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Review Article

Histopathological Effects of Environmental Pollutants on the Reproductive System of Zebrafish

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ABSTRACT

Environmental pollutants can affect living and non-living organisms in terrestrial and aquatic ecosystems and cause health problems. Many harmful substances that are increasingly used today directly or indirectly cause deterioration in the quality of life of living beings. Water-borne pollution primarily affects fish health. Due to pollutants, fish that feed, migrate, and reproduce in aquatic environments adversely affect their physiological, biochemical, and anatomical structure. Zebrafish are an essential model organism due to their development and easy reproduction. Many potential pollutants can reduce the quality of water in aquatic environments. Organic or inorganic pollutants released into marine environments from natural, industrial, domestic, etc., sources can be toxic to fish. Pesticides, one of these pollutants, are used to eliminate the effects of harmful pests. Still, they have also been shown to have negative effects on the reproductive system, as well as causing death in animals. Heavy metals can have a negative impact on the biological systems of fish by causing biological accumulations through the respiratory intake, affecting biological systems. Endocrine-disrupting chemicals (EDCs) can affect different tissues and organs, causing intergenerational impacts, and their results on the reproductive system increase due to their impact on the hormonal system. Nanoparticles also affect other biological systems in addition to the reproductive system. This review article discusses the evaluation of the histological effects of environmental pollutants on the reproductive system of zebrafish (*Danio rerio*) and the reasons for choosing zebrafish as a model organism.

Keywords: *Zebrafish, Toxicity, Pollution, Gonad, Histopathology*

Çevresel Kirleticilerin Zebra Balığı Üreme Sistemi Üzerindeki Etkileri

ÖZET

Çevresel kirleticiler, karasal ve sucul ekosistemlerdeki canlı ya da cansız varlıklara etki etmekle beraber sağlık sorunlarına da yol açmaktadır. Günümüzde kullanımı artan pek çok zararlı içerikli madde doğrudan ya da dolaylı olarak canlıların yaşam kalitesinde bozulmalara neden olmaktadır. Su kaynaklı meydana gelen kirlilikler öncelikle balık sağlığını etkilemektedir. Akuatik çevrede beslenen, göç eden, üreyen balıkların kirleticiler nedeni ile fizyolojik, biyokimyasal, anatomik yapılarında olumsuz etkiler gözlenmektedir. Zebra balığı, gelişimi ve kolay üremesi sayesinde önemli bir model organizmadır. Su ortamında suyun kalitesini düşüren pek çok potansiyel kirletici bulunmaktadır. Su ortamına doğal, endüstriyel, evsel vb. kaynaklardan atılan organik veya inorganik kökenli kirleticiler balıklar için toksik etkiye sebep olabilir. Bu kirleticilerden biri sayılan pestisitler, zararlı pestlerin etkilerini yok etmede kullanılırken canlılarda ölüme yol açmakla beraber pek çok sistem gibi üreme sistemindeki olumsuz etkileri ortaya konmuştur. Ağır metaller, balıkların en çok solunum yoluyla vücuda alınmasıyla biyolojik birikimlere yol açarak biyolojik sistemlerini olumsuz etkilemektedir. Endokrin bozucu kimyasallar (EBK), farklı doku ve organlarda etki göstererek nesiller boyu etkilenmeyi sağlamakla beraber hormonal sistemi etkilemesiyle üreme sistemi üzerindeki etkileri artmaktadır. Nanopartiküller, üreme sistemi ile birlikte farklı biyolojik sistemlere de etki etmektedir. Bu derleme makalede çevresel kirleticilerin zebra balığı (*Danio reiro)* üreme sistemi üzerindeki histolojik etkilerinin değerlendirilmesi ile beraber zebra balığının model organizma olarak seçilmesinin nedenleri anlatılmaktadır.

Anahtar Kelimeler: *Zebra balığı, Toksisite, Kirlilik, Gonad, Histopatoloji*

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I. INTRODUCTION

Today, with the acceleration of population growth, the needs of people are increasing in parallel. It is observed that the effects of chemicals that are harmful to the environment, used in agricultural and industrial areas, are increasing daily. Environmental pollutants are chemicals that pollute aquatic and terrestrial ecosystems, cause physical damage or affect biological systems. Examples of environmental pollutants are pesticides, heavy metals, nanoparticles, and endocrine-disrupting chemicals (EBC) [2]-[5]-[88].

Pesticides are used to destroy pests in agriculture and to obtain quality products. Pesticides can cause product increase by destroying target organisms and also cause damage to other non-target organisms. For this reason, pesticide types vary according to the living group that needs to be prevented [5]. Since ancient times, heavy metals have spread to the soil, atmosphere, hydrosphere, and pedosphere outside natural cycles. Water and air pollutants that occur during the discharge of industrial wastes are mixed with the ground by chemical means. As industrialization increases, heavy metal pollution increases [69]. These metals and their compounds are found in different concentrations in the earth’s crust [77]. Heavy metals, which are of great importance in environmental pollution, cannot be created or destroyed by humans and are separated from other toxic chemical elements [69]. Endocrine-disrupting chemicals (EBC) disrupt the function of the endocrine system, create negative effects on organs and systems and affect different systems with different mechanisms. When the effects of endocrine-disrupting chemicals (EBC) on the environment and people are investigated, they change the normal functioning of the endocrine system and cause harmful effects on the individual or the next generations [88]. Zebrafish play an essential role in evaluating the effects of endocrine disruptors and examining the mechanisms they affect. In addition to the positive effects of zebrafish being a model organism, it is easier to determine the molecular points of the effects of endocrine disruptors thanks to many studies on its toxicity. Understanding the endocrine system characteristics of zebrafish makes it possible to explain the effects of endocrine-disrupting substances on other vertebrate species and the transformation of changes in gene expression into diseases [91]. Nanoparticles are important substances used in industrial and scientific studies due to their particular structures and atomic arrangements that can be changed according to their purpose. The particles exhibit different chemical and physical properties at the nanoscale because they have different arrangement geometries, even though they have the same atoms in the macro-size particles. These particles enter the cell differently, creating a toxic effect [42].

II. MODEL ORGANISM: ZEBRAFISH AND ITS ADVANTAGES

Zebrafish is an important vertebrate model with many advantages. It can be produced in a laboratory environment, used in developmental biology studies, at low cost, without occupying much space, and continues its production [5]. Zebrafish, which are low in cost, are preferred more than other mammalian models in the laboratory environment due to their short life cycle, high reproductive capacity, and ability to develop outside the body, as well as their rapid embryonic development and many offspring. They allow the larval cycle to be monitored from the outside [3]-[37].

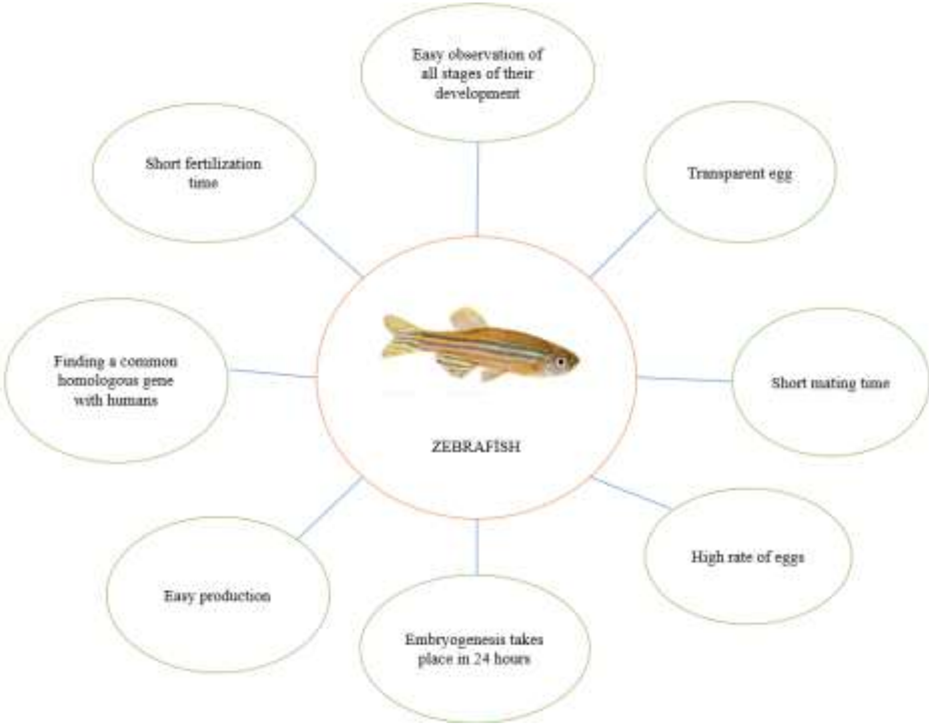

Figure 1. *Advantages of Zebrafish*

Zebrafish is preferred as a model organism in terms of its properties and is frequently used in organ, tissue, and cellular toxicity studies of environmental pollutants or drugs in toxicology [3]-[5]. The choice of zebrafish as a model organism for research is that it is estimated to have functional homologs of more than 70% of the genes related to human disease but are highly genetically similar to humans [5]. One of the most critical features in the selection of model organisms is that they are similar to human genes and are easy to produce under in vitro conditions [37].

