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RISKS INDUCED BY PESTICIDES ON FISH REPRODUCTION

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ABSTRACT

Pollution of water resources with agricultural water drainage has a great risk on fish reproduction. Organophosphrous pesticides such as Malathion and dimethaote are frequently used in Egypt due to their highly effectiveness for controlling agriculture pests. These pesticides were found that have endocrine disrupting effect on fish reproduction through lowering sex steroid hormones (estradiol and testosterone). Endocrine disrupting pesticides also have been implicated in the impairment of fish fecundity, semen quality, hatchability and survivability of fishes. Sex steroid hormones, vitellogenin, organosomatic index and histopathology are considered as biomarker tools used for assessing disrupting effect of pesticides on fish.

Keywords: Fish, reproduction, Pesticides, sex hormones

History of Endocrine disrupting pesticides:

There has been concern that uncontrolled uses of pesticides that reach the environment exert great and harmful effects on wildlife and human health since the publication of "Silent spring" book by Rachel Carson in 1962. Then term "endocrine disruptor" was introduced since publication of book entitled "Our Stolen Future, Are We Threatening Our Fertility, Intelligence and Survival? by **Colborn *et al*. (1996)**. In this book, she recorded that environmental chemicals disrupt the development of the endocrine system and exposure during development is often permanent.

Endocrine disrupting chemicals (EDCs) are defined as chemical substances that alter the normal endocrine function **(McKinlay *et al*., 2008)** including either naturally occurring chemicals as phytoestrogen or synthetic chemicals such as Pesticides, plasticizers, polychlorinated biphenyls (PCBs) and alkylphenolic compounds. EDCs exert their effect either through mimicking (act like a natural hormone) such as methoxychlor pesticide, certain polychlorinated biphenyls (PCBs) and bisphenol A (BPA) or antagonizing endogenous hormones such as tamoxifen or disrupt the synthesis and metabolism of hormones or interact with the hormone receptors **(Sonnenschein and Soto, 1998).**

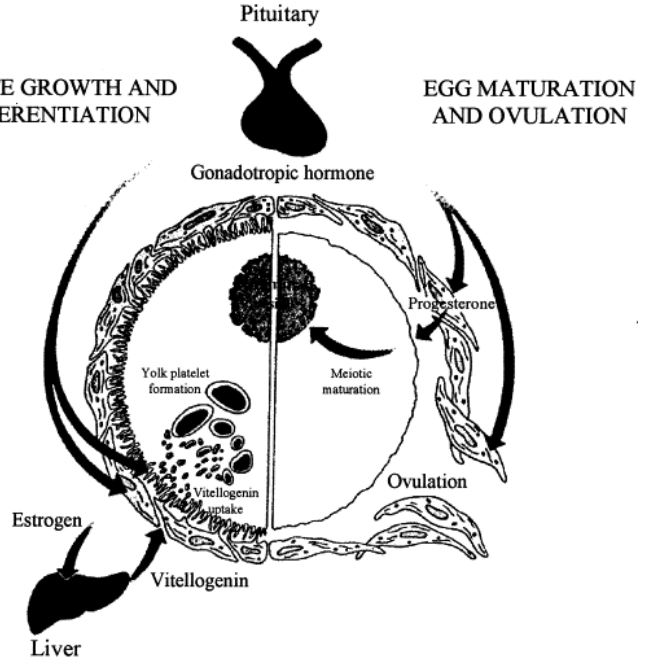
These chemicals can be found in environment (air, water, soil), Food products, household products, Pesticides, Plastics (bisphenol A, phthalates), Pharmaceutical drugs (birth control pills, DES, cimetidine) , industrial chemicals and heavy metals. Pesticides are used for controlling agriculture pest, flies in homes, gardens and on livestocks **(Srivastava *et al*., 2010**). The types and quantities of pesticides used are varies partly to types of crop **(Matthews, 1999).** The water resources are polluted with these pesticides from agricultural runoff or industrial effluents and their concentration in the ecosystem affected by many factories such microbial degradation which analyzes organic pesticides as part of their food or mineralization of pesticides to carbon dioxide, ammonia, water and inorganic salts **(Muller and Korte, 1975)** or photodegradation by (high temperatures, oxygen and hydrogen peroxide) which accelerate pesticides degradation **(Muszkat *et a*l., 2002).** In addition, high density of phytoplankton in water could absorb a high quantity of most pesticides in the water and so decreases its concentrations **(El-Nemaki *et al*., 2008).**

