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Effect of Endocrine Disrupting Chemicals Exposure on Reproduction and Endocrine Functions Using the Zebrafish Model

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ABSTRACT

An increasing concern has been rising about the number of chemical substances associated with anthropogenic activities that affect the environment as well as the health of humans and wild life. These chemical compounds can harm the proliferation of aquatic life causing several problems among which a reduction in population density, biodiversity of species and a variation in fish sex are considered. Due to the similarity between the function of hormone receptor systems in human beings and animals, there is a great concern about the possible human health effects. Endocrine disrupting chemicals (EDCs) are identified as a group of harmful natural and synthetic compounds that are able to mimic endogenous hormones altering their functions, metabolism and biosynthesis. Being lipophilic molecules, many EDCs bind to hydrophobic pouches in steroid receptors, such as the androgen and estrogen receptors, that play a crucial role in vertebrate development and reproduction. Certainly, reproductive dysfunction in fish such as deviated male to female sex ratios, reduced fertility, reproductive tract abnormalities, early puberty, obesity and numerous cancers represent some health effects related to EDCs exposure. EDCs affect a great number of genes transcription, which required the development of new tools to supervise the total EDCs effects. The appearance of immense parallel sequencing for examining gene transcription offers a delicate tool for evaluating the effects of EDCs on humans and vertebrates and elucidates EDCs mode of action. In aquatic life, fish are found to be the principal hazard organisms for EDCs exposure. Zebrafish preserve many developmental pathways similar to that found in humans. Consequently, zebrafish becomes an important model system to study endocrine disruptors, specifically in the early stages of organs development due to the translucence of their embryos.

INTRODUCTION

Water is indispensable to withstand life. A suitable, safe and available water supply must be presented to all living organisms and societies **(Caballero-Gallardoa *et al.*, 2016).** Globally, water suppliers are concerned to afford a good quality of drinking water with sufficient quantity. Nevertheless, one of the main problems regarding the quality of drinking water is the existence of numerous environmental contaminants including endocrine disrupting chemicals (EDCs) **(Padbye *et al.*, 2014)**. EDCs can be originated from several sources and are able to penetrate the

environment by various media, including air, soil, and water via disposed waste water and groundwater discharge, and accumulate in sediments and biota involving fish **(Soffker & Tyler, 2012)**. Approximately, the majority of these environmental contaminants are ubiquitous, and hence, water may not be purified during the potabilization using water treatment plants to provide suitable water for human consumption **(Schaider *et al.,* 2014)**. In fact, these pollutants represent a vital measure in risk assessment since they bioaccumulate in various aquatic organisms, especially fish, affecting the transfer of developing embryo during egg development **(Caballero-Gallardoa *et al.,* 2016)**. Various studies explained the alterations of numerous features of fish physiology due to EDCs exposure usually in aspects relating to development, growth and reproduction **(Arukwe *et al.,* 2014; Xu *et al.,* 2014).**

ENDOCRINE DISRUPTING CHEMICALS (EDCs)

EDCs are identified as a structurally group of exogenous agents that can interfere with the production, transport, release, metabolism, action, binding or elimination of hormones that maintain homeostasis and are responsible for the regulation of developmental processes **(EPA, 2014)**. EDCs can interact with the endocrine system affecting the health of humans, fish and wildlife. The main harmful EDCs effects are their interference with the hormonal system, which may alter the endocrine regulation of crucial physiological functions **(Morales *et al.,* 2014)**.

EDCs are able to mimic or block the endogenous hormones activity, which may cause serious impacts on the viability of exposed organisms during serious developmental and reproductive stages **(Scholz & Mayer, 2008)**. In an attempt to document the result of chemical exposure, several reports have mentioned prominent impairments in the development of neuroendocrine system **(Weber *et al.,* 2013)**. EDCs may also affect several processes involved in reproduction by interfering with enzymes or receptors involved in metabolism and steroid synthesis **(Ma *et al.,* 2012)**. EDCs can serve as agonists or antagonists of fish oocyte maturation **(Tokumoto *et al.,* 2005)**. Endocrine disruptors are able to affect many features of transcription and transcriptional regulation that influence gene expression **(Dominguez *et al.,* 2014)**.

The mode of action of EDCs can be classified into: (I) "hormone mimics": (agonistic/antagonistic effect), (2) disturbance of production, metabolism, secretion or transport of natural hormones, and (3) interruption in hormone receptors during production and function **(Rotchell & Ostrander, 2003)**. This division designates distinct and discrete paths of an EDC mechanism, reliant to the specific characteristics of the molecule in action **(Goksffyr, 2006)**. While, only one compound can certainly use all the mentioned modes of action, with respect to the dose presented to the organism **(Goksffyr , 2006)**.

It was found that EDCs can affect the aquatic organisms and impair human development if exposure arises during serious stages of development **(Wiegand *et al.*, 2001)**. Reports have connected the progress of testicular dysgenesis syndrome in humans to the EDC exposure **(Santos *et al.*, 2007)**. Furthermore, **Wiegand *et al.* (2001)** stated that the failure of environment detoxification by fish, and the absence of EDCs decomposition may affect fish development. Exposure to EDCs in water media is linked to various reproductive impacts in fish, including intersex induction (presence of both male and female sex organs) **(Barnhoorn *et al.*, 2004),** reducing levels of different hormones, and eleminating gamete production and fertilization capability **(Caballero-Gallardoa *et al.*, 2016)**. Natural and manufactured steroid estrogens, such as estrone (El), 17β -estradiol (E2), and 17 α -ethinylestradiol (EE2) are considered the most effective EDCs found in these effluents. Other industrial chemicals including nonylphenol (NP) and bisphenol A (BPA) are also responsible for the endocrine disruption in animals **(Xu *et al.*, 2014a)**. Several studies demonstrated that the exposure to these pollutants can lead to a typical modulation or interruption of fish development and reproduction. Being a strong estrogen, **Kidd *et al.* (2007)** mentioned that the impact of l7∞-ethinylestradiol (EE2) in the environment cause sex-reversal in male fish, generating a complete female population. **Baumann *et al.* (2013)** indicated that it can impair the gonadal maturation of both sexes. They also explained that these enormous aberrations in gonadal development were based on fish species, developmental stage and the exposure duration. In addition, it was found that the chronic exposure of zebrafish to estrogen affected growth, caused an induction in vitellogenin synthesis, a delay in onset of maturation, impair sex ratio and sexual differentiation and decreased the success of fecundity and fertilization as well **(Ankley *et al.*, 2009)**.

