDCs. Mechanistic pathways include: PPAR α activation (hepatotoxicity; lipid metabolism disruption); competition with thyroid hormone T4 for transthyretin (TTR) binding (thyroid axis disruption); interference with the sodium/iodide symporter (NIS); and immune cell dysregulation via PPAR and NF- κ B signalling.

Human epidemiological evidence links PFAS to: impaired couple fertility (Danish National Birth Cohort, increased involuntary infertility risk); altered sperm morphology (Danish male cohort); reduced birth weight and shorter gestational age (Project Viva, USA); and thyroid hormone alterations in pregnant women and neonates. PFOS and PFOA are listed under Annex B of the Stockholm Convention; PFOA and PFOS are subject to REACH restrictions in the European Union.

III.D.1. Animal Studies

Male rodents: Multigenerational studies in rats indicate that PFOA, even at very high doses (doses up to 30,000 times concentrations measured in humans), does not significantly impair male rat fertility or sperm production. However, PFOS at lower doses produces testicular histological changes. Studies on PFDoA (perfluorododecanoic acid) administered by oral gavage to rats demonstrate testosterone production decreases — with reduced expression of testosterone biosynthetic enzyme genes — at doses as low as 0.2 mg/kg/day. Over 40 testicular proteins involved in oxidative stress and the mitochondrial respiratory chain are modified after chronic PFDoA exposure. In the adult rat testis, PFDoA-induced decreased testosterone production is accompanied by increased apoptosis across multiple testicular cell types. Interspecies differences in PFAS half-lives (days in rodents versus years in humans) limit quantitative extrapolation of rodent dose–response data to human risk.

Female rodents: PFOA and PFOS do not appear to modify female fertility or ovarian morphology in rodent studies, but can alter ovarian steroidogenesis. In mice, in-utero exposure to PFOA (10 mg/kg/day) delays sexual maturation in female offspring: vaginal opening and first oestrus are retarded, and menstrual cycle regularity is impaired by PFOS (1–10 mg/kg/day) in adult rats. Effects are variable between species (rat vs. mouse).

Mammary gland: White et al. (2009) administered PFOA at 3–5 mg/kg/day to pregnant CD-1 mice and assessed female offspring mammary gland development (White et al., 2009). Delays in ductal elongation were observed from PND 1 through PND 63; terminal end bud (TEB) appearance was delayed; secondary and tertiary branching was reduced. At 18 months, exposed animals showed sparse epithelial arborisation, foci of ductal hyperplasia, inflammatory infiltration, and elevated stromal density. The mammary gland remained affected even when offspring body weight was normal, identifying it as a particularly sensitive endpoint. Late- gestational exposure alone (GD 15–17) was sufficient to produce persistent effects, establishing a narrow critical developmental window. Mice exposed to 0.3 mg/kg/day during puberty showed impaired ductal elongation indistinguishable from developmental exposure. Increased terminal bud formation is related to elevated ovarian progesterone synthesis induced by PFOA, with strain-specific differences noted.

In male minnows (*Phoxinus phoxinus*), increased vitellogenin production was observed after PFOA treatment — a biomarker of oestrogenic activity. Ovotestis signs were also described, confirming oestrogenic feminisation in

aquatic species. Female fish treated with PFOS showed reduced gonadal growth and impaired embryonic development.

Immune effects: Taylor et al. (2023) demonstrated that PFOA at 7.5 mg/kg/day for 15 days in C57BL/6 mice suppressed the T-cell-dependent IgM antibody response by 79% in females and 89.9% in males; plasmablast numbers were reduced 55%; overall B-cell counts fell 15%; splenic cellularity declined 20–27% in both sexes (Taylor et al., 2023). These immunotoxic effects have been confirmed in human populations — the Faroese children cohort (Grandjean et al., 2012) found dose-dependent suppression of tetanus and diphtheria vaccine antibody titres at PFAS concentrations within the observed population range.

III.D.2. Target Organs and Tissues

PFAS — Target Organs, Animal Models, and Human Cohort Evidence

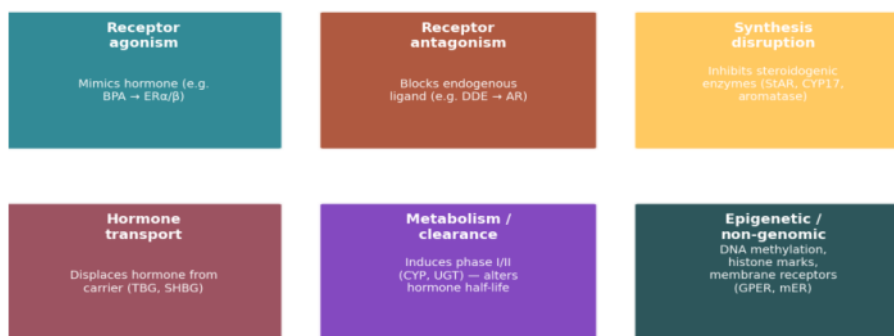
Target Organ, Animal Model Findings, Molecular Mechanism, Human Cohort Evidence

Mammary gland, PFOA: delayed ductal elongation; late-life hyperplasia (CD-1 mouse; White et al. 2009), ↑ Ovarian progesterone via PFOA; ↑ TEB; altered stromal–epithelial crosstalk, Reduced lactation duration in NHANES-derived analyses; Liver, Hepatomegaly (↑ 20–27% absolute liver weight); hepatocyte vacuolation, PPARα activation; ↑ ALT/AST; altered lipid metabolism, Liver enzyme elevations in occupational and general-population cohorts; Thyroid gland, ↓ TPO activity; ↓ NIS; ↑ metabolic T4 clearance; altered TTR binding, Competitive T4 displacement; PPARα-mediated glucuronidation acceleration, Project Viva: ↓ free T4 index in pregnant women; DNBC: OR=2.2 for miscarriage (PFOA highest quartile); Immune system (spleen/B-cells), ↓ IgM TDAR (–79 to –89.9%); ↓ plasmablasts; ↓ splenic cellularity (Taylor et al. 2023), PPARα and NF-κB-mediated B-cell maturation arrest, Faroese children: ↓ vaccine antibody titres (Grandjean et al. 2012); Fetal / neonatal (general), ↓ Birth weight; ↓ gestational age (Project Viva, rodent models), Altered placental development; ↓ insulin-like growth factor-1, Project Viva: OR=2.46 (1.05–5.80) for low birth weight (PFOS; low-folate women); Testis (rat), ↑ Apoptosis after PFDoA; ↓ testosterone biosynthetic enzymes, ↓ Star; ↓ Cyp11a1; ↑ oxidative stress proteins, Danish cohort: altered sperm morphology with PFOS/PFOA

Project Viva (n = 1,400 mother–singleton pairs; Eastern Massachusetts, USA; 1999–2002) provided important human evidence on PFAS and birth outcomes, particularly in the context of nutritional modulation (Zhang et al., 2023). Among women with the lowest dietary folate (<660 µg/day), a two-fold increase in plasma PFOA was associated with $\beta = -89.13$ g birth weight (95% CI: –166.84, –11.42). Among women with the lowest plasma folate (<14 ng/mL), doubling of plasma PFOS was associated with OR = 2.46 (95% CI: 1.05, 5.80) for low birth weight (<2,500 g). PFNA in low-folate women was associated with shorter gestational age ($\beta = -2.28$ days; 95% CI: –4.48, –0.08). These findings suggest folate status modulates PFAS- associated birth-outcome risk, with implications for interaction-based nutritional approaches to reproductive toxicant risk reduction.

For thyroid disruption, Coperchini et al. (2021) compiled evidence from more than 15 human biomonitoring studies and in-vitro mechanistic investigations, identifying competitive NIS inhibition, TTR-binding T4 displacement, PPARα-mediated acceleration of hepatic T4 glucuronidation, and direct TPO inhibition as the four principal thyrotoxic mechanisms of PFAS. Human cohort data from Project Viva confirmed PFAS mixture-associated reductions in maternal free T4 index, with PFOA, MeFOSAA, EtFOSAA, and PFHxS contributing most to the maternal effect; PFHxS and MeFOSAA contributed most to decreased neonatal T4 in males (Coperchini et al., 2021). The Danish National Birth Cohort found higher TSH in the highest quartiles of PFOS/PFOA/PFHxS at 5–10 gestational weeks, with OR $\approx$ 2.2 for miscarriage associated with the highest PFOA quartile.

In zebrafish larvae, PFOS induces structural thyroid follicular epithelial cell damage — reduced nuclear area, mitochondrial and endoplasmic reticulum ultrastructural modifications — and suppression of circulating thyroid hormones, with compensatory upregulation of TR-β.

Figure 18. Molecular modes of action of endocrine-disrupting chemicals (EDCs)

PFAS mechanisms of action on the thyroid axis. PFOA and PFOS compete with T4 for transthyretin (TTR) binding (increasing free T4 clearance), inhibit NIS-mediated thyroidal iodine uptake, inhibit thyroperoxidase, and activate PPARα to accelerate hepatic T4 glucuronidation and biliary excretion. The net result is a reduction in circulating free T4 and disruption of the T4/T3 ratio in maternal and fetal compartments, with downstream consequences for neuronal migration, myelination, and HPG axis programming.

III.E. Epidemiological Studies on Parabens

Parabens (para-hydroxybenzoic acid alkyl esters) are among the most widely used antimicrobial preservatives in cosmetics, personal care products, pharmaceuticals, and processed foods. The most commonly detected parabens in human biomonitoring are methylparaben (MeP), ethylparaben (EtP), propylparaben (PrP), and butylparaben (BuP). The alkyl chain length determines estrogenic potency: BuP > PrP > EtP > MeP. In yeast-based oestrogen-receptor binding assays (YES assay), parabens are approximately 10,000-fold less potent than 17β-oestradiol; in MCF-7 cell proliferation assays (the E-screen), potency is 10⁵–10⁷-fold lower. Despite this comparatively low intrinsic potency, high-frequency topical application (cosmetics applied multiple times daily), direct dermal absorption and systemic distribution, and cumulative exposure to multiple congeners simultaneously create continuous low-level systemic estrogenic load that has raised regulatory concern. European and international regulatory bodies (SCCS, US FDA) have reviewed maximum authorised concentrations for paraben species in cosmetics, leading to restrictions on BuP in leave-on products.

Detection rates for urinary MeP and PrP exceed 90% in general-population biomonitoring, including in pregnant women and infants. Urinary half-lives are in the range of 2–6 hours, placing parabens in the category of non-persistent compounds requiring multiple urine collections for valid exposure assessment.

Human epidemiological evidence on parabens and reproductive outcomes is limited but suggestive. A study in approximately 100 men attending a fertility clinic found that serum BuP was significantly associated with increased sperm DNA fragmentation, with a dose-dependent relationship (Li et al., 2004). A 2023 weighted quantile sum (WQS) analysis of reproductive-aged Chinese men (Shanghai Birth Cohort and affiliated andrological clinics) found that a paraben mixture was inversely associated with sperm quality parameters, with BuP exhibiting the highest index weight in the mixture — consistent with its greater estrogenic potency (Shen et al., 2023). Japanese cross-sectional data from subfertile couples (n = 109) showed urinary propylparaben negatively correlated with sperm concentration (r = −0.21; p = 0.04) (Iwamoto et al., 2017). In the Danish FEFO cohort (n = 315 men), men in the highest PrP and BuP quintiles had odds ratios of approximately 1.6–2.1 for below-reference sperm concentration, though CIs crossed unity — consistent with attenuation from single-void measurement error.

The detection in 2004 of five parabens (methyl, ethyl, n-propyl, n-butyl, isobutyl) in breast adipose tissue from carcinoma patients generated intense scientific and societal debate about whether deodorant/antiperspirant parabens could contribute to breast cancer risk. Two subsequent epidemiological studies (2002, 2003) — one case-control study and one cohort analysis — found no significant association between deodorant/antiperspirant use and breast cancer incidence, though the latter found a relationship between age of cancer onset and early commencement of deodorant use combined with axillary shaving. Current evidence

does not permit a conclusion on this question; the 2004 tissue study lacked controls and was considered methodologically insufficient by regulatory agencies.

III.E.1. Animal Studies

The animal literature on paraben reproductive toxicity distinguishes effects by compound, sex, and dose range. Methylparaben (MeP): An industrial consortium study confirmed the absence of effects on male reproductive organs after oral administration to juvenile rats at 1,000 mg/kg/day. Uterotrophic bioassay in immature Sprague-Dawley rats (Sun et al., 2016) demonstrated that MeP at 20 mg/kg/day induced significant uterine weight increases and ER α -mediated transcriptional responses including induction of Pgr (13.6-fold), CaBP-9k (254-fold), icabp (79-fold), and itmap1 (186-fold) — all blocked by the ER antagonist fulvestrant, confirming the ER-dependent mechanism (Sun et al., 2016). Molecular docking confirmed hydrogen bonding of the MeP p-hydroxyl group to ER α -LBD residues Glu353 and Arg394. The LOEL for uterotrophic response was 20 mg/kg/day for MeP. Ethylparaben (EtP): Exhibited a lower LOEL than MeP (4 mg/kg/day) in the same uterotrophic assay (Sun et al., 2016), consistent with its greater estrogenic potency relative to MeP. Oestrogen-responsive gene expression profiles in the uterus were qualitatively identical to those of MeP.

Propylparaben (PrP): A rat study reported spermatogenic effects without change in reproductive organ weight — specifically a decrease in testicular and epididymal sperm count (approximately 50% of controls at 1,000 mg/kg/day), reduced daily sperm production (approximately 70% of controls at all doses in the treated groups), and dose-dependent decreases in serum testosterone. This study was criticised by regulatory agencies for low animal numbers, incomplete reporting, high variability in organ weights and hormone assays, and insufficient study duration (not covering a complete rat spermatogenic cycle of 52 days). Propylparaben and isopropylparaben at high doses (1,000 mg/kg/day) in female rodents delay vaginal opening — the murine pubertal marker — and decrease oestrus length, ovarian weight, corpus luteum count, and increase cystic follicle formation.

Butylparaben (BuP): Hubbard et al. (2020) conducted a multigenerational reproductive assessment in Hsd:Sprague-Dawley rats with dietary BuP at 5,000–40,000 ppm (Hubbard et al., 2020). Findings included: 100% F1 survival at 40,000 ppm only with delayed weaning (to PND 28), reflecting general developmental toxicity; delayed vaginal opening in F1 females by approximately 3–9 days at 15,000–40,000 ppm (persisting after body-weight adjustment); accelerated testicular descent in the F2c 15,000 ppm group (14.8 ± 0.1 vs. 15.5 ± 0.2 days; $p < 0.05$); no significant effects on sperm motility, progressive motility, or spermatid count across generations; dose-related liver histopathology from 15,000 ppm (bile duct hyperplasia, periportal hepatocyte hypertrophy), consistent with metabolic burden rather than estrogenic hepatotoxicity. A separate study found that single-dose BuP (125–500 mg/kg intraperitoneal) in prepubertal rats induced spermatogenic cell apoptosis and disrupted Sertoli cell vimentin intermediate filaments, impairing the blood–testis barrier. In-utero n-butylparaben gestationally altered oestradiol levels and oestrogen receptor expression in adult male rat offspring, suppressing late spermatogenesis.

In mice, BuP at 1,000 mg/kg/day does not affect prostate, seminal vesicle, or preputial gland weight, but produces a slight increase in epididymis weight; dose-dependent reduction of spermatids in seminiferous tubules without change in spermatogonia or spermatocyte numbers; and significantly decreased serum testosterone at 1,000 mg/kg/day.

Histological analysis of female reproductive tracts from studies of methyl, ethyl, propyl, isopropyl, and isobutyl parabens reveals a consistent pattern: hypertrophy of the myometrium at the highest dose of propyl- and isopropylparaben (1,000 mg/kg/day) and at all doses of butyl and isobutyl parabens (62.5, 250, 1,000 mg/kg/day). Circulating oestradiol is significantly reduced in animals treated with methyl, ethyl, propyl, isopropyl, and isobutyl parabens — possibly due to negative feedback from enhanced uterotrophic oestrogen receptor stimulation, or to direct suppression of aromatase. Other studies predominantly report uterine weight increases.

Parabens — Animal Studies Summary

Compound, Model, Dose, Key Reproductive Finding, Regulatory Notes

Methylparaben (MeP), Immature SD rat (♀), 20 mg/kg/d oral, LOEL uterotrophic response; ↑ CaBP-9k, Pgr; ER α -dependent (blocked by fulvestrant), No male reproductive effects at 1000 mg/kg/d (industrial consortium); Ethylparaben (EtP), Immature SD rat (♀), 4 mg/kg/d oral, LOEL uterotrophic (< MeP); identical

Compound, Model, Dose, Key Reproductive Finding, Regulatory Notes

ER α transcriptional profile to MeP, Greater potency than MeP consistent with longer alkyl chain; Propylparaben (PrP), SD rat (σ), 1000 mg/kg/d oral, $\downarrow$ Sperm count (50%); $\downarrow$ daily sperm production (70%); $\downarrow$ testosterone, Study criticised for low n and short duration; SCCS flagged limitations; Butylparaben (BuP), Hsd SD rat (multigenerational), 5000–40000 ppm dietary, Delayed vaginal opening (F1 σ); $\uparrow$ liver histopathology; no sperm parameter effects, Hubbard et al. 2020; survival only maintained with extended weaning at top dose; Butylparaben (BuP), Prepubertal rat (σ), 125–500 mg/kg IP, Spermatocyte apoptosis; disrupted Sertoli cell vimentin; blood–testis barrier impairment, High dose / IP route limits extrapolation; Methyl + isopropyl PB, SD rat (σ), 1000 mg/kg/d, Delayed vaginal opening; $\downarrow$ oestrus length; $\uparrow$ cystic follicles; $\downarrow$ ovarian weight; myometrial hypertrophy, Consistent pattern across long-chain parabens

The overall animal evidence indicates that parabens exert uterotrophic and spermatogenic effects primarily at high doses — generally at or above 1,000 mg/kg/day for most endpoints — substantially exceeding estimated human exposure. However, the uterotrophic LOEL of 20 mg/kg/day for MeP and 4 mg/kg/day for EtP in immature rats, when converted to urinary metabolite equivalents, approaches concentrations within the high-percentile range of human population biomonitoring data, warranting continued epidemiological surveillance.

SYNTHESIS: COMPARATIVE SUMMARY OF REPRODUCTIVE TOXICANTS

The five chemical classes reviewed in this part — BPA, phthalates, polybrominated compounds, perfluorinated compounds, and parabens — share several cross-cutting properties of regulatory and biological significance.

Comparative Summary of Reproductive Toxicant Profiles**Chemical Class, Principal Mechanism, Most Sensitive Human Endpoint, Most Sensitive Animal Endpoint, Regulatory Status**

BPA (Xenoestrogen), ER α /ER β / GPR30 agonism; AR partial antagonism; thyroid disruption, Gestational exposure $\rightarrow$ child adiposity (MIREC); male sperm concentration; endometriosis association, Mammary gland dysplasia (25 μ g/kg/d in-utero; mouse); prostatic pre-neoplasia; transgenerational F3 social deficits, ECHA SVHC; banned from polycarbonate infant products in EU/Canada; Phthalates (Anti-androgens), PPAR α / NR5A1 competition $\rightarrow$ $\downarrow$ fetal testosterone; INSL3; PPAR γ in adipocytes, Prenatal MBP $\rightarrow$ $\downarrow$ AGD in boys (Swan et al. 2005); $\downarrow$ adult testosterone; $\downarrow$ sperm concentration and motility, Phthalate syndrome: $\downarrow$ AGD; cryptorchidism; hypospadias; focal testis dysgenesis (10–150 mg/kg/d rat), DEHP/DBP/DiBP/BBzP SVHC; REACH restriction in EU consumer articles; PBDEs (Thyroid disruptors), Competitive TPO inhibition; TTR/TBG displacement; PPAR α activation, $\downarrow$ Child TSH per 10 $\times$ prenatal Σ PBDE (HOME Study); cryptorchidism (Finnish/Danish cohort), $\downarrow$ Ovarian follicle pool; thyroid follicular cysts; spermatogenesis impairment; accessory gland atrophy, Stockholm Convention POP (penta/octaBDE); deca-BDE restricted; PFAS (Multi-target), PPAR α ; TTR–T4 competition; NIS inhibition; TPO inhibition; immune disruption, $\downarrow$ Birth weight / gestational age (Project Viva); $\downarrow$ vaccine antibody titres; $\downarrow$ couple fertility (DNBC), Mammary ductal delay (0.3–5 mg/kg/d PFOA; CD-1 mouse); $\downarrow$ IgM TDAR; hepatomegaly, Stockholm Convention POP (PFOS/PFOA); REACH restriction; Parabens (Weak xenoestrogens), ER α -mediated uterotrophic activity; spermatocyte apoptosis (high doses); AR-independent pathways, Sperm DNA fragmentation (BuP; fertility clinic cohort); $\downarrow$ sperm concentration (PrP; Japanese subfertile cohort), Uterotrophic LOEL 4–20 mg/kg/d (EtP/MeP; rat); spermatid reduction (BuP; mouse); myometrial hypertrophy, BuP under SCCS review (EU); concentration limits in cosmetics by directive 76/768/CEE

Non-Monotonic Dose–Response: BPA, phthalates, and parabens all exhibit NMDR relationships in experimental models. BPA at 25 μ g/kg/day produces greater mammary gland dysplasia than 250 μ g/kg/day in some rodent strain-dose-endpoint combinations. NMDR patterns are characteristic of ligand-activated receptor systems (ER α , AR) where receptor saturation, downregulation, and competing mechanisms produce maximal response at intermediate doses — challenging standard toxicological dose–response modelling and the linear extrapolation from high to low doses used in regulatory acceptable daily intake derivation.

Epigenetic Programming and Transgenerational Inheritance: BPA (F3 social and follicular deficits), DEHP (F3 follicular depletion; accelerated puberty), and PBDEs (F1 neurobehavioural alterations via maternal epigenetic imprinting) have all demonstrated transmission of reproductive effects beyond the directly exposed generation.

The probable mechanisms involve altered DNA methylation at imprinted loci, piRNA and miRNA dysregulation in the germline, and histone modification inheritance through the primordial germ cell programming window. These findings extend the scope of reproductive toxicological risk assessment beyond the directly exposed individual.

Mixture Toxicology: In-vivo studies demonstrate additive anti-androgenic effects of phthalate combinations at doses ineffective individually (DEHP + DBP at 100 mg/kg/day each vs. 500 mg/kg/day of either compound alone), consistent with the concept of dose addition within a mechanistically related class. Population-based mixture analyses (WQS, BKMR) consistently find joint EDC exposures produce reproductive associations absent for individual chemicals at ambient concentrations, with public health implications that exceed those suggested by single-compound risk assessments.

COMPLETE REFERENCE LIST — PART III

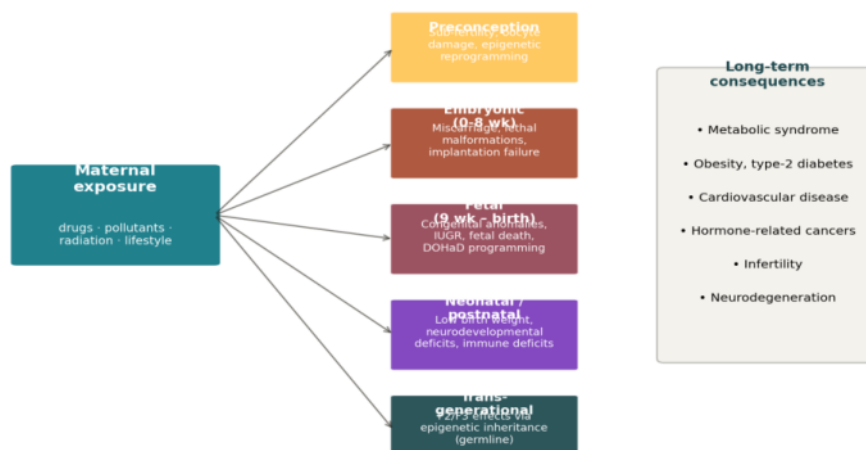
1. Braun JM, Li N, Arbuckle TE, Dodds L, Massarelli I, Fraser WD, et al. Association between gestational urinary bisphenol A concentrations and adiposity in young children: the MIREC study. *Environ Res.* 2019;174:112–119. doi:10.1016/j.envres.2019.02.038
2. Smarr MM, Grantz KL, Sundaram R, Maisog JM, Kannan K, Buck Louis GM. Parental urinary biomarkers of preconception exposure to bisphenol A and phthalates in relation to birth outcomes. *Environ Health.* 2015;14:73. doi:10.1186/s12940-015-0062-0
3. Markey CM, Luque EH, Munoz de Toro M, Sonnenschein C, Soto AM. In utero exposure to bisphenol A alters the development and tissue organization of the mouse mammary gland. *Biol Reprod.* 2001;65(4):1215–1223. doi:10.1093/biolreprod/65.4.1215
4. Tharp AP, Maffini MV, Hunt PA, VandeVoort CA, Sonnenschein C, Soto AM. Bisphenol A alters the development of the rhesus monkey mammary gland. *Proc Natl Acad Sci USA.* 2012;109(21):8190–8195. doi:10.1073/pnas.1120488109
5. Wolstenholme JT, Drobña Z, Henriksen AD, Goldsby JA, Stevenson R, Irvin JW, et al. Transgenerational bisphenol A causes deficits in social recognition and alters postsynaptic density genes in mice. *Endocrinology.* 2019;160(8):1854–1867. doi:10.1210/en.2019-00196
6. Guxens M, Ballester F, Espada M, Fernández MF, Grimalt JO, Ibarluzea J, et al. Cohort profile: the INMA—Infancia y Medio Ambiente—(Environment and Childhood) Project. *Int J Epidemiol.* 2012;41(4):930–940. doi:10.1093/ije/dyr054
7. Swan SH, Main KM, Liu F, Stewart SL, Kruse RL, Calafat AM, et al. Decrease in anogenital distance among male infants with prenatal phthalate exposure. *Environ Health Perspect.* 2005;113(8):1056–1061. doi:10.1289/ehp.8100
8. Wang Y, Ni C, Li X, Lin Z, Zhu Q, Li L, Ge RS. Phthalate-induced fetal Leydig cell dysfunction mediates male reproductive tract anomalies. *Front Pharmacol.* 2019;10:1309. doi:10.3389/fphar.2019.01309
9. Fletcher EJ, Santacruz-Márquez R, Mourikes VE, Neff AM, Laws MJ, Flaws JA. Effects of phthalate mixtures on ovarian folliculogenesis and steroidogenesis. *Toxics.* 2022;10(5):251. doi:10.3390/toxics10050251
10. Carnevali O, Tosti L, Speciale C, Peng C, Zhu Y, Maradonna F. DEHP impairs zebrafish reproduction by affecting critical factors in oogenesis. *PLoS One.* 2010;5(4):e10201. doi:10.1371/journal.pone.0010201
11. Talsness CE, Kuriyama SN, Sterner-Kock A, Schnitker P, Grande SW, Shakibaei M, et al. In utero and lactational exposures to low doses of polybrominated diphenyl ether-47 alter the reproductive system and thyroid gland of female rat offspring. *Environ Health Perspect.* 2008;116(3):308–314. doi:10.1289/ehp.10536
12. Vuong AM, Braun JM, Webster GM, Zoeller RT, Hoofnagle AN, Sjödin A, et al. Polybrominated diphenyl ether (PBDE) exposures and thyroid hormones in children at age 3 years. *Environ Int.* 2018;117:339–347. doi:10.1016/j.envint.2018.05.019
13. Zhang T, Zhou X, Xu A, Tian Y, Wang Y, Zhang Y, et al. Toxicity of polybrominated diphenyl ethers (PBDEs) on rodent male reproductive system: a systematic review and meta-analysis. *Environ Res.* 2020;187:109642. doi:10.1016/j.envres.2020.109642
14. Makey CM, McClean MD, Braverman LE, Pearce EN, Sjödin A, Weinberg J, Webster TF. Polybrominated diphenyl ether exposure and reproductive hormones in male participants of the Flame Retardant Exposure Study. *Reprod Toxicol.* 2016;62:46–52. doi:10.1016/j.reprotox.2016.04.009

