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Impact of High-Volume Chemicals on Androgen Signalling and Male Reproductive Health

Deepika Sehrawat and Gargi Bagchi*

Amity Institute of Biotechnology, Amity University, Gurgaon – 122413, Haryana, India; gbagchi@ggn.amity.edu

Abstract

Endocrine-disrupting chemicals, or EDCs, are the chemicals in the environment that can alter hormonal functions in humans and animals. Many industrial chemicals that act as EDCs are used and produced in large quantities worldwide. Because of their large-scale production, they are known as 'High volume chemicals' or HVCs. Humans and animals are exposed to these chemicals by day-to-day use of different products or through contaminated food or water. These HVCs greatly impact the hormone system of exposed living beings and their physiology. Many of these high-volume EDCs specifically interfere with male hormone (androgen) signalling by influencing biosynthesis, metabolism, and function of the Androgen Receptor (AR), resulting in a disruption of typical male developmental programming. As androgen is critical for male sexual differentiation, males are extremely susceptible to these androgen disruptors. Animal exposure studies demonstrate that these androgen disruptors lead to a decrease in sperm count, infertility, testicular dysgenesis, and prostate cancer, which is prevalent in many parts of the world today. Routinely used HVCs that can act as androgen-disruptors include Bisphenol (BPA), Phthalates, Dichlorodiphenyltrichloroethane (DDT), Glyphosate and Methoxyacetic Acid (MAA), among others. These chemicals disrupt androgen signalling by different mechanisms and result in the disruption of normal male physiological processes. This review highlights the toxicities and molecular mechanisms of hormone disruption induced by these routinely used high-volume chemicals that disrupt androgen signalling.

Keywords: Androgens, BPA, EDC, DDT, Glyphosate, MAA, Phthalate

1. Introduction

EDCs are natural or man-made compounds with the ability to influence hormones or the endocrine system. There are around 85,000 chemicals currently manufactured, of which more than thousand are potential EDCs. EDCs are found in significant quantities in routinely used consumer goods, including soaps, surface cleaners, facial cleansers, lotions, toothpaste, and children's products[1]. These chemicals belong to different classes of compounds, including organophosphate insecticides, herbicides, industrial chemicals such as PCBs (polychlorinated biphenyl), and cyclic hydrocarbons. Being non-biodegradable, they accumulate in the environment[2]. This bioaccumulation has serious consequences on soil, plant, and animal health.

The EDCs can enter the body through various pathways, including inhalation, placental transfer, and lactation. Once inside the body, these compounds can interfere with the endocrine system by mimicking, blocking, or altering normal hormonal signalling pathways[3]. This disruption can lead to a range of pathological conditions such as diabetes, obesity, neurodegenerative disorders, cardiovascular diseases, thyroid dysfunctions, etc. Moreover, EDCs profoundly affect the reproductive system, leading to sexual dysfunction and infertility in both males and females. In females, they are associated with conditions like subfertility, irregular menstrual cycles, Polycystic Ovarian Syndrome (PCOS), endometriosis, and the development of uterine fibroids. In males, EDCs are connected to reduced fertility, undescended testes (cryptorchidism), and abnormalities in the development of the urethra (hypospadias)[4] (Figure 1).