A full inhibition of fertilization success resulted from concentrations of 1.1 ng/L and 3-5 ng/L of EE2 leading to failure of population recruitment **(Fenske *et al.*, 2005**; **Schafers *et al.*, 2007)**. **Xu *et al.* (2008)** stated that the reproduction of zebrafish exposed to EE2 is harmaly affected leaving impairmaents in the performance of both male and female. In zebrafish, **Van der Kraak and Lister (2011)** also indicated that the natural 17β-estradiol inhibits its oocyte maturation. In addition, for zebrafish exposed to the anabolic androgen 17 β-trenbolone and the pharmaceutical estrogen EE2, an increase was noted in the concentration of vitellogenin and induced fish feminization at a concentration of 10 ng/L EE2. Additionally, a masculinization and a reduction were observed in the production of vtg after 50 ng/L of 17β-trenbolone exposure **(Orn *et al.*, 2006)**. Moreover, a decline in gonadal development, a reduction in fecundity accompanied by a decrease in fertility **(Xu *et al.*, 2008)** and impairments in gonadal differentiation **(Fenske & Segner, 2004)** were among the most frequently recorded bad effects. Nonylphenol (NP) can biologically stay active in the body for a long time compared to an endogenous estrogen **(Caballero-Gallardoa *et al.*, 2016)**.

Competing with estrogen, NP binds to the estrogen receptor, affecting reproduction **(Chaube *et al*., 2013)** and development in fish **(Puy-Azurmendi *et al*. 2014)**. Several studies linked between consumption of fish and human risk since fish are particularly exposed to EDCs such as organochlorine pesticides (heptachlor epoxide, dieldrin and hexachlorobenzene) **(Lee *et al*., 2014)** and triclosan **(Shanmugam *et al*., 2014)**, among others. Subsequently, the aquatic environment is considered a main basin for disrupting chemicals. A precise consideration is specified to the validation and development of studying approaches using zebrafish (*Danio rerio*) that introduces a very well characterized fish model.

ZEBRAFISH: A Model for Studying EDCS

Zebrafish (Hamilton) belongs to the Cyprinidae family, an undifferentiated gonochoristic fish with a polygenic sex determination system **(Anderson *et al*., 2012; Liew *et al*., 2012)**. It is a small tropical freshwater species which survives in northern Pakistan, Nepal, northern India and Bhutan rivers **(Sharma *et al*., 2014)**. It was designated as a juvenile hermaphrodite since males undergo a process of gonadal transformation "juvenile ovary-to- testis" **(Uchida *et al*., 2002; Maack & Segner, 2003)**. The zebrafish has been represented as a significant organism to study the biological effects of hormones and EDCs **(Naderi *et al*., 2014)**. This species is commonly selected in studies concerning toxicology **(Dai *et al*., 2014)** owing its small size, high fecundity, rapid *ex utero* development of embryos which are optically transparent, short generation period, and the presence of various mutant strains **(Segner, 2009)**. Thus, the beginning and the exposure course to EDCs can be studied on organ development *in vivo* and in real time. Currently, zebrafish is characterized as an ideal model for fast phenotypic assessment and toxicological studies due to the lab-dependent nature and the highly inbred of these animals, and the fact that its genome has been fully sequenced **(Lieschk & Currie, 2007)**. Genetically, *Danio rerio* is strictly similar to humans sharing a high resemblance in processes of physiology, behavior and development (**Howe *et al*., 2013)**. These similarities help those genes to be expressed by various disruptors in zebrafish to become orthologous to that found in human **(Segner, 2009)**. Consequently, research studies that employ zebrafish indicate how strictly development is affected and how these adverse effects may be translated to the health of human beings **(D'Angel & Freeman, 2017)**. A female zebrafish can lay approximately up to 200 eggs per week **(Vliegenthart *et al*., 2014)**. Furthermore, **Weber *et al*. (2013)** regarded that zebrafish is a brilliant model organism to study oocyte maturation since it undergoes processes that are highly well-maintained among other vertebrates. Being oviparous, zebrafish can be early exposed without touching either the mother or the maturation. Moreover, the visualization of embryonic developments are quite easy unlike mammalian models **(Yen *et al*., 2011)**. Zebrafish is used in quick experimentation being sexually mature three months post-hatching **(Clelland & Peng, 2009)**. In

addition, **Tokumoto *et al*. (2004)** stated that zebrafish is characterized by a great number of spawned eggs compared to other small fish models, and its oocytes are easily collected during different seasons. Spawning occurs every 4-7 days under appropriate environmental conditions **(Clelland & Peng, 2009)**. Reproductively, active female zebrafish can undergo some spawning activity level on almost a daily basis, but comparatively, large spawns occur every 5 to 10 days **(Ankley & Johnson, 2004)**. Zebrafish is a "broadcast" spawner, releasing eggs that settle at the bottom of the tank. Zebrafish embryos hatch in about 3 days at 28°C **(Ankley & Johnson, 2004)**. In addition, **Arukwe and Goksoyr (2003)** pointed out that zebrafish is characterized by the absence of eggshell or zona radiata proteins, which are synthesized in fish liver under estrogens control. Adult-based assays are found more appropriate than that of embryos for screening of EDCs because late stages can be used for assessing extra endpoints, such as body weight, sex ratios and gonadal-somatic index **(Dammann *et al.*, 2011; OECD 2012)**.

Zebrafish is recommended as test species in a number of existing ecotoxicological test protocols and modes of EDC action to evaluate toxicity of EDCs and explain their toxicity mechanisms **(Caballero-Gallardo *et al.*, 2016)**. Since many of the environmental EDCs affect the system of sex steroid belonging to vertebrates, the majority of EDC studies using zebrafish mentioned an interruption of its sexual differentiation **(Caspillo *et al.*, 2014)** and reproduction **(Han *et al.*, 2014)**. In the past, zebrafish model was primarily used in genetic and developmental research **(Hill *et al.*, 2005)**, but today, this species has become important in other new fields including toxicogenomics **(Williams *et al.*, 2014)**.

Despite the accumulation of toxicological, histological and gene expression data, there is a little information on the molecular mechanisms that regulate the process of the gonadal differentiation **(Wang *et al.*, 2011)**. **Sreenivasan *et al.* (2014)** explained that the exposure to EDCs can have deleterious effects on zebrafish as well as steroids and described the sex differentiation of this species as an extremely pliable.

BRAIN-PITUITARY-GONAD-LIVER AXIS

Fish reproduction, like all vertebrates, is controlled by the Hypothalamic-Pituitary-Gonad- Liver [HPGL] axis. The HPGL axis is the most important target of EDCs because multiple complex hormones control and regulate this axis **(Hachfi *et al.*, 2012)**. **Peter and Yu (1997)** explained that the hypothalamus secretes gonadotropin-releasing hormone (GnRH) as a result of the integration of some environmental signals through the brain. GnRH in vertebrates, is the crucial factor which controls the reproductive axis activity by stimulating the adenohypophysis to release gonadotropins (GtHs); follicle stimulating hormone (FSH) and luteinizing hormone (LH) from the pituitary (Fig.l).