15. White SS, Kato K, Jia LT, Basden BJ, Calafat AM, Hines EP, et al. Effects of perfluorooctanoic acid on mouse mammary gland development and differentiation resulting from cross-foster and restricted gestational exposures. *Reprod Toxicol*. 2009;27(3–4):289–298. doi:10.1016/j.reprotox.2008.11.054
16. Zhang Y, Mustieles V, Sun Q, Coull B, McElrath T, Rifas-Shiman SL, et al. Association of early pregnancy perfluoroalkyl and polyfluoroalkyl exposure with birth outcomes. *JAMA Netw Open*. 2023;6(5):e2314934. doi:10.1001/jamanetworkopen.2023.14934
17. Taylor KD, Woodlief TL, Ahmed A, Hu Q, Duncker PC, DeWitt JC. Quantifying the impact of PFOA exposure on B-cell development and antibody production. *Toxicol Sci*. 2023;194(1):101–114. doi:10.1093/toxsci/kfad043
18. Coperchini F, Croce L, Ricci G, Magri F, Rotondi M, Imbriani M, Chiovato L. Thyroid disrupting effects of old and new generation PFAS. *Front Endocrinol*. 2021;11:612320. doi:10.3389/fendo.2020.612320
19. Sun L, Yu T, Guo J, Zhang Z, Hu Y, Xiao X, et al. The estrogenicity of methylparaben and ethylparaben at doses close to the acceptable daily intake in immature Sprague-Dawley rats. *Sci Rep*. 2016;6:25173. doi:10.1038/srep25173
20. Hubbard TD, Brix A, Blystone CR, McIntyre BS, Shockley K, Cunny H, et al. Butylparaben multigenerational reproductive assessment by continuous breeding in Hsd:Sprague-Dawley SD rats. *Reprod Toxicol*. 2020;97:151–163. doi:10.1016/j.reprotox.2020.07.006
21. Burstyn I, Cox LA Jr, Cao Y, Eslick G, Harris S, Kheifets L, et al. Improving the design of epidemiology studies that use biomonitoring data. *BMC Med Res Methodol*. 2026;26:28. doi:10.1186/s12874-025-02753-5
22. McNabb-Kelada N, Burke T, Jayaraman S, Mills L, De La Torre A, Francoeur M, et al. Early-life PBDE flame retardant exposures cause neurobehavioral alterations in fish that persist into adulthood and vary by sex and route of exposure. *bioRxiv*. 2025. doi:10.1101/2025.04.28.651140
23. Shen X, Zhan M, Wang Y, Tang W, Zhang Q, Zhang J. Exposure to parabens and semen quality in reproductive-aged men. *Ecotoxicol Environ Saf*. 2023;265:115499. doi:10.1016/j.ecoenv.2023.115499
24. Iwamoto S, Miyashita C, Itoh S, Minatoya M, Araki A, Kishi R. Paraben exposure and semen quality of Japanese male partners of subfertile couples. *Environ Health Prev Med*. 2017;22(1):51. doi:10.1186/s12199-017-0618-7
25. Grandjean P, Andersen EW, Budtz-Jørgensen E, Nielsen F, Mølbak K, Weihe P, Heilmann C. Serum vaccine antibody concentrations in children exposed to perfluorinated compounds. *JAMA*. 2012;307(4):391–397. doi:10.1001/jama.2011.2034
26. Kortenkamp A. Low dose mixture effects of endocrine disruptors: implications for risk assessment and epidemiology. *Int J Androl*. 2008;31(2):233–240. doi:10.1111/j.1365-2605.2007.00862.x
27. Maffini MV, Rubin BS, Sonnenschein C, Soto AM. Endocrine disruptors and reproductive health: the case of bisphenol-A. *Mol Cell Endocrinol*. 2006;254–255:179–186. doi:10.1016/j.mce.2006.04.033

IV. Toxicology of Pregnancy

The reproductive success of a couple ultimately depends on a delicate biochemical equilibrium involving the father, the mother, and the fetus, and the interactions between them. The metabolic adaptations that occur during pregnancy — including increased pulmonary absorption and cardiac output, delayed gastric emptying, increased intestinal motility, and altered body fat distribution — can substantially modify the pharmacokinetics of xenobiotics and increase exposure of both the pregnant woman and the conceptus to potentially harmful substances. As illustrated in Figure 13, the consequences of maternal exposure to toxic agents for the offspring depend critically on the developmental stage at the time of exposure — whether during early or late embryogenesis, or during the fetal period.

Figure 13. Outcomes of maternal exposure to reprotoxicants for the offspring



Consequences for offspring of maternal exposure to toxic substances according to developmental stage. The figure illustrates differential outcomes (embryo lethality, teratogenicity, growth restriction, functional deficits) resulting from exposures at pre-implantation, organogenesis, and fetal periods of development.

The transported fertilised ovum requires two to six days before implantation. During this early pre-implantation stage, the embryo may be exposed to chemical substances that penetrate uterine fluids; absorption of xenobiotics can result in degenerative changes, alteration of the blastocyst protein profile, or implantation failure. The later period of embryogenesis is characterised by the differentiation, mobilisation, and organisation of cells and tissues into organ primordia. Pathogenic processes during this window can cause cell death, failure of cell-cell interaction, reduced biosynthesis, abnormalities in morphogenetic movements, mechanical disruption, adhesions, or oedema. The fetal period — beginning between days 54 and 60 of gestation when the crown-rump length reaches approximately 33 mm — is characterised by growth, histogenesis, and functional maturation. Toxic insults during this period may produce growth restriction, functional disorders, behavioural disturbances, transplacental carcinogenesis, or fetal death. The present part examines the biological, epidemiological, and mechanistic dimensions of these three cardinal categories of adverse pregnancy outcome: embryonic and fetal death; congenital anomalies; and intrauterine growth restriction.

IV.A. Embryonic and Fetal Death

IV.A.1. Definitions and Classification (ICD-11)

Precise, internationally standardised definitions of pregnancy loss are a prerequisite for epidemiological comparability across health systems, registries, and countries. The WHO International Classification of Diseases, 11th Revision (ICD-11), which came into effect on 1 January 2022, provides the current international framework for classifying gestational losses. National registration thresholds vary — gestational age cutoffs of 20, 22, 24, and 28 completed weeks are used by different countries — generating substantial heterogeneity in published rates that must be considered when interpreting surveillance data.

Table IV.A.1. ICD-11 Definitions of Fetal and Neonatal Death (WHO, 2022)

Category	ICD-11 Definition	Gestational Age / Weight Threshold	Notes
Early embryonic loss	Loss of conceptus before 6 completed weeks of gestation (post-LMP)	< 6 weeks	Often subclinical; may present as delayed or heavy menses
Miscarriage (spontaneous abortion)	Loss of conceptus before viability	< 22 completed weeks or < 500 g	ICD-11 subtypes: threatened, inevitable, incomplete, complete, missed (retained), recurrent (≥3 consecutive)
Stillbirth (fetal death)	Birth of a fetus showing no signs of life (no breathing, heartbeat, or voluntary muscle movement)	≥ 22 completed weeks or ≥ 500 g	WHO "reportable" late stillbirth threshold: ≥ 28 completed weeks (for international comparison)
Early neonatal death	Death of a liveborn infant	≤ 7 completed days (168 hours) of life	Included in perinatal death definitions
Late neonatal death	Death of a liveborn infant	> 7 days and ≤ 28 completed days of life	—
Perinatal death	Encompasses stillbirths (≥ 22 weeks) and early neonatal deaths (≤ 7 days)	—	For developing-country comparisons, intrapartum vs. antepartum distinction is important

An important methodological subdivision for research purposes distinguishes early fetal death (before 12 completed weeks of gestation, in which chromosomal anomalies predominate as cause) from late fetal death (after 12 weeks), in which placental, infectious, and environmental factors assume greater aetiological weight. The historical framework of Kline, Stein, and Susser (1989) — one of the foundational texts in reproductive epidemiology — used days from ovulation (DOV) to define developmental stages: blastocyst (days 1–6 DOV), pre-embryo and implantation (days 6–7 DOV), embryo (days 7–48 DOV), and fetal period (day 49 DOV onwards to the period of viability, approximately days 140–195). This granular periodisation remains useful in experimental and occupational toxicology settings.

IV.A.2. Global Epidemiology

Miscarriage and Early Pregnancy Loss

Miscarriage is the most common complication of pregnancy, affecting approximately 10–20% of clinically recognised pregnancies and up to 30–50% when biochemical pregnancies detected by early hCG testing are included. More than 90% of recognised pregnancy losses occur in the first trimester. When subclinical or "chemical" early losses — identified by urinary human chorionic gonadotropin (hCG) radioimmunoassay — are included, the total spontaneous abortion rate increases substantially: the landmark Wilcox et al. (1988) study found an incidence of subclinical fertilised-ovum loss following implantation of 22%. Chromosomal abnormalities in the conceptus account for approximately 50–70% of early spontaneous miscarriages; a 2023 study using whole-genome sequencing of products of conception found that up to two-thirds of all pregnancy losses may be attributable to fetal chromosomal abnormalities, with aneuploidy (principally autosomal trisomies) being the dominant class (Magnus et al., *Nature Medicine*, 2023).

In retrospective and cross-sectional surveys, the proportion of fetal deaths before 20 weeks of gestation ranges from 5.5 to 12.6%; when the definition is extended to 28 weeks, it reaches 6.2–19.6%. In prospective cohort studies using clinically confirmed pregnancies, spontaneous abortion rates are more consistently 11.7–14.6% up to 28 weeks of gestation. Recurrent pregnancy loss (RPL), defined as three or more consecutive miscarriages,

affects approximately 1–2% of couples attempting conception, with an identifiable aetiology in only about 50% of cases.

Stillbirth: Global Burden of Disease

Globally, stillbirths represent one of the most common yet systematically overlooked categories of preventable death. According to the GBD 2021 analysis published in *The Lancet* in 2024, the global stillbirth rate was **23.0** (95% UI: 19.7–27.2) per 1,000 births at ≥ 20 weeks' gestation in 2021, equivalent to approximately **3.04 million stillbirths** annually (GBD 2021 Stillbirths Collaborators, *Lancet*, 2024). Using the WHO "late stillbirth" threshold of ≥ 28 completed weeks — the standard used for global reporting and the WHO Every Newborn Action Plan (ENAP) — the 2021 rate was **16.1** (95% UI: 13.9–19.0) per 1,000 births. Approximately 30.5% of all stillbirths (some 926,000 in 2021) occurred between 20 and 28 weeks of gestation and are therefore invisible under late-stillbirth definitions.

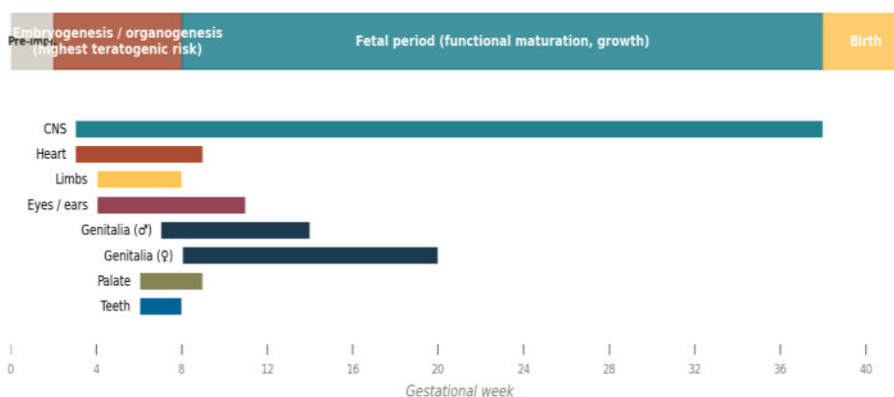
Regional disparities are stark and worsening in relative terms. Stillbirths in South Asia and sub-Saharan Africa comprised **77.4%** of the global total in 2021, up from 60.3% in 1990, indicating that geographic progress has been profoundly unequal. Despite a 39.8% reduction in global stillbirth rates between 1990 and 2021, this trajectory lagged behind the 45.6% concurrent reduction in neonatal mortality — the so-called "stillbirth paradox." The ENAP target is to reduce late-stillbirth rates to ≤ 12 per 1,000 births in every country by 2030; as of 2021, 139 mainly high-income countries had already met this target, while an estimated 56 countries will not achieve it without substantially accelerated intervention.

Table IV.A.2. Estimated Global and Regional Stillbirth Rates, GBD 2021 (≥ 28 Weeks' Gestation)

Region	Stillbirth Rate per 1,000 Births (≥ 28 weeks)	Approximate Absolute Numbers (2021)
High-income countries	~3–5	< 5% of global burden
Latin America and Caribbean	~8–12	—
North Africa and Middle East	~10–14	—
Global average	16.1 (95% UI: 13.9–19.0)	~2.1 million (≥ 28 wks)
East and Southeast Asia	~12–16	—
South Asia	~24–28	~40% of global burden
Sub-Saharan Africa	~28–35	~37% of global burden
Oceania / Pacific Islands	~18–20	—

Source: GBD 2021 Stillbirths Collaborators, *Lancet*, 2024.

Figure 21. Critical windows of developmental vulnerability in human pregnancy



Critical windows of developmental vulnerability. Timeline from fertilisation through birth showing the pre-implantation (all-or-nothing) period, the organogenesis window (weeks 3–8, peak teratogenic susceptibility), and the fetal period (functional maturation, growth, and CNS development), with key organ systems indicated at their sensitive periods.

IV.A.3. Developmental Mechanisms and Vulnerability Windows

The timing of a toxic or pathological insult relative to the developmental stage is the single most important determinant of the nature and severity of its effect. Three principal windows of vulnerability are recognised:

Pre-implantation period (days 0–14 post-fertilisation): During this window, undifferentiated cells retain totipotency and can compensate for limited cellular damage. The dominant consequence of chemical exposure is therefore either complete embryonic loss ("all-or-nothing" effect) — frequently manifesting as a delayed or abnormally heavy menstrual period — or no demonstrable effect. As Paul (1993) noted in the foundational occupational toxicology framework, the embryo is relatively resistant to teratogenic insults at this stage because cells have not yet entered the complex sequence of chemical differentiation; however, xenobiotic-induced alterations in blastocyst protein profiles and implantation failure represent genuine risks.

Organogenesis (weeks 3–8 post-fertilisation / days 18–56): This is the period of maximum susceptibility to structural teratogenesis. Neural tube closure occurs at days 22–28 post-fertilisation; cardiac septation at weeks 4–7; limb bud formation and differentiation at weeks 4–8; palatal fusion at weeks 6–9. Teratogenic exposures concentrated in this window carry the greatest risk of major structural anomalies: neural tube defects, congenital heart malformations, limb reduction defects, and orofacial clefts. Thalidomide's most critical teratogenic window is days 34–49 post-LMP (days 20–35 post-fertilisation).

Fetal period (week 9 to birth): Gross structural anomalies become substantially less likely because organogenesis is largely complete; however, functional disorders, organ-level toxicity (central nervous system, renal, endocrine), growth disturbances, and in extreme cases fetal death remain risks. The brain remains sensitive to toxic insult throughout gestation because myelination is not complete until the postnatal period; valproate, alcohol, and methylmercury all exert neurotoxic effects well into the fetal period.

The principal adverse reactions attributable to late embryogenesis exposures include spontaneous abortion, macroscopic structural anomalies, fetal death, growth restriction, and psychomotor developmental abnormalities. Fetal period insults more typically produce growth restriction, functional disorders, behavioural disturbances, and — with carcinogenic agents — transplacental carcinogenesis.

IV.A.4. Risk Factors for Embryonic and Fetal Death

Maternal Demographic and Physiological Factors

Maternal age: Advanced maternal age (AMA, defined as ≥ 35 years) is among the most consistently demonstrated risk factors for pregnancy loss. The rate of chromosomal aneuploidy in oocytes increases steeply with maternal age as a consequence of errors in meiotic chromosome segregation; trisomy 21 rates increase from approximately 1 in 1,000 births at age 30 to 1 in 100 at age 40. Women aged ≥ 40 have miscarriage rates approaching 40–50%. AMA is also independently associated with approximately a 1.5-fold increased risk of stillbirth after adjustment for confounders. Conversely, teenage pregnancy (< 20 years) confers elevated risks of placental abruption, preterm birth, and neonatal mortality. After adjustment for number of pregnancies and abortion history, the risk of fetal loss is approximately twice as high in women over 40 as in younger women, partly mediated by the increased frequency of trisomies.

Body mass index (BMI): Both extremes of BMI confer excess risk. Underweight women ($\text{BMI} < 18.5 \text{ kg/m}^2$) show higher rates of SGA births (OR 3.28, 95% CI: 1.51–7.05) and fetal growth restriction (OR 3.73, 95% CI: 1.54–8.37). Obese women ($\text{BMI} \geq 35 \text{ kg/m}^2$) face significantly elevated risks of gestational diabetes mellitus (OR 3.10, 95% CI: 1.95–4.89), pre-eclampsia, and stillbirth, with risk increasing with BMI category.

Parity and obstetric history: Grand multiparity is associated with increased risk, partly as a consequence of the association with maternal age and prior adverse obstetric outcomes. Prior spontaneous miscarriage is among the strongest predictors of subsequent pregnancy loss: women with one prior miscarriage have an approximately 20% recurrence risk; this rises to 30–40% after two consecutive losses. Obstetric history including prior fetal death, preterm birth, or a growth-restricted infant multiplies subsequent risk two- to four-fold.

Tobacco

Active smoking during pregnancy is significantly associated with miscarriage (OR 1.35, 95% CI: 1.18–1.55), with a clear dose-response relationship (OR 1.45 at ≥ 20 cigarettes/day) as demonstrated in the 2025 meta-analysis

by Rahimian et al. (*BMC Pregnancy Childbirth*, 2025). Passive (secondhand) smoking also confers measurable risk (OR 1.29, 95% CI: 1.15–1.45). Smoking increases stillbirth risk through multiple mechanisms: placental abruption, placental insufficiency via nicotine-mediated uteroplacental vasoconstriction, direct fetal hypoxia (elevated carboxyhaemoglobin and carboxymyoglobin), and reduced uterine and umbilical vascular diameter. Heavy smokers (≥ 20 cigarettes/day) show a 3.35-fold increased stillbirth risk in some cohort studies. A multigenerational effect has been documented, with maternal grandmaternal smoking in early pregnancy independently associated with increased stillbirth risk in grandchildren.

Alcohol

Alcohol consumption at any level during pregnancy carries no established safe threshold. Heavy first-trimester drinking is associated with increased rates of miscarriage and structural anomalies. Second-trimester heavy drinking is specifically associated with elevated risk of spontaneous abortion. A study of 9,236 births (Kaminski et al., 1978) demonstrated that maternal alcohol consumption exceeding 45.36 g/day was associated with an increased rate of stillbirths and growth-restricted infants. The teratological and neurodevelopmental consequences of alcohol are addressed in detail in Section IV.B.

Infections

TORCH complex (*Toxoplasma gondii*, Other agents, Rubella, Cytomegalovirus, Herpes simplex): These pathogens are the classical causes of congenital infection. First-trimester TORCH-positive maternal serology is associated with elevated rates of miscarriage, stillbirth, and congenital defects. Cytomegalovirus infection of the fetus causes neurological damage (sensorineural hearing loss, intellectual disability, microcephaly), stillbirth, and neonatal mortality. Maternal syphilis, mycoplasma genital infections, uterine infections, and maternal hyperthermia are similarly associated with fetal death.

Zika virus (ZIKV): Established as a human teratogen in 2016. Vertical transmission causes congenital Zika syndrome characterised by microcephaly, severe cerebral atrophy, ventriculomegaly, cerebellar hypoplasia, and arthrogryposis. The mechanism involves direct neurotropic infection of neural progenitor cells, causing impaired proliferation and increased apoptosis with resultant cortical developmental arrest.

SARS-CoV-2: COVID-19 infection during pregnancy — particularly during the Delta variant wave — was associated with a significantly increased risk of stillbirth (adjusted relative risk 1.90, 95% CI: 1.69–2.15 in a US cohort of 1,249,634 deliveries) (CDC *MMWR*, 2021). A large EHR cohort study found elevated odds of stillbirth following SARS-CoV-2 infection during early pregnancy (OR 1.75, 95% CI: 1.25–2.46) and mid-pregnancy (OR 2.09, 95% CI: 1.49–2.93), probably mediated by placental hypoperfusion driven by inflammatory and thrombotic pathology.

Chromosomal and Genetic Factors

Chromosomal abnormalities are the most common identifiable cause of first-trimester miscarriage, with trisomies (16, 22, 21, 18, 13) and monosomy X (45,X) constituting the most frequent aneuploidies. Balanced chromosomal rearrangements in either parent — particularly Robertsonian translocations — predispose to recurrent miscarriage through generation of unbalanced gametes.

Antiphospholipid Syndrome and Thrombophilias

Antiphospholipid syndrome (APS) is the most important acquired thrombophilic cause of recurrent pregnancy loss, characterised by antiphospholipid antibodies (lupus anticoagulant, anticardiolipin antibodies, anti- β_2 -glycoprotein I antibodies) causing placental thrombosis and infarction. APS accounts for approximately 15–20% of recurrent miscarriage cases. Inherited thrombophilias (Factor V Leiden, prothrombin G20210A mutation, protein C and S deficiency) are associated with placental vascular thrombosis; however, a Cochrane review and the ALIFE2 randomised controlled trial (2024) found no significant benefit of low-molecular-weight heparin over standard care for RPL in women with inherited thrombophilia (OR 1.08, 95% CI: 0.65–1.78).

Drug-Induced Embryo-Fetal Death

NSAIDs: A systematic review and meta-analysis of 38 observational studies found that NSAID use in early pregnancy was associated with a 28% increased risk of overall congenital anomalies (OR 1.28, 95% CI: 1.14–1.44) and a 19% increased risk of major birth defects (Schjøler et al., *Reproductive Toxicology*, 2024). After 20 weeks of gestation, NSAIDs inhibit prostaglandin-mediated renal vasodilation, causing fetal renal dysfunction and

oligohydramnios; at ≥ 30 weeks, they may cause premature ductal constriction. The FDA updated prescribing guidance in 2020 to recommend avoiding NSAIDs at ≥ 20 weeks gestation.

ACE inhibitors and ARBs: In the second and third trimesters, these agents block fetal renal prostaglandin synthesis, causing severe oligohydramnios, fetal renal tubular dysgenesis, skull hypoplasia (Potter sequence), neonatal renal failure, and fetal or neonatal death. These drugs are contraindicated throughout pregnancy.

Retinoids (isotretinoin): Oral isotretinoin is one of the most potent known human teratogens. Spontaneous abortion rates of 20–30% have been reported with first-trimester exposure; up to 40% of live-born infants with confirmed first-trimester exposure have major malformations. The mechanism involves retinoic acid receptor (RAR- α , RAR- β) dysregulation disrupting neural crest cell migration and anterior-posterior patterning.

IV.A.5. Environmental and Chemical Determinants

Lead

Prenatal lead exposure is associated with miscarriage risk, preterm birth, and growth restriction. Lead crosses the placenta readily; bone-stored lead is mobilised during pregnancy, potentially exposing the fetus to endogenous sources regardless of current environmental exposure. Lead mimics calcium in biological pathways, disrupts haem synthesis (affecting fetal oxygen transport), and causes epigenetic alterations in placental gene expression. A paternal occupational exposure effect of lead on fetal loss has also been documented (Savitz, Sonnenfeld, and Olshan, 1994), and a more consistently positive relationship has been observed between paternal mercury and anaesthetic gas exposure and fetal loss. No safe blood lead level during pregnancy has been established.

Mercury (Methylmercury)

Organic methylmercury crosses the placenta and accumulates in the fetal brain at concentrations exceeding maternal levels, because placental amino acid transporters (methionine transporters) actively concentrate it. The Minamata Bay epidemic (Japan, 1956) provided definitive proof of congenital methylmercury toxicity: children born to mothers who consumed contaminated seafood exhibited severe neurological damage including cerebral palsy, deafness, blindness, and intellectual disability (congenital Minamata disease). Mechanisms of neurotoxicity include disruption of the mitotic spindle (arresting neuroblast division), induction of apoptosis, generation of reactive oxygen species, and displacement of calcium in intracellular signalling.

PFAS (Per- and Polyfluoroalkyl Substances)

PFAS — so-called "forever chemicals" — persist indefinitely in biological systems. Prenatal PFAS exposure is associated with preterm birth (mothers with higher blood PFAS levels are approximately 1.5 times more likely to deliver preterm), IUGR, and reduced birth weight. PFAS cross the placenta by passive diffusion and have been detected in neonatal cord blood in the first published US detection study (Emory University, 2023). Mechanistically, PFAS disrupt maternal inflammatory cytokine balance (suppressing both IL-10 and TNF- α), alter placental development in a sex-specific manner, and interfere with hormonal regulation relevant to placentation. The relationship between PFAS and pregnancy loss is discussed further in Section IV.C.

Particulate Matter (PM_{2.5})

Ambient air pollution exposure during pregnancy is associated with fetal growth restriction and miscarriage through mechanisms of oxidative stress, systemic inflammation, placental disruption, and coagulation cascade activation. The placental uptake of fine and ultrafine particles has been demonstrated in histological studies. These mechanisms are elaborated quantitatively in Section IV.C.4.

Occupational Exposures

Several occupational exposures have been associated with adverse fetal outcomes in epidemiological studies. Women working in the paper pulp industry (laboratory or processing roles), in construction, or in transport and communications exposed to unspecified chemical mixtures showed significantly elevated risks of central nervous system anomalies, cardiac defects, and orofacial clefts in offspring. Veterinary professionals exposed to anaesthetic gases, radiation, insecticides, and zoonoses demonstrated significantly higher rates of congenital anomalies in offspring compared to control professions (Schenker et al., 1990). Paternal occupational exposures — to solvents, organic rubber manufacturing, certain pesticides, and anaesthetic gases — have been associated with adverse pregnancy outcomes, suggesting male-mediated fetal toxicity via sperm DNA damage, altered seminal plasma composition, or indirect maternal exposure.

IV.B. Congenital Anomalies

IV.B.1. Classification and Definitions

Congenital anomalies (birth defects, congenital disorders) are structural or functional abnormalities present at birth that arise during intrauterine development. Historically defined as "monstrosities" or visible structural malformations present at birth — whether internal or external, hereditary or not — the contemporary definition has been substantially broadened to encompass behavioural, functional, and biochemical abnormalities as well as gross anatomical defects.

A practical classification framework distinguishes:

- **Major anomalies:** Structural defects that cause significant morbidity, require medical or surgical intervention, or are life-threatening (e.g., neural tube defects, congenital heart defects, chromosomal syndromes).
- **Minor anomalies:** Isolated morphological variants without significant functional impairment (e.g., single palmar crease, attached earlobe). The clinical significance of minor anomalies escalates with number: the presence of three or more minor anomalies in a single individual indicates a substantially elevated probability of an underlying major anomaly or recognisable syndrome.
- **Structural anomalies:** Physically demonstrable malformations, deformations (mechanical constraint on otherwise normal tissue), or disruptions (destruction of previously normal tissue — as in vascular disruption sequences).
- **Functional and metabolic anomalies:** Metabolic, hormonal, or neurodevelopmental disorders without gross structural defect (e.g., congenital hypothyroidism, phenylketonuria, intellectual disability, fetal alcohol spectrum disorder).

Anomalies may be single or multiple; chromosomal alterations typically produce multiple system involvement, while single-gene mutations or environmental teratogen exposures may produce either an isolated defect or a characteristic syndrome. The proportion of malformations is approximately 41% higher in male than female offspring — largely explained by the higher frequency of genital organ anomalies (hypospadias, cryptorchidism) in males.