*Author for correspondence

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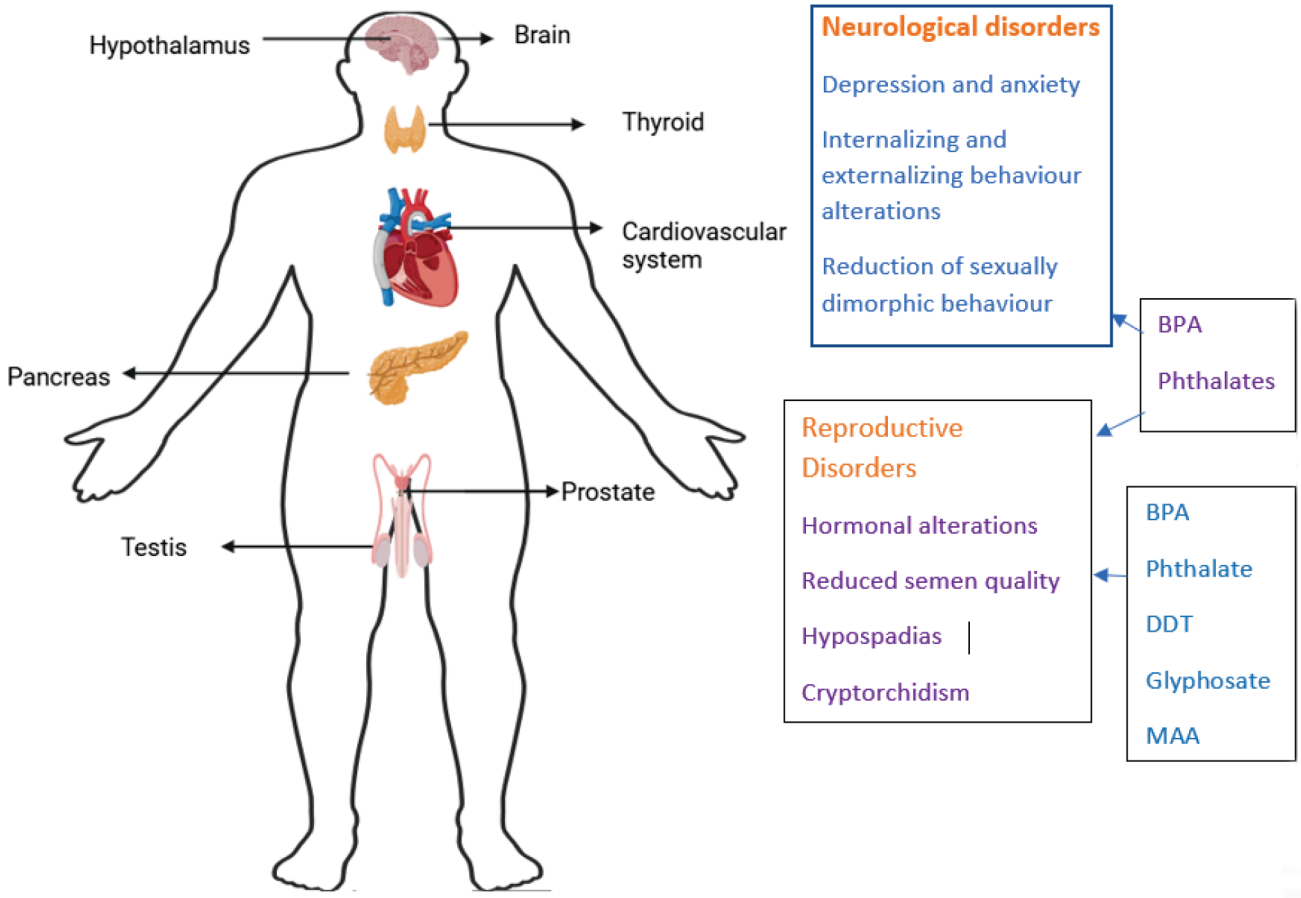


Figure 1. Impact of HVCs on male reproductive health.

Many EDCs have a serious impact on male reproductive health as they impact androgen signalling by blocking androgen synthesis, metabolism, clearance, or direct AR binding[5,6]. When androgens bind to the AR, this interaction induces a conformational change that facilitates the translocation of AR from the cytoplasm to the nucleus[7]. In the absence of androgens, AR remains in the cytoplasm in a complex with heat shock proteins, including HSP90, and other molecular chaperones, which maintain AR in a high-affinity conformation for ligand binding. Upon ligand binding, AR dissociates from HSP90 and associates with HSP27, initiating its translocation to the nucleus. After dissociation from HSP90, AR dimerizes and translocates into the nucleus, where it binds to Androgen Response Elements (AREs) located in the promoter regions of target genes, such as Prostate-Specific Antigen (PSA)[8]. Following DNA binding, AR recruits several transcriptional cofactors and these coregulators together coordinate the transcriptional activation of target genes.

