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REVIEW

THE TOXIC EFFECT OF 4-NONYLPHENOL ON MALE REPRODUCTIVE SYSTEM

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ABSTRACT

Reproductive system influences many environmental toxicants that present the serious risk to the reproduction of human and animals, including hormone-like compounds called endocrine disrupting chemicals (EDCs). The interest about EDCs increased during the last 50 years due to their interference with the development and functioning of the endocrine system in aquatic animals. One of these toxic contaminants is nonylphenol that has the negative impact on the reproductive parameters not only on fish, but also on amphibians and mammals. Nonylphenol can decrease male fertility following a disruption of testicular development, a damage of Sertoli and Leydig cells and a reduction of a count and motility of spermatozoa.

Keywords: endocrine disruptors, nonylphenol, Sertoli cells, Leydig cells, spermatozoa, toxicity

INTRODUCTION

The endocrine system is one of the body´s major homeostatic control system whose aim is to maintain normal functions and development in the face of constantly changing environment. Working in tandem with the nervous system, which mainly is responsible for rapid and immediate responses, the approximately 30 different glands comprising the

endocrine system tend to act in a slower and more sustained manner to regulate processes as diverse as the female reproductive cycle, bone growth, cell proliferation, and psychosocial behavior. Like all homeostatic control systems, the capacity to maintain physiological parameters within normal bounds is finite, and when this capacity is exceeded by chemical exposures, drugs, or environmental stressors, adverse consequences can ensue **(Marty *et al*., 2011).**

Endocrine disrupting chemicals (EDCs) or endocrine disruptors are compounds that can interfere with and alter the homeostasis of the endocrine system, by modulating its response, resulting in long-term adverse effects on human and animal health or their progeny. The effects also extend to the thyroid, nervous, immune system and metabolism in general (**Hotchkiss *et al*., 2008**). EDCs can be divided on the natural hormones normally occuring in humans or animals and plant derived metabolites. On the contrary, the second group includes various exogenous man-made substances such as synthetic hormones and drugs, industrial chemicals (organchloride pesticides, dioxins, polychloro biphenils (PCBs), polybromo biphenils (PBBs), and alkylphenols, parabens found in cosmeticas and other personal care products), maufactured industrial products such as plastic additives (bisphenol A, phthalates), antifouling paints, and chemicals used in farm animal production **(Pinto *et al*., 2004; Diamanti-Kandarakis *et al*., 2009).**

The endocrine reproductive effects of these chemicals are believed to be due to their ability to disrupt the synthesis and metabolism of endogenous hormones, disrupt the synthesis of hormone receptors, mimic the effect of endogenous hormones or antagonize the effect of endogenous hormones, by altering the hormonal and homeostatic systems (**Mueller, 2004**).

The biological actions of hormones, including estrogens, androgens, progesterone, thyroxine and the neurosteroids pregnenolone and dehydroepiandrosterone (DHEA), are mediated via high affinity protein receptors within the target cells. All steroids are fat-soluble and readily cross the cell membrane, interacting with dimeric receptor proteins; in the case of estrogens these are ER-α and ER-β, although recent evidence suggests a third class, putative-ER or ER-γ, is involved in fish and possibly mammals (**Dogde *et al*., 1996; McLachlan *et al*., 2001**).

The affinity of steroid for its receptor is so great that it is the equivalent of being able to taste a teaspoonful of sugar dissolved in the water of a swimming pool. The steroid-receptor complex binds to target regions of DNA termed “response elements”. This activates the cascade of reactions, which are the response to the presence of steroids (**Laws *et al*., 1995; Gray *et al*., 1999; Stroheker *et al*., 2004**). Impairment of endocrine functioning by

exogenous compounds affects predominantly the estrogen, androgen and thyroid receptors (**Mueller, 2004**).

Estrogenic effects

The principal mechanism for the effect of EDs on the reproductive system may be mediated through binding the estrogen receptor or androgen receptor, and mimicking the effects of estrogen and antagonizing the effects of androgen, which disrupts the normal effects of the sex hormones (**Kelce *et al*., 1998; Sonnenschein and Soto, 1998**).

Estrogen is a hormone that influences the development and maintenance of female sex characteristics, and the maturation and function of accessory sex organs. Estrogen is also produced in males and affects reproduction (**Alberts *et al*., 1983; Hess *et al*., 1997**). In males, it is present low concentration in blood but is present at extraordinarily high levels in semen. Estrogenic effects are mediated by 2 distinct nuclear receptors, estrogen receptor α (ER α) and ER β. The receptors are encoded by 2 different genes and are expressed in germ cells (**O´Donell *et al*., 2001**). ER α is present in postacrosomal, midpiece and tail regions and ER β in midpiece and tail regions and mitochondria of ejaculated human sperm (**Durke *et al*., 1998; Aquila *et al*., 2004; Solakidi *et al*., 2005**). Incubation of sperm with 17-β-estradiol affects capacitation, acrosomal exocytosis, and fertilizing ability (**Adeoya-Osigawa and Fraser, 2003**).