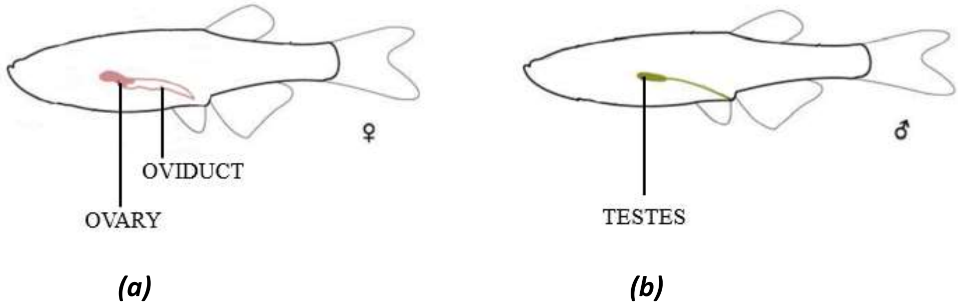

Figure 2. *(a) Zebrafish ovary, (b) Zebrafish testes.*

III. ENVIRONMENTAL POLLUTANTS

A. EFFECT OF ENDOCRINE DISRUPTORS

Since endocrine disruptors are very common in the aquatic environment, they can negatively affect the development and health of living things [91]. Endocrine-disrupting chemicals (EBC) are defined as substances or mixtures of substances taken from outside, which may affect the normal functioning of the endocrine system and have negative effects on organs and systems, as well as affect different systems with different mechanisms, altering the functioning of the endocrine system and causing health problems in the future generation or subpopulations of a healthy organism [15]-[88].

A. 1. Administration Of Endocrine Disruptors To Zebrafısh Affects Gonad Development:

A. 1. 1. Bisphenols

Bisphenol A (BPA), one of the strongest endocrine disruptors, is found in adhesives, cosmetics, paints, and food packaging, as well as in the structure of many industrial products [87]-[88]. In a study conducted with Bisphenol A, it was observed that certain degeneration signs and cell numbers decreased in the germinal epithelium and spermatozoa. In contrast, the number of Sertoli cells increased in light microscopy examination due to the increase in the Bisphenol A (BPA) dose in the testicular tissue of male zebrafish. In the examination with electron microscopy, it was observed that cell components changed, cell degeneration, an increase in Sertoli cells, and a decrease in spermatozoa cells and spermatogonia cells depending on the dose increase. And it was stated that the fish had no death during their experiment [50]. According to Akbulut and Yon [3], while an increase of atretic follicles was seen depending on the dose increase and the exposure day, oocyte development stopped deformities with reductions in the number of oocytes, irregularities in the nucleolus, and degenerations in the chromatin material and the oocytes of the ovaries were observed. Degeneration in oocyte morphology, enlargement of the region between the vitelline membrane and the zona radiata, and vacuolization in cortical alveoli were detected. However, in this study, it is said that BPA inhibits gametogenesis as a result of histopathological changes in the ovaries, according to the results of the treatment group. A different study by Yon and Akbulut [94] has reported the observation of similar effects. In addition to previous studies, it was observed that upon application of BPA to the ovary of zebrafish, fragmentation and cytoplasmic irregularities occurred in mature oocytes with an increase in dose. A slowdown in the oogenesis process accompanied these histopathological effects. Forner-Piquer et al. [24] observed that there was a decrease in the number of cells in the area where the spermatogonia cells are located, depending on the dose increase of the Bisphenol A (BPA) substance on the ECS (endocannabinoid system) in gonad tissues of zebrafish. At the same time, there was no change in cell number in the area occupied by the spermatozoa, and there was no increase or decrease in cells in the ovarian tissues. The changes did not affect the number of previtellogenic and mature oocytes in the vitellogenic stage but significantly increased the oocyte ratio. In their study with Bisphenol A, Molina et al. [95] reported that there was an increase in degeneration and vacuolization of the cytoplasm and structural changes in the nuclei of the eggs of female zebrafish, as well as a significant increase in the number of atretic follicles with an increase in the dose. In another study, Molina et al. [96] reported that atresia increased in cortical follicles, vitellogenic follicles, and mature follicles in ovarian tissue. Molina et al. [56] showed that by applying bisphenol A (BPA) to female zebrafish, vacuolization in the peripheral region of primordial follicles, and reductions in vitellogenic vesicles and lipid droplets in vitellogenic and mature follicles. In addition to these effects, a decrease in the ratio of mature follicles and vitellogenic follicles was observed. When looking at studies conducted with different substances along with Bisphenol A, Keiter et al. [97] examined the resulting gonadal changes in new generations resulting from the combination of Bisphenol A (BPA) and perfluorooctane sulfonate (PFOS). It was found that there was no change in testicular maturation and Leydig cells in male individuals in the F1 and F2 generations of zebrafish. In the F2 generation, a perinuclear oocyte was observed in only one male individual. No change was seen in F1 and F2 female individuals compared to the control group. In another study, Fang et al. [98] stated that there was no histopathological or stereological change in ovarian and testicular tissue upon application of different concentrations of Bisphenol A (BPA) in the presence of TiO2 [98]. In another research conducted by Giommi et al. [99], it was found that exposure to BPA caused a decrease in the number of spermatogonia in male individuals, but no change was observed in the number of developing oocytes in females. In a different study, it was observed that exposure to Bisphenol A (BPA) in male zebrafish resulted in an increase in the number of sperm as well as an increase in the number of apoptotic cells [100]-[101]-[102]. Bisphenol A (BPA) also affects spermatogenesis in male zebrafish [100]-[102]. According to Mostari et al. [107], applying BPA to zebrafish decreased the number of previtellogenic oocytes and spermatozoa-containing cysts in the male testes and shrinkage of the female ovaries. it has been reported that Bisphenol A (BPA) suppresses oogenesis and spermatogenesis. Molina et al. [108] reported that there were a large number of gonadotropic cells in the tissues of zebrafish exposed to BPA.

Bisphenol S (BPS), a derivative of the bisphenol family, is a xenoestrogens substance, but it is known that it disrupts the female reproductive system and adversely affects the health of living things. Qin et al. [66] observed that it causes lipid accumulation in female zebrafish. A decrease in oocytes and an increase in cortical follicles and vitellogenic oocytes were observed.

Bisphenol F (BPF), another bisphenol derivative, is used more and more for industrial production. Mu et al. [57], when looking at the gonads of zebrafish, it was observed that although there was no pathological finding in males, the fertility rate decreased. In contrast, transcriptional changes were observed in the gene axes of the reproductive endocrine system in female gonads. It causes reproductive toxicity in both sexes, affects the number of ovulations, reduces the reproductive rate, and thus reduces the number of embryos.

Yang et al. [103] have found that exposure to Bisphenol B (BPB) results in the expansion of the interstitial area between tubules and an acellular area in the testes of male zebrafish. At the same time, there is a significant decrease in the number of mature oocytes. In female individuals, post-vitellogenic oocytes were not observed in the ovary.

Yang et al. [104] have also studied Bisphenol AF (BPAF). In the results of the application, in male zebrafish exposed to high doses of BPAF, there were areas of no cells in the testis tissue and a decrease in the number of spermatids. In the ovarian tissues of female zebrafish, it was seen that the majority of cells were primary oocytes, with fewer being matured oocytes. It has been revealed that BPAF substance suppresses ovarian maturation.

Floren-9-bisphenol (BPFL), a member of the bisphenol family, is a substance used as an alternative to Bisphenol A [106]. In a study by Özkan-Kotiloğlu et al. [106], it was observed that degeneration of Leydig cells occurred in the testis tissue as a result of application to zebrafish.

A. 1. 2. Ethinylestradiol

Considering the substances in contraceptive drugs, Ethinylestradiol [36]-[88], which is a potent estrogen, 17α-Ethinylestradiol (EE2) and Fadrozole substances were applied to the gonads of zebrafish by Luzio [52]. Atretic oocyte and degenerative mineralization were observed in the ovaries with fluid deposits. Interstitial proteinaceous fluid, asynchronous development, enlargement of sperm ducts, adipocyte infiltration, and basement membrane separation were observed in testis tissue. Pathological severe findings such as intersex-interstitial changes associated with the separation of the basement membrane and asynchronous development were observed in high-dose Fadrozole exposure; when zebrafish is combined with EE2, it permanently impairs sexual development, causes enlargement of sperm ducts, triggers masculinization. Kernen et al. [38] observed that the sexual development of zebrafish exposed to EE2 is affected. Unlike other studies, it is observed that there is a significant decrease in thymocyte count during the period from puberty to sexual maturity and thymocyte loss with low levels of thymocyte count throughout this time. No histopathological effect was observed. In their study, Luzio et al. [113] reported that male individuals exposed to 17α- ethinylestradiol, Fadrozol, and their mixtures for 60 days from the second hour of zebrafish fertilization did not show the development of young ovaries. It was observed that individuals exposed to Fadrozol underwent masculinization. It was found that the binary mixtures of these substances play a role in supporting the growth of the gonad in female zebrafish, similar to estrogen. Örn et al. [109] stated that only previtellogenic oocytes in female zebrafish (38 dph) exposed to a dose of 10 ng/L of 17α-Ethinylestradiol were present in the ovary. In male individuals, it was stated that the percentage of spermatozoa increased compared to the control group after exposure to 50 ng/L of 17β-Trenbolone. Schuster et al. [110] found that adding the substance 17α-Ethinylestradiol (EE2) to zebrafish ovary tissue at rates of 100 ng L-1 and 25 ng L-1 resulted in fish mortality, with four deaths in the high dose and 1 in the low dose. In addition to the deaths, it was noted that the number of vitellogenic oocytes increased in the high dose. In their study, Silva et al. [111] observed that after applying Ethinylestradiol to zebrafish, all cells in the testis tissue of male fish were not observed, while Leydig cells were observed in the stroma. However, a decrease in the number of spermatozoa and spermatids was detected. Exposure to Ethinylestradiol suppressed egg maturation in females. Fibrosis, formation of protein-rich fluid, and inflammatory tissue were observed in the intestinal tissue. Intestinal fibrosis was observed to increase in both sexes. Exposure to the estrogen receptor agonist Ethinylestradiol caused a decrease in the proportion of cortical, vitellogenic, and mature oocytes, thereby affecting follicular development [112].