Non-canonical androgen signalling can occur when androgens or other hormones activate pathways that do not involve the AR directly. Instead, these pathways are mediated by other types of receptors, leading to different cellular responses than those typically triggered by AR signalling[8]. The EDCs that are produced and used in very large quantities, i.e., 1000,000 kg or more per year, called HVCs, have the potential to cause severe detrimental effects on man and animals.

In this review, we analyze the disruption of the androgen signalling pathway caused by these high-volume chemicals. The HVC are used ubiquitously in household activities or industry. For most of these chemicals, direct studies on the human endocrine system are lacking. The most commonly used high-volume chemicals that disrupt androgen signalling are Bisphenol A, Phthalates, DDT, Glyphosate, and MAA. As males are more vulnerable to these EDCs, a comparative analysis of the mode of action of these EDCs and the impact of these routinely used chemicals on male hormone signalling is presented in Table 1.

Table 1. Different EDCs and their mode of action

Chemicals	Category and uses	Yearly production worldwide	Mode of action	Health-associated effects	References
Bisphenol A	Plasticizers are used in plastics, epoxy resins, and other products. Commonly found in food packaging, water bottles, medical equipment, and electronics.	Around 6.5 million metric tons per year.	BPA inhibits the DHT-induced stabilization of AR and the DHT-induced dissociation of AR-Hsp90 complex.	Reduced sperm count and motility. Erectile Dysfunction and other sexual problems. Exposure during fetal development can lead to permanent changes in the male reproductive system.	9-13
Phthalates	Plastic: Used in consumer products like vinyl flooring, food packaging, and personal care products. Also used as a solvent, lubricant, and ink.	8 million tons	Interfere with androgen signalling involves inhibiting the activity of Enzyme 5-alpha reductase and aromatase.	Reduced sperm quality and quantity. Decreased testosterone levels and testicular dysfunction. Associated with an increased risk of developing conditions such as testicular cancer, prostate cancer, and reproductive birth defects.	14,15
DDT/DDE	Used for pesticides to control Insect-borne diseases and agriculture pests.	100 metric tons per year.	DDT can interfere with androgen signalling by Blocking the action of male hormones like testosterone. DDT can also have estrogen-like effects, which can further disrupt hormonal balance in the body.	Hypospadias and cryptorchidism. Reduced sperm quality and quantity. Decreased testosterone levels, and testicular dysfunction.	16,17
Glyphosate	Herbicides : Used in agriculture, forestry, and landscaping to control unwanted vegetation. Also used in home gardens and lawns as a weed killer.	0.7 to 0.8 million tons.	Glyphosate can inhibit the activity of an enzyme called 5-alpha reductase, which is involved in the conversion of testosterone to a more potent androgen called dihydrotestosterone (DHT).	Decreased sperm quality and quantity, altered hormone levels, and an increased risk of certain cancers, such as prostate cancer. Kidney disease, and non-Hodgkin's lymphoma.	18,19
Methoxyacetic acid	Paints: Used in a variety of industrial products including paints, inks and jet fuels.	>1 million tons.	MAA enhances the transcriptional activity of ligand-activated nuclear receptors and oxidative stress in spermatocytes. Depletes permatocytes by activating apoptosis.	Testicular toxicity, leucopenia, embryo lethality, and malformations.	20-24
Atrazine	Heribicide: Used to control weeds in crops such as corn, sugarcane and sorghum. Also used on golf courses and residential lawns to control weeds.	30 to 40 million pounds applied annually on crops.	Interferes with male hormone signalling by inhibiting the activity of enzyme aromatase.	Decreased testosterone levels. Altered sperm quality and quantity.	25

Table 1. Continued...