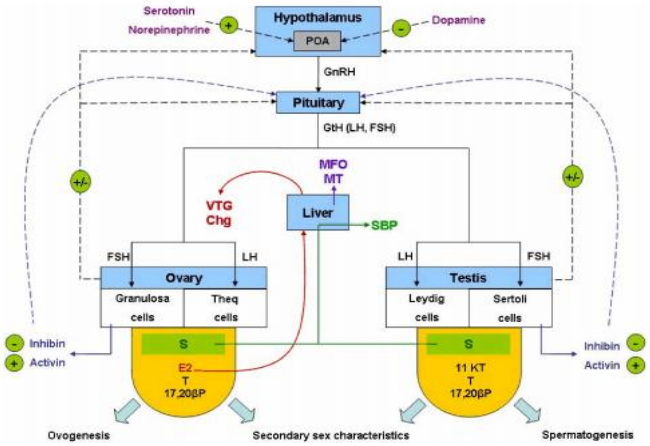

Fig. 1. Schematic presentation of the Hypothalamic-Pituitary Gonad-Liver axis in fish

Abbreviations: Chg = Choriogenin, E2 = 17-Estradiol, FSH=Follicle Stimulating Hormone, LH =Luteinizing Hormone, MFO =Mixed Function Oxydase, MT = Metallothionein, S = Steroides, SBP = Steroid Binding Proteins, T= Testosterone, VTG = Vitellogenin, 11KT = 11- Ketotestosterone,17,20*P = 17*, 20*-dihydroxy- 4- pregnen-3-one **(Hachfi *et al.*, 2012)**

In tum, both follicle stimulating hormone (FSH) and luteinizing hormone (LH) regulate gametogenesis and steroidogenesis in the gonads **(Clelland & Peng, 2009)**. In some vertebrates they include teleosts, ovarian steroid hormones and pituitary GtHs control oocyte maturation and growth. In the ovary, granulosa contains specific receptors to FSH (FSHR). l7∞-Estradiol (E2) is the main estrogen produced by the ovary in female teleosts, in addition to the production of great amounts of androgen and testosterone. Theca cells synthesize testosterone in the ovarian two-cell model which is then aromatized by means of cytochrome p-450 aromatase (CYP 19) to E2 by the granulosa cells as reviewed by **Nagahama (2000)**. **Arukwe and Goksffyr (2003)** reported that, E2 stimulates the eggshell Zr protein and vitellogenin (Vtg) production by female fish liver. The mechanism includes binding of the ligand (E2) to estrogen receptors (ER), which are then activated to form ER homodimers. These ER dimers bind to estrogen response elements (EREs) in the upstream regulatory region of the genes to undergo transcription of target genes **(Goksffyr, 2006)**.

In fish testis, follicle stimulating hormone receptor (FSHR) are localized to Sertoli cells. FSH can stimulate the proliferation of Sertoli cell and spermatogenesis. Luteinizing hormone receptor LHR is located on Leydig cells. The LH-receptor complex induces the synthesis of androgen. Then, the steroidogenic enzymes are induced and regulated by these pituitary LH and FSH hormones **(Themmen & Huhtaniemi, 2000)**. **Ankley *et al.* (2009)** stated that HPG axis is very vital system which can respond to environmental pollutants by numerous feedback mechanisms in order to sustain favorable conditions required to reproductive

process. The previous authors added that, these compensator responses can arise during the exposure to the stressor and even after the removal of this stressor. This explained the rapid and/or temporary variations in gene expression, which requires temporal studies to design predictive models and strong exposure indicators **(Ankley *et al.*, 2009)**. While diverse enzymes were affected, inhibitors of steroidogenesis such as fadrozole and prochloraz reduced the *Vtg* concentrations due to a decline in estradiol synthesis in female fish **(Ankley *et al.*, 2005; Villeneuve *et al.*, 2008)**. Steroid hormones are important in the preservation of HPG axis function, and feedback controls are accomplished on the system through variations in the production of steroids. Moreover, numerous recognized EDCs adversely affect steroid production through a direct modulation or inhibition of different enzymes included in steroid synthesis **(Ankley *et al.*, 2009)**. A sequence of factors, particularly sex steroids, can alter the function and the development of GnRH neurons. In turn, these neurons become targets for disrupting chemicals existing in the aquatic organisms **(Clelland & Peng, 2009)**. Gonadotropins released into the blood circulation provoke an increase in the production of androgen and estrogen by gonads. **Vosges *et al.* (2010)** indicated that a disruption in the GnRH system ontogeny of zebrafish occurred due to EE2 exposure by enhancing a rise in GnRH fibers and GnRH-ir neurons number, as well as a variation in the migration profile of GnRH-ir neurons and a decrease in the size of the GnRH-ir soma. E2, the major estrogen in female fish, is produced by follicular cells in the ovary. It was found that E2 synthesis in zebrafish ovary follows the same production pathways like vertebrates.

ENDOCRINE SYSTEM DISRUPTION IN ZEBRAFISH (Pituitary &Thyroid)

HPG axis has been selected as one of several pathways to illustrate the adverse effects of EDCs on fish endocrine system **(Caballero-Gallardo *et al.*, 2016)**. The endocrine system in fish is composed of numerous glands situated through the body which produce and secrete hormones to control biological processes. For example, the thyroid gland secretes thyroxine (T4) and triiodothyronine (T3) hormones, which help fish to acclimatize to changes in osmotic pressure and temperature. In addition, the thyroid and pituitary glands are targets for EDCs.

In vertebrates, the thyroid hormone system is secreted by the hypothalamus-pituitary-thyroid (HPT) axis as explained in the study of **Blanton and Specker (2007)** and **Zoeller *et al.* (2007)**. It was explained that the pituitary produces thyroid-stimulating hormone (TSH), which in turn, induces the thyroid gland to synthesize the thyroxine (T4) and some triiodothyronine (T3) after the exposure to environmental stimuli affecting the hypothalamus. Nevertheless, the circulating T4 found in the peripheral target tissues is concerned to produce the majority of the active T3 hormone through the action of 50-iodothyronine deiodinase. Moreover, **Crofton (2008)** mentioned that the complication of this system facilitates the impact of EDCs at

different levels, involving in transport, hormone synthesis and peripheral activation. In fish, **Matthiessen *et al*. (2018)** reported that the thyroid hormone is crucial for the regulation of early development, fundamental in metamorphosis and plays a major role in growth and reproduction. Furthermore, in higher vertebrates, they added that the thyroid hormone plays similar roles with slight differences.