EUROCAT (European Surveillance of Congenital Anomalies) maintains the most comprehensive European population-level registry system, covering approximately 1.5 million births annually across 22 countries, and provides standardised case definitions for major congenital anomalies used as the international benchmark for surveillance.

IV.B.2. Global Prevalence and Burden

Congenital anomalies affect approximately **3–6% of all live births** worldwide (WHO), though estimates vary with surveillance methodology, case ascertainment strategy, and inclusion or exclusion of chromosomal anomalies, stillbirths, and terminations of pregnancy for fetal anomaly.

The global birth prevalence of major congenital anomalies in spontaneous abortuses is approximately 19% — roughly ten times the rate observed in live-born infants; among stillborn infants weighing more than 500 g, the prevalence of anomalies reaches 32% (Shepard, Fantel, and Fitsimmons, 1989). The prevalence of major malformations in viable newborns is approximately 2.24% (Nelson and Holmes, 1989); minor malformations affect 3–15% (mean approximately 10%).

The EUROCAT historical dataset (2003–2007) recorded a total prevalence of major congenital anomalies of **23.9 per 1,000 births** (combining live births, stillbirths, and terminations for fetal anomaly) across its registry populations (Dolk, Loane, and Garne, *Adv Exp Med Biol*, 2010).

A systematic review and meta-analysis of 123 population-based studies encompassing 769 million births (Xie et al., *BMC Pediatrics*, 2025) provided updated specific prevalence data for eight major anomaly categories.

Table IV.B.1. Global Birth Prevalence of Selected Major Congenital Anomalies (Xie et al., 2025)

Congenital Anomaly	Pooled Prevalence per 10,000 Births	95% Confidence Interval
Anorectal malformation	3.11	2.77–3.50
Oesophageal atresia	2.46	2.29–2.63

Congenital Anomaly	Pooled Prevalence per 10,000 Births	95% Confidence Interval
Congenital diaphragmatic hernia	2.42	2.21–2.66
Omphalocele	2.23	2.02–2.46
Gastroschisis	1.79	1.55–2.07
Hirschsprung disease	1.34	1.10–1.63
Duodenal atresia	0.94	0.81–1.09
Intestinal atresia	0.86	0.72–1.01

Congenital heart defects (CHDs) remain the most common single category of major non-chromosomal anomaly at approximately 6.5 per 1,000 births in EUROCAT data. From the GBD 2021 cross-sectional study encompassing 204 countries (Li et al., *BMC Pediatrics*, 2025): globally, 31.64 million children aged 0–14 years were living with congenital birth defects in 2021 (prevalence rate 1,572.5 per 100,000), a 6.68% increase in absolute cases from 1990, though with a declining age-standardised rate (EAPC –0.17 per year); congenital heart anomalies accounted for 46.7% of all congenital birth defect deaths in 2021; and approximately 94% of severe congenital disorders occur in low- and middle-income countries.

The aetiological distribution of congenital anomalies is: genetic factors 10.1%; multifactorial inheritance 23%; uterine factors 2.5%; twinning 0.4%; teratogenic substances 3.2%; remainder of unknown cause. This large "unknown" category underscores the challenge of identifying specific chemical or environmental determinants.

IV.B.3. Teratogenic Mechanisms: Wilson's Six Principles (Updated Framework)

James G. Wilson's six principles of teratology (1959, refined 1977) remain the most widely cited conceptual framework in developmental toxicology. Their interpretation must, however, be updated to incorporate contemporary mechanistic knowledge.

Principle 1 — Susceptibility depends on the genotype of the conceptus and the manner in which it interacts with adverse environmental influences: Genetic variation in metabolic enzymes (CYP2C9, EPHX1, glutathione S-transferases), folate pathway genes (MTHFR C677T and A1298C polymorphisms), and developmental signalling receptor expression modulates individual teratogenic susceptibility. The same teratogenic dose of valproate, for instance, does not produce the same MCM rate in all exposed pregnancies.

Principle 2 — Susceptibility to teratogenic agents varies with developmental stage at the time of exposure: Pre-implantation (all-or-nothing effect; days 0–14); organogenesis (greatest risk of structural defects; weeks 3–8); fetal period (functional and growth effects). The temporal specificity of individual teratogens follows directly from this principle: thalidomide's limb defect window is days 34–49 post-LMP; valproate spina bifida risk peaks during neural tube closure at days 22–28 post-fertilisation.

Principle 3 — Teratogenic agents act through specific biochemical and molecular mechanisms on developing cells and tissues: Identified mechanisms include: (a) inhibition of DNA synthesis and folate antagonism (methotrexate, valproate via HDAC inhibition); (b) oxidative stress and reactive oxygen species generation (thalidomide, alcohol, methylmercury); (c) receptor-mediated disruption (retinoic acid receptors — isotretinoin; sex hormone receptors — DES; aryl hydrocarbon receptor — dioxins/PCBs); (d) inhibition of specific enzymes (warfarin: vitamin K carboxylase; NSAIDs: cyclooxygenase/prostaglandin synthesis); (e) disruption of cell signalling pathways (thalidomide via cereblon/SALL4; Zika virus via neural progenitor cell apoptosis); (f) vascular disruption (misoprostol, cocaine); (g) epigenetic modification (PFAS, arsenic, certain heavy metals).

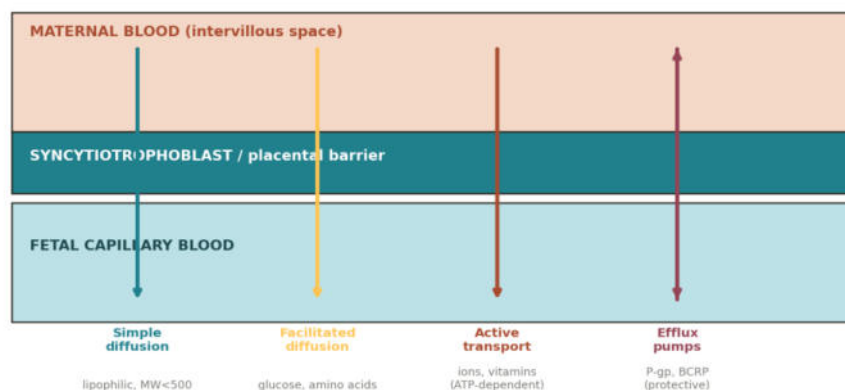
Principle 4 — Access of adverse influences to the developing tissues depends on the nature of the agent: Molecular weight, lipophilicity, protein binding, ionisation state, and expression of specific transporters in the syncytiotrophoblast collectively determine placental transfer. Small lipophilic molecules (most drugs, ethanol, organic mercury, organic solvents) cross freely by passive diffusion following Fick's law (rate proportional to concentration gradient, surface area, membrane thickness, and lipid-water partition coefficient). Transporter-mediated influx (SLC-family: OCT3, ENT1/2) mediates transfer of specific substrates. Protective efflux transporters (P-gp/ABCB1, BCRP/ABCG2, MRP2/ABCC2) pump certain agents back into maternal circulation, limiting fetal exposure; this partial barrier is overcome by high concentrations or by inhibition of these

transporters. Ion trapping also operates: because fetal blood pH is approximately 0.1 units lower than maternal blood, weak bases are concentrated in fetal circulation relative to maternal.

Principle 5 — Four manifestations of deviant development are death, malformation, growth retardation, and functional deficit: The type of effect is a function of dose, timing, and all preceding factors. At subthreshold doses, no effect may be discernible; just above threshold, the dose-response curve for structural teratogenesis is often steep. Valproate at $\geq 1,500$ mg/day monotherapy carries MCM rates up to 24%, versus approximately 5% at doses < 700 mg/day.

Principle 6 — Dose-response relationships always exist and must be characterised: Thresholds exist below which no embryotoxic effect is observed. The shape of the dose-response relationship — and particularly the threshold dose — varies with gestational timing, species, and individual genotype.

Figure 22. Mechanisms of placental transfer of xenobiotics



Structure of the human haemochorial placenta and transfer mechanisms at the syncytiotrophoblast. The figure shows passive transcellular diffusion, transporter-mediated influx (SLC-family), protective efflux transporters (ABCB1/P-gp, ABCG2/BCRP), endocytosis, and paracellular routes, with arrows indicating direction of transfer for representative xenobiotics.

IV.B.4. Known Pharmaceutical Teratogens

Valproate (Valproic Acid / Sodium Valproate)

Valproate is currently the best-characterised and most potent pharmaceutical teratogen in widespread clinical use worldwide. A Cochrane systematic review (Bromley et al., 2023) found major congenital malformations (MCMs) in **9.8%** (95% CI: 8.1–11.9%) of children exposed to valproate in utero, compared with 2.1% in women without epilepsy and 2.7% in women on lamotrigine monotherapy — the preferred alternative. The EURAP international pregnancy registry (Battino et al., 2024, covering 40 countries) reported MCMs in 9.9% (95% CI: 8.5–11.5%) of valproate-exposed pregnancies, entirely consistent with the Cochrane estimate.

Specific malformation risks from EUROCAT registry data (Jentink et al., *N Engl J Med*, 2010) are shown in Table IV.B.2:

Table IV.B.2. Valproate Teratogenicity — Specific Malformation Risk (EUROCAT Registry, Jentink et al., 2010)

Malformation	Adjusted Odds Ratio vs. Unexposed	95% Confidence Interval
Spina bifida	12.7	7.7–20.7
Cleft palate (isolated)	5.2	2.8–9.9
Craniosynostosis	6.8	1.8–18.8
Hypospadias	4.8	2.9–8.1
Atrial septal defect	2.5	1.4–4.4
Polydactyly	2.2	1.0–4.5

Beyond structural teratogenicity, valproate causes severe neurodevelopmental toxicity independent of visible malformations: IQ approximately 8–9 points lower than children unexposed or born to mothers with untreated epilepsy; 2–5-fold increased risk of intellectual disability (dose-dependent); autism spectrum disorder (adjusted HR 3.10, 95% CI: 2.24–4.28); ADHD (adjusted HR 1.62, 95% CI: 1.30–2.01); and by age 18 years, 42.1% absolute risk of any psychiatric disorder diagnosis.

The primary mechanism involves inhibition of histone deacetylase (HDAC), leading to hyperacetylation of H3 and H4 histones and epigenetic dysregulation of neural tube closure genes and neurodevelopmental gene networks. Valproate also inhibits folate metabolism and elevates homocysteine, contributing to neural tube defect risk. Folic acid supplementation at doses of 4–5 mg/day is recommended (rather than the standard 0.4 mg periconceptional dose), though a 2024 systematic review found no significant mitigation of structural anomaly risk specifically attributable to supplementation in valproate-exposed pregnancies.

Monotherapy at doses $\geq 1,500$ mg/day carries MCM rates up to 24%; polytherapy (valproate combined with other antiepileptics) further amplifies risk (OR monotherapy vs. unexposed 1.46, 95% CI: 1.11–1.91; polytherapy OR 2.06, 95% CI: 1.32–3.20; Kim et al., 2026).

Isotretinoin (13-cis-Retinoic Acid)

Isotretinoin (Accutane), used for severe acne, is one of the highest-risk pharmaceutical teratogens. Exposure during weeks 3–6 post-conception causes **retinoic acid embryopathy**: craniofacial malformations (microtia, cleft palate, nasal hypoplasia), CNS defects (hydrocephalus, cortical malformations), cardiac outflow tract defects, and thymic aplasia. Spontaneous abortion rates of 20–30% and major congenital anomalies in up to 40% of live-born infants have been reported with first-trimester exposure. The mechanism involves dysregulation of retinoic acid receptor (RAR- α , RAR- β) signalling, which governs anterior-posterior patterning, neural crest cell differentiation, and multiple organ developmental processes. The US iPLEDGE Risk Evaluation and Mitigation Strategy (REMS) programme — mandating monthly pregnancy testing and dual contraception — has demonstrably reduced but not eliminated inadvertent pregnancies. Topical retinoids, while structurally related, show no significantly elevated major congenital malformation risk in large Nordic cohort studies (aRR 1.1, 95% CI: 0.87–1.38; Puk et al., 2026).

Thalidomide

Thalidomide was withdrawn worldwide in 1961 following its association with over 10,000 cases of severe limb malformations (amelia/phocomelia, predominantly of the upper limbs), with infant mortality from associated internal organ malformations (cardiac, renal, gastrointestinal) reaching approximately 40%. It has since been re-introduced under strict risk management programmes for treatment of multiple myeloma and Hansen disease. The critical teratogenic window is days 34–49 post-LMP; phenotypic expression is tightly time-linked: exposure on days 20–24 post-fertilisation produces absent external ear; days 24–34 post-fertilisation produces inner ear, eye, and upper-limb damage (phocomelia/amelia). Humans are more than 1,000 times more susceptible than rats to thalidomide embryopathy — a disparity that allowed the drug's teratogenicity to be missed in initial rat reproductive toxicity testing.

The molecular mechanism, resolved in 2010, involves thalidomide binding to **cereblon** (CRBN), a substrate receptor of the CUL4A E3 ubiquitin ligase complex, causing proteasomal degradation of critical neosubstrate proteins including **SALL4** (a zinc-finger transcription factor essential for limb, ear, and eye development), IKZF1/3, and others. The SALL4 degradation hypothesis is the best-supported molecular explanation for thalidomide teratogenesis (Yamamoto et al., *eLife*, 2020). Additional mechanisms include anti-angiogenesis (disrupting limb bud vasculogenesis) and ROS-mediated oxidative stress.

Warfarin (Coumarin Anticoagulants)

Warfarin crosses the placenta freely; fetal serum concentrations may approach or exceed maternal values. **Warfarin embryopathy** (fetal warfarin syndrome) affects approximately **6%** of children born to mothers taking warfarin during the critical first-trimester window (weeks 6–12 post-LMP). Features include nasal hypoplasia, stippled epiphyses, bone and cartilage malformations, and growth restriction. The mechanism involves inhibition of vitamin K-dependent gamma-carboxylation of proteins essential for bone and cartilage matrix formation — principally matrix Gla protein (MGP) and osteocalcin. Second- and third-trimester exposure primarily causes CNS haemorrhagic defects (intracranial haemorrhage, hydrocephalus, Dandy-Walker

malformation) through interference with fetal haemostasis. Doses > 5 mg/day are associated with higher teratogenic risk, though cases have been reported at lower doses.

Methotrexate and Mycophenolate

Methotrexate, a folate antagonist and potent embryotoxin, causes miscarriage and **aminopterin/methotrexate embryopathy** (craniofacial abnormalities, limb reduction defects, CNS malformations) when administered systemically at rheumatological or oncological doses. It should be discontinued ≥ 3 months before conception. Paternal methotrexate exposure was not associated with increased congenital malformation risk (aOR 1.00, 95% CI: 0.62–1.61) in a 2025 systematic review.

Mycophenolate mofetil (MMF), used in transplantation and autoimmune disease, causes a characteristic syndrome including microtia, ear canal atresia, cleft palate, congenital heart defects, and digital/limb abnormalities in approximately 4–5% of exposed pregnancies. EULAR 2025 guidelines recommend discontinuing cyclophosphamide, methotrexate, and mycophenolate before conception.

Alcohol — Fetal Alcohol Spectrum Disorder (FASD)

Alcohol (ethanol) is the leading preventable cause of congenital disability worldwide and the most prevalent non-genetic intellectual disability. Ethanol and its principal metabolite acetaldehyde are direct cellular toxins that cross the placenta by rapid passive diffusion; the fetus cannot metabolise alcohol as efficiently as the adult, leading to prolonged fetal exposure relative to maternal blood alcohol concentrations. **There is no established safe level or safe gestational period for alcohol consumption during pregnancy.**

FASD encompasses a spectrum of permanent conditions:

- **Fetal Alcohol Syndrome (FAS):** The most severe form, characterised by the triad of facial dysmorphism (smooth philtrum, thin upper lip, small palpebral fissures), CNS damage (intellectual disability, behavioural problems), and prenatal/postnatal growth restriction. Prevalence in the USA: approximately **6–9 per 1,000 live births** (CDC, 2024).
- **Alcohol-related neurodevelopmental disorder (ARND):** Cognitive and behavioural impairments (executive function deficits, memory, attention) without the facial features of FAS.
- **Alcohol-related birth defects (ARBD):** Structural malformations of the heart, kidney, bone, and other organ systems.
- Overall FASD prevalence in the USA: estimated at **24–48 per 1,000 births**; worldwide prevalence approximately 7.7 per 1,000 (Front. Medicine, 2024).

Mechanistic pathways include: direct mitochondrial toxicity; inhibition of the neural cell adhesion molecule L1CAM (critical for neuronal migration); oxidative stress and ROS generation; suppression of retinoic acid synthesis; epigenetic dysregulation of imprinted genes and DNA methylation; and prostaglandin pathway disruption. The brain is sensitive to alcohol throughout gestation. First-trimester exposure preferentially causes facial dysmorphism and structural brain anomalies; second-trimester heavy drinking specifically elevates spontaneous abortion risk; third-trimester exposure causes growth restriction and brain volume reduction.

IV.B.5. Environmental Teratogens

Table IV.B.3. Known Human Teratogens: Pharmaceutical and Environmental Agents with Mechanisms

Agent	Critical Exposure Window	Primary Anomalies / Outcomes	Mechanism of Action
Thalidomide	Days 34–49 post-LMP	Amelia/phocomelia, cardiac, ear defects	Cereblon (CRBN) binding → SALL4 proteasomal degradation; anti-angiogenesis; ROS
Isotretinoin	Weeks 3–6 post-conception	Craniofacial, CNS, cardiac, thymic defects	RAR- α/β dysregulation; neural crest cell disruption
Valproate	Weeks 3–8 (structural); all trimesters (neurodevelopmental)	Spina bifida, cardiac, cleft palate;	HDAC inhibition; folate antagonism; histone hyperacetylation

Agent	Critical Exposure Window	Primary Anomalies / Outcomes	Mechanism of Action
		neurodevelopmental toxicity	
Warfarin	Weeks 6–12 (structural); 2nd–3rd trimester (CNS haemorrhage)	Nasal hypoplasia, stippled epiphyses; ICH, hydrocephalus	Vitamin K antagonism; MGP/osteocalcin carboxylation inhibition
Methotrexate	Weeks 4–10	Craniofacial defects, limb reduction, NTD	Folate antagonism; inhibition of dihydrofolate reductase and DNA synthesis
Mycophenolate	1st trimester	Microtia, cleft palate, limb/digital defects	IMPDH inhibition; guanosine nucleotide depletion
Ethanol	All trimesters	FAS, FASD (CNS, facial, growth restriction)	L1CAM inhibition; oxidative stress; mitochondrial toxicity; epigenetic
Methylmercury	Fetal period (especially 2nd trimester)	Cerebral palsy, deafness, cortical atrophy (Minamata disease)	Mitotic spindle disruption; ROS; neuronal apoptosis; amino acid transporter-mediated placental accumulation
Rubella virus	1st trimester (< 12 weeks most severe)	Congenital heart defect, cataracts, sensorineural deafness	Direct viral teratogenesis; vascular disruption; inflammation
Zika virus	1st–2nd trimester	Microcephaly, cortical malformations, arthrogryposis	Neural progenitor cell (NPC) tropism; apoptosis; cortical developmental arrest
Lead	Ongoing fetal exposure	NTD, growth restriction, neurocognitive deficit	Calcium mimicry; haem synthesis disruption; epigenetic (DNA methylation)
Dioxins (TCDD)	Fetal period	Endocrine disruption, craniofacial defects (cleft palate)	AhR activation; Wnt/ β -catenin pathway disruption; sex differentiation alteration
PFAS	Fetal period	Congenital heart defects (emerging evidence); growth effects	PPAR activation; endocrine disruption; epigenetic modifications

Methylmercury is the classical environmental teratogen proven definitively at Minamata, Japan (1956 epidemic). Methylmercury accumulates in the fetal brain via placental amino acid transport mechanisms. Low-level prenatal methylmercury exposure — from fish consumption in cohort studies of populations in the Faroe Islands and Seychelles — is associated with reduced performance in language, memory, attention, and fine motor tests in children.

Dioxins and PCBs: Polychlorinated dibenzo-*p*-dioxins and polychlorinated biphenyls activate the aryl hydrocarbon receptor (AhR), disrupting development of multiple organ systems. PCB exposure through contaminated rice oil (Yusho disease, Japan, 1968) caused a characteristic syndrome in newborns: growth restriction, brown skin pigmentation, premature tooth eruption, gingival hyperplasia, widening of the sagittal suture, facial oedema, and exophthalmos — a direct parallel to the Minamata syndrome in demonstrating mass chemical teratogenesis through contaminated food.

PFAS: In addition to growth restriction effects, PFAS are suspected developmental toxicants. Emerging evidence links gestational PFAS exposure to congenital heart defects, hypospadias, and limb anomalies, though dose-response data are less robust than for pharmaceutical teratogens.

IV.B.6. FDA Pregnancy Labelling: From Letter Categories to the Pregnancy and Lactation Labelling Rule (PLLR)

The FDA's former pregnancy letter classification system (Categories A, B, C, D, and X, introduced in 1979) was retired effective **30 June 2015** with the implementation of the Pregnancy and Lactation Labelling Rule (PLLR; FDA, 2014). The categorical letter system had been criticised on multiple grounds: oversimplification of complex risk data; prescriber confusion (particularly the widespread misinterpretation of Category C as implying mere "caution" rather than reflecting absence of adequate human data); inability to convey gestational-timing specificity; and failure to reflect dose-response relationships or newer pharmacokinetic and mechanistic evidence.

Table IV.B.4. FDA Pregnancy Labelling: Former Letter Categories versus PLLR Narrative Subsections

Former Category	Description	PLLR Section	Content Requirements under PLLR
A	Adequate controlled studies in women show no fetal risk	8.1 Pregnancy	Risk Summary (human and animal data); Clinical Considerations (dose adjustments, monitoring, adverse reactions, fetal/neonatal complications); Data (human and animal study details, pregnancy registries)
B	Animal studies show no risk; no adequate human studies OR animal studies show adverse effect not confirmed in controlled women studies	8.2 Lactation	Risk/benefit for breastfeeding; amount in milk; infant effects; monitoring recommendations
C	Animal studies show adverse effects; no adequate human studies OR no animal or human studies available	8.3 Females and Males of Reproductive Potential	Pregnancy testing requirements; contraception recommendations; fertility data (both female and male)
D	Evidence of human fetal risk; benefits may warrant use	—	—
X	Studies/experience show fetal risk that outweighs any benefit	—	—

The PLLR structured narrative format provides prescribers with mechanistically and temporally specific information for individualised risk-benefit counselling — including subgroup-specific gestational timing data (e.g., "avoid after 20 weeks" for NSAIDs), dose recommendations, and information from active pregnancy exposure registries. Old Category C drugs — a heterogeneous group ranging from agents with reassuring animal data and no human concern to genuinely poorly characterised agents — are now given nuanced narrative labelling that conveys this distinction clearly.

Table IV.B.5. Selected Drugs and Environmental Contaminants with Reproductive/Developmental Outcomes: Summary Table

Agent	Category (Historical FDA)	Birth Defect Prevalence / Risk Estimate	Mechanism	Special Risk Management
Isotretinoin	X	MCM in $\leq 40\%$ of 1st trimester exposures; SAB 20–30%	RAR pathway dysregulation	iPLEDGE REMS; monthly testing; dual contraception
Thalidomide	X	Phocomelia/amelia in $\sim 20\text{--}30\%$ of critical-window exposures	Cereblon/SALL4	STEPS/REMS; monthly testing; mandatory contraception
Valproate ($\geq 1,500$ mg/day)	D	MCM $\sim 10\%$ (all doses); $\sim 24\%$ at high dose	HDAC inhibition; folate antagonism	Avoid in women of childbearing potential; minimum effective dose; registry
Warfarin (1st trimester)	D	Warfarin embryopathy in $\sim 6\%$	Vitamin K antagonism	Switch to LMWH during weeks 6–12 if possible
Methotrexate	X	Embryopathy if used in pregnancy	Folate antagonism/DHFR inhibition	Cease ≥ 3 months before conception
ACE inhibitors/ARBs (2nd–3rd trimester)	D	Fetopathy (oligohydramnios, renal failure, skull hypoplasia)	Fetal RAS inhibition	Contraindicated; switch before or early in pregnancy
Methylmercury (food)	—	Congenital Minamata syndrome (cerebral palsy)	Mitotic spindle disruption; NPC apoptosis	Restrict high-mercury fish; seafood advisories
PFAS	—	LBW, preterm ($\sim 1.5\times$); emerging cardiac/urogenital data	PPAR activation; endocrine disruption; epigenetic	Exposure minimisation; phase-out of long-chain PFAS
Alcohol	—	FAS 6–9/1,000 (USA); FASD 24–48/1,000	L1CAM inhibition; mitochondrial toxicity; epigenetic	No safe dose; abstinence recommended throughout pregnancy

IV.C. Intrauterine Growth Restriction and Hypotrophy

IV.C.1. Definitions and Classification

Multiple partially overlapping terms are used in the literature to describe suboptimal fetal and neonatal growth; precision in their application is critical because they carry distinct pathophysiological, prognostic, and clinical management implications.

- **Low Birth Weight (LBW):** Birth weight < 2,500 g, regardless of gestational age (WHO, 1969). A global descriptor of neonatal risk, not inherently implying pathological cause. - *Very Low Birth Weight (VLBW):* < 1,500 g - *Extremely Low Birth Weight (ELBW):* < 1,000 g
- **Hypotrophe / Small for Gestational Age (SGA):** Birth weight or fetal abdominal circumference below the **10th percentile** for gestational age on a validated growth reference chart (e.g., INTERGROWTH-21st, WHO Fetal Growth Charts). SGA is a statistical descriptor — approximately half of SGA fetuses are constitutionally small but otherwise healthy and without evidence of pathology; the remainder exhibit pathological growth restriction.
- **Intrauterine Growth Restriction / Fetal Growth Restriction (IUGR/FGR):** Failure of a fetus to achieve its genetically predetermined growth potential, typically evidenced by sequentially declining growth velocity on ultrasound biometry, abnormal Doppler indices (elevated umbilical artery pulsatility index, brain-sparing reduced cerebro-placental ratio), and/or birth weight < 10th percentile with signs of fetal compromise. The Delphi consensus criteria (Gordijn et al., 2016) distinguish early FGR (< 32 weeks, associated with severe placental pathology) from late FGR ($\geq$ 32 weeks, more subtle Doppler changes but higher absolute prevalence).

The distinction between SGA without compromise and FGR is clinically significant: SGA neonates without Doppler abnormality have substantially lower perinatal mortality than those meeting FGR criteria, and management protocols differ accordingly.

Normal fetal weight progression: approximately 1 g at 8 weeks, 14 g at 12 weeks, 1,100 g at 28 weeks; thereafter approximately 1,100 g per 6 weeks to term. The normal term neonate weighs approximately 3,200 g. Growth restriction may be **symmetric (harmonious)** — proportionate reduction in all somatic dimensions, typically caused by a factor acting throughout gestation (e.g., chromosomal anomaly, early infection, severe chronic undernutrition) — or **asymmetric (dysharmonious)** — weight disproportionately reduced relative to skeletal dimensions, principally caused by late-acting placental insufficiency (uteroplacental blood flow restriction with relative sparing of brain growth, termed "brain-sparing").

IV.C.2. Global Prevalence and Public Health Burden

LBW affects approximately 15–20% of all births worldwide, with marked geographic heterogeneity: rates of 5–7% in high-income countries versus 25–30% in parts of South and Southeast Asia. The global LBW burden exceeds 20 million neonates per year (WHO).

SGA (< 10th percentile by definition affects 10% of any population); pathological SGA prevalence varies substantially. In low- and middle-income countries using the INTERGROWTH-21st growth standard, estimates of pathological SGA reach 19.3%.

