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# TOXIC EFFECTS OF POLLUTANTS ON TESTICULAR ACTIVITY IN THE FRESHWATER TELEOST, CLARIAS BATRACHUS (L.)

**Dr. Chandan Kumar**
Ph.D. (Science). VKSU. Ara. Bihar.

***ABSTRACT:***

*Catfish (Clarias batrachus L.) were exposed to selected sublethal concentrations of mercuric chloride (HgCl2; 0.05 mg l−1), methylmercuric chloride (CH3HgCl; 0.04 mg l−1) and emisan 6 (an organic mercurial fungicide; 0.5 mg l −1) for intervals of 45, 90 and 180 days from February to July (preparatory to spawning phase of the annual reproductive cycle). The gonadosomatic index showed a significant decrease after 90- and 180-day exposure to the mercurials. Histologically, the seminiferous tubules were smaller in size and contained mostly spermatids in comparison to the control fish in which they were greatly distended and full of spermatozoa. The Leydig cells showed pycnotic changes in Hg-treated fish after 90 and 180 days. In the Hg-treated testes, total lipid content and 32P uptake decreased significantly after 90 and 180 days. Levels of phospholipids and free cholesterol registered a significant reduction during all the durations. Esterified cholesterol level showed a significant decrease only in the 90-day HgCl2 and CH3HgCl groups, and in all 180-day Hg groups, while the level of free fatty acid decreased significantly only in the 180-day Hg groups. The observations suggest that impairment of testicular lipid metabolism by Hg is one of the possible factors that led to the inhibition of steroidogenesis and spermatogenesis.*

## INTRODUCTION:

Endocrine disruptors (EDs) are exogenous substances that alter the functions of endocrine system in living organisms causing adverse health effects to an individual or a population. EDs are known to provoke disarray of hormonally controlled physiological parameters (such as mineral and osmotic balances) or functions (such as growth, development and reproduction). There

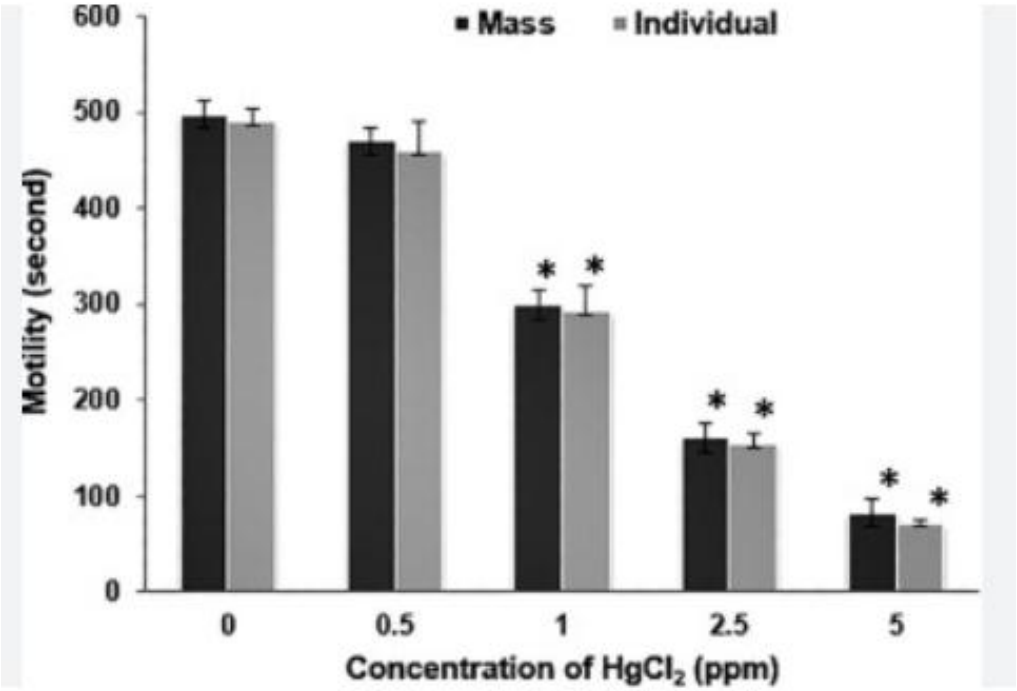
are different mechanisms through which EDs can act, either by binding to hormone receptor or altering the levels of endogenous hormones, or by modulating gene networks. This leads to stimulation/inhibition of downstream cellular and molecular pathways, thereby, affecting the normal parameters and functions. Lately, a large number of *in vitro* assays have been used extensively by several agencies to identify EDs, out of which a majority are organic molecules with low molecular weights. In the contrary, *in vivo* assays for ED detection are highly sensitive and provide biologically ecorelevant results than *in vitro* assays. Moreover, EDs lay among other complex contaminant families that easily arrive at aquatic system exerting adverse effects to aquatic fauna. EDs include pesticides, hormone mimics, heavy metals, polychlorinated biphenyls (PCBs), phthalates, organic solvents, flame retardants, surfactants, pharmaceuticals, etc. In addition to this, some EDs are naturally occurring, such as phyto-/myco-estrogens, which are synthesized naturally by fungi/plants (Liu, Kanjo, & Mizutani, 2010) whereas some other EDs are synthesized by chemical breaking down in industries such as polycyclic aromatic hydrocarbons (PAH). EDs are known to impart irreversible reproductive outcomes in wildlife fauna (Tubbs & McDonough, 2018). Feminization of males and infecundity are few of such outcomes (Gimeno, Gerritsen, Bowmer, & Komen, 1996). Apart from reproductive anomalies, EDs are also known to cause disarray of other endocrine systems across various axes, for example, hypothalamo-hypophyseal-gonadal (HHG) axis, thyroid and other cellular systems by exerting either antagonistic or agonistic effects upon binding to hormone receptors. Furthermore, some of these unfavourable effects (observed in wildlife fauna/experimental organisms) may also prevail in human if exposed at certain concentration and time causing endocrine dysregulation. Impact of ED exposure has been studied extensively in aquatic animals, particularly, in fishes. Hundreds of EDs have been detected till date, however, a very few have been tested. Nonetheless, the mechanism of action is still unknown for a wide range of chemicals.