Chemicals	Category and uses	Yearly production worldwide	Mode of action	Health-associated effects	References
PCBs	Organic pollutants: Used in electric equipment such as transformers and capacitors as well as in other products such as adhesives, paints and lubricants.	1.5 million tons.	PCBs can disrupt androgen signalling by interfering with the normal function of androgen receptors. PCBs in androgen signalling is that they can bind androgen receptors, either directly or indirectly, and interfere with the ability of androgens to bind to these receptors and activate cellular responses.	Reduced sperm quality and quantity. Decreased testosterone levels, Altered sexual development.	[26]
PBBs	Synthetic chemicals: Used in a variety of consumer products including electronics, textiles, and plastics.	1.5 million tons.	PBBs can interfere with androgen signalling by binding to or activating the androgen receptor. PBBs can also affect other Aspects of androgen signalling such as the synthesis and metabolism of androgens.	Reduced sperm quality and quantity. Reduced testosterone levels Impaired sexual function. Thyroid dysfunction. Immune system effects.	[27]
Arsenic	Pesticide: used in industry for wood preservation, and semiconductors.	~ 37 million tons.	Arsenic binds to the Zinc finger motifs of the AR and disrupts its ability to bind to DNA and regulate target gene expression.	Reduced fertility. Developmental abnormalities. Reduced sperm count, motility and viability. Urogenital cancers. Prostate cancer.	[28]
Lead	Metal: Used in industrial and consumer applications including batteries, and electronics.	4.8 million tons.	Lead can disrupt androgen signalling by binding to androgen receptors. Prevents the binding of natural hormones.	Reduced fertility, developmental abnormalities.	[29]

2. Bisphenol-A

BPA, an element of plastic, is one of the largest produced chemicals in the world, with its production increasing continuously[30] (Table 1). BPA was first synthesized in 1891 by Russian chemist Aleksandr Dianin. In 2020 the global production of BPA was approximately 10 million tons. In 2021, the production of BPA was predicted to surpass 10 million tons. The USA, South Korea, Taiwan, China and Japan are the top five countries in terms of annual production capacity of BPA[31].

It is estimated that 8 billion kilograms of BPA is produced every year and 100 tons is released into the environment[30,32]. Although there are currently no specific regulations for the use of BPA in consumer products, the Bureau of Indian Standards (BIS) has set a limit of 0.01 mg/l of BPA in drinking water in India. The USA has set a limit of 0.1mg/l in drinking water. BPA is used in the production of epoxy resins and plastic[33] and is found in food, toys, dental fillings, and medical tubing. It is additionally used to make vinyl chloride and thermal paper. Widespread BPA consumption is reflected by the fact that it has been detected in human maternal, fetal serum, and human placenta[34]. There has been a lot of public concern about the potentially harmful effects of BPA. As a result, many plastic products, especially those used for infants and young children, have been banned.

BPA interferes with the regular function of the (AR), which is crucial for sexual development and reproduction[35]. BPA impacts male reproduction by

different mechanisms that involve receptor stabilization, dissociation of Heat Shock Protein (HSP90), and nuclear translocation. BPA can work as both an agonist and an antagonist of the androgen receptor.

One important effect of BPA on AR nuclear translocation is to hinder the dissociation of AR from molecular chaperones. BPA inhibits the dissociation of AR from Hsp90, which renders AR inactive and unable to dimerize, resulting in the inability of AR to translocate into the nucleus[36]. This prevents the development of functional complexes that are essential for transcription. Disruption of AR transcription by BPA can have several negative health effects, including reduced testes size, decreased sperm production, cryptorchidism, reduced reproductive organ weights, and increased risk of certain cancers[37].