In a 2021 Brazilian cohort of 174,399 live births, the SGA rate was 5.5% and LBW rate was 6.8%; of LBW infants, 39.7% were SGA and 70% were preterm, illustrating the complex epidemiological overlap between growth restriction and prematurity. LBW premature SGA neonates carried the highest neonatal mortality in this cohort (78.1 per 1,000 live births), underscoring the additive adverse consequences of combined prematurity and growth restriction.

The proportion of LBW due to growth restriction (as opposed to prematurity) varies dramatically: in Sweden and the USA, approximately 45% of LBW births reflect IUGR rather than prematurity; in developing countries such as India, 79–96% of LBW is attributed to growth restriction (Villar and Belizan, 1982).

IV.C.3. Pathophysiology: Placental Insufficiency

The placenta is the principal determinant of fetal growth. Placental insufficiency — impaired maternal blood flow to the intervillous space — is the most common cause of IUGR in high-income countries, and its severest form is associated with pre-eclampsia.

Mechanisms of Placental Insufficiency

In normal pregnancy, extravillous trophoblasts (EVTs) invasively remodel the maternal spiral arteries during the first and early second trimester, replacing their musculo-elastic walls with fibrinoid material and transforming high-resistance arterioles into low-resistance, high-capacity conduits. This physiological remodelling ensures pressure-independent perfusion of the intervillous space.

In **pre-eclampsia-associated FGR** — the most common severe form:

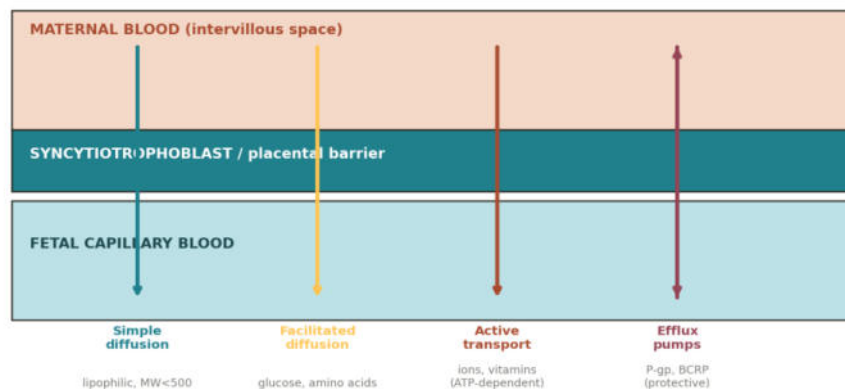
1. **Preclinical stage (first trimester)**: Incomplete EVT invasion → inadequate spiral artery remodelling → persistently high uteroplacental vascular resistance. 2. **Placental ischaemia**: Narrowed spiral arteries generate turbulent, pulsatile flow, causing oxidative stress, trophoblast apoptosis, and release of syncytiotrophoblast microparticles into maternal circulation. 3. **Clinical stage (≥ 20 weeks)**: The ischaemic placenta releases anti-angiogenic factors — principally **sFlt-1** (soluble fms-like tyrosine kinase-1, also known as soluble VEGF receptor-1) and **soluble endoglin (sEng)** — into the maternal circulation. sFlt-1 scavenges free VEGF and PlGF, profoundly impairing endothelial function system-wide. sEng antagonises TGF- β 1, further reducing endothelial nitric oxide (NO) synthesis. The net result is maternal hypertension, proteinuria, and systemic endothelial dysfunction (pre-eclampsia). 4. **Fetal consequences**: Reduced uteroplacental perfusion → fetal hypoxia → activation of adrenal stress responses (elevated glucocorticoids, catecholamines) → fetal blood flow redistribution to protect the brain (brain-sparing). Chronically deprived of glucose and oxygen, the fetus reduces anabolic activity, producing asymmetric growth restriction: abdominal circumference (reflecting hepatic glycogen store and splanchnic blood supply) reduced first; head circumference relatively preserved.

Additional molecular mediators:

- **Reactive oxygen species (ROS)**: Placental hypoperfusion → mitochondrial dysfunction → elevated superoxide and H_2O_2 → lipid peroxidation, DNA damage, trophoblast apoptosis.
- **Endothelin-1 elevation**: Potent vasoconstrictor released by dysfunctional endothelium, perpetuating vasoconstriction.
- **Nitric oxide (NO) reduction**: Impaired eNOS activity → increased peripheral vascular resistance.
- **Complement activation**: C5b-9 membrane attack complex deposition in spiral arteries.

Early-onset FGR (< 32 weeks) is associated with maternal pre-eclampsia in approximately 50% of cases; in early-onset pre-eclampsia, FGR is present in $> 90\%$ of cases.

Figure 22. Mechanisms of placental transfer of xenobiotics



Placental insufficiency mechanisms in FGR/pre-eclampsia. Schematic showing incomplete trophoblast invasion of spiral arteries, placental release of sFlt-1 and sEng into maternal circulation, scavenging of VEGF/PlGF, systemic endothelial dysfunction, fetal hypoxia, and brain-sparing adaptation.

IV.C.4. Environmental and Toxic Causes of IUGR and LBW

Tobacco Smoking

Tobacco smoking is the single most important modifiable environmental cause of SGA and LBW in high-income countries. Three principal mechanisms operate: (1) nicotine causes potent uteroplacental vasoconstriction, reducing uterine and umbilical arterial diameter; (2) carbon monoxide (CO) in cigarette smoke (20,000–60,000 ppm) binds fetal haemoglobin with 210 times the affinity of oxygen, forming fetal carboxyhaemoglobin and

carboxymyoglobin, severely impairing fetal oxygen delivery; (3) polycyclic aromatic hydrocarbons (PAHs) in tobacco smoke induce placental oxidative stress and DNA adduct formation.

The dose-response relationship is well-established: mean birth weight reduction of approximately 150–400 g per smoking pregnancy; LBW risk increased approximately **2–3-fold** among heavy smokers; SGA risk elevated approximately 1.5–2-fold. Passive (secondhand) smoking increases SGA risk (OR approximately 1.32, 95% CI: 1.14–1.55). Maternal smoking history (a multigenerational effect) and maternal grandmaternal smoking have also been associated with growth restriction in offspring beyond direct effects.

Alcohol

Third-trimester alcohol exposure is specifically associated with decreased fetal head circumference, reduced birth weight, and reduced brain volume — the growth components of fetal alcohol spectrum disorder. A study of 9,236 births (Kaminski, Rumeau, and Schwartz, 1978) showed that maternal alcohol consumption exceeding 45 g/day increased rates of both stillbirth and growth restriction. Maternal ingestion of alcohol is also accompanied by decreased birth length and head circumference.

Air Pollution (PM2.5 and NO2)

Ambient particulate matter exposure during pregnancy represents a major and underrecognised global cause of IUGR. A large meta-regression analysis published in *PLOS Medicine* (2021, encompassing 44 studies) found that each 10 µg/m³ increase in entire-pregnancy average PM2.5 exposure was associated with **–22 g birth weight** (95% UI: –32 to –12 g) (Szpiro et al., *PLOS Med*, 2021). Globally in 2019, ambient and household PM2.5 was attributable to an estimated 89 g (95% UI: 88–89 g) mean population-level reduction in birth weight. A Bayesian distributed-lag analysis identified critical sensitive periods: PM2.5 during gestational days 91–139 (–6 g, 95% CI: –11 to –1 g per µg/m³ per day) and days 249–272; NO2 during days 55–145 (–3 g, 95% CI: –5 to –1 g per ppb per day).

Biological mechanisms include: (1) oxidative stress and systemic inflammation impairing spiral artery trophoblastic remodelling; (2) direct transplacental particle transfer shown in histological placental studies; (3) autonomic nervous system dysregulation and increased blood viscosity; (4) endocrine disruption via PAH components that act as AhR ligands.

Heavy Metals

Lead: Prenatal blood lead levels are negatively and linearly correlated with birth weight across multiple cohort studies. A study of 202 pregnant women living near high-lead-exposure areas (Bornschein, Grote, and Mitchell, 1989) found that, after adjustment for gestational age, socioeconomic status, alcohol and tobacco use, maternal blood lead was associated with reduced birth weight and birth length but not head circumference. For Caucasian infants specifically, birth length was reduced by approximately 2.5 cm per log-unit increment in maternal blood lead. An age-dependent interaction was observed: at a blood lead of approximately 20 µg/dL, the infant of a 30-year-old woman weighed approximately 500 g less than that of a 20-year-old with the same lead level, suggesting additive vulnerability in older mothers. No safe blood lead level during pregnancy has been established.

Arsenic: A 2025 systematic review and Bayesian Kernel Machine Regression analysis (Rees et al., *Environment International*, 2025) found a dose-dependent relationship between maternal urinary arsenic and SGA: OR **2.86** (95% CI: 1.17–7.02) for one prospective cohort (China) and OR 3.63 (95% CI: 1.47–9.64) for the highest tertile in another cohort. Arsenic exposure was identified as the key metal significantly associated with preterm labour in the multi-metal mixture analysis.

Cadmium: Accumulates in the placenta and disrupts zinc-dependent enzymes; placental cadmium concentrations are inversely correlated with birth weight.

Table IV.C.1. Environmental Exposures and Key Birth Outcome Effect Estimates: Updated Synthesis (Modernised from Original Tableau 4)

Exposure / Factor	Category	Birth Outcome	Effect Estimate	Mechanism	Evidence Level
PM2.5 (10 µg/m ³ increment)	Air pollution	Birth weight reduction	–22 g (95% UI: –32 to –12 g)	Oxidative stress; inflammation;	Meta-analysis, 44 studies

Exposure / Factor	Category	Birth Outcome	Effect Estimate	Mechanism	Evidence Level
				placental disruption	
PM2.5 (global 2019 burden)	Air pollution	Population mean BW reduction	−89 g	As above	GBD / meta-regression
Tobacco smoking (active)	Behavioural	LBW risk; birth weight	2–3× LBW; −150 to −400 g	Nicotine vasoconstriction; CO hypoxia; PAH oxidative stress	Strong (consistent multi-cohort)
Tobacco smoking (passive)	Behavioural	SGA	OR ~1.32 (95% CI: 1.14–1.55)	Nicotine; CO	Cohort meta-analysis
Alcohol (> 45 g/day)	Behavioural	Stillbirth; IUGR; microcephaly	Increased stillbirth + IUGR	Mitochondrial toxicity; L1CAM inhibition; epigenetic	Cohort studies
Arsenic (urinary, 3rd tertile)	Heavy metal	SGA	OR 3.63 (95% CI: 1.47–9.64)	Mitochondrial toxicity; ROS; epigenetic	Prospective cohort
Arsenic (urinary, China cohort)	Heavy metal	SGA	OR 2.86 (95% CI: 1.17–7.02)	As above	Prospective cohort
Arsenic	Heavy metal	Preterm labour	Significant dose-response	BKMR mixture analysis	Multi-metal analysis
Lead (blood, elevated)	Heavy metal	Birth weight; birth length	Inverse linear; −2.5 cm/log(Pb)	Calcium mimicry; haem synthesis disruption; epigenetic	Multiple cohorts
Cadmium (placental)	Heavy metal	Birth weight	Inverse correlation	Zinc enzyme disruption	Placental tissue studies
PFAS (high vs. low blood)	Persistent pollutant	LBW; preterm birth	~1.5× preterm risk; LBW	PPAR-α/γ activation; endocrine disruption; epigenetic; placental dysfunction	Cohort studies; registry data
DEHP/phthalate metabolites	EDC	Birth weight; growth	Reduced BW; altered growth	THR downregulation; IGF2 methylation	Cohort and mechanistic studies
Malnutrition / folate deficiency	Nutritional	IUGR; NTD	IUGR; OR NTD 0.23 with folic acid suppl.	DNA synthesis impairment; placental growth	Intervention RCTs; cohort studies

Exposure / Factor	Category	Birth Outcome	Effect Estimate	Mechanism	Evidence Level
Protein-calorie undernutrition	Nutritional	Symmetric IUGR	Prevalent in developing countries	Substrate limitation; placental growth	Population-level data
Gestational hypertension / pre-eclampsia	Maternal disease	Asymmetric IUGR	FGR in > 90% early-onset PE	sFlt-1/VEGF imbalance; placental ischaemia	Strong (mechanism characterised)
Chromosomal anomaly (e.g., Down syndrome, T18)	Genetic/structural	Symmetric IUGR	Consistently associated	Intrinsic growth dysregulation	Registry data
Short interpregnancy interval (< 1 year)	Obstetric	LBW	3× increased risk	Uterine depleted reserves	Cohort studies

PFAS (Per- and Polyfluoroalkyl Substances)

PFAS cross the placenta by passive diffusion and have been detected in neonatal cord blood (Emory University study, 2023). Prenatal PFAS exposure is associated with reduced birth weight and LBW across multiple large cohorts. A nested case-control study in Guangxi, China, found that increasing maternal exposure to PFHpA, PFDoA, and PFHxS was associated with IUGR risk. An Emory University-led study found that mothers with higher blood PFAS levels were approximately 1.5 times more likely to deliver preterm.

Proposed mechanisms include:

- **Endocrine disruption:** PFAS bind and activate PPAR- α and PPAR- γ , disrupting lipid and glucose metabolism; suppress thyroid hormone synthesis, reducing fetal growth factors.
- **Systemic inflammation:** PFAS suppress both IL-10 (regulatory) and TNF- α (pro-inflammatory), disrupting the maternal immune milieu critical for placentation.
- **Placental dysfunction:** PFAS alter syncytiotrophoblast development in a sex-specific manner; the male placenta shows reduced syncytialisation and appears more vulnerable.
- **Epigenetic modifications:** Prenatal PFAS exposure is associated with altered H3K4me3, H3K9me3, and H3K27me3 histone methylation marks in offspring.

Phthalates and Other Endocrine-Disrupting Chemicals (EDCs)

Phthalates — plasticisers in flexible PVC, food packaging, and personal care products — are ubiquitous human exposures. Prenatal phthalate exposure (particularly DEHP and metabolites) is associated with reduced birth weight, shorter anogenital distance, and altered fetal growth. Mechanisms include:

- Downregulation of thyroid hormone receptor genes (Thra1, Thr β 1) in placental trophoblast, impairing placental development and nutrient transfer.
- Decreased expression of placental nutrient transporters (GLUT1, amino acid transporters).
- Altered IGF2 DNA methylation — IGF2 being the principal epigenetic regulator of placental and fetal growth — in association with high-molecular-weight phthalate urinary metabolites.

Bisphenol A (BPA) exposure in early pregnancy impairs uterine spiral artery remodelling in animal models, producing IUGR-like and pre-eclampsia-like phenotypes.

Maternal Nutrition

Maternal malnutrition remains the primary driver of IUGR in low-income settings, particularly where it produces symmetric (harmonious) growth restriction by limiting fetal substrate availability throughout gestation.

- **Folate deficiency:** Essential for DNA synthesis and one-carbon metabolism. Periconceptional folic acid supplementation reduces neural tube defect risk by approximately 50–70%; a meta-analysis found a

77% reduction in overall congenital anomaly risk with supplementation (OR 0.23, 95% CI: 0.16–0.32). Folate deficiency also impairs placental growth and trophoblast invasion.

- **Iron deficiency:** The most prevalent micronutrient deficiency globally; anaemia-related impairment of uteroplacental oxygen transport contributes to SGA.
- **Iodine deficiency:** Causes congenital hypothyroidism, cretinism, and fetal growth restriction.
- **Protein-calorie restriction:** Studies of the Dutch Hunger Winter (Stein, Susser, and Saenger, 1975) demonstrated that severe dietary restriction limited to the third trimester produces dysharmonious IUGR — reduced birth weight with relative sparing of head circumference — while restriction in the first trimester is primarily associated with later metabolic sequelae.
- **Dietary diversity:** Suboptimal dietary diversity (≤ 5 food groups per day) was associated with IUGR (aOR 2.18, 95% CI: 1.02–4.63) and preterm birth in a scoping review of low- and middle-income country settings.

IV.C.5. Methodological Challenges in the Study of IUGR and LBW

Several methodological problems complicate the study of toxic exposures and LBW. First, preterm birth must be treated as a potential mediator or competing outcome in any analysis of birth weight; longer pregnancies are inherently more exposed to cumulative toxic dose but also inherently produce larger infants (a survival or "depletion-of-susceptibles" artefact). Cox proportional hazards or survival analysis variants that account for time-varying covariates are required to address this.

Second, defining LBW as a binary variable ($< 2,500$ g vs. $\geq 2,500$ g) requires exposures of very large magnitude to produce statistically detectable signals; treatment of birth weight as a continuous variable in multiple regression models is substantially more sensitive for detecting subtle effects. The relative paucity of statistically significant findings in the occupational exposure literature may partly reflect failure to exploit this approach.

Third, the characterisation of exposures during short, biologically critical windows is essential. If a woman changed jobs or ceased working during the organogenesis period, or experienced variable environmental exposures during a critical gestational window, the exposure-effect relationship may be severely diluted by error in exposure assignment. This requirement for temporal precision in exposure characterisation is more stringent than for studies of chronic adult diseases, where exposure misclassification by months or even years may have minimal impact.

Finally, multiple adverse outcomes — IUGR, congenital anomalies, and spontaneous abortion — should ideally be analysed jointly in occupational or environmental cohort studies, because the relationship between a given exposure and a particular outcome may not be outcome-specific (e.g., lead, anaesthetic gases, and organic solvents all lack specificity for a single pregnancy endpoint).

V. Radiosensitivity of Germ Cells

Ionizing radiation represents one of the most intensively studied reproductive toxicants in human medicine and occupational health. Unlike most chemical agents — whose mechanisms of reproductive toxicity are often inferred indirectly — the biophysical basis of radiation-induced germ cell injury is understood at the molecular, cellular, and population levels with unusual precision. This combination of mechanistic clarity and robust epidemiological follow-up (particularly from the Japanese atomic bomb survivors, Chernobyl liquidators, and childhood cancer survivor cohorts) makes ionizing radiation an invaluable paradigm for reproductive toxicology as a whole.

The central challenge of this chapter is translating the language of radiation dosimetry into clinically actionable guidance: determining whether a given exposure — diagnostic radiograph, occupational dose, radiotherapy field — poses a meaningful risk to spermatogenesis, ovarian reserve, or a developing embryo, and communicating that determination in a framework that supports sound decision-making by patients, clinicians, and policymakers.

[FIGURE: fig23_radiation_dose | Caption: Schematic illustrating the relative magnitude of ionizing radiation doses from natural background, diagnostic medical imaging, occupational limits, and therapeutic exposures on a logarithmic scale (mGy). Key reproductive thresholds (ICRP 118) are marked for comparison: temporary male sterility (~150 mGy), fetal deterministic threshold (100 mGy), and permanent male sterility (3,500–6,000 mGy).]

V.A. Exposure to Ionizing Radiation

V.A.1. Conceptual Framework and Units

Before cataloguing sources of exposure, a working command of the dosimetric system is prerequisite. Three core quantities govern radiation protection:

Absorbed dose (D), measured in gray (Gy), expresses the mean energy imparted per unit mass of tissue:

$$D = dE / dm \text{ [J kg}^{-1} = \text{Gray (Gy)]}$$

The gray is the physically fundamental quantity for tissue injury assessment. When discussing gonadal thresholds — testicular doses for azoospermia, ovarian doses for follicle depletion — absorbed dose in Gy or mGy is always the operative metric.

Equivalent dose (H_T), measured in sievert (Sv), adjusts for the differential biological effectiveness of different radiation types through radiation weighting factors (w_R):

$$H_T = \sum_R w_R \cdot D_{T,R} \text{ [Sv]}$$

For the great majority of clinical and occupational exposures (X-rays, gamma rays, electron beams), w_R = 1, and 1 Gy equals 1 Sv. High-LET radiations such as alpha particles (w_R = 20) and neutrons (w_R = 2.5 to 20, energy-dependent) produce substantially greater biological damage per unit absorbed dose (ICRP, 2007).

Effective dose (E), also in sievert, is a whole-body risk-weighted quantity summing equivalent doses across organs, each multiplied by a tissue weighting factor (w_T) reflecting its relative contribution to stochastic risk:

$$E = \sum_T w_T \cdot H_T \text{ [Sv]}$$

Effective dose is appropriate for population-level risk communication, regulatory compliance, and comparison of procedures. ICRP Publication 147 (2021) explicitly states that effective dose is **not** appropriate for individual clinical risk estimation; for that purpose, absorbed dose to the specific organ (ovary, testis, uterus) is required (ICRP, 2021).

Linear energy transfer (LET) describes energy deposition density along a charged particle track:

$$L = dE / dl \text{ [keV } \mu\text{m}^{-1}\text{]}$$

Low-LET radiations (X-rays, γ-rays: ~0.3–2 keV/μm) produce predominantly single-strand DNA breaks, effectively repaired by base excision and strand break repair pathways. High-LET radiations (alpha particles: 80–200 keV/μm; heavy ions: >100 keV/μm) generate clustered, complex DNA double-strand breaks poorly amenable to repair, with substantially higher cytotoxicity and mutagenicity per absorbed dose. The **relative biological effectiveness (RBE)** formalizes this comparison:

$$\text{RBE} = D_{\text{reference}} / D_{\text{test}} \text{ (same biological effect)}$$

RBE peaks at approximately 100 keV/μm (optimal for clustered DSB induction) and declines above this due to overkill. For neutrons, RBE ≈ 20, hence w_R = 20 (ICRP, 2007). For gonadal radiosensitivity assessment, the

distinction between low-LET and high-LET matters clinically when alpha-emitting radionuclides (radium, polonium) become concentrated in gonadal tissue.

Table V.A.1. Radiation Weighting Factors (w_R) and Key Tissue Weighting Factors (w_T) per ICRP Publication 103 (2007)

Radiation Type / Tissue	Parameter	Value	Notes
Photons (X-rays, γ -rays, electrons)	w_R	1	Standard clinical and diagnostic radiation
Protons, charged pions	w_R	2	
Alpha particles, heavy ions	w_R	20	Radon decay products; internal emitters
Neutrons (< 1 MeV)	w_R	2.5–7.5	Energy-dependent continuous function
Neutrons (1–50 MeV)	w_R	20	Maximum weighting
Neutrons (> 50 MeV)	w_R	5	Decreasing effectiveness
Gonads (heritable + ovarian cancer)	w_T	0.08	Includes heritable effects contribution
Bone marrow, breast, lung, stomach	w_T	0.12 each	Highest w_T tissues
Thyroid, liver, oesophagus, bladder	w_T	0.04 each	
Bone surface, brain, salivary glands, skin	w_T	0.01 each	
Remainder tissues (13 organs)	w_T	0.12 total	Includes adrenals, gallbladder, uterus
Total	Σw_T	1.00	Normalized sum

Source: ICRP Publication 103 (2007), Table 3 and Table 5.

V.A.2. Natural Background Radiation

Every person on Earth is continuously exposed to ionizing radiation from natural sources. The global average effective dose from natural background is approximately **2.4 mSv/year**, though regional variation spans more than two orders of magnitude: from under 1 mSv/year in low-lying coastal areas to over 200 mSv/year in high-radon geological environments such as parts of Kerala (India) and Ramsar (Iran) (UNSCEAR, 2008).

The principal components of natural background are documented in Table V.A.2. Radon-222, a noble gas arising from uranium decay in soil and building materials, is the dominant contributor (~50% of total background in most countries). It delivers dose primarily to the bronchial epithelium and lungs through its short-lived decay products (polonium-218, -214), rather than to the gonads directly, and is therefore of limited direct relevance to reproductive dosimetry at ordinary residential levels. However, it serves as the dominant reference quantity for population background dose comparisons in radiation protection.

Table V.A.2. Principal Sources of Human Ionizing Radiation Exposure with Representative Effective and Gonadal Doses

Exposure Category	Approximate Annual Effective Dose	Typical Ovarian / Testicular Dose	Reference / Notes
Radon & thoron (inhalation)	~1.26 mSv/year	Negligible (lung dose dominant)	UNSCEAR 2008; ~50% of natural background
Cosmic radiation (sea level)	~0.39 mSv/year	~0.3 mSv/year	Increases ~15% per 1,000 m altitude
Terrestrial gamma radiation	~0.48 mSv/year	~0.48 mSv/year	Varies with geology
Internal radionuclides	~0.29 mSv/year	Varies	K-40, C-14 dominant contributors
Natural total	~2.4 mSv/year	—	UNSCEAR 2008/2020–2021
Chest X-ray (PA)	0.02–0.1 mSv/exam	0.01–0.1 mGy/exam	ACR 2018; ICRP 103
Abdominal X-ray	0.5–1.0 mSv/exam	1–3 mGy/exam	
Lumbar spine X-ray	1.5 mSv/exam	3–5 mGy/exam	Ovaries in scatter field
Pelvic CT	8–15 mSv/exam	10–50 mGy/exam	Highest routine diagnostic gonadal dose
Abdominopelvic CT	10–20 mSv/exam	20–40 mGy (ovaries)	UNSCEAR 2020/2021 Vol. I
Tc-99m bone scan	3–5 mSv/exam	4–5 mGy/fetus	ACOG 2017; ACR 2018
PET-CT (18F-FDG)	10–25 mSv/exam	~10–20 mGy/fetus	High-dose nuclear medicine
Fluoroscopy (complex, GI)	10–40 mSv/session	Variable, up to 100+ mGy	Requires individual assessment
Uterine artery embolization	40–100 mSv/session	20–100 mGy (direct)	Interventional procedure
Pelvic radiotherapy	Not applicable (therapeutic)	1–70+ Gy	Bowel/bladder/prostate/cervical Rx
Occupational (workers, avg.)	~1.1 mSv/year	Proportional	UNSCEAR 2020/2021 Vol. IV
Occupational limit (ICRP)	20 mSv/year (5-y avg.)	Organ-specific calculation needed	ICRP 103; max 50 mSv any single year
Chernobyl liquidators (high)	1,000–16,000+ mGy (acute)	Organ-specific	ARS cases; IAEA 1992
Chernobyl general population	10–60 mSv (lifetime estimate)	Low; internal I-131 dominant	UNSCEAR 2008
Fukushima general public	< 10 mSv (effective)	< 10 mGy estimated	UNSCEAR 2020/2021 Vol. II

Sources: UNSCEAR 2008; UNSCEAR 2020/2021; ICRP 103 (2007)
; ACOG Committee Opinion 723 (2017)
; ACR–SPR Practice Parameter (2018).

V.A.3. Medical Radiation

Medical exposure is the largest artificial source of human radiation dose at the population level. The UNSCEAR 2020/2021 Report (Volume I, Annex A) provides a comprehensive global assessment for the period 2009–2018 (UNSCEAR, 2022).

Key findings with reproductive relevance:

- **Diagnostic radiology** accounts for approximately 3.8 billion examinations annually worldwide. The global average per-capita effective dose from diagnostic radiology is 0.6–1.0 mSv/year in high-income countries.
- **Computed tomography (CT)** is the single largest medical contributor to population dose in developed countries, delivering effective doses of 2–20 mSv per examination. Abdominopelvic CT — the most common protocol of reproductive relevance — delivers 8–15 mSv effective dose with ovarian doses of 20–40 mGy and testicular doses of 10–30 mGy depending on field geometry and patient habitus (Lee et al., 2004).
- **Interventional radiology and fluoroscopy** can deliver dose-intensive exposures; complex fluoroscopically-guided procedures (uterine artery embolization, complex cardiac catheterization) may deliver gonadal doses of 20–100 mGy or more.
- **Radiation therapy (radiotherapy)** is used in approximately 50% of all cancer patients. For pelvic tumors (cervical cancer, prostate cancer, rectal cancer, para-aortic lymphoma), gonadal doses range from 1 to 70+ Gy depending on field placement and treatment intent, making fertility assessment an obligatory component of care (Siegel et al., 2022).