Alkylphenols

A well known class of environmental endocrine disruptors is the group of compounds known as alkylphenolpolyethoxylates (APEs), which are non-ionic surfactants, consisting of branched-chain alkylphenol which has been reacted with ethylene oxide, producing an ethoxylate chain (**White *et al*., 1994**). They are used in variety of products, including detergents, emulsifiers, pesticides, herbicides, paints, cosmetics, plastic ware (**Nimrod and Benson, 1996**) and intra-vaginal spermicides (**Bolt *et al*., 2001**). APEs undergo a biodegradation process to produce short side chain derivates such as nonylphenol, octylphenol and butylphenol in anaerobic conditions in water (**Montgomery-Brown and Reinhard, 2003**).

The estrogenicity of alkylphenols has been attributed to ability these chemicals have to mimic natural estrogen 17β-estradiol (E2) in binding to and activating the estrogen receptor

(**Routledge and Sumpter, 1997**). Most alkylphenols are able to bind to the estrogen receptors in a concentration-dependent manner and thus potentially act as activators (agonists) or inhibitors (antagonists) of cellular responses mediated through the estrogen receptor. The binding activity of alkylphenols is associated with the presence of and the size (length) of substituted alkyl group (**Tabira *et al*., 1999**). The hydrophobicity of organic compounds increases with alkylation (**Hansch *et al*., 1973**) and the increase in binding affinity of the alkylphenols is proposed to be partially caused by stronger hydrophobic interactions with the ligand-binding domain of the ER (**Routledge and Sumpter, 1997; Tabira *et al*., 1999; Hu and Azaiwa, 2003**).

Nonylphenol

Nonylphenol (NP) is the major degradation product of alkylphenol polyethoxylates **(Junk *et al*., 1974)** and is one of the most common chemical contaminants. NP is present in ambient air, water, soil, sediments and biota **(Ying et al., 2002)**. It is widely used as detergents, emulsifiers and surfactants in industrial and household products such as paints, plastics, cosmetics, construction materials, vulcanized rubber and paper **(USEPA, 2005).** NP can stay biologically active for a longer period of time in the body than endogenous estrogens (**Nimrod and Benson, 1996**) and can interfere with reproduction in fish, reptiles and mammals, and induces the cell death in gonads and changes to other reproductive parameters (**Nagao *et al*., 2001; Weber *et al*., 2002; Cardinali *et al*., 2004**).

Effect of nonylphenol on male reproductive system

There is an increasing concern that environmental contaminants affect the male reproduction of humans and wildlife. Male reproductive abnormalities may be due to the increased exposure to environmental contaminants such as organochlorine pesticides, polychlorinated biphenyls, dioxins, phytoestrogens, alkylphenol ethoxyxylates and other xenoestrogens that enter humans through food, drinking water, air and skin contact **(Toppari *et al*., 1996).**

Effect of nonylphenol on testis

The mammalian testis is a complex organ that serves two important functions: synthesis of steroids and production of spermatozoa that controlled by gonadotrophins and numerous locally synthesized factors **(Saez, 1994)** and among them oestrogens **(Carreau *et al*., 1999).**

NP profoundly impairs testicular function as evidenced by reduced testis size **(Chitra *et al*., 2002)**, low circulating testosterone, disturbed testicular structure and suppressed spermatogenesis (**Nagao *et al*., 2001; Tan *et al*., 2003; Cardinali *et al*., 2004**). NP influences an increasing incidence of testicular cancer, an increase in occurence of cryptorchidism and hypospodias during the past decades **(Carlsen *et al*., 1995; Toppari *et al*., 1996).** Various environmental contaminants can induce oxidative stress by generating reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) and superoxide anion (O_2^-) (**Chitra *et al*., 2003; Wang *et al*., 2003**). Low micromolar concentrations of NP showed to induce testicular oxidative stress and cytotoxicity *in vitro* (**Gong and Han, 2006**). NP administration increased ROS level and lipid peroxidation and depressed the activity of antioxidant enzymes such as superoxide dismutase and glutathione reductase in rat testis **(Chitra and Mathur, 2004).**

Effect of nonylphenol on epididymis

The epididymis is known to play an important role in providing the microenvironment for sperm maturation and storage of sperm **(Cornwall, 2009; Raymond *et al*., 2010)**. Numerous proteins synthesized and secreted by the epididymal epithelia cells are thought to be considerable members of epididymal microenvironment and be involved in male reproductive activities including the initiation of sperm maturation, sperm-oocyte recognition, the acrosome reaction directly or indirectly **(Yenuqu *et al*., 2006)**, interaction with *zona pellucida* and binding to and fuse with plasma membrane of the oocyte **(Cuasnicu *et al*., 2002)**. All these modifications necessitate a minimum period in which they can occur, a time in which the spermatozoa must stay in the epididymal caput and corpus **(Franca et al., 2005)**.