Other studies, including multigenerational studies utilizing oral exposures, have found effects on male reproduction[38]. When male rats are exposed to BPA at a concentration greater than 20 µg/kg bwt/day, a significant decrease in daily sperm production and epididymal sperm motility is observed. Exposure to low-dose BPA (lowest observed adverse effect level<50 mg/kg) was associated with decreased sperm counts in mice and adult rats[39] and impaired sperm motility in rats and mice.

3. Phthalates

Phthalates are industrial chemicals routinely used in consumer goods such as soap, shampoo, automobiles, children's products, nail polish, etc.[40] Phthalates are often called plasticizers, as they make the product soft and flexible. They are diesters of 1,2-benzene dicarboxylic acid (phthalic acid). Over 3 million metric tons of phthalates are produced annually on average, and more than 11 tons are used every minute worldwide for the manufacturing of plastics[41]. Phthalates were first introduced in the 1920s to replace the volatile and odorous camphor. In 1931, the commercial availability of polyvinyl chloride (PVC) and the development of di(2-ethylhexyl) phthalate (DEHP) initiated a boom in the plasticizer PVC industry. In 2020 the global production of phthalates approximates 3 million metric tons. While in 2021 worldwide phthalates production reached approximately 8 million metric tons.

Currently, the BIS has set a limit of 0.001mg/l for DEHP in drinking water. However, there are currently no specific regulations for other phthalates in India. The United States Environmental Protection Agency (EPA) has established a Maximum Contaminant Level (MCL) of 0.006 mg/l for DEHP, while there is no federal regulation for other phthalates. Several studies have shown that high levels of these compounds are found in body fluids. DEHP is most widely used in various Polyvinylchloride (PVC) products. Humans mainly get exposed through food products, mouthing children's products, or directly taking nutritional supplements and pharmaceuticals. Another important route of exposure is skin absorption through the use of cosmetics, sunscreen, and denture material.

Phthalates affect androgen signalling by activating the AR. Phthalates can bind to the AR, leading to changes in gene expression and cellular function. Recent studies show that some phthalates can activate the AR by binding to a specific binding pocket on the receptor[42]. Upon binding to this pocket, phthalates induce a conformational change in the receptor and subsequent changes in gene expression. This can lead to changes in androgen and altered androgen signalling, causing a variety of health issues[43].

In 2015 Cai and colleagues[44] showed that exposure to phthalate affects semen quality. Studies revealed that monobutyl phthalate (MBP) and monobenzyl phthalate (MBzP) cause reduced sperm concentration, and MBP and mono(2-ethylhexyl) phthalate (MEHP) cause decreased sperm motility and increased sperm DNA damage. Exposure to mono- and dibutyl phthalates at the concentration of 50 nM and 50 µM, respectively, can lead to AR inhibition in Leydig cells. Phthalate (500mg/kg) was associated with malformed seminiferous tubules. Moreover, in these rats' exposure to 10 and 100 mg/kg di (2-ethylhexyl), DEHP, could impact gene expression and hormone activity through DNA methylation, which leads to testicular dysgenesis syndrome or other reproductive disorders.

4. Dichlorodiphenyltrichloroethane

DDT and its metabolites, DDE, belong to organochlorine insecticides[45]. More than 1.8 million tons of DDT is produced globally every year (Figure 2). It has vast applications in the individual protection of crops and livestock. The use of DDT is now banned in the USA and some European countries. DDT is widely used for controlling insects that transmit human diseases such as leishmaniasis, malaria, dengue, and Chagas disease, although it is highly toxic to animals. In India, the Central Pollution Control Board (CPCB) is responsible

for the regulation of DDT. The maximum contaminant Level (MCL) for DDT in drinking water is set as 0.0005 mg/l. The USA has set an MCL of 0.0002 mg/l for DDT in drinking water. In Europe, the European Food Safety Authority (EFSA) is responsible for regulating DDT in food. The maximum residue limit for DDT in food is set at 0.01 mg/l. The main route of DDT exposure is direct contact or consumption of contaminated food. DDT and DDE are majorly harmful to organs, including the testis, kidney, liver, and immune system.