A. 1. 3. Progesterones

When progesterone is studied separately, a vast literature appears. In this review, we have examined some articles investigating the histopathological effects of zebrafish.

Progesterone, a natural steroid hormone produced by humans and animals, has affected oocyte maturation in the histological structure of the zebrafish ovary and caused changes such as an increase in the number of postovulatory follicles and the observation of protein-rich fluid with an increase in dose [124].

Medroxyprogesterone Acetate (MPA), one of the progesterone groups that pose potential androgenic risks for fish populations, was synthesized to show a similar effect of progesterone. It is used in birth control pills and hormone treatments [70]. As a result of the research of Shi et al. [70], they observed poses androgenic risks, and there are chronic elevations in the gonad tissues of zebrafish, however, it significantly affects sex differentiation and spermatogenesis. It was observed that the number of spermatocyte cells in testicular tissues increased. Zhao et al. [125] did not observe a significant difference in perinuclear oocytes, early vitellogenic oocytes, and late vitellogenic oocytes in female zebrafish exposed to the synthetic progestins Medroxyprogesterone Acetate (MPA) and Dydrogesterone (DDG) and their mixture. However, it was noted that there was a decrease in the proportion of cortical alveolar oocytes at high concentrations of Dydrogesterone and both high and low concentrations of the mixture. Atretic oocytes and degenerations, such as postovulatory oocytes, were observed at high concentrations of Dydrogesterone and the combination. Immature sperm was observed in male individuals at low levels.

Teigeler et al. [127] found that levonorgestrel did not cause hyperplasia, hypertrophy, or a change in the number of spermatogonia in the histological structure of the zebrafish testis. At a dose of 1.64 ng levonorgestrel/L in the female zebrafish ovary, atretic oocytes were formed. Shi et al. [128] found that exposure to dydrogesterone increased the number of atretic follicles in female zebrafish, while there was an increase in the proportion of mature sperm in male individuals.

To examine the effects of non-synthetic forms of progesterone on aquatic organisms and their impact on the endocrine system of fish due to their widespread presence in aquatic environments, Hou et al. [29], investigated that with the application of Norethindrone (NET) to the ovarian tissue of female zebrafish, it was observed that atretic follicular, late vitellogenic oocyte and cortical alveolar oocytes were formed. It was observed that the number of cortical alveolar oocyte cells decreased at 36.2 ng/L NET exposure, atretic follicles and late vitellogenic follicular cells increased, and the number of cortical alveolar oocytes decreased at 398.6 ng/L NET exposure. It has been shown that mating behavior is impaired by applying NET to male zebrafish. In their study, Liang et al. [126] exposed zebrafish to norethindrone (NET) for 90 days. As a result of exposure, perinuclear oocytes were observed in the 810 ng/L dose group, while no late/mature oocytes were observed in the ovary tissues of the 84 ng/L dose group. It has been reported that there was a significant expansion in the surface areas of mature spermatids, mature spermatozoa, and spermatocytes in the testis tissues.

A. 1. 4. Tributyltin

It has been determined that Tributyltin, which is used in dyeing the lower part of the ships and is found in antifouling paints [88], is applied to the testicular tissue of male zebrafish, causing an increase in spermatocyte and a decrease in spermatogonia and spermatozoa, depending on the dose increase [43]. However, it was observed that a few lacunae formations were formed. Phthalates, which are found in substances such as children's products, food packaging, cosmetics, drugs, and cleaning products, are endocrine-disrupting environmental pollutants that people are exposed to due to their plasticizing properties [87]-[88]. Xiao et al. [130] reported that TBT administered for 28 days reduced the number of eggs in female zebrafish, decreased the hatching ability, and increased the mortality rate of zebrafish larvae. In addition, it was observed that the number of early-stage follicles in various stages of development in the ovaries increased, while the number of mid/late-stage follicles decreased. A significant decrease in the number of follicles was observed between the fish treated with TBT and the control group.

A. 1. 5. Phthalates

Chen et al. [13], investigated the exposure of Dibutyl phthalate (DBP), Diisobutyl phthalate (DiBP), and their mixture, the MİX group, in testicular tissue of male zebrafish; delay in tissue development, increased in spermatocyte count and decrease in spermatozoa count are observed in DBP exposure; expansion of intracellular spaces was observed in DiBP exposure. Delayed development of testes of fish in the DBP-H experimental group and at the same time an increase in intercellular spaces occurred simultaneously. A reduction in spermatocyte and spermatozoa cells was observed. It was observed that the groups with the most testicular damage were in the MIX group. Hu et al. [115] showed in their study that dibutyl phthalate (DBP) suppresses male gonadal development in F1 offspring, reduces the number of sperm, and affects the seminiferous tubules, with an increase in Leydig cells. Chen et al. [116] studied the use of dibutyl phthalate (DBP) and 17α-ethynyl estradiol (EE_2) as a combination and individually was observed that there was a decrease in the number of spermatozoa and an increase in the number of spermatogonia and spermatocytes in the testis tissues of zebrafish exposed to DBP, and a decrease in the number of vitellogenic oocytes in the female ovary, with an increase in dose. Exposure to EE_2 resulted in a decrease in the number of mature sperm in male testis tissues and the absence of mature eggs in female ovaries. In the combination group, there was a decrease in the ooplasm and abnormalities in the ovary. Hu et al. [117] reported in their study that the application of di-n-butyl phthalate (DBP) to zebrafish resulted in a decrease in the number of eggs and impaired reproductive potential. It was observed that the number of vitellogenic oocytes in the female ovary tissue increased with an increase in dose, but the number of perinuclear oocytes decreased. When the testis tissues were examined, there was a significant increase in the number of spermatocytes and a significant decrease in the number of spermatids. Guo et al. [118] used a combination of three phthalate derivatives: di (2-ethylhexyl) phthalate, dibutyl phthalate, and acetyl tributyl citrate the development of perinucleolar oocytes and early cortical alveolar oocytes in ovarian tissues is not well observed. In addition, fragmentation, hyalinization, and hypertrophy of perifollicular cells in the zona radiata have been observed. Histopathological findings in testis tissues were more severe compared to those in ovarian tissues. Upon examination of these effects, impairments in spermatogonia and spermatocytes, undetermined cytoplasms, and a decrease in the number of Sertoli cells occurred. Spermatozoa are examined as the smallest cells within the spermatogenic cells.

Uren-Webster et al. [119] observed that in male zebrafish exposed to 50 and 5000 mg DEHP kg^{-1} concentration of di(2-ethylhexyl) phthalate (DEHP) alone, the number of germ cells and spermatozoa decreased while the number of spermatocytes increased. As a result, it has been reported that DEHP disrupts spermatogenesis. Santangeli et al. [120] found that when the female zebrafish were exposed to Di-isononyl phthalate (DiNP), a different type of phthalate, the number of vitellogenic oocytes decreased in low dose groups (0.42 mg/L, 4.2 mg/L, 42 mg/L), but no changes were observed in high dose groups (420 mg/L and 4200 mg/L). In the 0.42 mg/L, 420 mg/L, and 4200 mg/L dose groups, a smaller number of mature oocytes were observed compared to the other dose groups. Bis-(2-ethylhexyl) phthalate (DEHP) is a plasticizer that is used to increase the flexibility of plastic materials and has been shown to negatively affect reproductive and developmental processes in mammals [121]. Corradetti et al. [121] found that after three weeks of exposure to DEHP and 17β-Estradiol, there was an enlargement in the spermatid cysts and spermatogonial areas in male zebrafish. There has been a reduction in spermatocyte cysts.

A. 1. 6. Semircarbazide

Semircarbazide, a member of a large group of compounds called hydrazines, in which cancer-causing agents are commonly found, is known to cause toxic effects in male reproductive cells and have an estrogenic effect [90]. In a study done by Yu et al. [90], sperms packed with spaces in the seminiferous tubules of the testicular tissue of zebrafish were seen in exposure to the SMC substance. As the dose increased, it was observed that testicular tissues degenerated, spermatocytes were irregular, tunica propria, underwent structural deterioration and decreased sperm count at 1000 g/mL^{-1} exposure.

A. 1. 7. Methylparaben

It has been investigated that methylparaben, which is used as a preservative in various products and poses a threat to fish in the aquatic environment, inhibits testicular development by producing estrogenic and antispermatogenic activities in the testis tissue by applying it to zebrafish. Changes in the seminiferous tubules, atrophy of the germ cells, reduction in Sertoli cells, and signs of vacuolar degeneration were observed. As the dose increased, alteration in the seminiferous tubules, vacuolization in Sertoli cells, an increase in the number of gonocytes, and Leydig cells with small cytoplasm and small irregular nuclei were observed. It has been stated that there is an increase in the general atrophy of the testis [25]. In another study by Hassanzadeh [122], it was found that in the ovarian tissues of females, there were disruptions in the ooplasm in the interstitial space, disrupted yolk, granulomatous inflammation, and disruptions in the proteinaceous fluid. In addition to these findings, there was an increase in the number of atretic follicles and vitellogenic oocyte atrophy in the female ovarian tissues. Continuous exposure to methylparaben results in increased negative, estrogenic, and irreversible effects on the reproductive system of female zebrafish. In a different study, Hu et al. [123] observed that in male zebrafish, the number of spermatozoa decreased and the number of spermatogonia and spermatocytes increased with increasing doses of the chemical. In female zebrafish, the number of primary oocytes increases with dose, while the number of other oocytes (cortical-alveolar oocyte, early vitellogenic oocyte, and mature oocyte) decreases. When these effects are considered, it is observed that spermatogenesis and oogenesis stages are seriously disturbed and inhibited.