In Egypt, many authors detected presence of variant types of pesticides with different concentrations in the water and in musculature from different localities such as **Abbassy *et al*. (1999)**; **Mansour *et al*. (2001)**; **Abdel-Halim *et al*. (2006); El-Nemaki *et al*. (2008); Afifi (2009)** and **Eman *et al*. (2011).**

Hormonal control of fish reproduction:

Normal reproduction in vertebrate is controlled by two factors; extrinsic factors such as temperature and/or photoperiod or rainfall and intrinsic factor; the hypothalamic-pituitary gonadal (HPG) axis. These external stimuli act on hypothalamus resulted in secretion of gonadotrophin releasing hormone (GNRH) which in turn act on anterior pituitary that release gonadotrophin hormone (GTH) **(Browder *et al*., 1991)** which stimulate gonads to secrete steroid hormone. 11-ketotestosterone is the major androgen hormone in fish secreted from sertoli cells and responsible for sperm maturation **(Kime, 1993).** While, 17ß- estradiol is secreted from the follicular cells **(Nagler and Idler, 1992)** and stimulate liver to synthesize the egg yolk protein precursor (Vitellogenin)**.** This protein transported to the ovaries and incorporated into the oocyte, where it rapidly cleaved to form the yolk protein lipovitellin and phovitin **(Tyler *et al*., 1988).** Once

Oogenesis and spermatogenesis completed, the sex steroid hormone ceases and progestogen secreted which induce the final maturation of oocytes and sperm. **(Fig, 1)**



Fig, 1: Hormonal regulation of oocyte growth and differentiation (left) and egg maturation and ovulation (right). **(Browder *et al*., 1991)**

Effect of pesticides on gonadal hormones:

The hormonal level of both testosterone and estradiol are different according to the reproductive cycle of fish **(Rothbard *et al*., 1987);** during normal sexual ontogenesis in tilapia both testosterone and estradiol increase. Concentrations of plasma Sex steroid are considered as an indicator of gonadal status. In Ovary, estradiol must be synthesized in sufficient amount to stimulate liver to produce vitellogenin. In case of oocyte atresia; the estradiol hormone decrease lead to impaired vitellogenesis (**Ankley *et al*., 2002).**

Exposure of *Heteropneusts fossilis* to sublethal concentration of malathion for 72hr decreased the estradiol hormone (**Dutta *et al*. 1994)** and also showed low level of testosterone and estradiol with γ- hexachlorocyclohexane for 4 weeks (**Singh and Canario., 2004). Barber *et al*. (2007)** mentioned that estradiol and 11-KT in largemouth bass, *Micropterus salmoides* decreased after feeding on treated diet contain 46μg/g P,P-DDE and 0.8μg/g dieldrin for 4 month. Nile tilapia also showed decrease in sex steroid hormones upon exposure to organochlorine pesticide hexachlorobenzene (HCB) **(Rodas-Ortiz *et al*., 2008)**; acute dose (1.05 ppm) chronic dose (0.21 ppm) of butachlor **(Ghada, 2009)** and chlorpyrifos- ethyl for 15 days **(Özcan Oruc, 2010). Eman *et al*. (2011)** mentioned that *O. niloticus* fed on diet incorporated with malathion for 4 month exert endocrine disrupting effect on males than females through decreasing testosterone hormone; while dimethaote has

pronounced effect on females through decrease 17 ß- estradiol hormone. **Dogan and Can (2011)** reported that male *Oncorhynchus mykiss* exposed to sublethal concentration of dimethaote 0.735 mg/l for 30 day showed significant decrease in 17 ß- estradiol.

Many authors explained the decrease of sex steroid hormones as it may be due to some pesticides have mimic's effect and could stimulate negative feedback in gonadotrophin secretion, resulted in suppression the synthesis of endogenous estrogen **Folmar *et al*. (1996).** While **Garcia-Reyero *et al*. (2006)** explained the reduction as it may be due to interference with the production of free cholesterol, the sex hormone precursor which converted into testosterone (T) then converted either to E2 by the enzyme aromatase (CYP 19) or to 11- Ketotestosterone (11-KT) by the enzyme cytochrome P450 11ß- hydroxylase (CYP 11ß) and hence reduce steroid production and **Sijm and Opperhuizen (1989)** stated that rapid metabolic clearance of sex hormones by the liver through Mixed Function Oxidase (MFO) lead to decrease of sex hormones.

