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Endocrine, Reproductive and Developmental Toxicity of Butylated Hydroxyanisole: A Review

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Abstract

Butylated hydroxyanisole (BHA) is a widely used synthetic antioxidant found in processed foods, cosmetics, pharmaceuticals, and packaging materials. While its efficacy in preventing oxidative degradation is well-established, growing toxicological evidence has raised concerns about its potential effects on reproductive health. This review consolidates and critically examines findings from studies that explore BHA's impact on reproductive function in both males and females. Experimental studies have shown that BHA acts as an endocrine disruptor, leading to hormonal imbalances such as reduced levels of thyroxine and testosterone. At higher doses it has been observed to decrease the weights of reproductive organs, disrupt estrous cycles, delay sexual maturation, and impair sperm quality and motility. BHA exposure has been associated with a reduced male-to-female sex ratio, shortened anogenital distance, delayed sexual maturation, behavioral abnormalities, and reduced weight gain in offspring, indicating potential developmental and reproductive toxicity. This review highlights critical toxicological evidence and underscores the need for comprehensive, long-term research to better inform health risk assessments.

Key words : Butylated hydroxyanisole, endocrine disruption, reproductive toxicity, male and female fertility.

Food additives, also known as preservatives, are now a day added to foodstuffs for extending the shelf life of food and improving its flavor or retaining its appearance[8]. Food additives are a regular part of what we eat, helping to make food safer, easier to prepare,

and more convenient, despite some concerns about their safety[5]. Among these, preservatives play a key role in protecting high-risk foods like meat and dairy by preventing the growth of harmful microbes. However, some preservatives may pose health risks when consumed regularly or in large amounts[3].

Butylated hydroxyanisole (BHA), designated as E 320, is a synthetic antioxidant used since 1947 to prevent rancidity in fats and protect food, cosmetics, and pharmaceuticals from oxidative damage. It is a mixture of two isomeric compounds: 2-tert-butyl-4-hydroxyanisole and 3-tert-butyl-4-hydroxyanisole[4]. BHA can be used to create a synergistic combination of antioxidants with butylated hydroxytoluene (BHT), propyl gallate, and tert-butylated hydroquinone[29]. Due to their widespread use in the food business, particularly in the production of foodstuffs, cosmetics, medications, rubber, and petroleum products[7], this chemical is chosen for the study. Routes of human exposure to BHA are ingestion, der-mal contact, and inhalation. Some personnel in the petroleum, food, animal feed, and livestock production, cosmetics, rubber, and end product handling industries may be exposed to BHA. Employees working in fast-food restaurants that typically prepare and serve fried and fatty dishes may be exposed to high concentrations of BHA because the chemical is easily dissipated through thermal operations that produce steam and is volatile between 150°C and 170°C[28].

Because of their widespread usage, hundreds of articles and investigations from food and health authorities have expressed concerns about their potential health impacts. The combination of several exposure and ingestion sources leads to their cumulative effect.

Literature survey :

A comprehensive review of existing literature was conducted, encompassing both experimental and observational studies related to BHA (butylated hydroxyanisole). Relevant articles were sourced from major academic databases including Google Scholar, Science Direct, PubMed, WHO, and Research Gate. Peer-reviewed articles published between 1986 and 2024 were selected, with particular focus on studies examining the toxicological effects, regulatory status, and applications of BHA in food and cosmetic products. Special attention was given to studies involving endocrine disruption, reproductive toxicity, and bioaccumulation. The gathered evidence was critically compared to assess the potential health risks associated with BHA exposure.

Data extraction :

Search results were initially screened by title and abstract for relevance. Selected studies were categorized based on study design, objectives, methods, outcomes, and conclusions. Data extraction focused on health endpoints, exposure levels, regulatory decisions, and mechanistic insights, ensuring consistency and clarity across the reviewed literature.

Health and environmental concerns :

The national toxicology program in the USA stated in the report on carcinogens that BHA was reasonably anticipated to be a human carcinogen due to sufficient evidence of

carcinogenicity from research in experimental animals[20]. IARC categorized it as "possibly carcinogenic to humans" in Group 2B due to the development of fore stomach cancers in mice after long-term ingestion of high concentrations. The IARC classification of BHA as a possible carcinogen led to its inclusion on California Proposition 65, raising public concern about its safety. Despite established exposure limits by regulatory agencies, it has been restricted in children's products, excluded from programs like EPA's Safer Choice, and subject to mandatory labeling[6, 11]. BHA is listed as a chemical of possible concern under the OSPAR Convention due to its aquatic toxicity and bioaccumulation potential (log Kow = 3.29). It persists in surface waters and is not readily biodegradable. Its direct use in food preservation raises concern over human exposure[22,23].