V.A.4. Occupational Exposure

Radiation workers — nuclear power plant employees, industrial radiographers, medical radiation workers, interventional radiologists, nuclear medicine technologists, and airline crew — are subject to regulatory dose limits established in ICRP Publication 103. The occupational effective dose limit is **20 mSv/year averaged over any consecutive five-year period**, with no single year exceeding 50 mSv (ICRP, 2007). The UNSCEAR 2020/2021 Report (Volume IV, Annex D) documents that the global average annual effective dose to monitored radiation workers across all sectors is approximately **1.1 mSv/year** — well below regulatory limits in the vast majority of cases (UNSCEAR, 2022).

Interventional cardiologists and radiologists are among those with the highest occupational doses, sometimes approaching 5–10 mSv/year in effective dose terms. For reproductive counseling of occupationally exposed workers, the critical question is always the specific organ dose, not the effective dose.

V.A.5. Accidental and Emergency Exposures

Chernobyl (1986): The explosion at Chernobyl Unit 4 released approximately 5.2×10^{18} Bq of radioactivity. Among the 134 emergency workers who developed acute radiation syndrome (ARS), 28 died within weeks; doses to this high-exposure cohort ranged from approximately 1 to >16 Gy whole-body (IAEA, 1992). The general population of contaminated territories received lower effective doses (10–60 mSv for most residents), with the principal reproductive-age health concern being internal iodine-131 exposure to the thyroid. A 2025 analysis of 18 Italian birth cohorts exploiting regional variation in Chernobyl fallout found increased rates of miscarriage and stillbirth in women exposed in utero or in the first three years of life, emphasizing the heightened developmental radiosensitivity of early life (Grembi et al., 2025).

Fukushima Daiichi (2011): The accident resulted in substantial releases of cesium-137 and iodine-131. The UNSCEAR 2020/2021 Report Volume II (Annex B) — the most comprehensive reassessment — estimated effective doses to the general public in Japan at less than 10 mSv for the large majority of the population, with children generally receiving below 1–5 mSv effective dose (UNSCEAR, 2022). The Fukushima Health Management Survey Pregnancy and Birth Survey, which tracked over 115,000 pregnancies from 2012 to 2018, found no increase in preterm birth, low birth weight, or congenital anomalies attributable to radiation exposure over an 8-year follow-up (Kyojuka et al., 2022).

V.B. Types of Effects Considered

V.B.1. Classification Framework: Deterministic Versus Stochastic

Radiation-induced health effects are divided into two fundamentally distinct categories based on their dose–response relationship. This distinction has profound practical implications for reproductive risk assessment.

V.B.1.a. Deterministic Effects (Tissue Reactions)

Deterministic effects — renamed "tissue reactions" in ICRP Publications 103 (2007) and 118 (2012) to reflect their partial modifiability by biological response modifiers — result from the killing of cells in a tissue that exceeds that tissue's regenerative capacity. Their defining characteristics are:

1. **Threshold dose:** A practical dose threshold exists, below which the effect does not occur at a population level (defined by ICRP as the dose producing 1% incidence of the effect in the exposed population). Below threshold, cellular loss is compensated by proliferation of surviving cells.
2. **Dose-dependent severity:** Above threshold, the severity of the effect increases as a continuous function of dose.
3. **Potential modifiability:** Radioprotective drugs (e.g., amifostine), growth factors, and cytokines can modify the threshold or severity of some tissue reactions.

ICRP Publication 118 (2012) provides the current authoritative thresholds for tissue reactions (ICRP, 2012). The general principle from ICRP 118 remains: **acute doses up to approximately 100 mGy produce no functional impairment of any tissue** — confirming that stochastic effects are the primary risk concern for most occupational and public exposures. Gonadal deterministic thresholds are presented in detail in Tables V.B.1 and V.B.2 below.

V.B.1.b. Stochastic Effects: Cancer and Heritable Effects

Stochastic effects are probabilistic in nature. They result from a single DNA-damaging event in a surviving cell that initiates a mutation — either carcinogenic (in somatic cells) or heritable (in germ cells). Their defining characteristics are:

1. **No practical threshold:** Any dose may in principle initiate a mutation; the linear no-threshold (LNT) model is maintained for radiation protection purposes.
2. **Dose-proportional probability:** The probability of the effect increases linearly with dose (LNT model, with a dose and dose-rate effectiveness factor DDREF = 2 applied to convert from high-dose/high-dose-rate experimental data to low-dose/ low-dose-rate protection contexts).
3. **Dose-independent severity:** The severity of a radiation-induced cancer or heritable disease does not depend on the dose that initiated it.

ICRP Publication 103 (2007) established the foundational quantitative framework: combined cancer plus heritable effect detriment of approximately **5.5% per Sv** for the whole population (4.1% per Sv for adult workers). Tissue weighting factors were revised based on updated Life Span Study epidemiology (ICRP, 2007).

ICRP Publication 152 (2022) represents the most recent critical reassessment of the radiation detriment calculation methodology (ICRP, 2022). Key conclusions relevant to reproductive toxicology:

- Programming errors discovered in the Publication 103 working-age population calculation do not affect tissue weighting factors or the overall ~5% per Sv detriment for the whole population.
- The gonadal tissue weighting factor $w_T = 0.08$ is retained, incorporating both heritable effect risk and ovarian cancer risk contributions.
- DDREF = 2 for solid cancers is maintained; no compelling reason to revise from the Publication 103 value.
- The heritable risk estimate — approximately 20 cases per 10,000 persons per Gy for the first two generations — is based on UNSCEAR 2001 and is derived entirely from animal data by doubling dose extrapolation.

Table V.B.1. Deterministic Thresholds for Gonadal Tissue Reactions per ICRP Publication 118 (2012)

Endpoint	Threshold Dose (Gy)	Exposure Condition	Notes
MALE REPRODUCTIVE SYSTEM			
Detectable spermatogonial changes	~0.1 Gy	Single acute dose	FSH rise; morphological changes in type A

Endpoint	Threshold Dose (Gy)	Exposure Condition	Notes
Temporary oligospermia / sterility onset	~0.15 Gy	Single acute dose	ICRP 118 threshold (1% incidence); type A loss
Transient azoospermia	~0.8 Gy	Single acute dose	Recovery 9–18 months
Temporary sterility	0.15 Gy (clinical onset)	Single acute dose	Complete recovery expected
Prolonged azoospermia	2–3 Gy	Single acute dose	Recovery ~30 months; spermatocyte damage
Permanent sterility (single dose)	3.5–6 Gy	Single acute dose	High individual variability; 1–1.4 Gy may cause
			persistent azoospermia in some men
Permanent sterility (fractionated)	≥ 2.5 Gy	Fractionated	Reverse fractionation effect for testis
Leydig cell dysfunction (↓ testosterone)	> 20 Gy	Single/fractionated	Rare in adults; more sensitive in prepubertal boys
FEMALE REPRODUCTIVE SYSTEM			
Detectable follicle depletion	< 0.5 Gy	Single acute dose	Any dose causes some permanent follicle loss
Premature ovarian insufficiency (POI, 5%)	2–3 Gy	Fractionated	Age 20–30; 5% of exposed women
POI in 50% of exposed women	6–12 Gy	Fractionated	Age 20–30 at treatment
Effective Sterilizing Dose (ESD), age 20	16.5 Gy	Fractionated	Wallace formula; immediate POI in 97.5%
Effective Sterilizing Dose (ESD), age 30	14.3 Gy	Fractionated	Wallace formula
Effective Sterilizing Dose (ESD), age 40	~6 Gy	Fractionated	Wallace formula; declining reserve
Effective Sterilizing Dose (ESD), birth	20.3 Gy	Fractionated	Maximum reserve at birth

Sources: ICRP Publication 118 (2012); Wallace et al. (2005); Howell & Shalet (2005).

Table V.B.2. Dose–Risk Communication Framework for Reproductive Counseling

Dose Range	Primary Risk Category	Male Gonadal Effect	Female Gonadal Effect	Fetal Effect (if pregnant)
< 100 mGy	Stochastic (cancer)	No functional impairment	Minor follicle depletion; no clinical effect	No deterministic effects expected

Dose Range	Primary Risk Category	Male Gonadal Effect	Female Gonadal Effect	Fetal Effect (if pregnant)
100–500 mGy	Stochastic + early determ.	FSH rise; threshold zone for oligospermia	Measurable follicle loss; early threshold	Below teratogenic threshold
0.5–1.0 Gy	Deterministic emerging	Transient oligospermia; approaching azoospermia	Significant follicle depletion in older women	Below teratogenic threshold
1–3 Gy	Deterministic dominant	Azoospermia; recovery ~9–30 months	POI risk increasing; age-dependent	Potential teratogenic effects > 100 mGy
3.5–6 Gy	Severe deterministic	Risk of permanent azoospermia	POI in majority of women	Fetal death or severe malformations
> 6 Gy	Severe / irreversible	Permanent azoospermia likely	Permanent POI likely in most age groups	Incompatible with fetal survival

V.B.2. Heritable Effects: The Null Human Evidence Problem

Radiation-induced hereditary disease has never been definitively demonstrated in any human population despite intensive study of the largest natural experiment available: the 77,000+ offspring of Japanese atomic bomb survivors in the Life Span Study. The comprehensive analyses by Neel et al. (1990) and subsequent molecular studies (Yeager et al., 2021, using whole-exome sequencing of Chernobyl liquidator trios) have failed to demonstrate a statistically significant increase in heritable mutations at doses studied.

This null finding does not mean heritable effects are absent at any dose. Rather:

1. The biological plausibility is unambiguous: high-LET radiation is mutagenic in mouse germline studies, and the doubling dose approach yields a human heritable risk estimate of approximately 20 cases per 10,000 per Gy for the first two generations (ICRP 103; ICRP 152).
2. The statistical power to detect this signal in human cohorts is insufficient at the dose ranges studied (mean parental doses in atomic bomb survivor parents were typically < 0.2 Sv, insufficient to produce a detectable signal above the high background rate of spontaneous de novo mutations).
3. Practical clinical counseling acknowledges this: the dominant stochastic concern for low-to-moderate dose exposures is **cancer risk in the exposed individual**, not heritable disease in their offspring. For radiation protection purposes, $w_T = 0.08$ for gonads incorporates both heritable and ovarian cancer contributions (ICRP, 2007).

This situation contrasts sharply with several chemical reproductive toxicants (e.g., alkylating agents, ethylene oxide) where heritable effects have been documented experimentally at much lower doses in rodent models.

V.C. Analysis Procedure

V.C.1. Clinical Framework for Preconception Radiation Counseling

When a patient or healthcare worker presents for preconception counseling following radiation exposure — or a clinician must advise on the reproductive implications of a planned exposure — the evaluation follows a structured six-step framework (Hall & Giaccia, 2019; ICRP 147, 2021):

Step 1 — Characterize the Exposure Define: source type (external beam, sealed radionuclide source, diagnostic imaging, internal contamination), date, duration, fractionation schedule, anatomical field, and total prescribed or measured dose. For occupational workers, review badge dosimetry records and any bioassay results.

Step 2 — Dose Reconstruction to the Specific Organ Determine the absorbed dose to the ovary, testis, uterus, or developing embryo/fetus using treatment records, physicist calculations, or retrospective dosimetric models.

Effective dose from clinical records is insufficient for individual counseling; organ- level absorbed dose is required (ICRP 147). For radiotherapy patients, digital treatment plans yield dose-volume histograms (DVHs) for gonadal structures; if original plans are unavailable, phantom-based dosimetric reconstruction using contemporaneous treatment protocols may be employed.

Step 3 — Apply ICRP 118 Deterministic Thresholds Determine whether the reconstructed gonadal dose exceeds any relevant deterministic threshold for temporary or permanent sterility (see Tables V.B.1 and V.B.2). For fetal exposures, determine whether the dose exceeds the 100 mGy teratogenic threshold and the gestational window of exposure.

Step 4 — For Women: Apply the Wallace Formula For female patients who have received ovarian radiation, apply the Wallace formula to estimate the age at which premature ovarian insufficiency is expected (see Section V.D.3). This enables individualized fertility window counseling and urgency-of-fertility- preservation determination.

Step 5 — Stochastic Risk Communication Provide individualized cancer and heritable risk estimates using the ICRP framework (~5% per Sv excess absolute risk for the whole population). For most diagnostic exposures (< 20 mGy to gonads), the incremental stochastic risk is small relative to background lifetime cancer risk (~38% in developed countries). Emphasize: no threshold exists for stochastic effects; however, small incremental risks from diagnostic imaging are generally outweighed by the clinical benefit of the imaging procedure.

Step 6 — Fertility Preservation Counseling If reproductive capacity may be compromised by planned or already-received exposure, refer immediately to a reproductive medicine specialist for fertility preservation options (Section V.D.6). Timing is critical: cryopreservation options (sperm, embryo, oocyte, ovarian tissue) must be arranged before treatment initiation.

V.C.2. Dose Reconstruction Methodologies

Reconstruction of historical gonadal doses is technically demanding and requires multidisciplinary collaboration:

- **Radiotherapy treatment planning system review:** For patients treated in the digital era (typically post-1990), digital treatment plans yield DVHs for gonadal structures allowing precise dose quantification. For earlier treatment eras, phantom-based reconstruction using contemporaneous protocols is standard.
- **Childhood cancer survivor cohort dosimetry:** The Childhood Cancer Survivor Study (CCSS) and St. Jude Lifetime Cohort (SJLIFE) have systematically reconstructed individualized ovarian and testicular radiation doses using CT-based anthropomorphic phantom dosimetry. These reconstructions enabled the robust dose-response analyses that underpin current POI risk models (Im et al., 2023).
- **Population-level exposure estimation:** Epidemiological studies of accident-exposed populations (Chernobyl, Fukushima) use environmental monitoring data, individual bioassay (whole-body counting, thyroid measurement), and decay product modeling to estimate organ doses at the individual or cohort level.

V.C.3. Pregnancy and Radiation: The Hall Principles

The foundational clinical principles for managing radiation exposure in pregnancy — codified by Hall and Giaccia and reflected in ICRP Publication 84 (2000) — remain the standard of care (ICRP, 2000; Hall & Giaccia, 2019):

Principle 1: The risk to the embryo/fetus is **dose-dependent and gestational-stage- dependent**. There is no single threshold applicable across all developmental windows. The cell types and processes at risk change throughout gestation.

Principle 2: The most radiosensitive period for structural malformations is **organogenesis** (approximately 2–7 weeks post-conception). During preimplantation (< 2 weeks post-conception), the embryonic response follows an "all-or-nothing" rule: either sufficient cells die to cause embryonic loss, or normal development proceeds without detectable effect.

Principle 3: Radiation-induced severe intellectual disability (previously called "severe mental retardation") has its highest risk during neuronal proliferation at **8–15 weeks post-conception**, with a dose-response slope of approximately 40% per Sv for doses above 100 mGy at this stage (ICRP 103; ACOG, 2017).

Principle 4: The widely cited **100 mGy fetal threshold** refers to the practical threshold for teratogenic effects (structural malformations, microcephaly) and severe intellectual disability for exposures during organogenesis

and the fetal period respectively. Below 100 mGy, no deterministic fetal effects are expected and no indication for pregnancy termination arises from radiation risk alone (ICRP 103; ACOG Committee Opinion 723, 2017).

Principle 5: Carcinogenic risk in the offspring is a stochastic effect without a practical threshold. A 10–20 mGy fetal exposure may increase childhood leukemia risk by a factor of approximately 1.5–2.0 over the background rate of ~3.6 per 10,000 (Lin et al., 2012). This risk, while real and worth acknowledging, must be placed in context: the lifetime excess cancer risk from a 10 mGy fetal exposure is approximately 0.05% against a background lifetime cancer risk of ~38%.

[FIGURE: fig21_critical_windows | Caption: Critical developmental windows for radiation sensitivity in the human embryo and fetus. The preimplantation period (days 0–14 post-conception) is governed by the all-or-nothing rule. Organogenesis (weeks 2–7) represents the peak sensitivity for structural teratogenesis. The 8–15-week window is associated with maximum risk for severe intellectual disability. From ICRP Publication 84 (2000) and Hall & Giaccia (2019).]

V.C.4. ACR and ACOG Imaging Guidelines in Pregnancy

The American College of Radiology (ACR)–Society for Pediatric Radiology (SPR) Practice Parameter for Imaging Pregnant or Potentially Pregnant Adolescents and Women with Ionizing Radiation (revised 2018) and the ACOG Committee Opinion 723 (2017) provide the regulatory clinical framework (ACR–SPR, 2018; ACOG, 2017):

- **Ultrasound and MRI** are the modalities of choice during pregnancy, involving no ionizing radiation. MRI is preferred when diagnostic information cannot be obtained otherwise.
- **Plain radiography and CT** should **not be withheld** from pregnant patients when medically necessary. The fetal dose from most single diagnostic procedures is 1–50 mGy — well below the 100 mGy deterministic threshold.
- **Gadolinium MRI contrast** should be used only when expected diagnostic benefit outweighs theoretical risk; observational data suggest a possible association with stillbirth and neonatal adverse events at higher doses, and gadolinium is retained in fetal tissues.
- **Radioactive iodine (I-131)** is absolutely contraindicated after approximately 10–12 weeks of gestation because the fetal thyroid begins concentrating iodine by this time, and fetal thyroid ablation may result.
- **Tc-99m nuclear medicine** procedures (effective fetal dose typically < 5 mGy) are generally safe when clinically indicated and represent an acceptable risk-benefit balance for necessary procedures.

Table V.C.1. Estimated Fetal Dose by Medical Imaging Procedure and Clinical Risk Classification

Procedure	Estimated Fetal Dose (mGy)	Deterministic Risk?	Stochastic Risk	Clinical Guidance
Chest X-ray (PA)	0.01–0.1	None	Negligible	Safe; proceed if indicated
Abdominal X-ray (AP)	1–3	None	Very low	Safe; proceed if indicated
Lumbar spine X-ray	1–10	None	Very low	Safe
Pelvic X-ray	1–4	None	Low	Safe
IVP / contrast urography	5–10	None	Low	Consider US first; safe when needed
Pelvis CT (1st trimester)	10–50	None (< 100 mGy threshold)	Low–moderate	Proceed when indicated; inform patient
Abdominopelvic CT	15–50	None (< 100 mGy threshold)	Moderate (3–5 exams→100 mGy)	Proceed when indicated; dose tracking
Head CT	< 1	None	Negligible	Safe; low scatter to pelvis

Procedure	Estimated Fetal Dose (mGy)	Deterministic Risk?	Stochastic Risk	Clinical Guidance
Tc-99m bone scan	4–5	None	Low	Safe when indicated
Tc-99m V/Q lung scan	0.5–1.0	None	Very low	Preferred over CT-PA in some guidelines
PET-CT (18F-FDG)	10–20	None	Moderate	Individual clinical benefit-risk assessment
Fluoroscopy, complex	Variable, up to 100+	Possible if > 100 mGy	High if dose extensive	Requires individual real-time dosimetry
Barium enema	7–25	None	Low	Proceed if necessary
Radioiodine I-131	Thyroid ablation at ≥ 10 wk	Fetal hypothyroidism / aplasia	High	ABSOLUTE CONTRAINDICATION ≥ 10–12 wk
Gadolinium MRI	0 (no ionizing radiation)	None (ionizing radiation)	Possible (non-radiation)	Use only when essential; limited safety data

Sources: ACR–SPR Practice Parameter (2018); ACOG Committee Opinion 723 (2017); ICRP Publication 84 (2000).

V.D. Irradiation Effects on Germ Cells

V.D.1. Radiosensitivity of the Male Germ Cell Lineage

V.D.1.a. Biological Basis of Spermatogenic Radiosensitivity

The human testis is among the most radiosensitive organs in the body. The germinal epithelium — composed of the continuous spermatogenic lineage from self-renewing spermatogonial stem cells through spermatocytes, spermatids, and mature spermatozoa — is exquisitely sensitive to ionizing radiation by virtue of the rapid mitotic cycling of spermatogonial populations and the extreme vulnerability of meiotic cells to DNA double-strand break induction (Skourt et al., 2024).

The radiosensitivity hierarchy within the spermatogenic lineage is well-established (Howell & Shalet, 2005):

- 1. Type A spermatogonia (stem cells):** The most radiosensitive cells in the male reproductive tract — and among the most radiosensitive cells in the entire body. The D_0 for type A spermatogonia is approximately **0.5–1 Gy**, reflecting their extreme sensitivity to radiation-induced apoptosis. As committed progenitors of the entire spermatogenic program, damage at this level leads to progressive downstream depletion of all more mature cell types. Detectable changes occur at doses as low as 0.1 Gy.
- 2. Type B spermatogonia and primary spermatocytes:** Sensitive but less so than type A. Overt damage to primary spermatocytes occurs at 2–3 Gy, contributing substantially to spermatid and sperm production decline.
- 3. Secondary spermatocytes and round spermatids:** Relatively more resistant; overt lethality at doses ≥ 3–4 Gy. These cells are in post-meiotic differentiation phases and are less actively cycling.
- 4. Elongating spermatids and mature spermatozoa:** Relatively radioresistant for acute cell killing. Mature spermatozoa already released into the epididymis at the time of irradiation may still be used for fertilization but carry radiation-induced DNA damage — a critical consideration for the timing of attempted conception after exposure.
- 5. Leydig cells:** Considerably more radioresistant than germinal cells (clinical dysfunction threshold > 20 Gy), so that testosterone production and secondary sexual characteristics are generally preserved even when spermatogenesis is permanently impaired. Prepubertal boys are more sensitive, with some showing Leydig cell insufficiency at lower doses.

This differential radiosensitivity explains the dissociation frequently observed in radiotherapy patients: testosterone-mediated virilization and sexual function are maintained even in the face of profound azoospermia.

V.D.1.b. Quantitative Dose–Response for Male Fertility

Seminal quantitative data derive from the controlled irradiation studies of Rowley, Clifton, and Bremner conducted in the 1970s–1980s (landmark studies using therapeutic doses in adult volunteers), and from systematic analysis of clinical radiotherapy patient cohorts summarized by Howell et al. (2005). These data — corroborated and extended by subsequent childhood cancer survivor registry analyses — form the evidential basis for the ICRP Publication 118 thresholds:

Table

Single Acute Dose (Gy)	Spermatogenic Effect	Recovery Outlook
0.1	Detectable changes in type A; FSH rise	No significant sperm count impairment
0.15	ICRP 118 threshold for temporary sterility (1% incidence)	Normal counts expected; close follow-up
0.2–0.7	Dose-dependent transient oligospermia	Recovery within 12–24 months
≥ 0.8	Azoospermia (onset at 60–70 days)	Recovery in 9–18 months
2–3	Rapid azoospermia via spermatocyte damage	Recovery ~30 months
4–6	Rapid-onset azoospermia (< 60 d)	Recovery requires ≥ 5 years, uncertain
> 6	Risk of permanent azoospermia	Recovery unlikely in many cases

A critical clinical corollary: **testicular doses as low as 1.2–1.4 Gy may cause persistent azoospermia in some men**, reflecting individual variation in spermatogonial stem cell reserve and repair capacity. A large childhood cancer survivor study found that testicular doses above 7.5 Gy were independently associated with inability to sire a pregnancy (HR 0.12; 95% CI 0.02–0.64) (Kenney et al., 2012).

The so-called **reverse fractionation effect** of the testis deserves emphasis: for most tissues, fractionated radiation is less damaging than the same total dose delivered acutely, because interfraction repair reduces effective injury. For the testis, the opposite holds: fractionated doses are more gonadotoxic than equivalent single doses because fractionation prolongs the period of spermatogonial stem cell killing, reducing the probability of surviving stem cell repopulation. Thus, ICRP 118 sets the permanent sterility threshold lower for fractionated exposure (≥ 2.5 Gy) than for single acute exposure (3.5–6 Gy) (ICRP, 2012).

V.D.1.c. Temporal Kinetics of Spermatogenic Injury and Recovery

Because human spermatogenesis takes approximately 64–74 days from spermatogonial division to sperm release, the post-irradiation sperm count decline and recovery follow a highly predictable temporal pattern:

- **Days 0–14 post-irradiation:** Spermatogonia are killed; mature spermatozoa already in the epididymis are not immediately affected and appear normal in ejaculate analyses.
- **Days 14–60 (approximately):** Ejaculated sperm reflect the pre-irradiation spermatocyte/spermatid pool; counts remain normal or show only minor morphological changes. This "silent period" is clinically important: a normal semen analysis in the first weeks post-exposure does not exclude significant spermatogonial damage.

- **Days 60–70 (for doses 0.8–3 Gy) or earlier (for doses > 4 Gy):** Azoospermia develops as the depleted spermatogonial pool fails to supply new spermatids and spermatozoa.
- **Weeks 21 onward (for lower doses):** Surviving type A spermatogonial stem cells begin repopulating the seminiferous epithelium; recovery is dose-dependent and may extend from months to years.

A 2024 comprehensive review concluded that recovery after fractionated radiotherapy is significantly slower than after single-dose treatment, and that scattered testicular doses from pelvic/inguinal field radiotherapy (1.5–3 Gy) represent a clinically significant fertility risk even when the testes are not within the primary radiation field (Skourt et al., 2024).

V.D.1.d. Sperm DNA Damage and Conception Delay

Radiation induces dose-linear DNA strand breaks in all spermatogenic cells. Post-irradiation sperm DNA fragmentation is most severe 4–6 months after treatment completion — reflecting the transit of irradiated spermatids (present in the seminiferous tubules at the time of exposure) through to sperm release. Chromosomal aneuploidy in spermatozoa has been documented after both occupational and therapeutic doses; 30–40% of spermatozoa may demonstrate damaged DNA at the nadir following pelvic radiotherapy (Romerius et al., 2009).

This has direct clinical implications for the **recommended delay of at least 6–12 months (preferably 12 months) before attempting natural conception** following significant testicular radiation exposure, to allow clearance of irradiated spermatogenic waves and repopulation by stem cells that were either shielded or completed DNA repair.

V.D.2. Radiosensitivity of the Female Germ Cell Lineage

V.D.2.a. Fundamental Biological Differences from the Male System

The female reproductive system differs from the male in one biologically critical respect: **oocytes are not regenerated after birth**. The female is born with her complete endowment of primordial follicles — approximately 700,000 at birth (range 170,000–2,000,000 by some estimates) — which decline biexponentially throughout reproductive life to approximately 25,000 by age 37 and fewer than 1,000 at menopause (the biological threshold for ovarian failure) (Wallace et al., 2003).

This non-renewable reserve has a fundamental dosimetric consequence: **any radiation-induced follicle depletion is permanent and cumulative**. Unlike the male testis, where spermatogonial stem cell recovery restores fertility if sufficient stem cells survive, the female ovary cannot regenerate follicles after radiation-induced destruction. This biological asymmetry explains why the female reproductive system is, in practice, the more clinically vulnerable partner in radiation risk assessment.

[FIGURE: fig24_follicle_reserve | Caption: The biexponential decline in primordial follicle number across female reproductive life, from birth (~700,000) to menopause (< 1,000), modeled by the Faddy-Gosden equations. Superimposed curves illustrate how radiation-induced follicle depletion (at doses of 2, 6, and 14 Gy at age 25) shifts the menopause curve leftward, predicting premature ovarian insufficiency. Based on Wallace et al. (2003, 2005) and the Faddy-Gosden model.]

V.D.2.b. Oocyte Radiosensitivity: Quantitative Parameters

The D_0 for the human primordial oocyte — the dose that reduces survival to 37% on the exponential portion of the survival curve — is approximately **0.12 Gy**. This extraordinary radiosensitivity, lower by a factor of 4–8 than that of most somatic cells, was first established by Wallace et al. (2003) and has been corroborated by subsequent experimental and clinical data (Meirow et al., 2010).