The ratio between **(**E2/ T) hormones was used as biomarkers of contamination. In normal males the E2/T ratio overlap (1) while, in females was below (1). In contaminated fishes with pesticides**;** this ratio of both females and males often overlap due to alteration occur with hormone homestasis, either through impaired synthesis and/or metabolism resulted in incomplete or improper gonadal development **(Lavado *et al*., 2004)**.

Measurement of specific fish steroid hormones include 11-ketotestosterone and 17, 20 BP are of limited use specially by using RadioImmunoassay **(Kime, 1999)** and only the commercial mammalian steroids hormone kits was available.

Effect of pesticides on organosoamtic index:

Gonadosomatic and hepatosomatic indices are `simplest tools used for assessment of endocrine disruption. **Kime (1999)** mentioned that decrease of gonadosmatic index is indicative of decreased hypothalamic, pituitary or gonadal activity. GSI was decreased in *Clarias batrachus* after 6 month exposure to dimethoate **(Begum and Vijayaraghavan 1995)**; juvenile summer flounder exposed to O,P-DDT **(Mills *et al*., 2001)** and freshwater catfish *Heteropneustes fossilis* after 4 weeks of exposure to γ- hexachlorocyclohexane at concentration 10 mg/l **(Singh and Canario., 2004)**. Nile tilapia exposed to dimethaote (1.6 mg/kg) and malathion (0.17 mg/kg) showed significant decrease in GSI after 120 day **(Eman *et al*., 2011).** On the other hand some authors found that gonadosomatic index (GSI) of *O. massambicus* following exposure to 2µg/l and 5µg/l DDT showed no significant differences compared to control **(Mlambo *et al*., 2009)** and also *O. niloticus* exposed to sublethal concentrations (5, 10 and 15 ppb) of chlorpyrifos- ethyl for 15 and 30 days showed no significant difference **(Özcan Oruc, 2010)**.

Reduction in GSI may be due to oocyte atresia and degeneration of gonad **as** mentioned by **Scholz and Gutzeit (2000)** or may be impairment of lipid metabolism or restriction of their mobilization to the gonads during exposure to pesticides **(Singh, 1993)**. While **Kime (1999)** mentioned that decrease in GSI due to pollutant is not clear whether the primary dysfunction is at the gonad itself or as a result of deficiency of pituitary hormone secretion so the histological examination of pituitary gland with gonads and measurement of gonadotrophin releasing hormone is important to define the exact reason.

Hepatosomatic index showed significant decrease in in *Oncorhynchus mykiss* exposed to higher concentration of dimethaote (0.735 mg/ml) after 15 and 30 day of (**Dogan and Can (2011)** and in *O. niloticus* exposed to malathion and dimethaote fro 120 days

(Eman *et al*., 2011). Decline in HSI explained by **(Heath, 1995)** due to loss of energy stores as a result of elevated metabolic demand to deal with the chronic stress of pesticides.

Effect of pesticides on fecundity:

There are marked differences in fecundity among fish species which reflect often different reproductive strategies**.** Even within species, fecundity may vary as result of adaptation of environmental habitat **(Witthames *et al*., 1995)**. Absolute fecundity means total number of ripened eggs per female; while number of eggs in relation to weight or length of fish means relative fecundity.

Decline in fish fecundity may be due change environmental conditions such as temperature or contamination with pesticides. **Khallaf *et al.* (2003)** mentioned that *O. niloticus* collected from polluted Shanawan drainage canal, Al-Minufiya Governorate showed lower fecundity of ranged between 1234 to 3893 eggs for female with total length 12 to 23 cm and **Ghada (2009)** found that *O. niloticus* exposed to 1.05 and 0.21 ppm of butachlor for 6 days and 6 week showed highly significant decreases in absolute fecundity. In addition absolute fecundity in *O.niloticus* was significantly decreased after treatment with Dimethaote and malathion for 4 months (117.86±23.69 eggs/ female) and (156.59±32.78 eggs/ female) respectively compared to the control (245.25±23.69 eggs/ female) **(Eman *et al*., 2011)** due to oocyte atresia and decreased estradiol hormone. However **Mlambo *et al.* (2009)** found that *O. massambicus* exposed to sublethal concentration of DDT 2 and 5µg/l revealed no significant difference in absolute or relative fecundity.