Caballero-Gallardo *et al*. (2016) reported that an organism exposed to natural hormone levels or EDCs can impair or interfere with the proper function of the endocrine system affecting seriously the health of an organism and its progeny.

The majority of the environmental pollutants, which have been affected the thyroid system, such as perchlorate and some heavy metals are still being used. The strongest contaminant is that correlated with the oxidizing perchlorate mostly resulting from ammonium perchlorate and used in solid fuels **(Matthiessen *et al*., 2018)**.

Perchlorate inhibits the uptake of iodide via the thyroid gland; hence it interferes with the normal T4 synthesis, which in tum revokes negative feedback on TSH, thus leading inter alia to thyroid follicular hypertrophy and hyperplasia. Several laboratory experimentations studied and supported the inhibition of thyroid hormone synthesis by exposing fish to perchlorate **(Goleman *et al*., 2002; Bradford *et al*., 2005**).

Many pesticides are identified as EDCs in aquatic organisms, which are able to impact the development, reproduction and survival of different fish species. The effects of those substances on zebrafish exposed to EE2 **(Kazeto *et al*., 2004)**, TBP **(Deng *et al*., 2010)**, nicotine **(Kanungo *et al*., 2012)** and DE-71 **(Yu *et al*., 2014)** were reported. Variations in the expression of estrogen receptors; namely, BDE-209 **(Chen *et al*., 2012),** alpha and PFOA **(*Du et al*., 2013)** and TDCPP **(Wang *et al*., 2013)** due to exposure to EDCs such as EE2 **(Caspillo *et al*. 2014)** and DE-71**(Yu *et al*., 2014)** showed changes in *pax8a* gene expression involved in thyroid development. Moreover, **Shi *et al*. (2009)** stated that zebrafish embryos exposed to perfluorooctanesulfonate, a complex usually dispersed in the aquatic environment, can affect gene expression in the hypothalamic-pituitary-thyroid (HPT) axis, and also interrupt the thyroid gland at numerous stages, including the action of thyroid hormones, synthesis and regulation. Teleost hypothalamic hormones have similar activities to mammalian equivalents **(Caballero-Gallardo *et al*., 2016)**.

In the aquatic environment, **Caballero-Gallardo *et al*. (2016)** reported that phthalates are broadly used as plasticizers in cosmetics and food packaging. On applying a 200 µg/L concentration of mono-(2-ethylhexyl) phthalate (MEHP) on zebrafish larvae, **Zhai *et al*. (2014)** mentioned that genes required in the HPT system, such as *nis*, *tg* and *tshp* (thyroid hormone synthesis), *pax8* (thyroid development), *nkx2.1* (thyroid development), *diol* (thyronine deiodinase), *dio2* (thyroid hormone metabolism), and *ugtlab* (thyroid hormone metabolism) showed upregulation, while

ttr (thyroid hormones binding) exhibited downregulation. **Caballero-Gallardo *et al.* (2016)** examined if exposure to MEHP and the hydrolytic metabolite of di-(2-ethylhexyl) phthalate (DEHP) interrupt fish thyroid endocrine system by exposing embryos of zebrafish from 2 hours post-fertilization (hpf) to 168 hpf to various MEHP concentrations (1.6, 8, 40, and 200 mg/L). The whole-body content of thyroid hormone and transcription of genes included in the hypothalamic pituitary-thyroid (HPT) axis were examined. It was found that exposure to MEHP leads to an impairment in the thyroid endocrine system, which significantly increased whole-body T3 contents and decreased whole-body T4 contents **(Caballero-Gallardo *et al.*, 2016)**. In addition, the up-regulation of *Dio2* and *UGTlab* genes, linked with the metabolism of thyroid hormone, might be the cause of the decrease in T4 contents. In this study, elevated gene transcription of *Dio1* was also detected, which might be responsible for the degradation of the exceeded amount of T3. Moreover, treatment with MEHP enhanced genes transcription required in thyroid development (Nkx2.l and Pax8) and synthesis of thyroid hormone (NIS, TG and TSHb). While, the genes encoding proteins responsible for TH transport (transthyretin, TTR) showed a significant downregulation following treatment with MEHP. Generally, findings of **Caballero-Gallardo *et al.* (2016)** proved that acute exposure to MEHP disrupts the whole-body contents of thyroid hormones in embryos/larvae of zebrafish in addition to modifications in genes transcription involved in HPT axis, hence causing thyroid endocrine toxicity.

Zebrafish: a Model to Examine Aromatase as a Target of EDC Action

The particular role of the regulatory genes concerned with sexual differentiation, comprising sox9a and sox9b and anti-Műllerian hormone in sexual differentiation of zebrafish and in mediating EDC impact, is very limited (Schulz et al., 2007). Cytochrome p450 aromatase plays a major role because this enzyme defines the ratio of androgen/estrogen in the developing organism, since the action of EDC depends on the activity and/or expression of aromatase (Cheshenko *et al*., 2008). Caballero-Gallardo et al. (2016) indicated that, p450 aromatase is a vital steroidogenic enzyme which catalyzes the final phase in the transformation from androgens to estrogens. Moreover, this enzyme is important being involved in gonad development, reproduction, bone mineralization, behavior, glucose metabolism and other functions (Caballero-Gallardo et al., 2016). Expression of aromatase is mainly in gonads and slightly in the brain, adipose tissue, skin, placenta and endometrium as mentioned in the study of Goto-Kazeto et al. (2004). In the developing organism, Fenske and Segner (2004) and Vizziano et al. (2007) explained that, any variation of aromatase leads to a change in its phenotypic sex. This explanation clarifies the potential role of aromatase in determining sexual phenotype of zebrafish. Cheshenko et al. (2007) explained that the two isoforms are regulated differently; aromatase in the brain comprises in its promoter region an