Endocrine disrupting potential :

BHA has also been classified as a Category 1 priority substance by the European Commission on Endocrine Disruption due to evidence that it interferes with hormone function[23]. While an in vivo investigation discovered that it has antiestrogenic qualities, the available in vitro studies show that it has a modest estrogenic effect as well as anti-androgenic qualities. Concerns were raised about its potential endocrine disruptive effects[25].

Evidence from several studies indicates that BHA can weakly bind to estrogen receptors and act as an estradiol mimic in vitro. Although less potent than known environmental estrogens like octylphenol, BHA's ability to interact with hormonal pathways raises concerns about its potential cumulative effects alongside endogenous estrogens[14]. BHA has been shown to weakly interact with androgen receptors and moderately with thyroid receptors in a dose-dependent manner, producing additive effects on T3 transcriptional activity in vitro using yeast and HeLa cell models[12]. It also exhibits weak estrogenic activity, promoting estrogen receptor (ER)-dependent proliferation in MCF-7 cells and competing with estradiol for binding to ERα and ERβ. Additionally, BHA alters gene expression by reducing ERα levels and increasing progesterone receptor expression, mimicking the effects of estradiol, though only at much higher concentrations[21].

In vitro studies have shown that BHA exhibits partial anti-androgenic activity by inhibiting 5α-dihydrotestosterone-induced activation of the androgen receptor, while having little or no effect on its own—suggesting interference with androgen signaling without acting as an agonist[26]. It has demonstrated both estrogenic and anti-estrogenic properties, as seen in T47D-Kbluc and MCF-7 breast cancer cell assays, where it altered estrogen receptor signaling and inhibited estradiol-induced responses, highlighting its potential role in reproductive toxicity through hormonal disruption[24]. Additionally, it displays glucocorticoid-like and anti-glucocorticoid-like activity, and in combination with other cosmetic ingredients, has contributed to glucocorticoid-like effects, further supporting its classification as a potential endocrine disruptor[16].

BHA-Induced cellular effects :

Co-exposure of BHA and propylparaben in Vero cells resulted in enhanced cytotoxicity

compared to individual treatments, inducing oxidative stress, DNA damage, and G0/G1 cell cycle arrest. BHA was found to potentiate the pro-oxidant effects of propylparaben, with gene expression analysis implicating the TGF-β and ATM signaling pathways in the cellular response[19]. Further studies using H295R cells and Zebrafish demonstrated that BHA disrupts steroid hormone synthesis by upregulating key steroidogenic enzymes and altering estrogen and testosterone levels, suggesting endocrine disruption via the hypothalamic-pituitary-gonadal-liver axis[30]. In vivo findings in rats—rather than in pigs, rabbits, or rhesus monkeys—revealed reproductive and embryotoxic effects[27]. Additionally, BHA acts as a competitive inhibitor of steroid 5α-reductase 1 and 3α-hydroxysteroid dehydrogenase (AKR1C14) in rats. Docking analysis indicates that BHA binds to the dihydrotestosterone-binding site of AKR1C14, thereby reducing the formation of active neurosteroids[9].

Female Reproductive and Developmental effects of BHA :

BHA inhibited H_2O_2 -induced uterine activity, suppressed spontaneous contractions and prostaglandin production in the uteri of 21-day pregnant rats, and reduced H_2O_2 -induced chemiluminescence, indicating an antioxidant action and a potential modulatory effect on uterine function[2]. However, despite these seemingly protective effects, accumulating evidence highlights the reproductive and developmental toxicity of BHA. In its isomeric forms 2-BHA and 3-BHA, crosses the placenta in pregnant mice, leading to fetal exposure. Among these, 2-BHA exhibited greater accumulation in fetal tissues—particularly in the gastrointestinal tract—suggesting a higher developmental risk. The detection of radioactivity in both amniotic fluid and fetal tissues 24 hours after exposure indicates prolonged fetal contact[1]. Further contributing to developmental toxicity, BHA has been shown to inhibit placental 11β-hydroxysteroid dehydrogenase type 2 (HSD11B2), potentially increasing fetal exposure to glucocorticoids. This dysregulation is associated with impaired fetal growth and long-term reproductive and metabolic consequences[17].

BHA also disrupts estrogen signaling, a key component of reproductive health. In female CD-1 mice, dietary administration of 0.75% BHA for 2–3 weeks significantly reduced circulating and uterine levels of estradiol and estrone by 30–60%, inhibiting uterotropic responses such as uterine growth and estrogen-stimulated DNA synthesis[32]. Similar anti-estrogenic effects were observed in immature female rats, where BHA significantly reduced uterine and vaginal weights, both alone and in combination with 17β-estradiol, indicating interference with estrogen-mediated developmental processes[15].

