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TOXICOLOGICAL EFFECT OF CYPERMETHRIN IN EMBRYOS OF *DANIO RERIO*

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Abstract: The effect of the insecticide cypermethrin on the development of Danio rerio was analyzed. 80 recently hatched live fingerlings were used, which were placed in four Petri dishes with 20 organisms each: distilled water was placed in the control group; group 1, group 2, and group 3 were inoculated with cypermethrin at a dilution of 1 µL/L, 3 µL/L, and 5 µL/L, respectively. The fry were kept for 10 minutes and later they were transferred to other Petri dishes with water from the hatching aquarium, 24 hours later they were observed under a microscope. The control group presented normal development, however, groups 1, 2 and 3 presented damage to the notochord, when comparing the frequencies of malformations in the notochord with the control group 1 ($X2=21.5384$, $gl=1$, $p< 0.05$), group 2 ($X2=40$, $gl=1$, $p< 0.05$) and group 3 ($X2=32.7272$, $gl=1$, $p< 0.05$) presented significant differences with the control. However, when comparing the different dilutions, significant differences were only found between group 1 and group 2 ($X2=7.0588$, $gl=1$ $p< 0.05$), the latter being higher. It is concluded that exposure to cypermethrin in early stages causes damage to the notochord of *Danio rerio.*
Keywords: *Danio rerio*, toxicology, notochord, cypermethrin.

INTRODUCTION

A pesticide is any substance or mixture of substances intended to prevent, destroy or control any pest, including vectors of organisms that cause human or animal diseases, unwanted species of plants or animals that cause harm or interfere in any other way. way in the production, processing, storage, transportation or marketing of food, agricultural products, wood, derivatives thereof or animal feed. Pesticides are classified in various ways, either by the organism they control; insecticides, acaricides, fungicides

and herbicides, the mode of action in which they act, the uses for which they are intended or their chemical composition (Calva and Torres, 1998).

Pyrethroids are modified derivatives of pyrethrins, natural substances obtained from pyrethrum synthesized from the flowers of *Chrysanthemum cinerariaefolium* (Leng, 2008; Sayim et al., 2005). Pyrethroids are generally divided into two groups: Type II has a cyano group on the α-carbon of the rest of the alcohol, while in Type I pyrethroids it is absent (Saillenfait et al., 2015).

Currently, one of the most widely used insecticides is cypermethrin, a type II pyrethroid insecticide widely used in agriculture and veterinary medicine (Shukla and Taneja, 2002), synthesized in 1974 and introduced to the market in 1977 (Jiménez et al. al., 2008).

One of the main arguments for its wide use is its lower toxicity for groups of vertebrates such as mammals compared to organophosphate and organochlorine pesticides (Xie et al., 2022). Unfortunately, the application of these synthetic pyrethrin derivatives in addition to target pests also negatively affects biodiversity both regionally and locally, alters ecosystem functions, and impacts interspecies communication, as it is highly toxic to organisms. non-target such as arthropods, amphibians, fish and other aquatic organisms (Ullah et al., 2018).

Upon contact with the soil, cypermethrin reaches the groundwater table through runoff or leaching and can reach bodies of water such as lakes, streams, streams and other aquatic systems, causing catastrophic effects (Ullah et al., 2018). Cypermethrin is one of the most common contaminants in freshwater systems, where it can reach levels of up to 3 g/L in surface waters (Paravani et al., 2019). In aquatic ecosystems, fish are a group that is especially sensitive to pyrethroid pesticides due to their neurotoxic effects. It has been reported that it negatively affects their central nervous system, causes endocrine toxicity and is a promoter of tumorigenesis, in addition to being lethal in concentrations. much lower than the corresponding values for mammals and birds (Kaur and Singh, 2021). Even if the direct contact time with these chemicals is short, it is enough to produce changes in the erythrocytes in the blood of the fish, generating nuclear and cellular abnormalities such as the formation of micronuclei among others (Khatun et al., 2021).