DDT/ DDE acts at multiple sites and interferes with androgen signalling[40]. In vivo studies show that DDT reduces the binding of DHT to AR[46]. At high concentrations, DDT as well as DDE inhibit 5α-reductase which converts testosterone to DHT. This interference impacts gene expression and signalling pathways regulated by androgen, ultimately affecting processes such as development, reproduction, and other physiological functions. Further, this can result in a high incidence of epididymal and testicular lesions[47], reduced prostate growth and inflammation.

At high doses, DDT causes a reduction of seminiferous tubules in the testis. In humans, the available DDT toxicity data indicate reproductive toxicity, an increase in tetrazoospermia and oligozoospermia, low sperm counts, and motility. Administration of DDT in male rates at 100mg/kg/day permanently reduces androgen-dependent organ weight.

5. Glyphosate

Glyphosate is the largest-selling herbicide and most widely used pesticide in the market today, marketed by Monsanto as Roundup[48]. Over 5,000 tons of Glyphosate per year isproduced globally. It is a widely used herbicide administered on multiple food and non-food crops. Foods that are high in glyphosate include almonds, peas, sugar, and beetroots (Table 1). It is a competitive inhibitor of 5-enol pyruvyl shikimate 3phosphate synthase (EPSPS), a key enzyme in the shikimic acid pathway. The shikimic acid pathway is essential for the formation of aromatic amino acids, phenol, and metabolites. The endocrine-disrupting potential of glyphosate herbicides has been found in various species[49]. Toxicities include genotoxicity, cytotoxicity, and reproductive toxicity in animals and humans.

Glyphosate may interfere with androgen signalling by various mechanisms, including direct interaction with hormone receptors or interference with hormone synthesis, metabolism or transport. Some studies have suggested that Glyphosate has been shown to inhibit the activity of aromatase, an enzyme that is responsible for converting androgens into estrogens. This inhibition can alter the balance between androgen and estrogen levels in the body, potentially impacting androgen signalling pathways. This can result in a variety of health effects. Glyphosate exposure has been associated with epigenetic changes, including alterations in DNA methylation patterns and histone modifications. These epigenetic changes can influence gene expression, including genes involved in androgen signalling.

Studies have shown that Glyphosate exposure in male rats causes reproductive toxicity, such as decreased sperm counts and testosterone levels, along with an imbalance in the antioxidant defence system. Exposure of zebrafish to 5 or 10mg/l of glyphosate compromised the integrity of sperm DNA and reduced sperm vitality and mitochondrial function, significantly reducing the fertility of zebrafish[50].

6. Methoxyacetic Acid

Ethylene glycol monomethyl ether (EGME) is a high-volume chemical used in paints, inks, and jet fuels[51,52]. MAA is a metabolite of EGME, and MAA/ EGME is a highly used chemical (Table 1) that causes endocrine disruption as a hormone sensitizer. MAA exposure causes various developmental and reproductive toxicities (Table 1). Exposure to MAA is also associated with blood and immune disorders and limb degeneration. MAA induces changes in multiple genes and proteins of spermatocytes and Sertoli cells[53]. Both *in vivo* and *in vitro* studies show that MAA enhances the transcriptional activity of multiple ligand-activated nuclear receptors, such as estrogen receptor, progesterone receptor and AR. MAA disrupts androgen signalling at multiple sites[54].

Bagchi *et al.*[55] have shown that MAA enhances AR-dependent transcription without altering the receptor's intrinsic androgen responsiveness. This potentiation of AR activity is mediated through tyrosine kinase signalling that is independent of the Ras → MEK → ERK signalling pathway but requires PI3 kinase activity. This interference disrupts normal androgen signalling pathways and leads to downstream effects on cellular processes regulated by

androgens, such as cell proliferation, differentiation, and survival.