Male Reproductive Toxicity of BHA :

BHA has been shown to significantly inhibit androgen production in Leydig cells. Zhang *et al.*[31] demonstrated that it directly inhibits rat testicular 3β-hydroxysteroid dehydrogenase (HSD3B) with an IC_{50} of approximately 50 μM[31]. Further supporting this, Li *et al.*[17] found that BHA disrupts testosterone biosynthesis in immature rat Leydig cells by directly inhibiting the steroidogenic enzymes CYP17A1 (17α-hydroxylase) and HSD3B1,

Table-1. Reproductive Toxicity of Butylated Hydroxyanisole

Model Organism	Dosage (Duration)	Inference	Reference
Female			
Mice (pregnant)	IV 50 μCi/100 g (Day 11 and Day 18 of gestation)	Crosses the placenta, showing higher fetal accumulation and potential developmental toxicity	Ahmed *et al.*,[1]
CD-1 Mice	0.75% BHA-supplemented diet (2-3 weeks)	Lowers estrogen levels and suppresses estrogen-dependent uterine responses.	Zhu *et al.*,[32]
20-days-old immature female rats	50, 100, 250, 500 mg/kg	Reduced uterine and vaginal weights	Kang *et al.*,[15]
Sprague-Dawley Rats	10, 100, and 500 mg/kg/day (13 weeks)	Shortened estrous cycles, decreased mating rates, delayed sexual maturation	Jeong *et al.*,[13]
Rat and Human tissue	-	Inhibit placental HSD11B2	Li *et al.*,[17]
Male			
Sprague-Dawley Rats	10, 100, and 500 mg/kg/day (13 weeks)	Decreased serum testosterone levels, weight reductions in ventral prostate, and testes, reduced sperm motility and size	Jeong *et al.*,[13]
Rat	-	Inhibits rat testicular HSD3B	Zhang *etal.*,[31]
Rat	Leydig cells cultured with BHA (50 μM)	Inhibiting the steroidogenic enzymes CYP17A1 and HSD3B1	Li *et al.*,[18]
Mouse	100 μM	Induced cell cycle arrest	Ham *et al.*,[10]

HSD11B2- 11β-hydroxysteroid dehydrogenase type 2; HSD3B- 3β-hydroxysteroid dehydrogenase; CYP17A1- 17α-hydroxylase; HSD3B1- 3β-hydroxysteroid dehydrogenase 1

and by downregulating the expression of *Hsd17b3* and *Srd5a1*. BHA interferes with testicular steroidogenesis and may hinder male reproductive development, as evidenced by the significant reduction in androgen production caused by these disruptions[18].

In addition to enzyme inhibition, BHA has been demonstrated to affect the viability of testicular cells. Ham *et al.*[10] reported that treatment with 100 μM BHA reduced the viability of TM3 (Leydig) and TM4 (Sertoli) cells to 50% and 65%, respectively, compared

to controls. Apoptosis, compromised cell proliferation, and mitochondrial dysfunction were linked to this decrease. Such cytotoxic effects suggest that BHA might interfere with spermatogenesis and lead to male fertility impairments[10]. Collectively, these findings indicate that BHA alters the expression of testicular developmental genes and interferes with the normal function and survival of testicular cells.

Transgenerational effects of BHA :

Jeong *et al.*[13] investigated the reproductive and developmental toxicity of BHA using Sprague-Dawley rats (both male and female) exposed via gavage during pre-gestation, gestation, lactation, and up to 13 weeks postnatally. Doses included 0, 10, 100, and 500 mg/kg/day. Hormonal changes were noted, including lower blood thyroxine levels at 100 and 500 mg/kg/day, and decreased serum testosterone levels in both mature males and male offspring. At higher doses, weight reductions were observed in the vagina, ventral prostate, and testes. Reproductive function was adversely affected at 500 mg/kg, as evidenced by shortened estrous cycles, decreased mating rates, delayed sexual maturation (vaginal opening and preputial separation), reduced sperm motility and size, and slower sperm movement[13]. A one-generation rat study by Petersen *et al.*[23] revealed more developmental abnormalities, which supported these findings. These included a lowered male-to-female sex ratio, shorter anogenital distances, and delayed sexual development markers. Sperm characteristics like motility, length, and width were also adversely affected. Behavioral abnormalities, including impaired righting reflex, were observed alongside reduced weight gain and altered neonatal behaviour[23].

Evidence from *in vitro* and *in vivo* studies demonstrates that BHA adversely affects reproductive health in both males and females. It interferes with estrogenic and androgenic signaling pathways, interferes with gonadal function, and disrupts hormone biosynthesis. In females, BHA alters estrogen levels, impairs uterine development, and crosses the placenta, increasing fetal exposure and potentially compromising long-term reproductive health. By blocking important testicular enzymes and lowering testosterone production, it interferes with steroidogenesis in males. It also induces apoptosis and mitochondrial dysfunction in testicular cells, thereby threatening spermatogenesis and fertility. Additional findings from multigenerational studies reveal delayed sexual maturation, hormonal imbalances, and behavioral abnormalities in offspring. These findings show that BHA may harm reproductive health and highlight the need for ongoing safety regulation.

Conflict of Interest

None.

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