MUTAGENIC, TERATOGENIC AND CARCINOGENIC EFFECT OF CYPERMETHRIN

When cypermethrin comes into contact with the skin and eyes of an individual it produces irritation and when ingested symptoms of poisoning include hypersensitivity, profuse salivation, abnormal facial sensations, dizziness, headache, nausea, anorexia, fatigue, vomiting, discharge increased stomach upset and in severe cases muscle strains and seizures may occur. It has been reported that the population exposed to cypermethrin in crop fields can develop harmful health effects, such as intense dizziness, nervous, skin, and eye disorders, neonatal deaths, and birth defects (Triana-Velásquez et al., 2017; Hussien at al., 2013).

At a physiological level, cypermethrin produces alterations in the transport of sodium ions, by keeping the channels of this cation open in the nerve cells for seconds when they normally only open a few thousandths of a second after the signal is transmitted, which generates that there is an elongation of the sequence of repetitive impulses in the sensitive organs (Vergara-Chen et al., 2019; Triana-Velásquez et al., 2017). Histologically, cypermethrin has been reported to cause ischemia-associated malformations and

pyknosis generated in the cytoplasm of neurons in rat brain tissues. Likewise, it can form DNA monoadducts and cross-links between DNA strands, which induces damage to it (Hussien at al., 2013; Sayim et al., 2005).

Studies carried out in females exposed to doses of 20 mg/kg/day-1 through oral ingestion of cypermethrin reported damage to the uterus and ovaries, in the latter with loss of oocytes and follicular cells, when intake is prolonged for 4 weeks there is a reduction in mass in the ovaries, in addition to causing toxicity in the liver and kidneys, which makes it difficult to detoxify and break down harmful chemicals such as pesticides (Kaur and Singh, 2021). It is also capable of generating teratogenic effects and hereditary defects caused by mutations in germ cells, these mutagenic effects may not manifest immediately but after several generations, which can increase the genetic load of the population, and subsequently generate carcinogenesis, due to multiple genetic alterations that trigger uncontrolled cell proliferation (Henao et al, 2005).

THE ZEBRAFISH AS A MODEL FOR TERATOGENIC STUDIES

Due to their sensitivity to toxicological agents in the environment, fish are considered an excellent model to evaluate the mutagenic effects of pollutants (Ullah et al., 2018). The Danio rerio or zebrafish is one of the outstanding vertebrate animal models, because it allows us to analyze the genotoxic effects in developmental biology and thus evaluate the changes suffered in the development of organ systems, tissues, neural networks and human diseases. respectively (Yesudhanson et al., 2020). Its use as a model for the study of embryonic developmental toxicity has been steadily increasing. Because it has proven to be a predictive model for the evaluation of the teratogenic and neurotoxic effects of various genotoxic agents (García et al., 2018).

In the early stages of their development they are particularly sensitive to water contamination. For example, the presence of heavy metals in water affects various developmental processes during the embryonic period, resulting in reduced pup quantity and quality, viability, or exerting a direct toxic influence on developing embryos (Jezierska et al., 2009). For example, exposure to the genotoxic tiemetoxan at a concentration of 1.30µg/ml produced malformations in the notochord, lordosis of up to 30°, as well as alterations at the cardiac level and hypertrophy of the caudal fin in newly hatched fry (García et al., 2018). For their part, Jin et al (2011), report that adult Danio rerio organisms suffered hepatic oxidative stress and the activation of genes related to apoptosis such as p53, Apaf1 and Cas3, when exposed to concentrations of 1 µg/L of cypermethrin. during 4 and 8 days, when the insecticide concentration was 3 µg/L or higher, severe DNA damage occurred.