Key quantitative parameters of female gonadal radiosensitivity:

- **D_0 of the individual oocyte:** 0.12 Gy — the most radiosensitive cell type in the human body for permanent irreversible damage
- **LD_{50} for primordial follicles (human):** < 2 Gy (the dose eliminating 50% of non-growing follicles)
- **5% POI risk:** ~2–3 Gy fractionated, in women aged 20–30
- **50% POI risk:** ~6–12 Gy fractionated, in women aged 20–30

A 2021 experimental study using single-dose irradiation from 0.02 to 8 Gy in a mouse model found an LD_{50} for primordial follicles of **< 50 mGy** for gamma/X-rays — with growing follicles surviving doses up to 8 Gy — and documented that surviving oocytes produced embryos with elevated ploidy errors (25.5%) and fetal abortion

rates (23%), cautioning against sole reliance on natural conception from a radiation-depleted oocyte reserve (Stringer et al., 2021).

V.D.2.c. Follicular Stage Dependence of Radiation Sensitivity

A nuanced and often misunderstood aspect of ovarian radiosensitivity is the **stage-dependence**: radiosensitivity is not uniform across follicle developmental stages.

- **Primordial follicles (quiescent, dictyotene arrest):** Despite the extremely low D_0 for the oocyte itself, quiescent primordial follicles are paradoxically more radioresistant than growing follicles in terms of **immediate acute cell killing**, because oocytes in G0/G1 have minimal active DNA replication and reduced apoptotic signaling. The primary mechanism of primordial follicle depletion by radiation is **indirect**: ionizing radiation kills rapidly cycling granulosa cells, disrupting the bidirectional oocyte-granulosa cell signaling (c-Kit/SCF, PTEN/PI3K pathways) essential for oocyte survival maintenance, leading to indirect primordial oocyte loss (Zelinski et al., 2011).
- **Growing follicles (primary, secondary, antral):** More directly vulnerable to radiation-induced apoptosis because of active DNA replication and proliferating granulosa cell populations. Growing follicle destruction contributes to acute menstrual cycle disruption and transient amenorrhea.

This staging distinction has clinical importance: it explains why the radiation-induced loss of long-term ovarian reserve (measured in primordial follicle pool depletion) is not simply predicted by the immediate cycle-disrupting effects observed acutely after low doses.

The source French text (Jacquet et al., 1994) provides an important comparative biology observation: the guinea pig oocyte, unlike the mouse and other rodents, shares morphological and radiation sensitivity characteristics much closer to those of the human oocyte. Specifically, the immature oocyte of the guinea pig displays a "diplotene" nuclear chromatin configuration similar to the human, whereas the mouse immature oocyte has a "dictyate" pulverized chromatin pattern. This explains why a few hundred mGy suffice to sterilize a mouse — by directly killing the majority of immature oocytes — whereas several Gy are required to induce permanent sterility in a woman. The guinea pig therefore represents one of the most appropriate experimental models for human female gonadal radiobiology.

V.D.3. The Wallace Formula and Age-Dependent Ovarian Failure Prediction

The most clinically applied quantitative model for predicting the age of radiation- induced premature ovarian failure is the **Wallace formula**, developed by Wallace, Thomson, and Kelsey (2003, 2005):

The model derives from two mathematical foundations:

1. The **Faddy-Gosden biexponential model** of physiological primordial follicle decline with age (initial population at birth: ~701,200; at natural menopause: ~1,000; biexponential decline constants derived from histological counts).
2. The oocyte radiosensitivity parameter $D_0 = 0.12 \text{ Gy}$, yielding an exponential survival fraction for oocytes surviving a fractional dose z :

$$\text{Survival fraction} = \exp(-z / D_0) = \exp(-z / 0.12)$$

From these parameters, the model computes the **Effective Sterilizing Dose (ESD)** for each age at treatment — defined as the dose at which immediate premature ovarian insufficiency occurs in 97.5% of women of that age:

Table V.D.1. Effective Sterilizing Dose (ESD) by Age at Treatment from the Wallace Formula for Fractionated Radiotherapy

Age at Radiotherapy (years)	ESD (Gy, fractionated)	Remaining Follicles at Treatment	Notes
Birth	20.3	~701,200	Maximum reserve; high ESD
5	~19.3	~500,000 (estimated)	Very high reserve
10	18.4	~360,000	Pre-pubertal; high reserve
15	17.5	~200,000 (estimated)	Pubertal transition

Age at Radiotherapy (years)	ESD (Gy, fractionated)	Remaining Follicles at Treatment	Notes
20	16.5	~150,000	Early reproductive age
25	~15.5	~100,000 (estimated)	
30	14.3	~72,000	Peak fertility years
35	~10.0	~37,000 (estimated)	Declining reserve
40	~6.0	~13,000	Markedly reduced reserve; low ESD

Source: Wallace WHB et al. (2003, 2005).

The pronounced age-dependence of ESD reflects the declining ovarian reserve: an older woman with fewer primordial follicles requires a smaller absolute depletion to reach the <1,000 follicle menopause threshold, and therefore a lower radiation dose exhausts her reserve. This explains the clinical urgency of fertility preservation counseling in women ≥ 35 years approaching any pelvic radiation treatment.

A simplified linear prediction equation from a 2022 clinical model for patients under 40 years and ovarian doses below 5 Gy provides a practical counseling tool (Donadon et al., 2022):

$$\text{LOF (\%)} = 2.70 + 11.08 \times \text{Dose (Gy)}$$

where LOF is the percentage reduction in time to expected ovarian failure compared to an unirradiated woman of the same age.

V.D.4. Uterine Radiation Effects

Beyond ovarian damage, radiation to the uterus independently compromises reproductive outcomes through several mechanisms (Jeruss & Woodruff, 2009):

- **Endometrial damage and fibrosis:** Reducing endometrial receptivity. Doses above 20 Gy to the uterus in prepubertal girls markedly impair uterine growth and volume, predisposing to miscarriage and intrauterine growth restriction in later pregnancies.
- **Uterine vascular damage:** Radiation damages endometrial microvasculature. Doses above 14 Gy to the uterus confer significant risk of spontaneous pregnancy loss, preterm labor, and placental abnormalities (abruption, accreta spectrum).
- **Total body irradiation (TBI) effects:** Women who received TBI as conditioning for haematopoietic stem cell transplantation have markedly elevated rates of obstetric complications — including gestational hypertension, premature labor, and low birth weight — even when ovarian function is preserved through donor oocyte programs. This reflects direct uterine injury independent of ovarian function.

V.D.5. Childhood Cancer Survivor Cohort Data

V.D.5.a. Premature Ovarian Insufficiency in Female Survivors

The **Childhood Cancer Survivor Study (CCSS)** — a multi-institutional North American cohort of >24,000 five-year survivors diagnosed 1970–1999 — and the **St. Jude Lifetime Cohort (SJLIFE)** provide the most extensive and methodologically rigorous data on radiation-related reproductive late effects in survivors of childhood and adolescent cancers (Chemaitilly et al., 2017).

Key findings on premature ovarian insufficiency from the SJLIFE cohort:

- Overall POI prevalence: **10.9%** compared to ~1–2% in the general population.
- Independent risk factors: ovarian radiotherapy at any dose (HR 13.85 for doses < 10 Gy; HR 132.34 for doses ≥ 10 Gy), and cyclophosphamide equivalent dose $\geq 8,000$ mg/m² — demonstrating that radiation and alkylating agents independently and synergistically damage ovarian reserve.

A landmark 2023 Lancet Oncology report by Im et al. developed and validated age- specific POI risk prediction models incorporating ovarian radiation dosimetry in 7,891 CCSS survivors and 1,349 SJLIFE validation cohort survivors (Im et al., 2023). POI prevalence increased from 7.9% at age 21 to 18.6% by age 40 in the CCSS cohort.

Models incorporating dosimetric data achieved AUC values of 0.76–0.78 in derivation and 0.88–0.95 in the SJLIFE validation cohort. The resulting individualized risk calculator is publicly available (ccss.stjude.org).

A 2025 CCSS-SART CORS linkage study of ART outcomes in 9,885 female survivors found that, among those using autologous oocytes, live birth rate per ovarian stimulation cycle (31.6%) was not significantly different from siblings (38.3%), **except** in those who received pelvic radiotherapy (RR 0.33; 95% CI 0.15–0.73) or cranial radiotherapy (RR 0.44; 95% CI 0.20–0.97). No survivor who had received pelvic RT doses > 20 Gy achieved a live birth using donor oocytes, reflecting uterine as well as ovarian damage at this dose level (Dull et al., 2025).

V.D.5.b. Male Childhood Cancer Survivor Gonadal Outcomes

For male childhood cancer survivors, the critical gonadal dose threshold for impaired fertility is lower than previously appreciated, and includes scattered dose from field arrangements that do not directly target the testes:

- Testicular doses > **7.5 Gy** are independently associated with male infertility and inability to sire a pregnancy (HR 0.12; 95% CI 0.02–0.64) in childhood cancer survivor cohorts (Kenney et al., 2012).
- Scattered testicular doses from abdominal/pelvic radiotherapy — commonly 0.5–3 Gy — cause dose-dependent temporary and potentially permanent oligospermia/azoospermia.
- Total body irradiation (TBI, typically 10–14 Gy) as conditioning for bone marrow transplantation results in permanent azoospermia in the majority of recipients; only approximately 15% of men given 10 Gy TBI regain any spermatogenesis.
- The Children's Oncology Group (COG) Long-Term Follow-Up Guidelines Version 6.0 (2023) provide evidence-based surveillance recommendations for spermatogenic impairment in male survivors who received alkylating agents or gonadal irradiation (COG, 2023).

V.D.6. Fertility Preservation Strategies in the Radiation Context

V.D.6.a. General Principles

Fertility preservation discussions must occur **before** any potentially gonadotoxic treatment (Oktay et al., 2025; ESHRE, 2025

). Both the 2025

ASCO update and the 2025 ESHRE good practice recommendations emphasize that all patients facing potential gonadotoxic treatment should be expeditiously referred to reproductive medicine for fertility preservation consultation, even when treatment is urgent.

V.D.6.b. Gonadal Shielding

Testicular shielding using lead clamshell devices or custom-fabricated alloy blocks is a low-cost, first-line intervention for male patients receiving inguinal, pelvic, or abdominal irradiation where the testes lie outside but near the primary beam. Correctly applied shielding can reduce scattered testicular dose by approximately 50–95% depending on technique, proximity to the field edge, and photon beam energy (Malone et al., 2022).

Important limitations apply:

- Shielding reduces direct and primary scattered dose but cannot eliminate internal scatter from beams passing through overlying pelvic tissues.
- Modern intensity-modulated radiotherapy (IMRT) and volumetric arc therapy (VMAT) are reducing gonadal scatter independently of external shielding, prompting some centres to reassess routine shielding protocols.
- The American Association of Physicists in Medicine (AAPM) recommends individualized, case-by-case assessment of shielding based on treatment technique and computed testicular dose.

Ovarian shielding is anatomically less effective for female patients due to the deep pelvic location of the ovaries, and scattered dose from surrounding pelvic tissues substantially limits shielding efficacy.

V.D.6.c. Ovarian Transposition (Oophoropexy)

Surgical transposition of the ovaries out of the planned radiation field — performed laparoscopically before pelvic radiotherapy — can reduce ovarian dose by 50–90% (Wiechert et al., 2023). A 2023 meta-analysis of laparoscopic oophoropexy in women undergoing pelvic radiation for anorectal malignancies demonstrated preserved ovarian function in **66.9%** (95% CI 55.0–79.0%) of patients at follow-up.

Key clinical points:

- Effectiveness is greatest for laterally directed beams; circumferential pelvic irradiation (rectal adenocarcinoma, cervical cancer) leaves greater residual scattered dose to transposed ovaries.
- Transposed ovaries retain their ability to respond to controlled ovarian hyperstimulation for future embryo or oocyte cryopreservation.
- Because the procedure does not protect the uterus, most women who conceive after oophoropexy alone will require gestational surrogacy.

V.D.6.d. GnRH Agonist Co-treatment

Gonadotropin-releasing hormone (GnRH) agonists theoretically protect the ovary by inducing a reversible prepubertal gonadal state through FSH/LH suppression, thereby reducing the number of actively cycling follicles exposed to radiation damage. The evidence base for radiation-specific gonadoprotection remains limited and inconsistent:

- Evidence supporting GnRHa protection is stronger for **chemotherapy** (alkylating agents) than for **radiation**, reflecting an important mechanistic distinction: the quiescent primordial follicles most at risk from radiation are **not** gonadotropin-dependent, limiting the biological plausibility of this intervention for radiation gonadotoxicity.
- Current ASCO 2025 and ESHRE guidelines do not recommend GnRHa as a replacement for established fertility preservation methods; it may be offered as an adjunct for reducing menorrhagia during chemotherapy phases with thrombocytopenia (ASCO, 2025; ESHRE, 2025).

V.D.6.e. Gamete and Tissue Cryopreservation

For male patients: Sperm cryopreservation is the gold standard for all postpubertal males facing gonadotoxic radiation. It is non-invasive, highly effective, and should be offered as standard of care. For prepubertal boys unable to provide semen samples, **testicular tissue cryopreservation** (biopsy and liquid nitrogen storage) is offered at specialized centres, with autologous transplantation or in-vitro spermatogenesis remaining experimental but promising approaches (Davis et al., 2023).

For female patients: Three established options exist (ESHRE, 2025; ASCO, 2025):

1. **Embryo cryopreservation** — the most established technique; requires a partner or donor sperm; typically 2–3 weeks for controlled ovarian hyperstimulation and oocyte retrieval; success rates per thawed embryo transfer of 40–50% per cycle in young women.
2. **Mature oocyte cryopreservation (vitrification)** — no partner required; success rates now comparable to fresh cycles in experienced centres; the preferred option for single women or those unwilling to use donor sperm.
3. **Ovarian tissue cryopreservation and autotransplantation** — no longer classified as experimental by ESHRE (2023) and ASCO (2025 update); over 200 live births reported worldwide from transplanted cryopreserved ovarian cortex strips; the only option for prepubertal girls. Risk of reintroducing malignancy with reimplantation must be assessed for each cancer diagnosis: highest risk for haematological malignancies (leukemia); low risk for solid tumors. Orthotopic reimplantation into the pelvic peritoneum restores both ovarian endocrine function and natural fertility.

Table V.D.2. Summary of Quantitative Dose Thresholds for Reproductive Endpoints (ICRP 118; Clinical Data)

Endpoint	Threshold / Key Dose	Exposure Condition	Source Reference
Detectable spermatogonial changes (male)	0.1 Gy	Single acute	ICRP 118; Howell 2005
Temporary oligospermia onset (male)	0.15–0.2 Gy	Single acute	ICRP 118
Transient azoospermia (male)	~0.8 Gy	Single acute	Rowley et al.; Howell 2005
Temporary sterility threshold (male, ICRP)	~0.15 Gy	Single acute	ICRP Publication 118 (2012)

Endpoint	Threshold / Key Dose	Exposure Condition	Source Reference
Permanent sterility (male, single dose)	3.5–6 Gy	Single acute	ICRP 118
Permanent sterility (male, fractionated)	≥ 2.5 Gy	Fractionated	ICRP 118; reverse fractionation effect
Measurable follicle depletion (female)	Any dose (no threshold)	Any	Wallace 2003; Stringer 2021
Oocyte D_0 (radiosensitivity parameter)	0.12 Gy	Individual cell	Wallace 2003
LD_{50} for primordial follicles (human est.)	< 2 Gy	Single / fractionated	Wallace 2003; Meirow 2010
POI risk 5%	~ 2 –3 Gy	Fractionated; age 20–30	Wallace 2005; CCSS data
POI risk 50%	~ 6 –12 Gy	Fractionated; age 20–30	Wallace 2005
ESD (age 20, fractionated)	16.5 Gy	Fractionated	Wallace formula (2005)
ESD (age 30, fractionated)	14.3 Gy	Fractionated	Wallace formula (2005)
ESD (age 40, fractionated)	~ 6 Gy	Fractionated	Wallace formula (2005)
Fetal teratogenesis / SMR threshold	100 mGy	Acute; organogenesis/8–15 wk	ICRP 103; Hall; ACOG 2017
Fetal childhood leukemia risk $\times 1.5$ –2.0	10–20 mGy	Fetal exposure	Linnet 2012; ACOG 2017
No functional tissue impairment (any tissue)	< 100 mGy acute	Single acute	ICRP 118

Sources: ICRP 103 (2007); ICRP 118 (2012); ICRP 152 (2022); Wallace et al. (2003, 2005); Howell & Shalet (2005); Linet et al. (2012); ACOG (2017); Im et al. (2023).

V.D.7. Species Differences and Experimental Models

The source French text (Benghali, Chapter V) includes an important methodological discussion of experimental models for female gonadal radiobiology, drawing on the research of Jacquet et al. (1994, 1995), and contextualizing the significance of inter-species differences for risk extrapolation.

The mouse model: The immature (diplotene-arrested) mouse oocyte has a "dictyate" pulverulent chromatin configuration. A few hundred mGy suffice to kill the majority of immature oocytes in mice, producing rapid sterility. Mouse immature oocytes are thus far more radiosensitive than their human counterparts; direct dose extrapolation from mouse sterilization doses to human doses would grossly overestimate human female gonadal risk.

The guinea pig model: Studies conducted at the authors' laboratory demonstrated that the guinea pig oocyte, unlike those of the mouse and other rodents, possesses morphological and radiation sensitivity characteristics closely resembling those of the human. The immature guinea pig oocyte has a "diplotene" configuration typical of the human — not the dictyate pattern of the mouse — and requires several Gy for sterilization induction, mirroring human susceptibility.

These observations establish the guinea pig as one of the most appropriate experimental models for assessing genetic and cytogenetic risks to the human female germline from ionizing radiation (Jacquet et al., 1994).

Chromosomal translocation analysis (experimental method): The French source describes the specific cytogenetic methodology used in guinea pig studies:

- Female adult guinea pigs (3–4 months) were mated, and newborn females were irradiated with 1 or 2 Gy X-rays to the ovaries within the first 2 days of life (when oocytes are predominantly in the immature diplotene stage).
- Oocytes were collected at 1 year of age — ensuring that the oocytes analyzed were indeed in the immature stage at the time of irradiation.
- "Meiotically competent" oocytes were obtained by puncture of follicles on the ovary surface on day 10 of the cycle, cultured in vitro for 6 hours to resume meiosis and reach metaphase I (MI).
- At MI, the 64 guinea pig chromosomes appear as 32 bivalent pairs; reciprocal translocations between non-homologous chromosomes produce characteristic aberrant association figures (trivalents, quadrivalents, chains or rings) visible by light microscopy.
- Radiosensitivity across pre-ovulatory stages (15, 8, 4, 3, 2, 1 week and 2 days before ovulation) was examined by irradiating adult ovaries with 1, 2, or 4 Gy.
- Transmission of chromosomal translocations to offspring was studied by irradiating females 2 days before ovulation (the pre-ovulatory stage most susceptible to translocation induction), then mating with males and analyzing oocytes from female offspring at 4 months of age.

The general principles derived from this experimental framework align with and underpin the clinical dosimetry thresholds: namely, that structural chromosomal aberrations — particularly reciprocal translocations — constitute the principal category of heritable genetic damage from ionizing radiation in germ cells.

V.D.8. Chernobyl and Fukushima: Evidence on Reproductive Outcomes

V.D.8.a. Chernobyl Reproductive Data (Selected Post-2020 Studies)

A 2020 retrospective cohort study from Israel (Muhsen et al., 2020) followed immigrant women exposed to Chernobyl fallout in Belarus/Ukraine versus matched non-exposed immigrants. Chernobyl-exposed women were more likely to be nulliparous, had fewer children (mean 2.1 vs. 3.1; $P < 0.001$), and more often required fertility treatments (adjusted OR 1.8; 95% CI 1.04–3.2). These findings are consistent with radiation-induced ovarian reserve reduction but cannot be definitively attributed to radiation alone given confounding factors (selection bias, migration stress, behavioral changes).

The 2025 Italian cohort analysis of 18 birth cohorts (1976–1993) by Grembi et al. found increased miscarriages and stillbirths in women exposed in utero or in the first three years of life to Chernobyl fallout — providing new long-term evidence of developmental radiosensitivity sequelae (Grembi et al., 2025).

Crucially, no evidence of increased heritable genetic disease — Mendelian disorders, chromosomal abnormalities, or increased de novo mutation rate — has been demonstrated in offspring of Chernobyl liquidators through multilocus genotyping and whole-exome sequencing analyses (Yeager et al., 2021, *Nature*), confirming the absence of detectable transgenerational heritable effects at the doses studied.

V.D.8.b. Fukushima Reproductive Outcome Data

The UNSCEAR 2020/2021 Report Volume II Annex B concluded that radiation doses to Fukushima Prefecture residents were too low to produce detectable non-cancer health effects, including reproductive effects, in the general population (UNSCEAR, 2022). The Fukushima Health Management Survey Pregnancy and Birth Survey found no increase in preterm birth, low birth weight, or congenital anomalies in over 115,976 pregnancies tracked from 2012 to 2018 (Kyojuka et al., 2022). These negative findings at population level are entirely consistent with the dose estimates: with mean effective doses < 10 mSv to the general public — well below any deterministic threshold — no excess reproductive effects would be biologically expected.

Conclusion

Ionizing radiation exemplifies several principles central to reproductive toxicology:

1. **Threshold-based deterministic effects** govern sterility outcomes; quantitative dose thresholds are established with unusual precision (ICRP 118) and form the foundation for clinical counseling.
2. **The non-renewable female oocyte reserve** makes the female reproductive system uniquely vulnerable: any follicle depletion is permanent and cumulative, and the Wallace formula provides individualized, age-specific prediction of premature ovarian failure.
3. **The male spermatogenic epithelium** — with type A spermatogonia as the most radiosensitive cells in the body — is also highly sensitive, but the stem cell repopulation potential permits recovery at moderate doses; permanent azoospermia requires doses above 3.5–6 Gy acutely or ≥ 2.5 Gy fractionated.
4. **Heritable effects**, while biologically plausible, have not been demonstrated in any human population despite intensive study; risk estimates remain extrapolated from animal models.
5. **Fertility preservation** — sperm banking, oocyte/embryo cryopreservation, ovarian tissue banking — must be offered to all patients before gonadotoxic treatment. The 2025 ASCO and ESHRE guidelines represent the current clinical standard of care.
6. **The diagnostic imaging threshold** of 100 mGy for fetal deterministic effects means that the vast majority of diagnostic medical imaging poses no clinically significant teratogenic risk; rational, evidence-based communication of this to patients prevents harmful procedure avoidance.

References

References are listed in order of first appearance throughout the volume (Vancouver style). Digital Object Identifiers (DOIs) and direct URLs are provided wherever available.

1. Bonde JP, Tøttenborg SS, Andersson AM, et al. Reproductive toxicology: keeping up with our changing world. *Front Toxicol*. 2024;6:1456687. <https://doi.org/10.3389/ftox.2024.1456687>
2. International Council for Harmonisation. ICH Guideline S5(R3): Detection of Reproductive and Developmental Toxicity for Human Pharmaceuticals. FDA CDER. 2020. <https://www.fda.gov/media/108894/download>
3. Welsh M, Saunders PTK, Fisker M, et al. Identification in rats of a programming window for reproductive tract masculinization, disruption of which leads to hypospadias and cryptorchidism. *J Clin Invest*. 2008;118(4):1479–1490. <https://doi.org/10.1172/JCI34241>
4. Monniaux D, Clément F, Dalbiès-Tran R, et al. The ovarian reserve of primordial follicles and the dynamic reserve of antral growing follicles: what is the link? *Biol Reprod*. 2014;90(4):85. <https://doi.org/10.1095/biolreprod.113.117077>
5. Skakkebaek NE, Rajpert-De Meyts E, Main KM. Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. *Hum Reprod*. 2001;16(5):972–978. <https://doi.org/10.1093/humrep/16.5.972>
6. Levine H, Jørgensen N, Martino-Andrade A, et al. Temporal trends in sperm count: a systematic review and meta-regression analysis of samples collected globally in the 20th and 21st centuries. *Hum Reprod Update*. 2023;29(2):157–176. <https://doi.org/10.1093/humupd/dmac035>
7. Adamovsky O, et al. Advances in understanding the reproductive toxicity of endocrine disrupting chemicals. *Front Cell Dev Biol*. 2024;12:1390247. <https://doi.org/10.3389/fcell.2024.1390247>
8. Maranghi F, et al. Investigating the impact of environmental pollutants on embryo-fetal development. *Reprod Toxicol*. 2025. PMC12705484. <https://pmc.ncbi.nlm.nih.gov/articles/PMC12705484/>
9. Völkel W, Colnot T, Csanádi GA, et al. Metabolism and kinetics of bisphenol A in humans at low doses following oral administration. *Chem Res Toxicol*. 2002;15(10):1281–1287. <https://doi.org/10.1021/tx025548t>
10. Hamers T, et al. Exposures, Mechanisms, and Impacts of Endocrine-Active Flame Retardants. *Curr Opin Pharmacol*. 2014;19:125–133. <https://pmc.ncbi.nlm.nih.gov/articles/PMC4252719/>
11. EFSA CEP Panel. Re-evaluation of the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs. *EFSA Journal*. 2023;21(4):e06857. <https://doi.org/10.2903/j.efsa.2023.6857>
12. European Environment Agency. Human exposure to Bisphenol A in Europe. EEA Publications. 2023. <https://www.eea.europa.eu/en/analysis/publications/human-exposure-to-bisphenol-a>
13. Kolatorova L, et al. Mechanisms of bisphenol A and its analogs as endocrine disruptors. *Endocrine Connections*. 2025. PMC12185661. <https://pmc.ncbi.nlm.nih.gov/articles/PMC12185661/>
14. Khodasevich D, et al. Biomonitoring of Environmental Phenols, Phthalate Metabolites and Related Compounds. *Toxics*. 2024. PMC12736981. <https://pmc.ncbi.nlm.nih.gov/articles/PMC12736981/>
15. Sueiro A, et al. A health conundrum of bisphenol A and its alternatives: charting a safer path forward. *J Environ Exposure Anal*. 2025. <https://doi.org/10.20517/jeea.2025.39>
16. Apel P, et al. Human biomonitoring guidance values for selected phthalates and DINCH (HBM4EU). *Int J Hyg Environ Health*. 2021;234:113722. <https://doi.org/10.1016/j.ijheh.2021.113722>
17. Vogel N, et al. Current exposure to phthalates and DINCH in European children and adolescents — HBM4EU Aligned Studies 2014–2021. *Int J Hyg Environ Health*. 2022. <https://doi.org/10.1016/j.ijheh.2022.114005>
18. SCHEER. Update of the guidelines on the benefit-risk assessment of phthalates in certain medical devices. European Commission. June 2024. https://health.ec.europa.eu/document/download/831f9b60-88de-4197-858f-d001c5b0cc26_en
19. Swan SH, Main KM, Liu F, et al. Decrease in anogenital distance among male infants with prenatal phthalate exposure. *Environ Health Perspect*. 2005;113(8):1056–1061. <https://doi.org/10.1289/ehp.8100>