A. 1. 8. Tris Phosphate

TBOEP is an increasingly used and important environmental contaminant due to its negative effects on reproductive toxicity and disruption of the endocrine system. According to the study conducted by Huang et al. [131], TBOEP has negative effects on the development of the male and female reproductive systems. In female zebrafish exposed to tris(2-butoxy ethyl) phosphate (TBOEP), the number of previtellogenic oocytes decreased while the number of oogonia increased. In male zebrafish, spermatogonia counts increased with dose, while spermatid counts decreased in the 100 mg/L dose group. In a study conducted by Xu et al. [85], after the application of Tris (2-butoxy ethyl) phosphate (TBOEP) to zebrafish, the concentration of 17β-estradiol increased in both sexes. In addition, testosterone increase was observed only in male zebrafish. Depending on the dose increase, an increase in the number of oogonia and previtellogenic oocytes in females, no change in the number of spermatogonia in males, an increase in the number of spermatocytes, and a decrease in the number of spermatids were observed.

Tris (2-chloroethyl) phosphate (TCEP) is one of the environmental pollutants that occur in aquatic environments and belongs to the group of tris phosphates. According to Sutha et al. [105], in their study, increasing doses of TCEP in female zebrafish caused atresia, vacuolization, an increase in atretic oocytes, and degenerate oocytes. In male zebrafish, there were decreases in the number of spermatozoa and spermatocytes, and distortion and atrophy of seminiferous tubule epithelium were reported. Exposure to Tris (2-chloroethyl) phosphate (TCEP) resulted in histopathological changes in egg and testis tissues in both male and female zebrafish.

According to a study conducted by Yang et al. [114] using a different phosphate derivative, 2-ethylhexyl diphenyl phosphate (EHDPHP), the number of vitellogenic oocytes decreased with increasing dose, while the number of perinucleolar oocytes increased. EHDPHP application resulted in damage to the ovaries and suppression of oogenesis. In male zebrafish, disturbances have been observed in seminiferous tubules and interstitial cells. Additionally, in all dose groups, there was a decrease in the number of spermatids. Anomalies in the development of sperm cells were observed after exposure to EHDPHP.

A. 1. 9. Dichloroaniline And Methylenedianiline

The substances 3,4-Dichloroaniline (3,4-DCA) and 4,40-Methylenedianiline (4,40-MDA), which are put to use in the production of most consumer or industrial outputs, are miscible with water. Considering the toxicological effects of these two aniline substances used in this research by Bhujyan et al. [8], testosterone and 17β-estradiol (E_2) levels of both female and male zebrafish decreased and their reproductive potential could be impaired.

A. 1. 10. Metochlors

Ou-yang et al. [60], stated that metolachlor and S-metolachlor substances were applied to 5-month-old zebrafish. It was observed that estradiol (E_2) levels increased with changes in reproductive gene expression in female zebrafish and testosterone levels decreased in male zebrafish.

A. 1. 11. Tebuconazole

Tebuconazole is a common fungicide that affects the endocrine system of organisms in aquatic ecosystems [45]. In this study, Li et al. [45] applied 3 different life stages and their effects on the endocrine system were examined. In juvenile zebrafish exposed to tebuconazole, a reduction in germ cells of sexually mature zebrafish and male-directed sex differentiation were observed. It has been revealed that the 17β-estradiol/testosterone ratio decreases at all life stages and therefore inhibits Cyp19 gene expression.

According to a study conducted by Jiang and colleagues [129], after the application of a mixture of Tebukonazol (TEB) and Difenokonazol (DIF) for 21 days, the number of perinuclear oocytes and cortical alveol oocytes increased in female zebrafish, the number of early vitellogenic oocytes decreased only in the DIF and mixture group, and the number of late/mature vitellogenic oocytes decreased in the TEB, DIF, and mixture group. "In the testis tissue of male zebrafish, an increase in the number of spermatogonia occurred only in the group treated with DIF, while a decrease in the number of spermatocytes occurred. The number of spermatocytes increased in the TEB and mixture groups. In addition to these effects, a decrease in the number of spermatids and spermatozoa was observed in the TEB, DIF, and mixture groups. Except for the single exposure of the TEB and DIF groups, late oogenesis and late spermatogenesis occurred in the mixture group.

A. 1. 12. Cypermethrin

Beta-Cypermethrin is a Type-2 synthetic pyrethroid insecticide frequently used in pest control. Lu et al. [51], in their research, observed differences in 17β-estradiol (E2) and testosterone (T) in the gonads of zebrafish. With the results obtained, it has been observed that it harms the reproductive system of zebrafish by impairing sex hormone secretion and affecting HPG gene expression, as it delays gonadal development.

We can directly or indirectly take endocrine disruptors into our body system in many ways, especially in the environment we live in and the tools and equipment we use in our daily life. Thanks to the use of zebrafish as a model organism in the studies, these effects can be revealed to some extent (Table 1).

Table 1. *Histopathological effects of some endocrine disruptors on zebrafish gonads.*

ENDOCRINE INSTRUCTIONS USED	ORGAN-TISSUE	DAMAGES SEEN	SOURCE
Bisphenol A	Ovary	Increase in atretic follicles, oocyte development arrest, deformation with a decrease in oocytes, irregularities of nucleolus and degenerations of chromatin material, degeneration of oocytes, enlargement of the region between the vitelline membrane and the zona radiata, vacuolization in cortical alveoli	Akbulut and Yon, 2014
	Ovary	Fragmentation and cytoplasmic irregularities occurred in mature oocytes	Yon and Akbulut, 2014
	Ovary	There was an increase in degeneration and vacuolization of the cytoplasm and structural changes in the nuclei of the eggs, a significant increase in the number of atretic follicles	Molina et al., 2013
	Ovary	Atresia increased in cortical follicles, vitellogenic follicles, and mature follicles	Molina et al., 2018
	Ovary	Vacuolization in the peripheral region of primordial follicles, decrease in vitellogenic vesicles in vitellogenic and mature follicles, decrease in lipid droplets, decrease in the ratio of mature follicles and vitellogenic follicles	Molina et al., 2021
	Ovary	Significant increase in oocyte rate	Forner-Piquer et al., 2020
	Testes	Decreased spermatogonia cell numbers	
	Testes	Certain degenerations in the germinal epithelium and spermatozoa and decrease in cell number, an increase in the number of Sertoli cells, a change in cell component ratios, cell degeneration, a decline in spermatozoa and spermatogonia cells	Lora et al., 2016
	Testes-Ovary	A decrease in the number of previtellogenic oocytes and spermatozoa-containing cysts	Mostari et al., 2012

	Testes	A decrease in the number of spermatogonia in male individuals	Giommi et al., 2021
	Ovary-Testes	Female orientation of gonad tissues, increased number of atretic follicles	Song et al., 2020
Bisphenol S	Ovary	Lipid accumulation in tissues, decrease in the number of oocytes, increase in the number of cortical follicles and vitellogenic oocytes	Qin et al., 2021
Bisfenol F	Ovary-Testes	Decreased reproductive potential in males, transcriptional changes in the gene axes of the endocrine system in female gonads, causing reproductive toxicity, affecting the number of ovulations, decrease in reproductive rate, decrease in the number of embryos	Mu et al., 2022
Bisphenol AF(BPAF)	Testes	There were areas of no cells in the testis tissue and a decrease in the number of spermatids	Yang et al., 2016
	Ovary	The majority of cells were primary oocytes with fewer being matured oocytes.	
Bisphenol B(BPB)	Testes	In the expansion of the interstitial area between tubules and an acellular area	Yang et al., 2017
	Ovary	A significant decrease in the number of mature oocytes	
Floren-9-bisphenol (BPFL)	Testes	Degeneration of Leydig cells occurred	Özkan-Kotiloğlu et al., 2022
Ethinylestradiol (EE_2) and Fadrozole	Ovary	Observation of interstitial proteinaceous fluid deposits, increase in atretic oocytes, observed degenerative mineralization	Luzio, 2016
	Testes	Observation of interstitial proteinaceous fluid deposits, enlargement of sperm ducts, adipocyte infiltration, separation of the basement membrane, the spread of sperm ducts, masculinization, interstitial alterations, asynchronous development	
17α-Ethinylestradiol (EE_2)	Gonad	Effect on sexual development, decrease in thymocyte count	Kernen et al., 2022
	Ovary	The number of vitellogenic oocytes increased	Schuster et al., 2019
	Testes	A decrease in the number of spermatozoa and spermatids	Silva et al., 2012
	Ovary	Fibrosis, formation of protein-rich fluid, and inflammatory tissue were observed in the intestinal tissue	
17α-Ethinylestradiol and 17β-Trenbolone	Ovary	Only previtellogenic oocytes were present in the ovary	Örn et al., 2006
Medroxyprogesterone	Gonad	Gender differentiation	