**DISCUSSION:**

Fishes are well known bio-indicators to understand toxicity of EDs as they (Hutchinson, Ankley, Segner, & Tyler, 2006) show sexual plasticity and sensitivity towards sex steroids or xenobiotic compounds (Nagahama, Nakamura, Kitano, & Tokumoto, 2004). Incidentally, many sex steroid analogues or pesticides are known to cause estrogenic effects on variety of physiological systems in fish. In this context, model organisms, such as, zebrafish, medaka and fathead minnow, have been scrutinised. This review highlights valuable inferences of ED toxicity gained using model as well as non-model organisms including food fishes like carps (Cirrhinus mrigala, Catla, and Labeo rohita), followed by murrels (Channa *marulius*, *C. punctatus* and *C. striatus*) and catfishes (Clarias gariepinus, *C. batrachus* and Heteropneustes fossilis). In most of the cases, mere resemblance of certain molecular structures of EDs is sufficient enough to act like native steroid hormones and hence targeting the gonads. However, several EDs often alter HHG axis to impart deleterious effects on gonads eventually. Furthermore, EDs have the potential to affect higher vertebrates owing to biomagnification (Borga, Gabrielsen, & Skaare, 2001; Vives, Moreira, Brienza, Zucchi, & Nascimento Filho, 2006; Schäfer et al., 2015). In light of this, several observations suggesting adverse effects of EDs in fish in terms of sexual plasticity, neuroendocrine mechanisms, thyroid and immune modulation, gonadal development and maturation along with transcriptome/genome profile modulations are discussed in this review. Furthermore, advancements in ecological risk assessments of EDs are also highlighted.

Fishes pose diverse reproductive strategies, most of which are poorly understood (Kime, 1998). Sexual plasticity is one of the reproductive strategies in certain fish species wherein individuals can reversibly change their from one functional sex to the other in response to various environmental and social cues. Fish reproduction has been an ecologically relevant indicator of endocrine disruption since last four decades (Arcand-Hoy & Benson, 1998). Exogenous exposure of sex steroid such as 11-ketotestosterone (11-KT), a potent fish androgen, is known to act agonistically on androgen receptor in adult

female goldfish (Kobayashi, Aida, & Stacey, 1991). EDs alter the expression of cytochrome P450 (CYP) enzymes which play critical roles in sex differentiation and development in teleosts (Kazeto, Place, & Trant, 2004).