20. US EPA. Non-cancer Human Health Hazard Assessment for DEHP. EPA Technical Support Document. December 2025. <https://www.epa.gov/system/files/documents/2025-12/18-dehp-non-cancer-human-health-hazard-tsd-public-release-hero-dec-2025.pdf>
21. Mandal PK. Health toxicity effects of brominated flame retardants. *Environ Pollut.* 2021;286:117500. <https://doi.org/10.1016/j.envpol.2021.117500>
22. Stockholm Convention Secretariat. Polybromodiphenyl ethers (BDEs) — Overview. UNEP. <https://www.pops.int/implementation/industrialpops/bdes/overview/tabid/5371/default.aspx>
23. Siddique S, et al. Has Regulatory Action Reduced Human Exposure to Flame Retardants? A Meta-Analysis of PBDEs and HBCDDs in Human Breast Milk. *Environ Sci Technol.* 2023;57(47). <https://doi.org/10.1021/acs.est.3c02896>
24. Hamers T, et al. Exposures, Mechanisms, and Impacts of Endocrine-Active Flame Retardants. *Curr Opin Pharmacol.* 2014. <https://pmc.ncbi.nlm.nih.gov/articles/PMC4252719/>
25. Gaballah S. Exposure to Legacy and Alternative Brominated Flame Retardants in Maternally and Fetally Derived Placental Tissue in Rats and Humans [dissertation]
26. EFSA CONTAM Panel. Risk to human health related to the presence of perfluoroalkyl substances in food. *EFSA Journal.* 2020;18(9):e06223. <https://doi.org/10.2903/j.efsa.2020.6223>
27. European Commission. PFAS pollution — chemicals regulation and restriction proposal. DG Environment. 2023. https://environment.ec.europa.eu/topics/chemicals/pfas-pollution_en
28. US EPA. National Primary Drinking Water Regulation for PFAS. 2024. <https://www.epa.gov/sdwa/drinking-water-health-advisories-genx-chemicals-and-pfbs>
29. Chen Q, et al. Reproductive toxicity of PFOA, PFOS and their substitutes: a review. *Chemosphere.* 2024. <https://doi.org/10.1016/j.chemosphere.2024.141528>
30. SCCS. Scientific Committee on Consumer Safety Opinion on Methylparaben (CAS No. 99-76-3). European Commission Health. December 2023. https://health.ec.europa.eu/system/files/2023-12/sccs_o_276_final.pdf
31. Darbre PD, Harvey PW. Paraben esters: review of recent studies of endocrine toxicity, absorption, esterase and human exposure, and discussion of potential human health risks. *J Appl Toxicol.* 2008;28(5):561–578. <https://doi.org/10.1002/jat.1358>
32. Błaszczyk M, et al. Ecotoxicological impacts of parabens on flora and fauna. *npj Sustainable Agriculture.* 2025. <https://doi.org/10.1038/s44454-025-00012-2>
33. CIR Expert Panel. Amended Safety Assessment of Parabens as Used in Cosmetics. *Cosmetic Ingredient Review.* 2018. <https://www.cir-safety.org/sites/default/files/Parabens.pdf>
34. European Commission. Regulation (EC) No 1223/2009 on Cosmetic Products. OJ L 342/59. 2009. <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32009R1223>
35. Levine H, Jørgensen N, Martino-Andrade A, Mendiola J, Weksler-Derri D, Mindlis I, Pinotti R, Swan SH. Temporal trends in sperm count: a systematic review and meta-regression analysis. *Hum Reprod Update.* 2017;23(6):646–659. [doi:10.1093/humupd/dmx022](https://doi.org/10.1093/humupd/dmx022)
36. Levine H, Jørgensen N, Martino-Andrade A, Mendiola J, Weksler-Derri D, Jollès M, Pinotti R, Swan SH. Temporal trends in sperm count: a systematic review and meta-regression analysis of samples collected globally in the 20th and 21st centuries. *Hum Reprod Update.* 2023;29(2):157–176. [doi:10.1093/humupd/dmac035](https://doi.org/10.1093/humupd/dmac035)
37. Lewis K, Cannarella R, Liu F, Roth B, Bushweller L, Millot JC, Kuribayashi S, Kuroda S, Aguilar Palacios D, Vij SC, Cullen J, Lundy SD. Sperm concentration remains stable among fertile American men: a systematic review and meta-analysis. *Fertil Steril.* 2024. [doi:10.1016/j.fertnstert.2024.08.322](https://doi.org/10.1016/j.fertnstert.2024.08.322)
38. Szabó A, Forintos A, Váncsa S, Ács J, Váradi A, Filipov T, Szarvas T, Nyirády P, Hegyi P, Kopa Z, Ács N. Lifestyle-, environmental-, and additional health factors associated with an increased sperm DNA fragmentation: a systematic review and meta-analysis. *Basic Clin Androl.* 2023;33:6. [doi:10.1186/s12958-023-01054-0](https://doi.org/10.1186/s12958-023-01054-0)
39. Aizer A, Baccarelli AA, Dye CK, Jackson GL, Wu H, Kanz KG, Manz KM, Pennell K, Chillrud LG, Kioumourtzoglou MA, Hoffmann Dishon N, Machtinger R, Orvieto R, Kalia V. Exposome profiling of environmental pollutants in seminal plasma and novel associations with semen parameters. *Environ Sci Technol.* 2024;58(29). [doi:10.1021/acs.est.3c10314](https://doi.org/10.1021/acs.est.3c10314)

40. De Angelis A, Chioccarelli T, Manfredola F, Ferraro B, Cappetta D, Moggio M, Berrino L, Fasano S, Telesca M, Porreca V, Mele VG, Diano N, Cobellis G. Variation of sperm quality and circular RNA content in men exposed to environmental contamination with heavy metals in "Land of Fires", Italy. *Hum Reprod.* 2024;39(7). doi:[10.1093/humrep/deae109](https://doi.org/10.1093/humrep/deae109)
41. Charmandari E, Mastorakos G, Stathori G, Valsamakis G, Hatzigapiou K, Vlahos N. Endocrine-disrupting chemicals, hypothalamic inflammation and reproductive outcomes: a review of the literature. *Int J Mol Sci.* 2024;25(21):11344. doi:[10.3390/ijms252111344](https://doi.org/10.3390/ijms252111344)
42. Enangue Njembele AN, Bailey JL, Tremblay JJ, Demmousse ZB. Mechanism of action of an environmentally relevant organochlorine mixture in repressing steroid hormone biosynthesis in Leydig cells. *Int J Mol Sci.* 2022;23(7):3997. doi:[10.3390/ijms23073997](https://doi.org/10.3390/ijms23073997)
43. Kaur G, Dufour JM, Washburn RL, Hibler T. Sertoli cell immune regulation: a double-edged sword. *Front Immunol.* 2022;13:913502. doi:[10.3389/fimmu.2022.913502](https://doi.org/10.3389/fimmu.2022.913502)
44. Venditti M, Minucci S. New insight on the in vitro effects of melatonin in preserving human sperm quality. *Int J Mol Sci.* 2022;23(9):5128. doi:[10.3390/ijms23095128](https://doi.org/10.3390/ijms23095128)
45. Gaskins AJ, Chavarro JE, Meeker JD, Braun JM, Mínguez-Alarcón L. Endocrine disrupting chemicals and male reproductive health. *Fertil Steril.* 2023;120(6). doi:[10.1016/j.fertnstert.2023.10.008](https://doi.org/10.1016/j.fertnstert.2023.10.008)
46. Estienne A, Vasseur C, Dupont J, Serra L, Froment P. Review: mechanisms of glyphosate and glyphosate-based herbicides action in female and male fertility in humans and animal models. *Cells.* 2021;10(11):3079. doi:[10.3390/cells10113079](https://doi.org/10.3390/cells10113079)
47. Gorga A, Centola CL, Marín-Briggiler C, Sobarzo C, Cohen D, Pellizzari E, Rindone G, Camberos MC, Galardo M, Riera M, Meroni SB. Low doses of glyphosate/Roundup alter blood-testis barrier integrity in juvenile rats. *Front Endocrinol.* 2021;12:615678. doi:[10.3389/fendo.2021.615678](https://doi.org/10.3389/fendo.2021.615678)
48. Nerozzi C, Bucci D, Galeati G, Spinaci M, Yeste M, Recuero S. Effects of Roundup and its main component, glyphosate, upon mammalian sperm function and survival. *Sci Rep.* 2020;10:10988. doi:[10.1038/s41598-020-67538-w](https://doi.org/10.1038/s41598-020-67538-w)
49. Skakkebaek NE, Rajpert-De Meyts E, Buck Louis GM, Toppari J, Andersson AM, Eisenberg ML, Jensen TK, Jørgensen N, Swan SH, Sapra KJ, Ziebe S, Priskorn L, Juul A. Male reproductive disorders and fertility trends: influences of environment and genetic susceptibility. *Physiol Rev.* 2016;96(1):55–97. doi:[10.1152/physrev.00023.2015](https://doi.org/10.1152/physrev.00023.2015)
50. Li D, Ping H, Lin J, Li K, Zhang X, Jiang X-W. Environmental oestrogens disrupt testicular descent and damage male reproductive health: mechanistic insight. *J Cell Mol Med.* 2023;27(15). doi:[10.1111/jcmm.17837](https://doi.org/10.1111/jcmm.17837)
51. Jorgensen A, Miles H, Stukenborg JB, Mitchell RT, Svingen T, Chetty T. Environmental impacts on male reproductive development: lessons from experimental models. *Horm Res Paediatr.* 2022;95(2). doi:[10.1159/000519964](https://doi.org/10.1159/000519964)
52. Roychoudhury S, Chakraborty S, Choudhury A, Das A, Jha NK, Sláma P, Nath M, Massányi P, Ruokolainen J, Kesari KK. Environmental factors-induced oxidative stress: hormonal and molecular pathway disruptions in hypogonadism and erectile dysfunction. *Antioxidants.* 2021;10(6):837. doi:[10.3390/antiox10060837](https://doi.org/10.3390/antiox10060837)
53. Trasande L, Andersson AM, Kortenkamp A, Toppari J, Heindel JJ, Juul A, Skakkebaek NE, Hauser R, Hass U. Male reproductive disorders, diseases, and costs of exposure to endocrine-disrupting chemicals in the European Union. *J Clin Endocrinol Metab.* 2015;100(4):1267–1277. doi:[10.1210/jc.2014-4325](https://doi.org/10.1210/jc.2014-4325)
54. Radke EG, Cooper GS, Braun JM, Meeker JD. Phthalate exposure and male reproductive outcomes: a systematic review of the human epidemiological evidence. *Environ Int.* 2018;121(Pt 1):764–793. doi:[10.1016/j.envint.2018.07.029](https://doi.org/10.1016/j.envint.2018.07.029)
55. Doea AO, Makrigiannakis A, Tsatsakis A, Zachariou A, Mamoulakis C, Calina D, Carvalho F, Sofikitis N, Kalantzi O, Amir S, Shah ST. Endocrine disruptors acting on estrogen and androgen pathways cause reproductive disorders through multiple mechanisms: a review. *Int J Environ Res Public Health.* 2021;18(4):1464. doi:[10.3390/ijerph18041464](https://doi.org/10.3390/ijerph18041464)
56. Shulhai A-M, Street ME, Esposito S, Bianco V, Donini V. Which is the current knowledge on man-made endocrine-disrupting chemicals in follicular fluid? An overview of effects on ovarian function and reproductive health. *Front Endocrinol.* 2024;15:1435121. doi:[10.3389/fendo.2024.1435121](https://doi.org/10.3389/fendo.2024.1435121)

57. Polizzi A, Rives C, Cadoret F, Frenois F, Giton F, Lasserre F, Loison G, Gamet-Payrastre L, Dopavogui L, Gatimel N, Loiseau N, Leandri R, Ellero-Simatos S, El Fouikar S. Pre- and postnatal dietary exposure to a pesticide cocktail disrupts ovarian functions in 8-week-old female mice. *Int J Mol Sci.* 2022;23(14):7525. [doi:10.3390/ijms23147525](https://doi.org/10.3390/ijms23147525)
58. Damdimopoulou P, Björvang RD. Persistent environmental endocrine-disrupting chemicals in ovarian follicular fluid and in vitro fertilization treatment outcome in women. *Ups J Med Sci.* 2020;125(2):85–94. [doi:10.1080/03009734.2020.1727073](https://doi.org/10.1080/03009734.2020.1727073)
59. Monteiro CS, Caetano J, Marinho R, Becker de Sousa Xavier E. A critical analysis of the impact of endocrine disruptors as a possible etiology of primary ovarian insufficiency. *JBRA Assist Reprod.* 2020;24(2):223–230. [doi:10.5935/1518-0557.20200005](https://doi.org/10.5935/1518-0557.20200005)
60. Munari EV, Persani L, Bonomi M, Frixou M, Rossetti R, Moleri S, Federici S. Primary ovarian insufficiency: update on clinical and genetic findings. *Front Endocrinol.* 2024;15:1464803. [doi:10.3389/fendo.2024.1464803](https://doi.org/10.3389/fendo.2024.1464803)
61. Komsky-Elbaz A, Kalo D, Roth Z. Effect of environmental and pharmaceutical exposures on fetal testis development and function: a systematic review of human experimental data. *Anim Reprod.* 2020;17(3):e20200041. [doi:10.1590/1984-3143-AR2020-0041](https://doi.org/10.1590/1984-3143-AR2020-0041)
62. Gao F, Shen H, Guan J, Fu M, Zhang Q, Zhang S. Reproductive toxicology of environmental endocrine-disrupting chemicals in women: a cohort study protocol. *Front Cell Dev Biol.* 2024;12:1335028. [doi:10.3389/fcell.2024.1335028](https://doi.org/10.3389/fcell.2024.1335028)
63. Patton BK, Madadi S, Pangas SA. Control of ovarian follicle development by TGF β family signalling. *Curr Opin Endocr Metab Res.* 2021;18:102–108. [doi:10.1016/J.COEMR.2021.03.001](https://doi.org/10.1016/J.COEMR.2021.03.001)
64. Ozawa H. Kisspeptin neurons as an integration center of reproductive regulation. *Reprod Med Biol.* 2021;20(4):369–381. [doi:10.1002/rmb2.12419](https://doi.org/10.1002/rmb2.12419)
65. Sadeghpour S, Farrokh-Eslamlou HR, Behrooz Lak T, Maleki F, Bahadori R, Ghasemnejad-Berenji H. The impact of sociodemographic factors, lifestyle, and medical history on semen quality in young men. *Russ Open Med J.* 2025;14. [doi:10.15275/rusomj.2025.0419](https://doi.org/10.15275/rusomj.2025.0419)
66. Kalamatov R, Paizildaev T, Ismailov I, Omorova A, Seitova A. Hormonal and sexual dysfunctions in men residing in environmentally unfavorable conditions: a clinical-functional analysis using the ICF framework. *Bull Sci Pract.* 2026;12(4). [doi:10.33619/2414-2948/125/27](https://doi.org/10.33619/2414-2948/125/27)
67. Abbara A, Koysombat K, Dhillo WS. Assessing hypothalamic pituitary gonadal function in reproductive disorders. *Clin Sci.* 2023;137(11):863–884. [doi:10.1042/CS20220146](https://doi.org/10.1042/CS20220146)
68. Montjean D, Khorsi-Cauet H, Ben Ali H, Abou Diwan M, Lahimer M, Benkhalifa M, Ajina M, Cabry R, Bach V. Endocrine disrupting chemicals and male fertility: from physiological to molecular effects. *Front Public Health.* 2023;11:1232646. [doi:10.3389/fpubh.2023.1232646](https://doi.org/10.3389/fpubh.2023.1232646)
69. Whorton MD, Krauss RM, Marshall S, Milby TH. Infertility in male pesticide workers. *Lancet.* 1977;2(8051):1259–1261. [doi:10.1016/S0140-6736\(77\)91958-5](https://doi.org/10.1016/S0140-6736(77)91958-5)
70. Plowchalk DR, Meadows MJ, Mattison DR. Reproductive toxicity of 2-bromopropane in female and male B6C3F1 mice. *Reprod Toxicol.* 1992;6(1):49–56. [doi:10.1016/0890-6238\(92\)90052-7](https://doi.org/10.1016/0890-6238(92)90052-7)
71. Zaneveld LJD. Physiology of reproduction. In: Dixon RL, ed. *Reproductive Toxicology*. New York: Raven Press; 1985:81–98.
72. Braun JM, Li N, Arbuckle TE, et al. Association between gestational urinary bisphenol A concentrations and adiposity in young children: the MIREC study. *Environ Res.* 2019;174:112–119. [doi:10.1016/j.envres.2019.02.038](https://doi.org/10.1016/j.envres.2019.02.038) | Li D-K et al. Urine bisphenol-A (BPA) level in relation to semen quality. *Fertil Steril.* 2011;95(2):625–630. [doi:10.1016/j.fertnstert.2010.09.026](https://doi.org/10.1016/j.fertnstert.2010.09.026)
73. Markey CM, Luque EH, Munoz de Toro M, Sonnenschein C, Soto AM. In utero exposure to bisphenol A alters the development and tissue organization of the mouse mammary gland. *Biol Reprod.* 2001;65(4):1215–1223. [doi:10.1093/biolreprod/65.4.1215](https://doi.org/10.1093/biolreprod/65.4.1215) | Tharp AP, Maffini MV, Hunt PA, VandeVoort CA, Sonnenschein C, Soto AM. Bisphenol A alters the development of the rhesus monkey mammary gland. *Proc Natl Acad Sci USA.* 2012;109(21):8190–8195. [doi:10.1073/pnas.1120488109](https://doi.org/10.1073/pnas.1120488109) | Wolstenholme JT, Drobňá Z, Henriksen AD, et al. Transgenerational bisphenol A causes deficits in social recognition and alters postsynaptic density genes in mice. *Endocrinology.* 2019;160(8):1854–1867. [doi:10.1210/en.2019-00196](https://doi.org/10.1210/en.2019-00196)

74. Maffini MV, Rubin BS, Sonnenschein C, Soto AM. Endocrine disruptors and reproductive health: the case of bisphenol-A. *Mol Cell Endocrinol*. 2006;254–255:179–186. [doi:10.1016/j.mce.2006.04.033](https://doi.org/10.1016/j.mce.2006.04.033)
75. Wang Y, Ni C, Li X, Lin Z, Zhu Q, Li L, Ge RS. Phthalate-induced fetal Leydig cell dysfunction mediates male reproductive tract anomalies. *Front Pharmacol*. 2019;10:1309. [doi:10.3389/fphar.2019.01309](https://doi.org/10.3389/fphar.2019.01309)
76. Swan SH, Main KM, Liu F, Stewart SL, Kruse RL, Calafat AM, et al. Decrease in anogenital distance among male infants with prenatal phthalate exposure. *Environ Health Perspect*. 2005;113(8):1056–1061. [doi:10.1289/ehp.8100](https://doi.org/10.1289/ehp.8100)
77. Fletcher EJ, Santacruz-Márquez R, Mourikes VE, Neff AM, Laws MJ, Flaws JA. Effects of phthalate mixtures on ovarian folliculogenesis and steroidogenesis. *Toxics*. 2022;10(5):251. [doi:10.3390/toxics10050251](https://doi.org/10.3390/toxics10050251)
78. Carnevali O, Tosti L, Speciale C, Peng C, Zhu Y, Maradonna F. DEHP impairs zebrafish reproduction by affecting critical factors in oogenesis. *PLoS One*. 2010;5(4):e10201. [doi:10.1371/journal.pone.0010201](https://doi.org/10.1371/journal.pone.0010201)
79. Talsness CE, Kuriyama SN, Sterner-Kock A, et al. In utero and lactational exposures to low doses of polybrominated diphenyl ether-47 alter the reproductive system and thyroid gland of female rat offspring. *Environ Health Perspect*. 2008;116(3):308–314. [doi:10.1289/ehp.10536](https://doi.org/10.1289/ehp.10536) | McNabb-Kelada N, Burke T, Jayaraman S, et al. Early-life PBDE flame retardant exposures cause neurobehavioral alterations in fish that persist into adulthood and vary by sex and route of exposure. *bioRxiv*. 2025. [doi:10.1101/2025.04.28.651140](https://doi.org/10.1101/2025.04.28.651140)
80. Vuong AM, Braun JM, Webster GM, et al. Polybrominated diphenyl ether (PBDE) exposures and thyroid hormones in children at age 3 years. *Environ Int*. 2018;117:339–347. [doi:10.1016/j.envint.2018.05.019](https://doi.org/10.1016/j.envint.2018.05.019) | Zhang T, Zhou X, Xu A, et al. Toxicity of polybrominated diphenyl ethers (PBDEs) on rodent male reproductive system: a systematic review and meta-analysis. *Environ Res*. 2020;187:109642. [doi:10.1016/j.envres.2020.109642](https://doi.org/10.1016/j.envres.2020.109642) | Makey CM, McClean MD, Braverman LE, et al. Polybrominated diphenyl ether exposure and reproductive hormones in male participants of the Flame Retardant Exposure Study. *Reprod Toxicol*. 2016;62:46–52. [doi:10.1016/j.reprotox.2016.04.009](https://doi.org/10.1016/j.reprotox.2016.04.009)
81. Zhang Y, Mustieles V, Sun Q, et al. Association of early pregnancy perfluoroalkyl and polyfluoroalkyl exposure with birth outcomes. *JAMA Netw Open*. 2023;6(5):e2314934. [doi:10.1001/jamanetworkopen.2023.14934](https://doi.org/10.1001/jamanetworkopen.2023.14934) | Taylor KD, Woodlief TL, Ahmed A, et al. Quantifying the impact of PFOA exposure on B-cell development and antibody production. *Toxicol Sci*. 2023;194(1):101–114. [doi:10.1093/toxsci/kfad043](https://doi.org/10.1093/toxsci/kfad043) | Coperchini F, Croce L, Ricci G, et al. Thyroid disrupting effects of old and new generation PFAS. *Front Endocrinol*. 2021;11:612320. [doi:10.3389/fendo.2020.612320](https://doi.org/10.3389/fendo.2020.612320) | White SS, Kato K, Jia LT, et al. Effects of perfluorooctanoic acid on mouse mammary gland development and differentiation resulting from cross-foster and restricted gestational exposures. *Reprod Toxicol*. 2009;27(3–4):289–298. [doi:10.1016/j.reprotox.2008.11.054](https://doi.org/10.1016/j.reprotox.2008.11.054) | Grandjean P, Andersen EW, Budtz-Jørgensen E, et al. Serum vaccine antibody concentrations in children exposed to perfluorinated compounds. *JAMA*. 2012;307(4):391–397. [doi:10.1001/jama.2011.2034](https://doi.org/10.1001/jama.2011.2034)
82. Sun L, Yu T, Guo J, Zhang Z, Hu Y, Xiao X, et al. The estrogenicity of methylparaben and ethylparaben at doses close to the acceptable daily intake in immature Sprague-Dawley rats. *Sci Rep*. 2016;6:25173. [doi:10.1038/srep25173](https://doi.org/10.1038/srep25173) | Hubbard TD, Brix A, Blystone CR, et al. Butylparaben multigenerational reproductive assessment by continuous breeding in Hsd:Sprague-Dawley SD rats. *Reprod Toxicol*. 2020;97:151–163. [doi:10.1016/j.reprotox.2020.07.006](https://doi.org/10.1016/j.reprotox.2020.07.006) | Shen X, Zhan M, Wang Y, Tang W, Zhang Q, Zhang J. Exposure to parabens and semen quality in reproductive-aged men. *Ecotoxicol Environ Saf*. 2023;265:115499. [doi:10.1016/j.ecoenv.2023.115499](https://doi.org/10.1016/j.ecoenv.2023.115499) | Iwamoto S, Miyashita C, Itoh S, et al. Paraben exposure and semen quality of Japanese male partners of subfertile couples. *Environ Health Prev Med*. 2017;22(1):51. [doi:10.1186/s12199-017-0618-7](https://doi.org/10.1186/s12199-017-0618-7)
83. Magnus MC, Wilcox AJ, Willer CJ, et al. Chromosomal alterations in first-trimester miscarriages. *Nature Medicine*. 2023;29(11):2936–2941. [doi:10.1038/s41591-023-02645-5](https://doi.org/10.1038/s41591-023-02645-5)
84. GBD 2021 Stillbirths Collaborators. Global, regional, and national stillbirths at 20 weeks' gestation or longer in 204 countries and territories, 1990–2021. *Lancet*. 2024;404(10466):1955–1988. [doi:10.1016/S0140-6736\(24\)01925-1](https://doi.org/10.1016/S0140-6736(24)01925-1)
85. Rahimian P, et al. Tobacco exposure and risk of spontaneous abortion: a dose-response meta-analysis. *BMC Pregnancy Childbirth*. 2025;25(1). [doi:10.1186/s12884-025-07135-5](https://doi.org/10.1186/s12884-025-07135-5)
86. Centers for Disease Control and Prevention. Risk for stillbirth among women with and without COVID-19 at delivery hospitalization. *MMWR Morb Mortal Wkly Rep*. 2021;70(47):1640–1645. [doi:10.15585/mmwr.mm7047e1](https://doi.org/10.15585/mmwr.mm7047e1)

87. Schiøler AL, et al. Pregnancy outcomes and birth defects following maternal use of non-steroidal anti-inflammatory drugs. **Reprod Toxicol**. 2024;123:108521. [doi:10.1016/j.reprotox.2024.108521](https://doi.org/10.1016/j.reprotox.2024.108521)
88. Emory University. Emory-led research first to detect 'forever chemicals' in newborns. September 2023. https://news.emory.edu/stories/2023/09/hs_PFAS_in_newborns_14-09-2023/story.html
89. Savitz DA, Sonnenfeld NL, Olshan AF. Review of epidemiologic studies of paternal occupational exposure and spontaneous abortion. **Am J Ind Med**. 1994;25(3):361–383.
90. Schenker MB, Samuels SJ, Green RS, Wiggins P. Adverse reproductive outcomes among female veterinarians. **Am J Epidemiol**. 1990;132(1):96–106.
91. Dolk H, Loane M, Garne E. The prevalence of congenital anomalies in Europe. **Adv Exp Med Biol**. 2010;686:349–364. [doi:10.1007/978-90-481-9485-8_20](https://doi.org/10.1007/978-90-481-9485-8_20)
92. Xie X, Pei J, Zhang L, Wu Y. Global birth prevalence of major congenital anomalies: a systematic review and meta-analysis. **BMC Pediatr**. 2025. [doi:10.1186/s12887-025-05244-4](https://doi.org/10.1186/s12887-025-05244-4)
93. Li Y, He C, Yu H, et al. Global, regional, and national epidemiology of congenital birth defects in children from 1990 to 2021. **BMC Pediatrics**. 2025. [doi:10.1186/s12887-025-05398-1](https://doi.org/10.1186/s12887-025-05398-1)
94. Bromley RL, Weston J, Marson AG. Maternal use of antiepileptic agents during pregnancy and major congenital malformations in children. **Cochrane Database Syst Rev**. 2023;(10). [doi:10.1002/14651858.CD004848.pub4](https://doi.org/10.1002/14651858.CD004848.pub4)
95. Jentink J, Loane MA, Dolk H, et al. Valproic acid monotherapy in pregnancy and major congenital malformations. **N Engl J Med**. 2010;362(23):2185–2193. [doi:10.1056/NEJMoa0907328](https://doi.org/10.1056/NEJMoa0907328)
96. Briggs GG, Freeman RK, Towers CV, Forinash AB. *Drugs in Pregnancy and Lactation*. 12th ed. Philadelphia: Wolters Kluwer; 2021
97. Yamamoto J, et al. Thalidomide binding to cereblon and SALL4 degradation. **eLife**. 2020;9:e57473. [doi:10.7554/eLife.57473](https://doi.org/10.7554/eLife.57473)
98. Centers for Disease Control and Prevention. Data and Statistics on FASDs. Atlanta: CDC; 2024. <https://www.cdc.gov/fasd/data/index.html>
99. Engle EC, et al. Actions to prevent and identify fetal alcohol spectrum disorders. **Front Med**. 2024;11:1278973. [doi:10.3389/fmed.2024.1278973](https://doi.org/10.3389/fmed.2024.1278973)
100. US Food and Drug Administration. Pregnancy and Lactation Labeling Rule (PLLR). December 2014; effective June 30, 2015. <https://www.fda.gov/drugs/labeling-information-drug-products/pregnancy-and-lactation-labeling-resources>
101. Szpiro AA, et al. A meta-regression and analysis of attributable global burden for PM2.5 and birth weight. **PLOS Med**. 2021;18(9):e1003718. [doi:10.1371/journal.pmed.1003718](https://doi.org/10.1371/journal.pmed.1003718)
102. Rees KA, Machin MA, Sheen K. Heavy metal exposure during pregnancy and its association with low birth weight, SGA, and preterm birth. **Environ Int**. 2025. [doi:10.1016/j.envint.2025.107980](https://doi.org/10.1016/j.envint.2025.107980)
103. GBD 2021 Stillbirths Collaborators. Global, regional, and national stillbirths at 20 weeks' gestation or longer in 204 countries and territories, 1990–2021: findings from the Global Burden of Disease Study 2021. **Lancet**. 2024;404(10466):1955–1988. [doi:10.1016/S0140-6736\(24\)01925-1](https://doi.org/10.1016/S0140-6736(24)01925-1)
104. Lawn JE, Blencowe H, Waiswa P, et al. Stillbirths: rates, risk factors, and acceleration towards 2030. **Lancet**. 2016;387(10018):587–603. [doi:10.1016/S0140-6736\(15\)00837-5](https://doi.org/10.1016/S0140-6736(15)00837-5)
105. World Health Organization. ICD-11 for Mortality and Morbidity Statistics. Geneva: WHO; 2024. <https://icd.who.int/browse11>
106. World Health Organization. Congenital disorders. Fact sheet. Geneva: WHO; updated February 2023. <https://www.who.int/news-room/fact-sheets/detail/birth-defects>
107. Wilcox AJ, Weinberg CR, O'Connor JF, et al. Incidence of early loss of pregnancy. **N Engl J Med**. 1988;319(4):189–194.
108. Centers for Disease Control and Prevention. Risk for stillbirth among women with and without COVID-19 at delivery hospitalization — United States, March 2020–September 2021. **MMWR Morb Mortal Wkly Rep**. 2021;70(47):1640–1645. [doi:10.15585/mmwr.mm7047e1](https://doi.org/10.15585/mmwr.mm7047e1)
109. Schiøler AL, et al. Pregnancy outcomes and birth defects in offspring following maternal use of non-steroidal anti-inflammatory drugs during pregnancy: systematic review and meta-analysis. **Reprod Toxicol**. 2024;123:108521. [doi:10.1016/j.reprotox.2024.108521](https://doi.org/10.1016/j.reprotox.2024.108521)