MATERIALS AND METHODS

STUDY ORGANIZATIONS

This research work was carried out in the laboratories of the Xalapa Biology Faculty of the ``Universidad Veracruzana``. The Danio rerio embryos used in this experiment were bred in the facilities of the Xalapa Biology Faculty, of the ``Universidad Veracruzana``. The reproductive organisms were kept in 20-liter fish tanks (40 cm x 20 cm x 25 cm) in a ratio of 3 females and 6 males until spawning (48 hours). to the eggs in the tanks. The physicochemical parameters at which both the parents and the eggs were maintained until hatching were: pH 7, constant temperature 27 °C, with a light/dark photoperiod of 10/14 h. The adult fish were fed twice a day ad libitum with food Lomas basic flakes.

INSECTICIDE

To carry out the experiment, the commercial insecticide Cipermethrin 200 of the Agro IQC brand was used, whose active ingredient is Cipermethrin ((±)-Alpha-Cyano-3-phenoxybenzyl(±)-cis, trans-3-(2,2-dichlorovinyl)-2,2-dimethyl cyclopropane carboxylate)), with a composition of 21.05% by weight equivalent to 200 g of active ingredient. For the experiment, three solutions were made: 1 µL/L, 3 µL/L and 5 µL/L.

EXPERIMENTAL MODEL

Once hatched, using plastic pipettes, 80 live fingerlings were separated into four groups of 20 organisms each, one control and three experimental groups. For the experiments, three different concentrations of cypermethrin (1 µL/L, 3 µL/L and 5 µL/L) were prepared in beakers and distributed in Petri dishes (Figure 1b). In the control group, the fry were kept in a Petri dish in 10 ml of distilled water for 10 minutes; in group 1, the fish were placed in 10 ml of water with cypermethrin in a 1 µL/L dilution for 10 minutes; in group 2 the fry were placed in 10 ml of water with cypermethrin in a 3 µL/L dilution for 10 minutes and in group 3 the fry were kept in 10 ml of water with cypermethrin in a 5 µL/L dilution for 10 minutes.

Subsequently, the fry were placed in another Petri dish with water from the aquarium where they were kept for 24 hours. The fry were observed with an optical microscope to describe if there was any malformation, the organisms were photographed and later preserved in glycerin (Modified from Tellez-Mora et al., 2016).

STATISTIC ANALYSIS

For the analysis of the frequencies obtained, non-parametric chi-square tests were performed using the SigmaPlot 13 software from Softonic.

RESULTS AND DISCUSSION

80 newly hatched fingerlings of Danio rerio divided into 4 groups of 20 organisms were analyzed: in the control group no malformations or phenotypic changes were observed in the 20 fingerlings (figure 1a); in group 1 that was exposed to a concentration of 1 µL/L of cypermethrin, 6 organisms with normal development were observed for 14 that presented damage to the notochord (figure 1c); in group 2 the organisms were exposed to a concentration of 3 µL/L, in the 20 organisms damage was observed in the notochord and one of them died, so the damage in the notochord was observed post mortem; Group 3 was exposed to a concentration of 5 µL/L of cypermethrin, where 2 organisms presented normal development while the remaining 18 presented damage to the notochord.

When performing the statistical analysis with the chi-square test, significant differences were found between the control and the three dilutions, the frequency of organisms with damage to the notochord being significantly higher in the 1 µL/L solutions ($X2=21.5384$, $gl=1$, $p< 0.05$), 3 µL/L ($X2=40$, $gl=1$, $p< 0.05$) and 5 µL/L ($X2=32.7272$, $gl=1$, $p< 0.05$). When comparing the different dilutions, significant differences were only found between the 1 µL/L and 3 µL/L dilutions = $X2=7.0588$, $gl=1$ $p< 0.05$, but not between 1 µL/L and 5 µL/L ($X2= 2.5$, $gl=1$ $p= 0.05$) nor between 3 µL/L and 5 µL/L ($X2= 2.10$, $gl=1$ $p= 0.05$).