As discussed earlier, the scientific community worldwide has been able to establish the induction of VTG production using juvenile or male fish as one of the most notable and convincing biological response linked to EDs exposure that are mostly estrogenic in nature (Harries et al., 1999; Tyler & Routledge, 1998). Several reports in various fish species depicted similar effects (Flammarion et al., 2000; Nichols, Miles-Richardson, Snyder, & Giesy, 1999; Purdom et al., 1994). Studies on concomitant implications of these responses in terms of gonadal impairment and reproductive success are very crucial. Several such research evidences, to the best of our knowledge, are briefly reviewed below.

Alterations in VTG levels was observed upon exposure of synthetic estrogen such as EE2 and natural estrogens like estrone and E2 along with EDs such as DDT, BPA, methyl paraben, alkylphenols, its derivatives and several pharmaceutical drugs in teleosts (Celius, Haugen, Grotmol, & Walther, 1999; Chen, Wu, Tsai, Hsien, & Huang, 2019; Dambal, Selvan, Lite, Barathi, &

Santosh, 2017; Hylland & Haux, 1997; Ibor et al., 2016; Johnson & Lema, 2017; Kim et al., 2012; Larsson et al., 1999; Leatherland, 1993; Nimrod & Benson, 1997; Jobling, Sumpter, Sheahan, Osborne & Matthiessen, 1996). Induction of VTG production is often associated with developmental disruptions and anomalies in fishes such retarded testicular growth and formation of egg cells in testis (Gray & Metcalfe, 1997; Harries et al., 1996; Lye, Frid, & Gill, 1998; Lye, Frid, Gill, Cooper, & Jones, 1999; Lye, Frid, Gill, & McCormick, 1997).

**CONCLUSION:**

Apart from exerting estrogenic activities, inducing VTG production and causing testicular abnormalities, a number of reports on EDs elucidated occurrences of intersex, wherein, the riverine fish roach, Rutilus rutilus; the sturgeon, Scaphirhynchus platyorynchus; the male flounder, P. flesus; the Nile tilapia, O. niloticus; the common dab, Limanda limanda and the gudgeon, Gobio gobio were scrutinised (Aerle et al., 2001; Harshbarger, Coffey, & Young, 2000; Jobling et al., 1998, 2002; Kosai, Jiraungkoorskul, Sachamahithinant, & Jiraungkoorskul, 2011; Stentiford & Feist, 2005; Tyler & Routledge, 1998), a few of which were confirmed by histology of gonads. Exposure of DDT in the Japanese medaka, O. latipes, led to an intersex condition of gonad in male (Metcalfe et al., 2000) and altered gonadal morphology in O. mossambicus (Mlambo, Van Vuren, Barnhoorn, & Bornman, 2009). A report using the African catfish, C. gariepinus, depicted that early exposure of EE2 and diethylstilbestrol (DES) induced morphological changes and altered ovarian steroidogenic pathway (Sridevi et al., 2015). Upon treatment with EE2, reduction in spermatids was observed in males whereas MT treatment led to precocious ovarian development in females which were confirmed by gonadal histology (Swapna & Senthilkumaran, 2009). EE2 exposure altered the expression of cyp19a1a and cyp19a1b in zebrafish (Kazeto et al., 2004). Similar effects were reported upon exposure to nonylphenol, octylphenol, BPA and benzo[a]pyrene (an ED as well as a known carcinogen) in zebrafish (Alharthy, Albaqami, Thornton, Corrales, & Willett, 2017; Kazeto et al., 2004) which was

also reported in other fish species such as Rivulus marmoratus (Lee, Seo, Kim, Yoon, & Lee, 2006) and S. salar (Meucci & Arukwe, 2006).

Synthetic progestins (such as drospirenone, gestodene, norgestrel) and pharmaceutical drug such as (atrazine, fadrozole and metformin) altered sex steroid levels, induced reproductive behavioural changes, pertured steroid synthesis and affected gonadal morphology in several fish species (Ayobahan et al., 2020; Blanco, Fernandes, Medina, Blázquez, & Porte, 2016; Frankel et al., 2016; Hou et al., 2018; Niemuth & Klaper, 2015; Vasanth, Bupesh, Vijayakumar, Subramanian, & Ramasamy, 2018). Trenbolone exerted androgenic effects in fathead minnow (Ankley et al., 2003) like the effects imparted by pesticides such as linuron, fenitrothion, and vinclozolin (Katsiadaki et al., 2006; Makynen et al., 2000).