Acetate (MPA)	Teste	Effect on spermatogenesis, increase in spermatocyte cell number	Shi et al., 2019
Medroxyprogesterone Acetate (MPA) and Dydrogesterone (DDG)	Ovary	there was a decrease in the proportion of cortical alveolar oocytes at high concentrations of Dydrogesterone and both high and low concentrations of the mixture.	Zhao et al., 2015
Levonorgestrel	Ovary	At a dose of 1.64 ng levonorgestrel/L in the female zebrafish ovary, atretic oocytes were formed.	Teigeler et al., 2021
Dydrogesterone	Ovary	An increase in the number of atretic follicles	Shi et al., 2018
	Testes	An increase in the proportion of mature sperm	
Tributyltin	Testes	Increase in spermatocyte count, decrease in spermatogonia and spermatozoa count	Lan et al., 2020
	Ovary	The number of early-stage follicles in various stages of development in the ovaries increased, while the number of mid/late-stage follicles decreased	Xiao et al.,2018
Dibutyl phthalate (DBP)	Testes	Reduces the number of sperm, and affects the seminiferous tubules, with an increase in Leydig cells	Hu et al., 2010
	Ovary	The number of vitellogenic oocytes increased	Hu et al., 2020
	Testes	A significant increase in the number of spermatocytes and a significant decrease in the number of spermatids	
Dibutyl phthalate (DBP) and Diisobutyl phthalate (DiBP)	Testes (DBP)	Delay in developmental stages, increase in spermatocyte count, decrease in spermatozoa count	Chen et al., 2020
	Testes (DBP-H)	Decreased number of spermatocytes and spermatozoa cells	
	Testes (DiBP)	Enlargements in intracellular spaces	
Dibutyl phthalate (DBP) and 17α-ethynyl estradiol (EE_2)	Testes	Decrease in the number of spermatozoa and an increase in the number of spermatogonia and spermatocytes	Chen et al., 2015
	Ovary	Decrease in the number of vitellogenic oocytes	
Di (2-ethylhexyl) phthalate, dibutyl phthalate, and acetyl tributyl citrate.	Ovary	The development of perinucleolar oocytes and early cortical alveolar oocytes in ovarian tissues is not well observed, fragmentation, hyalinization, and hypertrophy of perifollicular cells in the zona radiata	Guo et al., 2016
	Testes	Impairments in spermatogonia and spermatocytes, undetermined cytoplasms, and a decrease in the number of Sertoli cells. Spermatozoa are examined as the smallest cells within the spermatogenic cells.	
Di(2-Ethylhexyl) phthalate (DEHP)	Testes	The number of germ cells and spermatozoa decreased while the number of spermatocytes increased	Uren-Webster et al., 2010

Di-isononyl phthalate (DiNP)	Ovary	The number of vitellogenic oocytes decreased in low-dose groups, but no changes were observed in high-dose groups. In the 0.42 mg/L, 420 mg/L, and 4200 mg/L dose groups, a smaller number of mature oocytes were observed compared to the other dose groups	Santangeli et al., 2017
Bis-(2-Ethylhexyl) phthalate (DEHP) and 17β-Estradiol	Testes	An enlargement in the spermatid cysts and spermatogonial areas, a reduction in spermatocyte cysts.	Corradetti et al., 2013
Semircarbazide(SMC)	Testes	Sperm packed with spaces in the seminiferous tubules, degeneration, irregularities in spermatocytes, structurally deformed tunica propria and decreased sperm count	Yu et al., 2017
Methylparaben	Testes	Inhibition of testicular development, atrophy of germ cells, Decreased number of Sertoli cells, vacuolar degeneration, structural changes in seminiferous tubules, vacuolization of Sertoli cells, increased number of gonocytes, small cytoplasm, and small irregular nuclei in Leydig cells, increased atrophy	Hassanzadeh, 2017
	Ovary	Disruptions in the ooplasm in the interstitial space, disrupted yolk, granulomatous inflammation, and disruptions in the proteinaceous fluid. An increase in the number of atretic follicles and vitellogenic oocyte atrophy	Hassanzadeh 2016
	Testes	The number of spermatozoa decreased and the number of spermatogonia and spermatocytes increased	Hu et al., 2023
	Ovary	The number of primary oocytes increases with dose, while the number of other oocytes decreases.	
Tris (2-butoxy ethyl) phosphate (TBOEP)	Ovary	Increased number of oogonia and previtellogenic oocytes	Xu et al., 2017
	Testes	An increase in spermatocyte count, a decrease in spermatid count	
	Ovary	The number of previtellogenic oocytes decreased while the number of oogonia increased	Huang et al., 2019
	Testes	Spermatogonia counts increased with the dose, while spermatid counts decreased	
Tris (2-chloroethyl) phosphate (TCEP)	Ovary	Caused atresia, vacuolization, an increase in atretic oocytes, and degenerate oocytes	Sutha et al., 2022
	Testes	Decreases in the number of spermatozoa and spermatocytes and distortion and atrophy of seminiferous tubule epithelium	
2-Ethylhexyl diphenyl phosphate (EHDPHP)	Ovary	The number of vitellogenic oocytes decreased while the number of perinucleolar oocytes	Yang et al., 2022

		increased	
	Testes	Disturbances have been observed in seminiferous tubules and interstitial cells, a decrease in the number of spermatids	
Norethindrone (NET)	Ovary	Decrease in cortical alveolar oocyte cells, increase in atretic follicles, and late vitellogenic follicular cells, anomalies in the development of sperm cells	Hou et al., 2020
	Ovary	Perinuclear oocytes were observed in the 810 ng/L dose group, while no late/mature oocytes were observed in the ovary tissues of the 84 ng/L dose group	Liang et al., 2020
	Testes	A significant expansion in the surface areas of mature spermatids, mature spermatozoa, and spermatocytes	
Tebukonazol (TEB) and Difenokonazol (DIF)	Ovary (TEB and DIF)	The number of perinuclear oocytes and cortical alveoli oocytes increased	Jiang et al., 2021
	Ovary (DIF and MIX)	The number of early vitellogenic oocytes decreased	
	Ovary (TEB, DIF, and MIX)	The number of late/mature vitellogenic oocytes decreased	
	Testes (DIF)	An increase in the number of spermatogonia and a decrease in the number of spermatocytes	
	Testes (TEB and MIX)	The number of spermatocytes increased	
	Testes (TEB, DIF and MIX)	A decrease in the number of spermatids and spermatozoa	
	Testes (MIX)	Late oogenesis and late spermatogenesis	

B. SILENT POLLUTANTS: PESTICIDES

Pesticides are the chemicals, which are known as plant protection products used to control disease-causing vectors such as mice and rats, and reduce the disruptive effects of living organisms like insects, weeds, fungi that live on or around animals, human bodies, and plants, decrease the nutritive value of food sources when production, storage, and consumption[5]-[68]. In pesticides, while target and desired pests show selective and specific toxicity; it has minimal toxicity to humans, plants, and animals. However, every pesticide has some degree of toxicity. For this reason, no pesticide is safe for health. But when they are used under certain conditions, their risks are reduced [79].

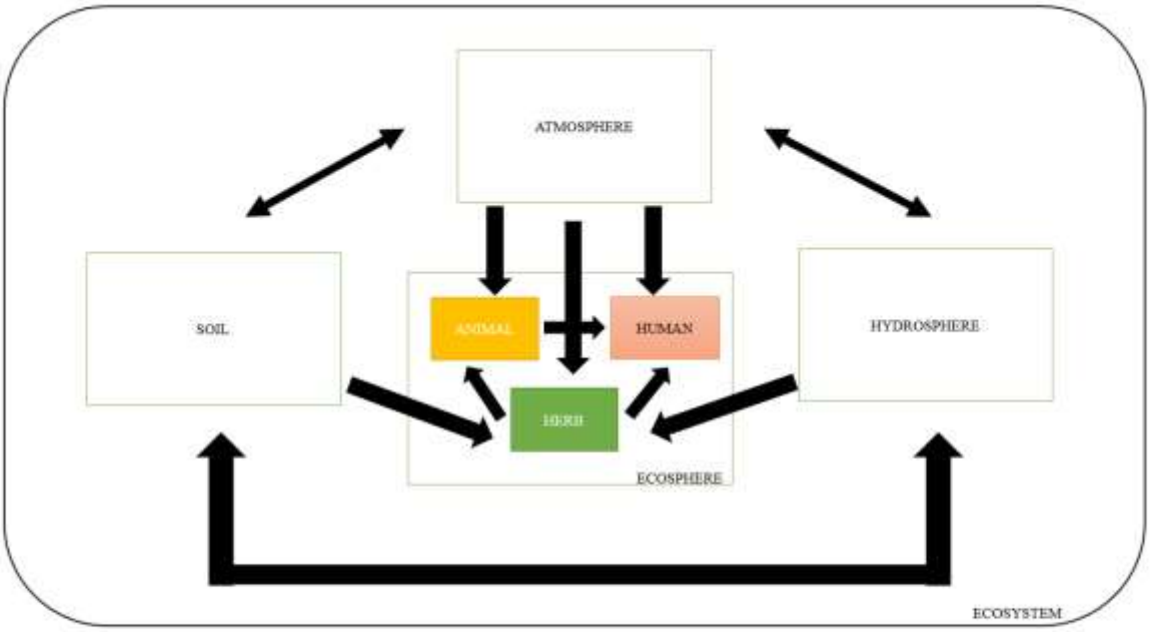

Figure 3. *Transport of Pesticides in the Ecosystem.*

B. 1. HISTOLOGICAL EFFECTS OF PESTICIDES, WHICH ARE INDISPENSABLE FOR AGRICULTURAL LIFE, ON GONADAL TISSUES OF ZEBRAFISH

B. 1. 1. Chlorpyrifos

Chlorpyrifos (CP), which is included in the organophosphorus group of carbamates, is not easily broken down in nature and is also used in the agricultural struggle. Fish is also a substance that is metabolized [71]. In a study about this substance, it was observed the level of vacuolization in the ovary and testes tissues increased depending on the exposure time when applied to adult zebrafish. In addition, the elongation of the seminiferous tubules in male fish was investigated. It was observed that vacuolization increased in both gonad tissues of the ovaries at 96 hours of exposure and degenerative oocyte and follicle atresia occurred in the ovarian tissue of female fish [55].