estrogen response element which is responsive to estrogens, whereas the gonadal form cannot respond to estrogens. This can be pointed to the impact of the estrogenic pollutants on the sexual differentiation of zebrafish in the environment that are not due to a direct impact on the gonads, while indirectly through aberrations in the metabolism of cerebral steroids (Segner, 2009). It is important to mention that the gonadal aromatase Cyp 19ala is down-regulated during the gonadal transformation of zebrafish. Although the expression of this gonadal aromatase in adults (sexually dimorphic) is found to be high in ovary and low in testis (Wang &Orban, 2007; Jorgensen et al., 2008), it has been postulated that Cyp l9a l a plays a crucial physiological role in ovarian differentiation in fish, and its down-regulation is essentially required for the differentiation of fish testis (Guiguen et al., 2010). Prior to gonadal differentiation, Trant *et al*. (2001) showed that the brain aromatase was differentially expressed in zebrafish larvae and recommended that gonads differentiation depends on the brain aromatase dimorphically expressed. On the contrary, the study of Kallivretaki *et al*. (2007) on the expression of cyp l9a2 in zebrafish through sexual differentiation of gonads didn't note any indication on the dimorphic expression of brain aromatase, which contradicts the role of the brain in triggering the sexual phenotype of gonads in zebrafish. Moreover, Kazeto *et al*. (2004) mentioned that cyp19a2 gene was up-regulated due to treatment of zebrafish with BaP (10 µM). Tributyltin (TBT) is an organotin chemical used in industry and agriculture as a biocide and can induce the masculinization of fish (Shimasaki *et al*., 2003). In addition, McGinnis and Crivello (2011) studied the effects of TBT as an EDC on zebrafish. They recorded that genes including daxl, so.x9a, sfl (brain), cyp19a, daxl, zfERPI (liver), and cyp19a (gonads) were overexpressed, and a decrease was noted in some genes expression such as fst and so.x9a in the hepatic tissue. In addition, Kazeto *et al*. (2004) proved that para-nonyl phenol, can induce the cyp l9a2 gene expression in a dose-dependent manner.

It was shown that fish group exposed to an elevated concentration of para-nonylphenol (1µM) increased the transcript abundance of cyp19a2 with approximately sixty times higher than that of the control group (Kazeto et al., 2004). After treatment with NP and 33-octylphenol, Puy-Azurmendi *et al*. (2014) observed that the expression of cyp19alb was up-regulated, suggesting that cyp19 genes would promote powerful transcription for EDC targets. Furthermre, E2 can induce both estrogenic biomarkers in fish embryos, while Halm *et al*. (2002) and Hinfray *et al*. (2006) found that E2 application in adults is restricted to cyp 19a1b males. Chen et al. (2018) obtained the inverse pattern when applying BPA, as E2 expression was severely increased in adults, while it didn't regulate embryonic vtg. Therefore, these variable responses demonstrated that these estrogenic chemicals can modify gene expression via diverse mechanisms, and the severity of the molecular targets varies according to fish developmental stage. Moreover, Baker and Hardiman (2014) explained that, those compounds alter the process of steroidogenesis, which is crucial through development

in early stages, although disruptors that entirely impair reproduction would affect expression in the sexually mature fish individuals. Being gender-dependent, some reproductive impairments induce the expression of genes in embryos and males (Pawlowski *et al*., 2004), but they may repress the expression in females (Kang *et al.*, 2008). Kazeto *et al*. (2004) evaluated the roles of EDCs on fish reproductive physiology and investigated the impact of numerous types of EDC on the transcript amount of two CYPI9 isoforms, CYPI9A I and CYPI9A2 in juveniles of zebrafish. It was found that the expression of CYPI9 genes were not affected by clofibrate or atrazine. While, NP and ethinylestadiol induce the expression of CYPI9A2 gene in a dose-dependent manner. Moreover, Baumann *et al.* (2014) investigated the discontinued exposure to the feminizing effects of EE2 on zebrafish. Treatment with estrogens normally increases cypl9b mRNA levels, since estrogen-responsive elements are present in the promoter region of the aromatase gene (Diotel *et al*., 2010; Brion *et al*., 2012). Growth and sexual maturation are strictly related to each other, thereby affect genes expression involved in the hormonal system (Baumann, 2008; Chen & Ge, 2013). In zebrafish, Fenske and Segner (2004) mentioned that, the expression of aromatase in individuals with undifferentiated gonads is relatively low, while its level rises during gonadal differentiation. This may elucidate the high expression levels of aromatase in zebrafish individuals, which are sexually undifferentiated at 60 dph, while group of immature females showed low expression levels at 100 dph in Baumann et al. (2014) study. Those data proposed that the developmental stage is of pivotal role to analyze EDCs effects on the expression of aromatase.

Polycyclic aromatic hydrocarbons (PAHs) are abundant organic pollutants getting up from the incomplete combustion of organic materials (Caballero-Gallardo *et al*., 2016). Kazeto *et al*. (2004) found that exposure to benzo [a] pyrene (BaP) significantly increased CYP19A2 transcript abundance. They suggested that different EDC classes may alter developmental and reproductive physiology in fish through modulation of differential transcriptional of the CYP19 genes with impairment in neural tissue.

In summary, the estrogenic EDCs as well as BaP induced CYPI9A2 gene expression only in zebrafish juveniles. Moreover, BaP was found to be anti-estrogenic in estrogen-induced transcriptional response of CYPI9A2. Consequently, many EDCs may impair reproductive steps of the brain-pituitary gonadal-axis in fish via an impairment in CYP19A2 expression without affecting CYP19Al gene in the gonads (kazeto *et al*., 2004).

Effects of EDC Exposure on Zebrafish Oocyte Maturation and Ovarian Follicles

Oocyte maturation is known as an essential process that synthesizes viable eggs and consequent fertilization in the sexually reproducing animals. This process is started when the GnRH is produced by brain, which triggers the pituitary to secrete LH and forces ovarian follicles for producing the maturation-inducing hormone (MIH) (Clelland

& Peng, 2009; Rime et al., 2010). In the vertebrate ovary, production of MIH involves an interaction between the epithelial cell types, including the thecal and the granulosa cell layers (Nagahama & Yamashita, 2008). In many fish species, the natural MIH is 17a-hydroxy ,20b-dihydroprogesterone (Tokumoto *et al*., 2005). On the oocyte membrane, MIH binds to receptors triggering a protein cascade that promotes the activation of maturation-promoting factor (MPF) in the oocyte cytoplasm (Tokumoto *et al*., 2008). This MIH binding enhances the formation of MPF by producing cyclin B; a regulatory protein essential in MPF activity (Nagahama & Yamashita, 2008; Tokumoto *et al*., 2012). Being active, MPF releases mature oocytes from meiotic arrest occurring during prophase I to initiate the resumption of the meiotic cycle. Tokumoto et al. (2008, 2011) observed some morphological signs during fish growth. Oocyte maturation includes the breakdown of the germinal vesicle at the prophase/metaphase transition as well as the conversion from opaque to transparent ovarian follicles.