110. Vargesson N. Thalidomide-induced teratogenesis: history and mechanisms. **Birth Defects Res C Embryo Today**. 2015;105(2):140–156; updated *Am J Med Genet A*. 2025. [doi:10.1002/ajmg.a.63987](https://doi.org/10.1002/ajmg.a.63987)
111. Briggs GG, Freeman RK, Towers CV, Forinash AB. *Drugs in Pregnancy and Lactation*. 12th ed. Philadelphia: Wolters Kluwer; 2021.
112. Li Y, He C, Yu H, Wu D, Liu L, Zhang X. Global, regional, and national epidemiology of congenital birth defects in children from 1990 to 2021: a cross-sectional study. **BMC Pediatrics**. 2025. [doi:10.1186/s12887-025-05398-1](https://doi.org/10.1186/s12887-025-05398-1)
113. Rees KA, Machin MA, Sheen K. Heavy metal exposure during pregnancy and its association with low birth weight, SGA, and preterm birth: systematic review and BKMR analysis. **Environ Int**. 2025. [doi:10.1016/j.envint.2025.107980](https://doi.org/10.1016/j.envint.2025.107980)
114. Chen J, Zhao X, Wang L, et al. Association between gestational arsenic exposure and infant growth. **Ecotoxicol Environ Saf**. 2024;282:116763. [doi:10.1016/j.ecoenv.2024.116763](https://doi.org/10.1016/j.ecoenv.2024.116763)
115. Grandjean P, Landrigan PJ. Neurobehavioural effects of developmental toxicity. **Lancet Neurol**. 2014;13(3):330–338. [doi:10.1016/S1474-4422\(13\)70278-3](https://doi.org/10.1016/S1474-4422(13)70278-3)
116. Bornschein RL, Grote J, Mitchell T. Effects of prenatal lead exposure on infant size at birth. In: Smith M, Grant L, eds. *Lead Exposure and Child Development*. Boston: Kluwer Academic; 1989.
117. Kline J, Stein Z, Susser M. *Conception to Birth — Epidemiology of Prenatal Development*. Monograph in *Epidemiology and Biostatistics* vol. 14. New York: Oxford University Press; 1989.
118. Stein ZA, Susser MW, Saenger G. *Famine and Human Development. The Dutch Hunger Winter of 1944/45*. New York: Oxford University Press; 1975.
119. Kaminski M, Rumeau C, Schwartz D. Alcohol consumption in pregnant women and the outcome of pregnancy. **Alcoholism: Clinical and Experimental Research**. 1978;2:155–163.
120. Villar J, Belizan JM. The relative contribution of prematurity and fetal growth retardation to low birth weight in developing and developed societies. **Am J Obstet Gynecol**. 1982;143(7):793–798.
121. Paul M. *Occupational and Environmental Reproductive Hazards: A Guide for Clinicians*. Baltimore: Williams and Wilkins; 1993.
122. *Part IV prepared as a component of the Master-level textbook: Reproductive Toxicology, by Dr. BENGHALI Sofiane Med el Amine, USTO-MB, Algeria. Literature reviewed 2020–2026 with foundational historical references retained where definitive. All quantitative estimates are drawn from primary sources or systematic reviews; 95% confidence intervals and uncertainty intervals reflect published estimates from original studies. DOIs provided for primary literature; URLs provided for regulatory and surveillance sources.*
123. International Commission on Radiological Protection. The 2007 Recommendations of the International Commission on Radiological Protection. ICRP Publication 103. *Ann ICRP*. 2007;37(2–4). [DOI: https://doi.org/10.1016/j.icrp.2007.10.003](https://doi.org/10.1016/j.icrp.2007.10.003)
124. International Commission on Radiological Protection. Use of Dose Quantities in Radiological Protection. ICRP Publication 147. *Ann ICRP*. 2021;50(1). [DOI: https://doi.org/10.1177/0146645321993563](https://doi.org/10.1177/0146645321993563)
125. International Commission on Radiological Protection. ICRP Publication 103. *Ann ICRP*. 2007;37(2–4). [DOI: https://doi.org/10.1016/j.icrp.2007.10.003](https://doi.org/10.1016/j.icrp.2007.10.003)
126. United Nations Scientific Committee on the Effects of Atomic Radiation. *Sources and Effects of Ionizing Radiation: UNSCEAR 2008 Report, Volume I*. United Nations, New York, 2010. https://www.unscear.org/unscear/en/publications/2008_1.html
127. https://www.unscear.org/unscear/en/publications/2008_1.html
128. <https://doi.org/10.18356/9789213589205>
129. <https://doi.org/10.1016/j.icrp.2007.10.003>
130. <https://doi.org/10.1097/AOG.0000000000002355>
131. <https://gravitas.acr.org/PPTS/GetDocumentView?docId=23>
132. United Nations Scientific Committee on the Effects of Atomic Radiation. *UNSCEAR 2020/2021 Report Volume I: Evaluation of Medical Exposure to Ionizing Radiation*. United Nations, New York, 2022. [DOI: https://doi.org/10.18356/9789213589205](https://doi.org/10.18356/9789213589205)

133. Lee CI, Haims AH, Monico EP, Brink JA, Forman HP. Diagnostic CT scans: assessment of patient, physician, and radiologist awareness of radiation dose and possible risks. *Radiology*. 2004;231(2):393–398. DOI: <https://doi.org/10.1148/radiol.2312030767>
134. Siegel R, Miller K, Fuchs HE, Jemal A. Cancer statistics, 2022. *CA Cancer J Clin*. 2022;72(1):7–33. DOI: <https://doi.org/10.3322/caac.21708>
135. United Nations Scientific Committee on the Effects of Atomic Radiation. UNSCEAR 2020/2021 Report Volume IV: Evaluation of Occupational Exposure to Ionizing Radiation. United Nations, 2022. https://www.unscear.org/unscear/en/publications/2020_2021_4.html
136. International Atomic Energy Agency. The Chernobyl Accident: Updating of INSAG-1. Safety Series No. 75-INSAG-7. Vienna: IAEA; 1992. https://www-pub.iaea.org/MTCD/Publications/PDF/Pub913e_web.pdf
137. Grembi JA, et al. Fallout and health: Chernobyl's legacy, early-life exposure, and protective behavior. *Journal of Public Economics*. 2025. DOI: <https://doi.org/10.1016/j.jpubeco.2025.105289>
138. United Nations Scientific Committee on the Effects of Atomic Radiation. UNSCEAR 2020/2021 Report Volume II: Annex B — Levels and Effects of Radiation Exposure due to the Accident at the Fukushima Daiichi Nuclear Power Station. United Nations, 2022. https://www.unscear.org/unscear/uploads/documents/publications/UNSCEAR_2020_21_Annex-B-CORR.pdf
139. Kyojuka H, Yasuda S, Murata T, et al. Effects of the Great East Japan Earthquake on perinatal outcomes: results of the Pregnancy and Birth Survey in the Fukushima Health Management Survey. *J Epidemiol*. 2022;32(Suppl XII):S57–S63. DOI: <https://doi.org/10.2188/jea.JE20210444>
140. International Commission on Radiological Protection. ICRP Statement on Tissue Reactions and Early and Late Effects of Radiation in Normal Tissues and Organs. ICRP Publication 118. *Ann ICRP*. 2012;41(1–2):1–322. DOI: <https://doi.org/10.1016/j.icrp.2012.02.001>
141. International Commission on Radiological Protection. Radiation Detriment Calculation Methodology. ICRP Publication 152. *Ann ICRP*. 2022;51(3). DOI: <https://doi.org/10.1177/01466453221090724>
142. <https://doi.org/10.1016/j.icrp.2012.02.001>
143. Wallace WHB, Thomson AB, Saran F, Kelsey TW. Predicting age of ovarian failure after radiation to a field that includes the ovaries. *Int J Radiat Oncol Biol Phys*. 2005;62(3):738–744. DOI: <https://doi.org/10.1016/j.ijrobp.2004.11.038>
144. Howell SJ, Shalet SM. Spermatogenesis after cancer treatment: damage and recovery. *J Natl Cancer Inst Monogr*. 2005;34:12–17. DOI: <https://doi.org/10.1093/jncimono/lgi032>
145. Hall EJ, Giaccia AJ. *Radiobiology for the Radiologist*. 8th ed. Philadelphia: Lippincott Williams & Wilkins; 2019
146. Im C, Ehrhardt MJ, Robinson LL, et al. Development and validation of age-specific risk prediction models for primary ovarian insufficiency in long-term survivors of childhood cancer: a report from the Childhood Cancer Survivor Study and St Jude Lifetime Cohort. *Lancet Oncol*. 2023;24(12):1281–1294. DOI: [https://doi.org/10.1016/S1470-2045\(23\)00510-7](https://doi.org/10.1016/S1470-2045(23)00510-7)
147. International Commission on Radiological Protection. Pregnancy and Medical Radiation. ICRP Publication 84. *Ann ICRP*. 2000;30(1). <https://www.icrp.org/publication.asp?id=icrp+publication+84>
148. American College of Obstetricians and Gynecologists. Guidelines for Diagnostic Imaging During Pregnancy and Lactation. Committee Opinion 723. *Obstet Gynecol*. 2017;130(4):e210–e216. DOI: <https://doi.org/10.1097/AOG.0000000000002355>
149. Linet MS, Slovis TL, Miller DL, et al. Cancer risks associated with external radiation from diagnostic imaging procedures. *CA Cancer J Clin*. 2012;62(2):75–100. DOI: <https://doi.org/10.3322/caac.21132>
150. American College of Radiology; Society for Pediatric Radiology. ACR–SPR Practice Parameter for Imaging Pregnant or Potentially Pregnant Adolescents and Women with Ionizing Radiation. Revised 2018. <https://gravitas.acr.org/PPTS/GetDocumentView?docId=23>
151. <https://gravitas.acr.org/PPTS/GetDocumentView?docId=23>
152. <https://doi.org/10.1097/AOG.0000000000002355>
153. <https://www.icrp.org/publication.asp?id=icrp+publication+84>

154. Skourt Y, Mouine N, Boulgana T, et al. Radiotherapy and testicular function: a comprehensive review of radiation-induced effects on spermatogenesis and Leydig cell function, with a focus on clinical implications and fertility preservation. *Cancers*. 2024;16(14):2507. DOI: <https://doi.org/10.3390/cancers16142507>
155. Kenney LB, Cohen LE, Shnorhavorian M, et al. Male reproductive health after childhood, adolescent, and young adult cancers: a report from the Children's Oncology Group. *J Clin Oncol*. 2012;30(27):3408–3416. DOI: <https://doi.org/10.1200/JCO.2011.38.6938>
156. Romerius P, Ståhl O, Moëll C, et al. Hypogonadism risk in men treated for childhood cancer. *J Clin Endocrinol Metab*. 2009;94(11):4180–4186. DOI: <https://doi.org/10.1210/jc.2009-0337>
157. Wallace WHB, Thomson AB, Kelsey TW. The radiosensitivity of the human oocyte. *Hum Reprod*. 2003;18(1):117–121. DOI: <https://doi.org/10.1093/humrep/deg016>
158. Meirow D, Biederman H, Anderson RA, Wallace WHB. Toxicity of chemotherapy and radiation on female reproduction. *Clin Obstet Gynecol*. 2010;53(4):727–739. DOI: <https://doi.org/10.1097/GRF.0b013e3181f96b54>
159. Stringer JM, Alesi LR, Winship AL, Hutt KJ. Beyond loss of fertility: characterizing the long-term molecular and cellular signatures of DNA damage in irradiated oocytes. *Hum Reprod*. 2021;37(3):554–572. DOI: <https://doi.org/10.1093/humrep/deab251>
160. Zelinski MB, Murphy MK, Lawson MS, et al. In vivo delivery of FTY720 prevents radiation-induced ovarian failure and infertility in adult female nonhuman primates. *Fertil Steril*. 2011;95(4):1440–1445. DOI: <https://doi.org/10.1016/j.fertnstert.2010.11.027>
161. Jacquet P et al. Radiosensitivity of the guinea pig oocyte compared with the mouse and human. 1994
162. Wallace WHB, Thomson AB, Kelsey TW. The radiosensitivity of the human oocyte. *Hum Reprod*. 2003;18(1):117–121. DOI: <https://doi.org/10.1093/humrep/deg016>; Wallace WHB, Thomson AB, Saran F, Kelsey TW. Predicting age of ovarian failure after radiation to a field that includes the ovaries. *Int J Radiat Oncol Biol Phys*. 2005;62(3):738–744. DOI: <https://doi.org/10.1016/j.ijrobp.2004.11.038>
163. Donadon MF, Lima RS, Baracat EC, Soares Junior JM. Practical prediction model for ovarian insufficiency after radiation. *Rev Assoc Med Bras*. 2022;68(5):659–664. DOI: <https://doi.org/10.1590/1806-9282.20220102>
164. Jeruss JS, Woodruff TK. Preservation of fertility in patients with cancer. *N Engl J Med*. 2009;360(9):902–911. DOI: <https://doi.org/10.1056/NEJMra0801454>
165. Chemaitilly W, Li Z, Huang S, et al. Premature ovarian insufficiency in childhood cancer survivors: a report from the St. Jude Lifetime Cohort. *J Clin Endocrinol Metab*. 2017;102(7):2242–2250. DOI: <https://doi.org/10.1210/jc.2016-2988>
166. Dull AM, Brodeur MN, Smith MA, et al. Assisted reproductive technology use and outcomes in childhood cancer survivors: a report from the Childhood Cancer Survivor Study. *Fertil Steril*. 2025;123(2):e1–e10. DOI: <https://doi.org/10.1016/j.fertnstert.2024.11.012>
167. Children's Oncology Group. Long-Term Follow-Up Guidelines for Survivors of Childhood, Adolescent and Young Adult Cancers. Version 6.0. October 2023. https://www.survivorshipguidelines.org/pdf/2023/COG_LTFU_Guidelines_Only_v6.pdf
168. Oktay K, Harvey BE, Loren AW. Fertility preservation in patients with cancer: ASCO clinical practice guideline update 2025. *J Clin Oncol*. 2025. DOI: <https://doi.org/10.1200/JCO-24-02782>
169. European Society of Human Reproduction and Embryology. Good practice recommendations on fertility preservation for medical reasons. *Hum Reprod*. 2025;40(8):1391–1420. DOI: <https://doi.org/10.1093/humrep/deae143>
170. Malone S, Donaldson K, Dahele M, et al. Fertility preservation is an imperative goal in the clinical practice of radiation oncology: a narrative review. *Ecancer*. 2022;16:1461. DOI: <https://doi.org/10.3332/ecancer.2022.1461>
171. Wiechert L, Hanker L, Schwab R, et al. Laparoscopic ovarian transposition prior to pelvic radiation in young female patients with anorectal malignancies: systematic review and meta-analysis. *Colorectal Dis*. 2023;25(7):1355–1365. DOI: <https://doi.org/10.1111/codi.16571>
172. Davis S, Khizir L, Lichtbroun B, et al. Sexual function and fertility preservation in testicular cancer survivors. *J Mens Health*. 2023;19(2):1–8. DOI: <https://doi.org/10.31083/jomh.2022.011>

173. Jacquet P et al. Radiosensitivity characteristics of the guinea pig oocyte compared with the mouse and human. 1994
174. Muhsen K, Shoob H, Maimon N, et al. Reproductive effects of exposure to low-dose ionizing radiation: a long-term follow-up of immigrant women exposed to the Chernobyl accident. *J Clin Med*. 2020;9(6):1786. DOI: <https://doi.org/10.3390/jcm9061786>
175. International Commission on Radiological Protection (ICRP). The 2007 Recommendations of the International Commission on Radiological Protection. ICRP Publication 103. *Ann ICRP*. 2007;37(2–4). DOI: <https://doi.org/10.1016/j.icrp.2007.10.003>. Available at: <https://www.icrp.org/publication.asp?id=ICRP+Publication+103>
176. International Commission on Radiological Protection (ICRP). ICRP Statement on Tissue Reactions and Early and Late Effects of Radiation in Normal Tissues and Organs — Threshold Doses for Tissue Reactions in a Radiation Protection Context. ICRP Publication 118. *Ann ICRP*. 2012;41(1–2):1–322. DOI: <https://doi.org/10.1016/j.icrp.2012.02.001>. Available at: <https://www.icrp.org/publication.asp?id=ICRP+Publication+118>
177. International Commission on Radiological Protection (ICRP). Radiation Detriment Calculation Methodology. ICRP Publication 152. *Ann ICRP*. 2022;51(3). DOI: <https://doi.org/10.1177/01466453221090724>. Available at: <https://www.icrp.org/publication.asp?id=ICRP+Publication+152>
178. International Commission on Radiological Protection (ICRP). Use of Dose Quantities in Radiological Protection. ICRP Publication 147. *Ann ICRP*. 2021;50(1). DOI: <https://doi.org/10.1177/0146645321993563>. Available at: <https://www.icrp.org/publication.asp?id=ICRP+Publication+147>
179. International Commission on Radiological Protection (ICRP). Pregnancy and Medical Radiation. ICRP Publication 84. *Ann ICRP*. 2000;30(1). Available at: <https://www.icrp.org/publication.asp?id=icrp+publication+84>
180. United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR). Sources and Effects of Ionizing Radiation: UNSCEAR 2008 Report to the General Assembly, with Scientific Annexes. Volume I: Sources. United Nations, New York,
181. Available at: https://www.unscear.org/unscear/en/publications/2008_1.html
182. United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR). UNSCEAR 2020/2021 Report Volume I: Evaluation of Medical Exposure to Ionizing Radiation. United Nations, New York, 2022. DOI: <https://doi.org/10.18356/>
183. Available at: https://www.unscear.org/unscear/en/publications/2020_2021_1.html
184. United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR). UNSCEAR 2020/2021 Report Volume II: Annex B — Levels and Effects of Radiation Exposure due to the Accident at the Fukushima Daiichi Nuclear Power Station. United Nations, New York, 2022. Available at: https://www.unscear.org/unscear/uploads/documents/publications/UNSCEAR_2020_21_Annex-B-CORR.pdf
185. United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR). UNSCEAR 2020/2021 Report Volume IV: Evaluation of Occupational Exposure to Ionizing Radiation. United Nations, New York, 2022. Available at: https://www.unscear.org/unscear/en/publications/2020_2021_4.html
186. International Atomic Energy Agency (IAEA). The Chernobyl Accident: Updating of INSAG-1. Safety Series No. 75-INSAG-7. Vienna: IAEA; 1992. Available at: https://www-pub.iaea.org/MTCD/Publications/PDF/Pub913e_web.pdf
187. Grembi JA, et al. Fallout and health: Chernobyl's legacy, early-life exposure, and protective behavior. *Journal of Public Economics*. 2025. DOI: <https://doi.org/10.1016/j.jpubeco.2025.105289>
188. Kyoizuka H, Yasuda S, Murata T, et al. Effects of the Great East Japan Earthquake on perinatal outcomes: results of the Pregnancy and Birth Survey in the Fukushima Health Management Survey. *J Epidemiol*. 2022;32(Suppl XII):S57–S63. DOI: <https://doi.org/10.2188/jea.JE20210444>
189. Hall EJ, Giaccia AJ. Radiobiology for the Radiologist. 8th ed. Philadelphia: Lippincott Williams & Wilkins; 2019.

190. Lee CI, Haims AH, Monico EP, Brink JA, Forman HP. Diagnostic CT scans: assessment of patient, physician, and radiologist awareness of radiation dose and possible risks. *Radiology*. 2004;231(2):393–398. DOI: <https://doi.org/10.1148/radiol.2312030767>
191. Howell SJ, Shalet SM. Spermatogenesis after cancer treatment: damage and recovery. *J Natl Cancer Inst Monogr*. 2005;34:12–17. DOI: <https://doi.org/10.1093/jncimono/lgi032>. Available at: <https://academic.oup.com/jncimono/article/2005/34/12/905249>
192. Skourt Y, Mouine N, Boulgana T, et al. Radiotherapy and testicular function: a comprehensive review of radiation-induced effects on spermatogenesis and Leydig cell function, with a focus on clinical implications and fertility preservation. *Cancers*. 2024;16(14):2507. DOI: <https://doi.org/10.3390/cancers16142507>. Available at: <https://pmc.ncbi.nlm.nih.gov/articles/PMC11274587/>
193. Romerius P, Ståhl O, Moëll C, et al. Hypogonadism risk in men treated for childhood cancer. *J Clin Endocrinol Metab*. 2009;94(11):4180–4186. DOI: <https://doi.org/10.1210/jc.2009-0337>
194. Kenney LB, Cohen LE, Shnorhavorian M, et al. Male reproductive health after childhood, adolescent, and young adult cancers: a report from the Children's Oncology Group. *J Clin Oncol*. 2012;30(27):3408–3416. DOI: <https://doi.org/10.1200/JCO.2011.38.6938>
195. Meirow D, Biederman H, Anderson RA, Wallace WHB. Toxicity of chemotherapy and radiation on female reproduction. *Clin Obstet Gynecol*. 2010;53(4):727–739. DOI: <https://doi.org/10.1097/GRF.0b013e3181f96b54>
196. Zelinski MB, Murphy MK, Lawson MS, et al. In vivo delivery of FTY720 prevents radiation-induced ovarian failure and infertility in adult female nonhuman primates. *Fertil Steril*. 2011;95(4):1440–1445. DOI: <https://doi.org/10.1016/j.fertnstert.2010.11.027>
197. Stringer JM, Alesi LR, Winship AL, Hutt KJ. Beyond loss of fertility: characterizing the long-term molecular and cellular signatures of DNA damage in irradiated oocytes. *Hum Reprod*. 2021;37(3):554–572. DOI: <https://doi.org/10.1093/humrep/deab251>
198. Donadon MF, Lima RS, Baracat EC, Soares Junior JM. Practical prediction model for ovarian insufficiency after radiation. *Rev Assoc Med Bras*. 2022;68(5):659–664. DOI: <https://doi.org/10.1590/1806-9282.20220102>. Available at: <https://pmc.ncbi.nlm.nih.gov/articles/PMC9948280/>
199. Im C, Ehrhardt MJ, Robinson LL, et al. Development and validation of age-specific risk prediction models for primary ovarian insufficiency in long-term survivors of childhood cancer: a report from the Childhood Cancer Survivor Study and St Jude Lifetime Cohort. *Lancet Oncol*. 2023;24(12):1281–1294. DOI: [https://doi.org/10.1016/S1470-2045\(23\)00510-7](https://doi.org/10.1016/S1470-2045(23)00510-7)
200. Dull AM, Brodeur MN, Smith MA, et al. Assisted reproductive technology use and outcomes in childhood cancer survivors: a report from the Childhood Cancer Survivor Study. *Fertil Steril*. 2025;123(2):e1–e10. DOI: <https://doi.org/10.1016/j.fertnstert.2024.11.012>. Available at: <https://pmc.ncbi.nlm.nih.gov/articles/PMC10841316/>
201. Children's Oncology Group (COG). Long-Term Follow-Up Guidelines for Survivors of Childhood, Adolescent and Young Adult Cancers. Version 6.0. October 2023. Available at: https://www.survivorshipguidelines.org/pdf/2023/COG_LTFU_Guidelines_Only_v6.pdf
202. Muhsen K, Shoob H, Maimon N, et al. Reproductive effects of exposure to low-dose ionizing radiation: a long-term follow-up of immigrant women exposed to the Chernobyl accident. *J Clin Med*. 2020;9(6):1786. DOI: <https://doi.org/10.3390/jcm9061786>. Available at: <https://pmc.ncbi.nlm.nih.gov/articles/PMC7356322/>
203. Wiechert L, Hanker L, Schwab R, et al. Laparoscopic ovarian transposition prior to pelvic radiation in young female patients with anorectal malignancies: systematic review and meta-analysis. *Colorectal Dis*. 2023;25(7):1355–1365. DOI: <https://doi.org/10.1111/codi.16571>
204. Malone S, Donaldson K, Dahele M, et al. Fertility preservation is an imperative goal in the clinical practice of radiation oncology: a narrative review. *Ecancer*. 2022;16:1461. DOI: <https://doi.org/10.3332/ecancer.2022.1461>. Available at: <https://ecancer.org/en/journal/article/1461>
205. Oktay K, Harvey BE, Loren AW. Fertility preservation in patients with cancer: ASCO clinical practice guideline update 2025. *J Clin Oncol*. 2025. DOI: <https://doi.org/10.1200/JCO-24-02782>
206. European Society of Human Reproduction and Embryology (ESHRE). Good practice recommendations on fertility preservation for medical reasons. *Hum Reprod*. 2025;40(8):1391–1420. DOI: <https://doi.org/10.1093/humrep/deae143>

- 207.** Davis S, Khizir L, Lichtbroun B, et al. Sexual function and fertility preservation in testicular cancer survivors. *J Mens Health*. 2023;19(2):1–8. DOI: [https://doi.org/ 10.31083/jomh.2022.011](https://doi.org/10.31083/jomh.2022.011). Available at: <https://www.jomh.org/articles/10.22514/jomh.2023.011>
- 208.** American College of Obstetricians and Gynecologists (ACOG). Guidelines for Diagnostic Imaging During Pregnancy and Lactation. Committee Opinion 723. *Obstet Gynecol*. 2017;130(4):e210–e216. DOI: <https://doi.org/10.1097/AOG.0000000000002355>. Available at: <https://www.acog.org/clinical/clinical-guidance/committee-opinion/articles/2017/10/guidelines-for-diagnostic-imaging-during-pregnancy-and-lactation>
- 209.** American College of Radiology (ACR); Society for Pediatric Radiology (SPR). ACR–SPR Practice Parameter for Imaging Pregnant or Potentially Pregnant Adolescents and Women with Ionizing Radiation. Revised 2018. Available at: <https://gravitas.acr.org/PPTS/GetDocumentView?docId=23>
- 210.** National Research Council. Health Risks from Exposure to Low Levels of Ionizing Radiation: BEIR VII Phase 2. Washington, DC: National Academies Press; 2006. Available at: <https://doi.org/10.17226/11340>
- 211.** Jacquet P, et al. Radiosensitivity of the guinea pig oocyte compared with the mouse and human. 1994. [Original French-language research cited in source text, Benghali Sofiane Med el Amine, Chapter V.]