B. 1. 2. Roundup Wg

The effects on different target and non-target organisms for glyphosate-based herbicides, which are frequently used in global agriculture, are toxicologically significant. In a study by Davico et al. [17], an increase in first ovary follicles, a decrease in late ovarian follicles, and a smaller ovarian follicle diameter were observed for female zebrafish exposed to the glyphosate-based herbicide Roundup WG®(RWG). It was observed that the vitelline membrane in the ovarian follicle became thinner and the vitelline protein content increased in the high-dose groups. In addition, vacuolization in ovarian follicle cells, increases in perivitelline spaces, and impaired mitochondria in oocytes were observed.

B. 1. 3. Spirotetramat

In studies with Spirotetramate, a keto-enol insecticide, it has been reported that it has a toxic effect on living things in aquatic ecosystems [74]. Wu et al. [84], stated that Spirotetramate substance in the ovarian tissues of female zebrafish decreased the diameter of the oocytes, the diameter of the oocytes of female zebrafish exposed to high concentrations became even smaller, and the oocytes in the ovarian tissues became less mature, and most oocytes showed the first three oocyte phases (primary oocyte, cortical-alveolar oocyte, vitellogenic oocyte) but did not show the fourth and fifth oocyte stages (maturation oocyte, mature egg).

B. 1. 4. Pyriproxyfen

Pyriproxyfen is a type of insecticide used to protect cotton crops or other crops from pests. Maharajan et al. [54], stated that the Pyriproxyfen (PPF) substance applied to zebrafish is thought to delay egg development in the decreased E_2/T (estrogen-testosterone) ratio in the reproductive system of zebrafish, and it has been stated that the number of mature oocytes begins to decrease with low E_2 concentration level and insufficient VTG (vitellogenin) production. For this reason, it has been observed that male and female zebrafish gonads can impair the reproductive success of the next generation by showing histopathological destructive effects. A reduction in ripe spermatozoa and vitellogenic oocytes was observed in zebrafish. It has been explained that the number of spermatogonia and spermatid cells changes depending on the dose increase, the number of spermatid cells and spermatozoa cells decreases, and the number of spermatogonia and spermatocyte cells increases. Perinuclear oocytes and alveolar oocytes increased in ovarian tissues, while the number of vitellogenic oocytes decreased with increasing doses.

B. 1. 5. Deltamethrin

In a study conducted with Deltamethrin, a synthetic pyrethroid insecticide, fragmentation of the vesicle structures of the oocytes, irregularities in the cytoplasm, irregularity in the chromatin material of the primary oocytes, shrinkage in the nucleoli, swelling, and a decreased in the number of cortical alveoli, and degeneration in the ooplasm were observed in the ovary tissue of adult female zebrafish in 2 different dose groups. Depending on the dose increase, while the chromatin material degenerated, it was observed that the ooplasm was in a granular structure and there were spaces between it and the vitelline membrane. There was a decline in primary oocytes, their growth arrest, autolysis, degeneration, and deterioration in cortical granules. With these findings, it was observed that the chromatin material was degenerated compared to the zebrafish in the control group, with an increment in the intraovarian connective tissue and atretic follicles, and deterioration of oocyte morphology [40].

Petrovici et al. [63], observed that there was a decrease in the number and size of spermatocytes, and a slight increase in spermatogonia, depending on the dose increase in the testicular tissue of male zebrafish. In some experimental groups, mature spermatozoa were found in the seminiferous tubules. However, in the group with the highest dose, a significant reduction in the seminiferous tubules was observed, as well as a decrease in the size and quantity of sperm cells. While few spermatozoa were observed in the lumen of the seminiferous tubules, infiltration with an acidophilic fluid was observed on the testicular edge of only one zebrafish.

B. 1. 6. Cypermethrin

Cypermethrin, a different substance belonging to the synthetic pyrethroid group, is one of the most common pollutants in freshwater recently and is generally used to control harmful organisms in domestic, agricultural, and industrial areas [92]. Pitchika et al. [64], observed that when 10 $\mu g/l^{-1}$ of cypermethrin was applied to zebrafish, sperm count decreased in male fish and there were no significant changes in plasma vitellogenin levels in both sexes. In conclusion, it was specified that cypermethrin caused spermatotoxicity in zebrafish.

Beta-cypermethrin is a type-2 synthetic pyrethroid insecticide frequently used in pest control. In a study by Lu et al. [51], cortical alveoli increased in the gonads of zebrafish, while early vitellogenic oocytes and mature oocytes decreased in the ovaries. A decrease in spermatids and an increase in spermatocytes and spermatogonia were observed in the testicles.

B. 1. 7. Mancozeb

Abar Gurol et al. [6], applied Mancozeb substance, a type of fungicide, to male zebrafish, and observations were made at 5 ppm exposure. Fusion and fibrosis in the seminiferous tubules, degeneration in growing spermatogenic cell groups, bleeding, and intertubular space formations were observed in the parts between sperms and spermatogenic cells. It was stated that as the dose increased, the seminiferous tubules were irregular and their borders could not be distinguished. Significant signs of vacuolization and edema were observed. In some tubules, very thin basement membranes and hypertrophy of spermatocytes were observed. Clusters of degenerative spermatogenic cells were selected, and some of these cells were found to contain pycnotic or karyolitic nuclei. In some tubules, İt was seen that the developing spermatogenic cells decreased and there were sperm in these tubules only in the lumen region and the sperm count decreased.

B. 1. 8. Flutolanil

Flutolanil, another type of fungicide, was reported by Teng et al. [78], to apply to zebrafish, it was observed that as the dose increased, the number of spermatozoa in the testicular tissue decreased and the seminiferous tubule lumen was destroyed at a dose of 1000 mg/L.

B. 1. 9. Climbazole

Climbazole (CBZ), a different type of fungicide, was reported by Liao et al. [47] and is used in fungal infections and various personal care products. In this study with CBZ substance, it was observed that it significantly affected purine and glutathione metabolism in male zebrafish and accordingly induced testicular cell apoptosis. In addition, it has been observed that it can decrease sperm production by inhibiting the synthesis of basic sex hormones.

B. 1. 10. Tebuconazole And Diphenol Conazole

In a study done by Jiang et al. [33], the MIX group consisting of triazole fungicides tebuconazole (TEB) and diphenol conazole (DIF) substances and mixtures were applied to zebrafish. It was noticed that vitellogenic oocytes decreased in DIF and MIX groups applied in female zebrafish, while late vitellogenic oocytes decreased in TEB, DIF, and MIX groups. In male zebrafish, it was seen that spermatogonia increased and spermatocyte decreased in the applied DIF group, while the spermatids and spermatozoa decreased in TEB, DIF, and MIX exposure. It has been said that fish exposed to the MIX group have rates of late oogenesis and late spermatogenesis.

B. 1. 11. Diazinon

Diazinon, a different pesticide type, is a common pesticide that can affect aquaculture. In the study conducted by Darvishi et al. [16], atrial and hyperplasia follicles were observed along with a decrease in oocyte development depending on the dose increase in female fish with diazinon application to zebrafish. In addition, when the endocrine effect was examined, it was observed that progesterone decreased significantly, but the ratios of 17β-estradiol and testosterone did not change.

Pesticides affect all living forms due to their excessive use in the agricultural sector. It affects the aquatic ecosystem, the terrestrial ecosystem around agricultural areas, the people who are the practitioners, the balance of the habitat, and even all the beneficial or harmful organisms in the environment by passing from the soil to the seas and lakes. These effects may occur after long-term or short-term exposure, or there may be no known effects. For this reason, Zebrafish, a model organism, has been a role model for many researchers to try pesticides. Studies have shown that while pesticides have beneficial effects on their pests, they negatively affect gonad development in zebrafish. Since pesticides are indispensable in our lives, they silently affect all living things (Table 2).