Effect of pesticides on sperm:

Teleosts are differed from mammals that have immotile sperm and it attains motility only on contact with water for only a few minutes and it enters the eggs via the micropyl. Most chemicals present in the aquatic environment exert their effect within the testis during spermatogenesis resulted in malformed sperm and abnormal sperm motility and so affecting fertilizing ability **(Kime, 1999).** For example**;** Cypermethrin reduced sperm motility in *Heteropneutes fossilis* after 45 days exposure (**Singh and Singh., 2008a)** and *O. niloticus* exposed to butachlor and malathion **(Ghada, 2009 and Musa 2010)** respectively**.** It was found that malathion and dimethoate have great effect on semen quality of *O. niloticus* after 120 day treatment. They significantly decrease sperm cell concentration and sperm motility with significant increase in tail deformity **(Eman *et al*., 2011).**

Significant decrease of individual sperm motility may be due to the effect of organophosphorus pesticides on mitochondria that alter ATP production or may be due to oxidative stress which lead to production of lipid peroxidation in spermatozoa affecting its motility (**Piña-Guzmán *et al*., 2006**). Sperm cell concentration and sperm quality decrease due to degenerative changes and lacking of germ cell lining to seminiferous tubules associated with organophosphorus pesticides treatment and deformity of sperm resulted from a decrease in acetylcholinesterase inhibitors activity that impaired function of caput of epididymis **(Okamura *et al*., 2009)**.

Effect of pesticide on gonadal and liver tissue:

Gonads and liver play an important role in the concentration of plasma sex steroid hormone either through gonadal secretion or the rate of hepatic deactivation and excretion. In addition liver is the organ in which synthesis of vitollogenin (egg yolk precursor) occur which used as biomarkers in male exposed to environmental contaminates has estrogenic effect. Many of histopathological changes due to pesticides exposure was detected in gonads such as vacuolation with presence of vitollogenic fluid in the ovarian parenchyma and necrosis of primary oocytes, atretic oocytes, degenerative and necrotic changes in the seminiferous tubules **(Jiraungkoorskul *et al*., 2003; Figueiredo-Fernandes *et al*., 2006; Mlambo *et al*., 2009** and **Eman et al., 2011**). The oocyte atresia explained the decrease of estradiol hormone and vitellogenic oocytes (**Van den Belt *et al*., 2002)** also necrosis of hepatopancrease will be reflected in low plasma concentration of vitellogenin. In addition activation of kupffer cells as well as focal hepatic haemorrhage associated with deposition of golden brown haemosiderin pigment was recorded in *O.niloticus* exposed to malathion for 90 days **Eman et al. (2011)** as a result of internal bleeding in the hepatic tissue and this considered as biomarker of stress induced by the toxic pesticides **(Suresh, 2009)**. Necrosis and vacuolization of hepatocytes occur due to excessive exhaustion of liver during detoxification process to get rid the pesticides from the body and the inability of fish to regenerate new liver cells **(Ayoola, 2008)**.

CONCLUSION

Our aquaculture are at great risk from these endocrine disrupting pesticides which drained to water either directly or indirectly affecting the reproductive performance of fish through decreased sex steroid hormones, fecundity and semen quality; so we recommended that the agricultural drainage water containing endocrine disrupting pesticides must be avoided to use in rearing brood stock.

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المخاطر الناجمة عن المبيدات على تكاثر الاسماك

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تلوث مصادر المياه بالصرف الزراعى له خطورة كبيرة على تكاثر الاسماك. الملاثيون والدايمواثيت من اكثر المبيدات العضوية الفسفورية التى تستخدم فى مصر بكثرة لتاثيرها العالى فى التحكم فى الافات الزراعية. وقد وجد ان هذه المبيدات لها تاثير اضطرابى على الغدد الصماء من خلال خفض مستوى الهرمونات الجنسية (الاستراديول والتستيرون). المبيدات المسببة اضطراب فى الغد الصماء ايضا تسببت فى ضعف خصوبة الاسماك، جودة السائل المنوى،نسبة الفقس ومعدل البقاء على قيد الحياة. تعتبر هرمونات الجنس، الفيتلوجنين، دليل الاعضاء والهستوبثولوجى من الدلالات البيولوجية لتقييم تاثير الخلل للمبيدات على الاسماك.