A study of **Uguz *et al*. (2009)** proved that NP in high concentrations negatively influences motility, mitochondrial membrane potencial and chromatin integrity of epididymal rat spermatozoa.

Effect of nonylphenol on Leydig cells and steroidogenesis

The testicular Leydig cells are the predominant source of the male sex steroid hormone testosterone, express estrogen receptors (ERs) and are subject to regulation by estrogen **(Sherrill *et al*., 2010).** In mammalian species, Leydig cell development is regulated by the pituitary gonadotropin luteinizing hormone (LH) and by steroid hormones (i.e. androgen and estrogen) **(Abney, 1999).**

NP have the effect on testosterone biosynthesis **(Gong and Han, 2006)** and can inhibite hCG-induced testosterone release in rat Leydig cells **(Wu *et al*., 2010).**

Effect of nonylphenol on Sertoli cells

The normal onset of spermatogenesis and the eventual production of a sufficient sperm number to insure fertility depends at least in part on Sertoli cells. They are the somatic cells that provide physical reinforcement to germ cells and mediate the movement of growth factors, hormones and signals into seminiferous tubules where spermatogenesis commences. Sertoli cells contribute to the consumption of excess byproducts after sperm cells have fully developed (**Martincic *et al*., 2001; Sinha and Swerdloff, 1999**). Specific impairment of Sertoli cells will produce a parallel dysfunction in sperm production (**Weber *et al*., 2002**).

The exposure to NP can induce hypertrophy of Sertoli cells **(Miura *et al*., 2005)** and increase the levels of intracellular ROS and lipid peroxidation markers **(Gong and Han, 2006)**, which may plays an important role in the induction of apoptosis **(Gong *et al*., 2009).**

Apoptosis occur under the direction of an active, intracellular death program that can be stimuled or inhibited by environmental agents **(Jacobson *et al*., 1997).** It is a form of cell death by a characteristic set of morphological and biochemical changes. Apoptosis is recognized as an early cellular inductor of toxicity (**Weber *et al*., 2002**).

The endoplasmatic reticulum, which is the site for folding and assembly of proteins, lipid biosynthesis, vesicular traffic, and cellular Ca^{2+} storage, is sensitive to alterations in homeostasis. Several stimuli, including alterations of Ca^{2+} homeostasis and accumulation of unfolded proteins in the endoplasmatic reticulum, can cause oxidative stress of the endoplasmatic reticulum and prolonged oxidative stress of the endoplasmatic reticulum will lead to apoptosis (**Kadowaki *et al*., 2004; Gong and Han, 2006**). NP can induce apoptosis in rat testicular cells, particularly in Sertoli cells by inhibiting the activity Ca^{2+} pump in the endoplasmatic reticulum (**Hughes *et al*., 2000**).

Effect of nonylphenol on spermatozoa

The mamalian spermatozoon is a deceptively simple, and terminally differenciated cell. A functional spermatozoon is composed of three main regions: the head, the midpiece and the tail. The sperm midpiece consists of a variable number of mitochondria wrapped helically around the anterior portion of the flagellum (**Ramalho-Santos *et al.*, 2002**).

Mitochondria are important organelles in sperm homeostatis and the activity of sperm mitochondria is correlated with sperm motility, and thus with fertilization potential (**Gravance *et al.*, 2000**). The role of these mitochondria might be provide ATP for sperm movement though oxidative phosporylation. The necessary ATP is produced mostly by glycolitic pathways thoughout the flagellum, with mitochondrial ATP required only in specific circumstances (**Ramalho-Santos *et al.*, 2002**).

Mitochondria are the referential target of many toxic compounds (**Higgins and Rogers, 1974; Bragadin and Dell'Antone, 1996; Bragadin and Marton, 1997; Bragadin and Viola, 1997; Bragadin *et al.*, 1998, 1999**) since a damage to mitochondria which synthesize ATP gives rise to a corresponding cell damage. The exposure to low-level doses of NP inhibits ATP synthesis in mitochondria **(Bragadin *et al.*, 1999).**

CONCLUSION

The variety of man-made chemicals and their degradation products, on which the modern human life is dependent, can act as endocrine disruptors, enter humans and animals through food, drinking water, air and skin contact and so affect the health and reproduction. One of these chemicals is nonylphenol that can cause the abnormalities in male reproduction. Many authors confirm toxic ability of NP to disrupt a structure and development of testis and epididymis, decrease male fertility, including a decline of spermatozoa count, decreased spermatozoa motility parameters, production of oxidative stress and Sertoli cells apoptosis. Therefore it is very important the presence and the effects of nonylphenol on living organisms in the environment further monitor and investigate.

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