Table 2. *Effects of some pesticide types on zebrafish ovary-testicular tissues.*

PESTICIDE	ORGAN-	DAMAGES SEEN	SOURCE

USED	TISSUE		
Chlorpyrifos (CP)	Ovary	Advanced vacuolization, degenerative oocytes, follicle atresia	Manjunatha, 2015
	Testes	Elongation, vacuolization in seminiferous tubules	
Roundup WG®(RWG)	Ovary	Increase in the number of primary follicles, decrease in the number of late ovarian follicles, decrease in follicle diameter, thinning of the follicle vitelline membrane, increase in vitelline protein content, vacuolization in follicle cells, increase in perivitelline cavities, deterioration in the structure of mitochondria in oocytes	Davico et al., 2021
Spirotetramate	Ovary	Reduction in diameter of oocytes, late maturation in oocytes	Wu et al., 2018
Pyriproxyfen	Ovary	Decreased vitellogenic oocytes, increased perinuclear oocytes, and alveolar oocytes decreased vitellogenic oocytes	Maharajan et al., 2020
	Testes	Decrease in mature spermatozoa, changes in spermatogonia and spermatid cells, decrease in spermatid cells and spermatozoa cells, increase in the number of spermatogonia and spermatocyte cells	
Deltamethrin	Ovary	Disintegration in vesicle structures, irregularities in the cytoplasm, irregularity in the chromatin material of primary oocytes, shrinkage of the nucleoli, degeneration in the ooplasm, degeneration of the chromatin material, decrease in primary oocytes and their growth arrest, autolysis, degeneration, deterioration in cortical granules, degeneration of chromatin material, increase in follicles in the connective tissue, atretic atrophy, impaired oocyte morphology	Koç et al., 2009
Deltamethrin	Testes	Decreased spermatocyte count, increased number of spermatogonia, decreased size and amount of sperm cells, greatly reduced seminiferous tubules, few spermatozoa in the lumen of seminiferous tubules, infiltration with an acidophilic fluid on the testicular edge of only one zebrafish	Petrovici et al., 2020
Cypermethrin	Testes	Decrease in sperm count	Pitchika et al., 2019
Beta-cypermethrin	Ovary	An increase in the number of cortical alveoli, a decrease in early vitellogenic oocytes, and mature oocytes	Lu et al., 2021
	Testes	Decreased spermatid count, increased spermatocyte and spermatogonia count	
Flutolanil	Testes	Decrease in sperm numbers, degeneration of seminiferous tubule lumen	Teng et al., 2020
Mancozeb	Testes	Fusion and fibrosis in the seminiferous tubules, degeneration in growing spermatogenic cell groups, bleeding and intertubular space formations, irregularity and indistinct borders in seminiferous tubules, vacuolization and edema, hypertrophy of spermatocytes, degenerative spermatogenic cell clusters and some of these cells in cytolytic pycnotic or pycnotic cells. In some tubules, there is a decrease in spermatogenic cells developing in some tubules	Arman et al., 2020

		and there is only sperm in the lumen region in these tubules and a decrease in sperm count	
Climbazole	Testes	Inducing testicular cell apoptosis, reducing sperm production by inhibiting the synthesis of essential sex hormones	Liao et al., 2020
Tebuconazole (TEB) Diphenolconazole (DIF) MIX	Ovary(DIF and MIX)	Decreased number of vitellogenic oocytes	Jiang et al., 2021
	Ovary(TEB, DIF, and MIX)	Decreased number of late vitellogenic oocytes	
	Testes (DIF)	Increase in spermatogonia cells, decrease in spermatocyte cells	
	Testes (TEB, DIF, and MIX)	Decreased number of spermatids and spermatozoa	
	Testes-Ovary	Late oogenesis and late spermatogenesis	
Diazinon	Ovary	Decreased oocyte development, increased atresia, and hyperplasia of follicles	Darvishi et al., 2022

C. HEAVY METALS- ACCUMULATION IN MODEL ORGANISM ZEBRAFISH GONADS

Heavy metals, one of the sources of chemical pollution in the aquatic environment, are transition elements in the periodic system. Heavy metals pass into aquatic environments with industrial wastes, fires in forests, dust carried by the wind, volcanic eruptions, residues carried by erosion, domestic wastes, and sewage, and affect the metabolism of living things. Heavy metals mixed with the aquatic environment such as seas, lakes, and rivers cause bioaccumulation in fish. The accumulation level of heavy metals in different tissues of fish varies according to different parameters. These parameters vary according to the amount of heavy metal in the environment, the interaction time of the fish with the heavy metal, the age of the fish, the type of heavy metal, the metabolic activity of the fish, the stage of development, the physicochemical state of the water as well as the tissues and organs. In the aquatic environment where they live, fish take heavy metals from the external environment through gills, food, and skin [4]-[61].

C. 1. Fluoride

Li et al. [44], stated that in a study conducted with fluoride, which is naturally found in the earth's crust, it caused the dissolution of the ovarian follicular membrane and hypertrophic oocytes in female zebrafish at 18.60 mg/L exposure for 30 days in the gonad tissues of zebrafish. And at 36.83 mg/L exposure, severe deformations and atretic follicles were observed in ovarian tissues. It was observed that atretic follicles increased at 18.60 mg/L exposure for 60 days and there were more serious dissolutions in the follicular membrane at 36.83 mg/L exposure. In male fish, on the other hand, as the day and exposure rate increased, it was observed that the cell membrane structure was loosened and destroyed, and there were acellular areas in the testis tissues at 36.83 mg/L exposure for 60 days.

C. 2. Copper

Copper (Cu) element, which is one of the vital heavy metals found in surface waters, negatively affects the living things in the aquatic ecosystem, and it is necessary for red blood cells and many oxidation and reduction processes in animals and humans [10]. Cao et al. [10], reported that it was found that the number of spermatogonia in the testis tissue of zebrafish exposed to copper decreased, and the number of primordial follicles increased, the number of primary follicles, secondary follicles, and mature follicles decreased in female fish depending on the dose increase. On the other hand, it was observed that the number of spermatozoa in the testis tissue decreased. It was observed that the number of mature follicles in the ovarian tissue of female zebrafish exposed to low doses for 30 days decreased, and as the dose increased, the number of secondary and mature follicles in the ovary tissue of female fish decreased, while the number of spermatocytes and spermatozoa in the testicular tissue of male fishes decreased. As the day and dose increased, it was stated that there was no death in the experimental groups. It has been observed that zebrafish exposed to copper(Cu) adversely affect gonadal development and damage the gonad structure. It has been said that it changes the gene expressions related to the endocrine system in the gonads and also affects the reproductive biology of zebrafish of both sexes. Observing the conclusion of this study shows was revealed that copper causes histopathological effects in zebrafish gonads.

C. 3. Uranium

Uranium [15] is included in the heavy metals group in endocrine-disrupting chemicals (EBC). Although there are not many studies on uranium, Barillet[35], stated in his study, that in the exposure of water-based uranium to the gonad tissue of adult zebrafish; uranium material was not found in the testes tissues of fish exposed to normal uranium, it was observed that there was uranium in the testicles of fish exposed to Depleted Uranium (DU) or DU-233U mixture. Nuclear vacuoles and changes in spermatozoa were observed in spermatozoa nuclei. In addition to these effects, it has been observed that it does not cause death since it is not very toxic to fish. Simon et al. [72], on the other hand, stated/observed that application of depleted uranium (DU) to zebrafish did not cause any histological effect in the ovaries of female fish, but it caused changes in the seminiferous tubule structure and cyst formation in the testicles of male fish. However, vacuolization in Sertoli cells and deterioration in Leydig cells, and cluster formations in the intertubular space were observed. When the fish were re-treated, the intensity of uranium's impact decreased, but its negative effects continued to be seen.

C. 4. Thallium

The element thallium, which is very rare in nature, is widely found in the aquatic ecosystem. Thus, it has negative effects on human health, especially on aquatic organisms [28]. Research done by Hou et al. [28], observed that there was a decrease in degenerated oocytes and mature oocytes in zebrafish ovaries administered thallium. According to the studies of the researchers, heavy metals that mixed with the aquatic ecosystem and accumulated caused negative effects on the zebrafish gonads. In addition to their histopathological effects, some heavy metals (copper) have been observed to affect gene expressions related to the endocrine system in the gonads. In this review, in which various heavy metals are discussed, it can be said that heavy metals in nature have a negative effect, considering the effect on zebrafish. (Table 3)

Table 3. *Histological effects of some heavy metals on zebrafish tissues.*

HEAVY METAL USED	ORGAN-TISSUE	DAMAGES SEEN	SOURCE
Fluoride(F^-)	Ovary	Increase in hypertrophic and atretic oocytes, Dissolution in follicular membrane	Li et al., 2015
	Testes	Loose and damaged tissues in the cell membrane, increase in acellular areas	
Copper (Cu)	Ovary	Decrease in the number of follicles	Cao et al., 2019
	Testes	Decreased spermatozoa count, decreased spermatozoa count, decreased spermatocyte count	

Uranium	Testes	No pathological findings	Barillet, 2010
Depleted Uranium (DU) DU-233U	Testes	Nuclear vacuoles in spermatozoa nuclei, changes in spermatozoa	
Depleted Uranium (DU)	Ovary	No histopathological findings	Simon et al., 2018
	Testes	Cyst formation with changes in the seminiferous tubule structure over time, vacuolization in Sertoli cells, deterioration in Leydig cells, cluster formations in the intertubular space	
Thallium	Ovary	Degenerated oocytes, decreased number of mature oocytes	Hou et al., 2017

D. NANOPARTICLES- TOXIC EFFECTS OF VARIOUS NANOPARTICLES ON MODEL ORGANISM ZEBRAFISH

For more than two decades, work in the field of nanotechnology has attracted attention with its revolutionary promises, including medicine [30]. Nanotechnology is an important technology used in various industrial fields today [11]. In the agriculture and food industry, its applications are increasing day by day, from phytosanitary products to ingredients included in foodstuffs or food packaging [30]. Commercial use and uncontrolled release of nanoparticles may pose significant risks to environmental pollution [11]. Nanoparticles are deliberately surrounded by bioconjugates that target specific cells. These bioconjugates can be DNA or proteins. Uncoated or coated nanoparticles cause biodegradation in the cell environment and disrupted nanoparticles cause cellular responses. Accumulation of biodegradable nanoparticles in cells may cause pathological changes within the cell, such as disruption of organelle integrity [20]. Although nanoparticle measurement is an important parameter in reproductive toxicity, zebrafish is one of the best model organisms for evaluating reproductive toxicity with the advantage of a high reproductive rate. Thus, nanoparticles may have effects on the reproductive system and fetal development [9]-[75]. Nanotoxicology also is a discipline that reveals important studies that connect many different fields of science like biology, medicine, and toxicology [11]-[19]-[83]. Zebrafish is an important model organism for toxicity measurement of nanoparticles due to its high reproductive rate, and these nanoparticles can affect fetal development in both male and female fish [11]-[81].