Follicle clearing takes place during the transformation of vitellogenin into yolk proteins. In follicle clearing assays, this translucent appearance is an indication of maturation (Clelland & Peng, 2009). The pathway included in oocyte maturation may be disturbed by EDCs that can inhibit reproductive development. Being localized on follicle-enclosed oocytes, Nagahama and Yamashita (2008) and Tokumoto *et al.* (2011, 2012) indicated that membrane progestin receptors (mPRs) and G-protein-coupled receptors share similar characteristics and possess high affinity for MIH. Thus, they considered them as probable MIH receptors. Tokumoto *et al.* (2005) and Rime *et al.* (2010) stated that, mPRa and mPRb were identified as candidates for this binding affinity in zebrafish. Reports concerning zebrafish revealed that inhibition of its oocyte maturation is due to the block of the mPRa with antisense oligonucleotides, suggesting that mPRs served as EDCs and MIH mediators that prevent aquatic organisms development (Tokumoto *et al*., 2012). In spite of EDC-specific variations in their modes of action, one of the potential mechanisms of action is the disruption of cyclin B production through the pathway of oocyte maturation (Kortner & Arukwe, 2007). Tokumoto et al. (2008) and Ogawa et al. (2011) recommended that, mPRs can represent a mutual relationship between MIH and EDCs effects, and fish oocyte maturation can serve as a model to understand the impact of EDCs exposure on marine environment.

Many EDCs antagonize and inhibit maturation as well as impair reproductive development such as ethynyl estradiol, o, p-DDD (lysodren) and methoxychlor (Rime *et al*., 2010; Ogawa *et al*., 2011). Tokumoto *et al.* (2004) explained that some EDCs exert their inhibitory or stimulatory effects by using mPRs since they are structurally similar to endogenous hormones. Tokumoto *et al.* (2007) suggested that the mode of action of diethylstilbestrol includes activation of mPRa to stimulate maturation of oocyte. EDCs can harmfully affect tissue formation and fecundity, increasing the probability of cancer development (D'Angelo & Freeman, 2017).

D'Angelo and Freeman (2017) assessed the effects of atrazine and diazinon (used agriculturally as examples of herbicides and pesticides) classified as EDCs by impairing and reducing the frequency of oocyte maturation of zebrafish. They cultured and exposed ovarian follicles of zebrafish to different atrazine especially 2,4-D and diazinon concentrations. Follicles were assessed at 2, 3 and 4 hours for viability and size, while clearing was only evaluated at 4 hrs post-exposure. It was found that follicle clearing was significantly decreased at the examined atrazine and diazinon concentrations, while no effect was detected with 2,4-D exposure (D'Angelo & Freeman, 2017). Ghodageri and Katti (2013) observed that male gonads in fish exposed to atrazine were poorly developed. In addition, atrazine can increase oxidative stress causing poisonous effects in fish (Jin *et al*., 2009). Hayes *et al.* (2002) reported that low doses of atrazine can harm fish viability by disrupting reproductive function and development leading to fish demasculinization.

Chlorophenols are used as wood treatments, biocides and also for bleaching paper mills. 2,4-D is an herbicide, similar to atrazine and is considered to be the most popular chlorophenols present in the environment (Bukowska, 2006). Its by-product has been reported to threat humans and wildlife. In nontarget organisms, it was noted that 2,4-D disrupts reproduction including steroidogenesis and the HPG axis, which are essential in reproduction and development processes. Ma *et al*. (2012) recorded a decrease in the number of spawned eggs as well as in the number of successfully hatched eggs by exposing zebrafish to 2,4-DCP. Koc and Akbulut (2012) mentioned that oogenesis was slow in rate, and the number of nonviable oocytes were increased. The afore- mentioned authors explained that interruption in oogenesis was related to the slowness in oocytes development and growth inhibition. Wiegand *et al.* (2001) indicated that teratogenic effects observed during organogenesis in developing zebrafish embryos were due to atrazine exposure, affecting the reproduction and survival of next generations in nontarget organisms. In addition, adverse effects have been examined due to exposure of zebrafish to diazinon, such as a low rate of hatchability and an increase in mortality (Osterauer & Kohler, 2008), a reduction of motility in zebrafish exposed to 10 lM diazinon (Yen *et al.*, 2011), and a disruption of steroidogenesis and a reduction in the number of eggs spawned and hatched by zebrafish (Ma *et al*., 2012). Additionally, more adverse effects were observed, including a delay in oogenesis noted by the small number of oocytes and the high incidence of the deformed and atretic oocytes in their ovaries (Koc & Akbulut, 2012).

Gonadal Histopathology as Indices of Endocrine Disruption

Histopathology is broadly used to assess impact of EDC on endocrine system (van der Ven *et al*., 2003). Caballero-Gallardo *et al*. (2016) explained that interruption of hormonal functions and metabolism in fish exposed to EDC will provoke pathological impairments of cellular and tissue structures in organs involved in action and synthesis of

hormones. Exposure to EDC may cause alteration in gametogenesis progression or result in tissue damage, such as induction of apoptosis, necrosis, cellular hypertrophy and hyperplasia. In vertebrate fish, gonadal histopathology can be demonstrated by the presence of granulomatomous lesions in zebrafish ovaries, indicating EDC alterations **(Rossteuscher *et al*., 2008)**. Using light microscopy, **Maack and Segner (2003)** investigated the gonadogenesis of zebrafish to examine the appropriateness of gonadal histology in EDCs risk assessment. It was found that primordial germ cells were dorsocaudally located in the cavity at 2 weeks post-fertilization (pf) age. While, the majority of fish had a pair of gonads including meiotic germ cells at 4 weeks pf; these gonads indicated presumptive ovaries **(Maack & Segner, 2003)**. At week 5pf, 87% of examined fish possessed perinucleolar oocytes in their ovaries. In addition, gonads of females zebrafish belonging to week 11 pf. showed a rise in gonadal size and an increase in perinucleolar oocytes number and size **(Maack & Segner, 2003)**. Under the effect of EDCs at the beginning of week 5, some fish displayed gonad alterations, including a reduction in the size and number of the oocytes. The oocytes appeared irregular in shape and enhanced basophilia and they disintegrated into small residual bodies. It was found that the stromal cells were more numerous infiltrating the gonadal matrix since the number of oocytes was decreased **(Maack & Segner, 2003)**. In 7week-old, the appearance of higher gonial cells number arranged in cyst was observed in zebrafish with impaired gonadal morphology. These gonads were known as presumptive testes. Adding to the gonial cells, **Maack and Segner (2003)** noticed spermatocytes in one fish out of 32 studied individuals. During the succeeding weeks, especially at 11 weeks pf, fish percentage presenting early testes augmented to reach 40%. They also found a series of gonadal impairments in various fish specimens from week 5 pf, which explain the transformation of altered ovaries into testes. Therefore, **Maack and Segner (2003)** identified zebrafish as a 'juvenile hermaphrodite'. Their study results are used as a guide to prevent incorrect diagnosis of hermaphroditism in zebrafish.