Other abnormalities included impaired milt production (Jobling et al., 1998) and altered spermatogenesis (Lye et al., 1998) in European flounder. Reduced gonodopodium (modified anal fin) size was observed in the mosquito fish, G. affinis (Batty & Lim, 1999) which is very critical for sperm transfer and is formed under the influence of testosterone (T). On the contrary, earlier reports suggested strong masculinization of the anal fin in mosquito fish upon exposure to phytosterols (such as ß-sitosterol, campestrol, and stigmastanol) and other naturally occurring androgenic chemicals (Denton, Howell, Allison, McCollum, & Marks, 1985; Howell & Denton, 1989; Howell, Black, & Bortone, 1980). Long-term exposure to cythion (an organophosphorus pesticide) affected reproduction in C. punctatus by biochemical changes in gonad and degeneration of immature oocyte (Ram & Sathyanesan, 1987). Recent studies on the delta smelt, Hypomesus transpacificus and the Japanese medaka, O. latipes, reported alteration in endogenous steroid levels and developmental toxicity upon exposure to herbicides like glyphosate and fluridone (Jin et al., 2018, 2020). Apart from chemical compounds mentioned in the previous sections, heavy metals are also known to exert toxic effects on gonads in their native/compound as well as in nano-particulate forms which are briefly reviewed further.

A report using two fish species, C. punctatus and Aorichthys aor demonstrated occurrence and bioaccumulation of heavy metals in the river

Ganges, Allahabad, India wherein metals accumulation in fish muscles was found to be in the order as follows: Zn > Pb > Cu > Cr > Cd (Gupta et al., 2009). A study using the shark catfish, Pangasianodon hypophthalmus and the Japanese eel, Anguilla japonica, suggested that heavy metals such as Pb, Mo, Rb and As might exert inhibitory effects on spermatogenesis (Yamaguchi et al., 2007). Concomitant to the findings in these reports, earlier reports suggested that Zn and its compounds accumulation in fish tissues might result in increased mortality rate, growth retardation and hypoxia conditions (Shaffi, 1979). Pb(NO3)2 caused hormonal imbalances and disarray in normal follicular steroidogenesis in stinging catfish, H. fossilis (Chaube, Mishra, & Singh, 2010). Toxic effects of CdCl2 were observed on rainbow trout's embryos (Lizardo-Daudt & Kennedy, 2008). Another study using O. mossambicus depicted estrogen-like growth-promoting property of Cd in juveniles, yet in adult fish it negatively affected reproduction (Amutha & Subramanian, 2013). MeHg and compounds like HgCl2 exerted androgenic affect and inhibited gonadal development in several fish species (Baldigo et al., 2006; Dey & Bhattacharya, 1989; Friedmann, Costain, MacLatchy, Stansley, & Washuta, 2002; Friedmann, Watzin, Brinck-Johnsen, & Leiter, 1996; Kime, 1998; Kirubagaran & Joy, 1988).

In the last two decades, increased production and usage of metallic nanoparticles (NPs) have inevitably led to heavy discharge into different compartments of environment including aquatic ecosystems resulting in accumulation at various levels of the food chain. A study, in this light, depicted morphological disarray in testis of C. batrachus upon treatment with Cu/Cu-NPs which was further confirmed by transmission electron microscopy (Gupta et al., 2016; Murugananthkumar, Rajesh, & Senthilkumaran, 2016). ZnO-NPs and ZnSO4 exposure to common carp exhibited defective testicular lumen which was confirmed through histology (Deepa et al., 2019). Furthermore, ZnO-NPs induced developmental malformations and decreased hatching rates in zebrafish embryos (Han, Zhai, Liu, Hao, & Guo, 2017). In conclusion, various categories of EDs led to intersex condition, caused alterations in sex steroid levels and steroidogenic enzyme gene expression, and induced anomalies in gonadal morphology along with several other cellular dysfunctions wherein hormone biomarkers were used

to detect EDs induced aberrations. However, identification of genetic biomarkers will ensure toxicity detection elegantly.

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