D. 1. Titanium Dioxide

Titanium dioxide (TiO2) nanoparticle is used in the purification of water by cleaning micropollutants, pesticides, dyes, and organic pollutants. In addition to its benefits, it can pose a health risk to humans and animals as a result of contact. Toxic and degenerative effects have been observed in animal experiments [22]-[93]. Titanium dioxide nanoparticles are the most common and commercial substance used in personal care products that we use in daily life such as sunscreens, soap, and shampoo. Chronic exposure to titanium dioxide nanoparticles can cause toxic effects on the zebrafish reproductive system [7]-[58]-[76]. In a different study, it was observed that there was DNA damage in zebrafish treated with high doses of titanium dioxide [7]-[67]. Considering the studies in which Titanium dioxide (TiO2) was applied to zebrafish testis tissues; Wang et al. [81], in their research, observed a defect in egg production and follicle formation in female zebrafish. With the findings obtained at the end of the study, they suggested that titanium dioxide could pass to the ovary through blood circulation and directly impair oocyte development. In a different titanium dioxide research, Fang et al. [23] observed an increase in eggs in females during the exposure period. It was observed that egg production decreased in the group treated with titanium dioxide and BPA. In the study conducted by Akbulut and Yön [2], it was observed that when testicular tissues were exposed to low doses of TiO2, there was vacuolization in the seminiferous tubules, the number of spermatogonia decreased, and the number of sperm cells increased. As the dose increased, it was observed that many seminiferous tubules merged and the seminiferous tubules were disrupted, the severity of degeneration increased, the spaces between sperm cells increased, and spermatogonia, spermatocyte, and spermatic cells decreased in TiO2 exposure. In another study, Kotil et al. [41] stated that, due to the increase in the dose of titanium dioxide (TiO2) in testicular tissues, it was observed that in the exposure to TiO2, loss of cristae in the cytoplasm of Sertoli cells, mitochondrial degeneration in spermatogenic cells and spermatozoa, enlargement of perinuclear spaces in spermatids, cytoplasmic organelles degenerated into membrane residues and lysis in Sertoli cells. In these studies, in which TiO2 was applied, changes were observed in the testis tissues of zebrafish, while no death was observed. In a different study by Cheng et. al. [14], the growth effect of Microcystin- LR (MCLR) in the presence of titanium dioxide was examined. Accumulation of both substances has been observed in the gonads of zebrafish. While no significant changes were observed in ovarian and testicular morphology, a significant decrease in estradiol (E2) and testosterone (T) levels was observed, along with a dysregulated gene transcription in the Hypothalamic-Pituitary-Gonadal (HPG) axis. As a result, there was no change in the developmental stages of the gonads of female and male zebrafish. Wang et al. [80] in their study, titanium dioxide (TiO2) resulted in ultrastructural demount of the intercellular junctions in germ cells in male zebrafish testes and a decrease in transepithelial electrical resistance in TM4 cells induced by the PBDEs congener 2, 20, 4, 0 tetrabromodiphenyl ether (BDE47) stated that the damage was exacerbated.

D. 2. Gold Nanoparticles

Gold nanoparticles are widely used in the medical industry thanks to their chemical stability and unique optical properties, and for this reason, they are of great interest in the scientific world [11]-[73]. Besides being used in drug delivery [7]-[46]-[76], immunochromatography diagnostics, optical microscopy, and confocal laser microscopy are also used as imaging particles and are even important substances used for cancer therapy [11]-[21]-[31]-[39]-[48]-[81].

D. 3. Silver Nanoparticles

Dayal et al. [18] in their study, observe many nucleoli in the perinucleolar stage (PS) oocyte nuclei, which caused thread breaks in the ovaries of female zebrafish. It was observed that zona radiata separated in the membranes of oocytes at the vitellogenic and mature stages and became atretic oocytes. Silver nanoparticles are the most studied substances because they are widely used in the cosmetics industry, biosensors, drug delivery systems, and antimicrobial agents [11]-[34]-[62]-[65]-[89]. It is used in medical imaging, bactericidal applications as well as consumer products [1]-[7]. Chen et al. [12], in a study with silver nanoparticles (AgNP), AgNPs similar to oocyte maturation-inducing hormones were observed to induce germinal vesicle destruction (GVBD). It has been observed that $AgNO_3$ has cytotoxic effects on follicle cells. Zebrafish exposure to AgNP(30 mg/mL) and $AgNO_3$(10 mg/mL) doses revealed irregularities, nuclear condensation, and fragmentation in the oocyte cytoplasm. In $AgNO_3$ exposure, it was observed that there was acute vacuolization in the ovarian follicle cells, and swelling in the mitochondria, and due to these results, the follicle cells underwent apoptosis. A study conducted by Orbea et al. [59], observed the ratio of silver nanoparticles increased in the tissues of male zebrafish. Ma et al. [53] stated that the increasing use of silver nanoparticles (AgNP) can cause negative effects on aquatic organisms. As a result of the study, it was observed that the fertility of female zebrafish decreased significantly with the increase of apoptotic cells in the gonads.

D. 4. Silica Nanoparticles

The effects of Silica Nanoparticles (SNP) and Cadmium Chloride ($CdCl_2$), which are major environmental pollutants, Liu et al., [49] in their study observed ovary structure and function changed as a result of exposure of zebrafish to these substances, there were results in a decrease in ovarian quality, a decrease in the number of mature oocytes and a deterioration in offspring development.

Nanoparticle studies in model organism zebrafish are less than in other studies. Nanoparticles, which are included in our lives with the development of technology, can cause toxic effects in fish as well as affect humans by exposure over time. In these studies, the use of zebrafish has been a guide to make it easier for us to predict their effects on other living organisms (Table 4).

Table 4. *Effects of some nanoparticles on zebrafish gonads.*

NANOPARTICLES USED	ORGAN-TISSUE	DAMAGES SEEN	SOURCE
Titanium dioxide (TiO_2)	Ovary	Impaired egg production and follicle formation, impaired oocyte development	Wang et al., 2011
		Increase in the number of eggs	Fang et al., 2015
		In the group administered with BPA, however, egg production decreased	
	Testes	Vacuolization in the seminiferous tubules, decrease in spermatogonia, increase in sperm cells, union in the seminiferous tubules, deterioration of the seminiferous tubules, increase in the severity of degeneration, increase in the spaces between sperm cells, decrease in the number of spermatocyte and spermatic cells	Akbulut and Yön, 2016
		Loss of cristae in the cytoplasm of Sertoli cells, mitochondrial degeneration in spermatogenic cells and spermatozoa, enlargement of perinuclear spaces in spermatids, the degenerative transformation of cytoplasmic organelles into membrane remnants, and necrosis with lysis in Sertoli cells	Kotil et al., 2017
		High rate of autophagy and necrosis in Sertoli cells	Bai and Tang, 2019
		Ultrastructural localization of intercellular junctions of germ cells	Wang et al., (2021
	Ovary-Testes	Irregularity in hormonal level with no effect on the gonads	Cheng et al., 2018
Gold nanoparticles	Ovary	Separation of zona radiata in membranes of vitellogenic and mature oocytes, atretic oocytes	Dayal et al., 2016
Silver nanoparticles	Ovary	Observation of cytotoxic effect in follicle cells, irregularities in the oocyte cytoplasm, nuclear condensation and fragmentation, acute vacuolization in ovarian follicle cells, swelling in mitochondria, apoptosis in follicle cells	Chen et al., 2016
	Testes	Increase in silver nanoparticle ratio	Amaia Orbea et al., 2017
	Gonad	Increase in apoptotic cells	Ma et al., 2018
Silica nanoparticles (SiNP) and cadmium chloride ($CdCl_2$)	Ovary	Change in ovarian structure and function, decrease in ovarian quality, decrease in the number of mature oocytes, impaired offspring development	Pai et al., 2021

IV. CONCLUSIONS

Because of its advantages in determining the effects of environmental pollutants, which are a global problem, zebrafish is a preferred model organism. These pollutants released into the water, have become threatening to many living organisms' health through the ecological cycle. Research on reproductive toxicity in zebrafish may be important for toxicity studies. Toxic data developed on the zebrafish reproductive system due to chemical sources will be useful for applications to be made on this subject. The effects of these chemical pollutants can be determined through studies and the damages they may cause can be prevented.

Today, environmental pollution has increased so much that the results cannot be measured. Environmental pollution has become an important problem with the development and spread of industry; agriculture, industry, and technology areas and the increase of the negative effects of environmental pollutants on terrestrial or aquatic ecosystems. Because of its advantages in determining the effects of environmental pollutants, which are a global problem, zebrafish is a preferred model organism. These pollutants released into the water, have become threatening to many living organisms' health through the ecological cycle. Research on reproductive toxicity in zebrafish may be important for toxicity studies. Toxic data developed on the zebrafish reproductive system due to chemical sources will be useful for applications to be made on this subject. The effects of these chemical pollutants can be determined through studies and the damages they may cause can be prevented. The effects of these chemical pollutants can be determined through studies, and the damage they may cause can be prevented.

Consciously or unconsciously, the release of waste into nature and the pollution of aquatic ecosystems should be prevented. We must reduce the use of these harmful chemicals to provide a clean living environment for all living beings in the ecosystem.

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