Bisphenols induces Follicular Atresia and Impair Reproduction in Zebrafish

Bisphenol A (BPA) was used to assess the greatest risk on human health due to one's potential exposure **(Le Corre *et al.*,2015)**. BPA was selected since it is classified as an endocrine disrupting chemical due to its deleterious effects on the endocrine system. **Migliaccio *et al.* (2018)** studied the effect of low concentrations of BPA on the follicular development in zebrafish ovary by applying a qualitative and quantitative histomorphological method. Results of **Migliaccio *et al.* (2018)** showed that BPA exposure interrupted follicular development since it disrupted the previtellogenic and vitellogenic stages. Moreover, it increased follicular recruitment, enhanced the follicular change from stage III to stage IV producing enlarged follicles in stage I and induced follicular atresia. **Migliaccio *et al*. (2018)** suggested that BPA enhanced the follicular progression by producing big atretic follicles which undergo atresia. This led to a failure in oocyte maturation which may explain why the number of mature follicles was unchanged due to BPA exposure. In addition, their data revealed that zebrafish ovary is

very sensitive to different BPA concentrations by evaluating the size of late vitellogenic and atretic follicles histomorphologically.

Beside causing reproductive toxicity, most studies of BPA exhibit additional side effects including disrupted function of pancreatic β-cell, liver damage and thyroid hormone interruption **(Le Corre *et al.*, 2015)**. Results of **Lee *et al.* (2015)** on wild freshwater and marine fish and those of **Wang *et al.* (2015)** conducted on water revealed that, sediments and fish from Taihu Lake in China demonstrated the toxicity of BPA to aquatic organisms. Moreover, **Alenazi *et al.* (2015)** found that BPA which is generally present in plastic products affected expression of cytochrome cyp19 genes either by exhibiting an overexpression *in cyp19a* located in testis or a forming a decreased expression in *cyp19b* situated in the brain.

For bisphenol S (BPS), little evidence is available on its endocrinological effects. In **Ji *et al.* (2013)** study, adult zebrafish pairs were exposed to 0.5, 5, and 50 µg/L of BPS for 21 d, and the effects on reproduction, sex steroid hormones and transcription of the genes belonging to HPG axis were investigated. **Ji *et al.* (2013)** showed a significant decrease in the gonadosomatic index and egg production in female zebrafish exposed to ≥0.5 µg/L BPS. While, a significant increase in 17 β-estradiol plasma concentrations in both sex of fish was recorded **(Ji *et al.*, 2013)**. However, they observed that testosterone concentration was significantly decreased, *cyp 19a* was up-regulated and cyp 17 and 17βhsd transcripts were down-regulated in male zebrafish **(Ji *et al.*, 2013)**. Exposure of fish adults to BPS caused low rates of hatchability even in clean water. Moreover, Fl embryos exposed to continuous BPS showed poor hatchability and high malformation rates compared to untreated Fl embryos with BPS **(Ji *et al.*, 20l3)**. The observations of **Ji *et al.* (2013)** demonstrated that to low BPS exposure level impair HPG axis feedback regulatory circuits and affect the offspring development.

Additional Examples of Toxic EDCs affecting Zebrafish Reproduction

Numerous EDCs were reported with toxic effects on zebrafish. **Caballero-Gallardo *et al.* (2016)** mentioned that heptachlor is one of the EDCs which is also highly poisonous and persistent in the aquatic environment. At 96 hours post fertilization (hpf), they found that larvae of zebrafish exposed to EDCs showed that values of LC50 represented 0.24, 1.74 and 1.59 mg/L to endosulfan, heptachlor and methoxychlor, respectively. **Caballero-Gallardo *et al.* (2016)** reported that larvae of zebrafish were more sensitive to endosulfan.

Bromophenols are produced from the biodegradation of other contaminants and resulted as byproducts of some plastics decomposition. 4,6-tribromophenol (TBP) is considered the most commonly released brominated phenol among bromophenols **(Caballero-Gallardo *et al.*, 2016)**. **Deng *et al.* (2010)** assessed TBP effect on zebrafish embryos and they found an alteration in sex ratio towards males. The previous authors

suggested that TBP significantly affect fish population in marine environment since FI-generation larvae showed high malformations, delayed growth and reduced survival rates. In zebrafish, embryonic treatment with butachlor (an herbicide generally used to regulate weed of vital crops) noted that butachlor had a toxic effect represented by delaying fish hatchability and resulting in a sequence of malformations including yolk sac and pericardiac edema in a concentration-dependent manner followed by mortality **(Caballero-Gallardo *et al*., 2016)**. Zebrafish exposed to 16 µM and 20 µM butachlor, **Tu *et al.* (2013)** recorded that the mortalities were 93.3% and 100%, respectively. In addition, butachlor LC50 value was found to be 14 µM at 84 hpf. Triadimefon classified as a pesticide disturbs the reproduction of zebrafish **(Liu *et al*., 2014)**. At 120 days, **Caballero-Gallardo *et al.* (2016)** observed that length and weight of female fish exposed to 0.5 µg/mL triadimefon were significantly lower than those of the control. While, exposure of male fish to 0.25 and 0.5 µg/mL triadimefon caused a decrease in weight and length compared to the control group. For a four month, **Santos *et al.* (2006)** investigated the exposure effect of tributyltin (TBT) and the synthetic estrogen EE2 exposure on the larvae of zebrafish at 5 days post-fertilization. They showed that fish exposed to TBT represented 62.5% males in control group, while its percentage was equal to 86 and 82 in TBT 25 ng/g and TBT 100 ng/g, respectively, indicating a male sex bias. Complete blockage of the TBT masculinizing effect was obtained due to EE2 co-exposure, which supports the idea that the existence of estrogens in the aquatic environment may neutralize the masculinizing effect of TBT on fish. However, **Morthorst *et al*. (2010)** exposed zebrafish to appropriate androgenic steroid trenbolone acetate concentrations and presented an irreversible masculinization of Tb which pays attention to its androgenic discharge consequences on the aquatic organisms. Finally, exposure to chemicals such as TBP (male) **(Deng *et al*., 2010)**, TBBA **(Chow *et al*., 2013)** and EE2 **(Caspillo *et al*., 2014)**, exhibits an overexpression of *vtg*. While, TBP (female) **(Deng *et al*., 2010)**, nicotine **(Kanungo *et al*., 2012)** and DE-71 **(Yu *et* al.,2014)** produces a decreased expression in zebrafish.