In male rats, it was observed that within 24 hours, a single dose of MAA at 100mg/kg/day caused degeneration of pachytene spermatocytes. At 500 mg/kg/day, MAA exposure resulted in a decrease in testicular weight. Continuous dosing leads to progressive depletion in spermatocytes. Studies indicate that humans are much more sensitive as testicular toxicity occurs at 2.6 mg/m^3, whereas toxicity observed in rats is at 167mg/m^3. Exposure of rat pachytene spermatocytes to 5 mM MAA causes up-regulation of a thiol-specific antioxidant (TSA) protein at 30 minutes and down-regulation of polo-like kinase-1 (Plk1) after 6 hrs[56]. In Sertoli cells, induction of stress protein A170 occurs within 30 min, whereas cyclic AMP phosphodiesterase splice variant PDE 3.1 is repressed in 60 min duration, suggesting that the oxidative balance of cells was hampered by MAA. Upon MAA exposure, spermatocytes activate their defence mechanism by producing antioxidants resulting in apoptosis. Up-regulation of A-170 correlates with a change in the redox status of cells. In meiosis, microtubule organization and spindle formation are regulated by Plk1 protein. Therefore, a decrease in Plk1 protein upon MAA treatment leads to DNA damage and disassembly of spindle fibres in spermatocytes. In short, EGME and MAA cause severe reproductive, developmental, and blood-related toxicities in several species. These include testicular toxicity, embryonic lethality, leucopenia, and malformations. Testicular damage is a hallmark of acute EGME exposure[54].

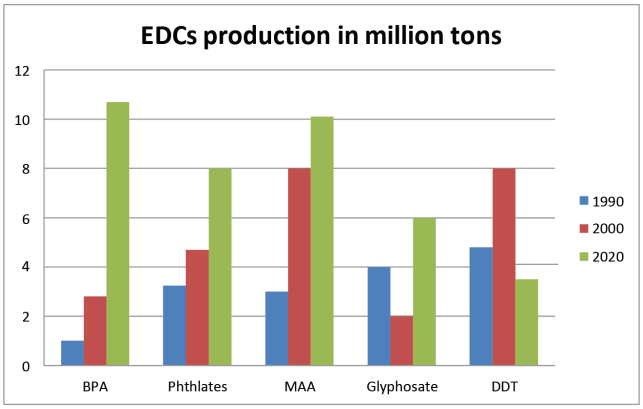


Figure 2. Production of high-volume chemicals in the last three decades.
(Sources: BPA: [57,58], Phthalates: [59,60], MAA: [61], Glyphosate: [62,63], DDT: [64])

7. Conclusion

This review presents an analysis of the sources of HVCs, routes of exposure, and the impact of high-volume chemicals on androgen signalling, male physiology, and reproduction. Many studies have demonstrated that these chemicals cause detrimental effects on male reproductive health through their ability to act as AR antagonists or by influencing AR signalling otherwise. While the mechanistic information is derived from *in vitro* tests and *in vivo* animal models, several reports demonstrate the negative effects of these EDCs in humans. EDCs interfere with testicular development by disrupting hormone signalling, particularly androgen and estrogen pathways, leading to impaired spermatogenesis, reduced testosterone levels, and increased risk of conditions like cryptorchidism and testicular dysgenesis syndrome. These disruptions are linked to long-term reproductive health issues and fertility problems in males. Phthalates and BPA have been shown to impair Leydig and Sertoli cell function, contributing to reduced testosterone production and abnormal sperm development. While additional research at all levels is required to gain a thorough understanding of the mechanisms and effects of these chemicals worldwide, it is necessary to be cautious about their production and use because of their potential to cause significant harm to male reproductive health. Continued studies are essential to monitor the production and use of these HVCs and their impact on male reproductive health, as well as screen other potential HVCs for AR disrupting potential so that caution might be applied in their continued use.

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