EDC Toxicogenomic Studies Using Zebrafish

Concerning toxicogenomic approaches, zebrafish is used as a model in numerous studies reported for various EDCs. One of the most studied compounds of these pollutants applied on zebrafish were cited for organochlorine insecticides **(Chow *et al*., 2013),** chloroacetanilide herbicides **(Tu *et al*., 2013),** fungicides **(Jiang *et al*.,2014)** and phosphomethyl amino acids herbicides **(Uren Webster *et al*., 2014)**. **Uren Webster *et al*. (2014)** stated that zebrafish exposed to10 mg/L glyphosate (used as herbicide and contaminate water surfaces) changed the gonadal transcript profiling with repect to genes, including *esrl* and *cyp19al* in ovarian tissue as well as *cat, sold* and *hsd3b2* in testicular tissue. The outcomes of **Uren Webster *et al*. (2014)** explained that toxicity mechanisms comprise oxidative stress and interruption in the biosynthesis pathway of the steroidogenic enzymes.

Exposure to genistein (2200 µg/L) showed a series of developmental alterations of 48 hpf zebrafish embryos represented by spontaneous movement, head and tail malformations, edema and a reduced blood circulation **(Schiller *et al.*, 2013)**. Moreover, **Schiller *et al.* (2013)** indicated that the results of zebrafish microarrays in 2 days old detected a difference expression in about 881 genes, which significantly affected several molecular pathways. According to **Schiller *et al.* (2013)** microarray results, there was an upregulation in genes such as *cyp19al b* and *vtg 1* (responding to estradiol stimulus), and *sc4mol* (biosynthesis of steroids), while *nkx2.1* and *pa.x2a* (development of thyroid gland), and a downregulation was detected in *hoxa9a*, *hoxalOb,* and *hoxallb* by applying 2400 µg/L genistein concentration. In addition, **Santos *et al.* (2014)** reported that the hormone receptor genes *(ar, esr2a* and *esr2b)* were downregulated at 0.2 and 2 µg/L concentrations of genistein. In developing zebrafish embryos, **Ren *et al.* (2012)** explained that ≥1 µM of benzo(a)pyrene (BaP) selected as PAH can cause deleterious malformation and mortality. Using microarray analysis, **Huang *et al.* (2014)** also demonstrated that BaP perturbed correlated genes of photoreceptor development by inducing dysfunction and developmental defects in the visual system defects.

Recovery of Zebrafish from Estrogenic Effects

Administration of estrogenic substances during sexual differentiation of zebrafish results in a reversible feminization which indicates an arrest of testicular differentiation taking place at the nonfunctional ovarian-like period **(Fenske *et al.*, 2005)**. It was demonstrated that the arrested gonads of male are transformed from the ovarian like into a testicular tissue by stopping estrogen exposure. This depends on the duration and the concentration of exposure to estrogen (**Segner *et al.*, 2006; Schafers *et al.*, 2007)**.

Baumann *et al.* (2014) exposed zebrafish to the discontinued l7∞-ethinylestradiol (EE2) effects through the phase including sexual differentiation and the end of gonad maturation. **Baumann *et al.* (2014)** reported that EE2 can exert feminization and inhibition affecting zebrafish sexual development. In addition, they showed no response on the mRNA expression of brain aromatase (cyp 19b), but they record a significant elevation in vitellogenin levels, an inhibition in body growth and maturation of gonads that occurred in both fish sex. Besides, **Baumann *et al.* (2014)** indicated that sex ratios were directed to undifferentiated and female individuals. After 40 days of recovery, all those EE2 effects were found reversed under zebrafish development and feminization **(Baumann *et al.*, 2014)**. Undifferentiated zebrafish possesses a more pliable gonad development since once estrogen exposure has been stopped, fish can resume development consistent with its genetic sex **(Fenske *et al.*, 2005)**. **Larsen and Baatrup (2010)** and **Morthorst *et al.* (2010)** added that this process depends on the specific mechanism of EDC since the androgenic effects is more persistent than estrogenic effects. Moreover, **Schafers *et al.* (2007)** stated that

zebrafish exposed to 10 ng/L EE2 can be recovered until 75 dpf, however prolonged exposure to 177 dpf led to a continuous inhibition of reproduction **(Baumann *et al.*, 2014)**. It can be concluded that the inhibitory and feminizing impact of EE2 due to endocrine disruption are considered reversible, while lethal pathological alterations are irreversible. In addition, the recovery process in zebrafish is determined by time, period and concentration of exposure **(Baumann *et al.*, 2014)**. Remarkably, the recorded reversibility occurring at mRNA and protein levels is linked to morphology of gonads. Although zebrafish had the obtained female sex ratio of 70:30, the declined maturity indices as well as the elevation of VTG levels in zebrafish during continuous exposure of EE2 at higher concentrations, a regular 50:50 ratio and normal levels of VTG were obtained in their corresponding belonging to recovery groups. Furthermore, **Baumann *et al.* (2013)** investigated the reversibility phenomenon of endocrine disruption in zebrafish at various impacts by using three types of EDCs selected consistent with their mechanisms of action. These EDCs were EE2 selected as a semi-synthetic estrogen 17β-trenbolone chosen as an anabolic steroid and prochloraz which is a fungicide, is characterized by the inhibition of p450 evaluated along the different stages of zebrafish development. Half of the fish were subjected to a continuous exposure until 100 days post hatch, and the other half was kept after 60 days of exposure in clean water. For prochloraz and trenbolone, **Baumann *et al.* (2013)** observed that nearly all effects didn't change in clean water after 40 days of depuration. However, these chemicals affected sex ratio creating an irreversible shift towards fish males, in addition to the permanent effects on VTG, growth and aromatase levels. It was shown that EE2 can only exert a strong reversibility of those effects. These findings revealed that the sexual growth of zebrafish is a very sensitive process that can easily be impaired by environmental pollutants. Consequently, **Baumann *et al.* (2013)** assessed that the intervallic exposure to those chemicals can provoke serious alterations for humans and wildlife.

CONCLUSION

The current review supports the necessity to control the exposure to EDCs in order to avoid their harmful effects on aquatic organisms as well as on humans and wildlife. Concerning the variety of test approaches, the usage of biomarkers and the evaluation of various biological effects seem to be very meaningful to study endocrine disruption. While, "classic" end-points such as gonadal histopathology in addition to effects on growth and sex ratio must be examined to clarify the adverse consequence of an EDC pathway. In this article, it was confirmed that the exposure of zebrafish to EDCs at various developmental stages can explain important insights on the effects of EDC on human health.

It can be concluded that the research on EDC using zebrafish model remains mostly underexplored. Currently, the enormous technical opportunities presented by

zebrafish to study the role of functional genome on the interactions between gene and environment and to associate between the alteration in gene expression in zebrafish and disease used in biomedical research seem to be poorly used in the research of EDC. It is expected that the utilization of zebrafish model in the field of EDC study will ultimately receive more attention.

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