Prolonged exposure to cypermethrin has been extensively analyzed, which is why various studies have reported that among its effects are: the development of hereditary genetic diseases, carcinogenesis, and birth defects (Hassien et al., 2013). However, in this work we were able to observe that cypermethrin can induce damage in specialized tissues such as the notochord, and that these damages occur in a short time of exposure to the toxin.

The notochord is a structure that plays

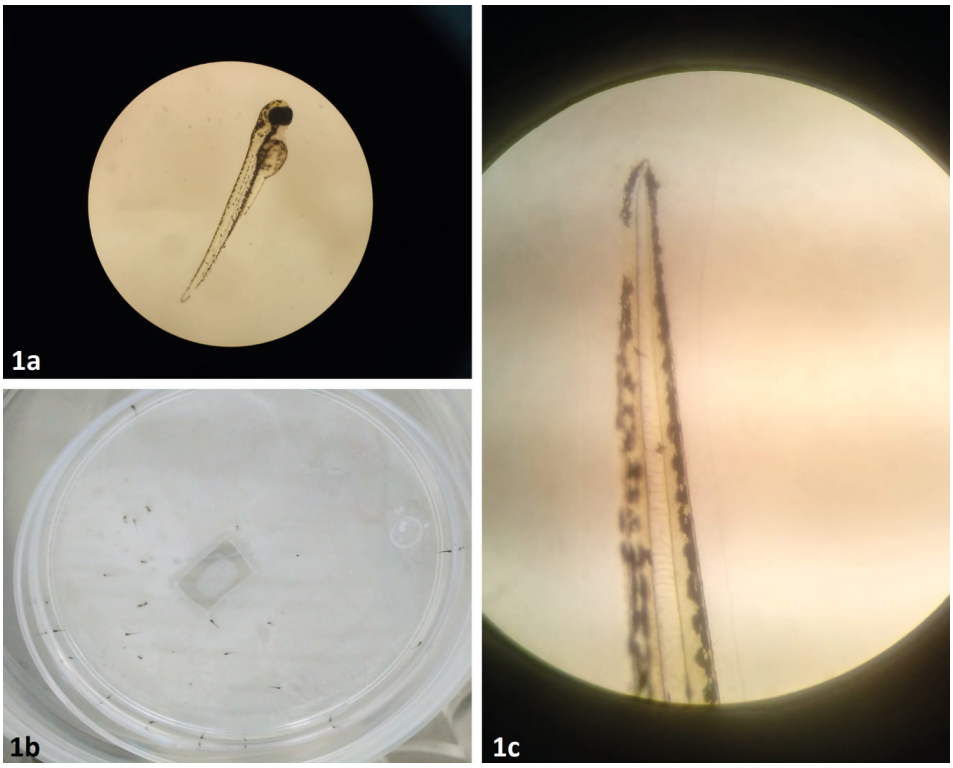

Figure 1. Danio rerio fingerlings during the different treatments. Figure 1a shows a fingerling that was maintained for 10 minutes in the control treatment; Figure 1b shows one of the Petri dishes where the 20 fingerlings were deposited; Figure 1c shows the damage that exposure to cypermethrin produced in the notochord of a fingerling exposed to a concentration of 3 µL/L.

a crucial structural role in embryonic development, since it acts as a hydrostatic skeleton that facilitates locomotion. In the case of Danio rerio, the presence of a differentiated notochord seems to be necessary for the induction of pioneer muscle cells. (Lim et al., 2017; Odenthal et al., 1996). In addition, recent studies have shown that the embryonic notochord has a signaling function that is essential for the correct formation of the vertebral column (Seleit et al., 2020).

Cypermethrin had already been reported as causing malformations in embryos of the Mystus cavasius catfish, Ali et al (2018) report that from a concentration of 8 µg L−1 and in a period of at least 24 hours of exposure, several malformations, such as dark brown and dark yolk sac, eggshell rupture, notochord rupture, as well as unhatched eggs. This teratogenic effect of cypermethrin had already been previously reported by Shi et al (2011) who noted that eggs exposed for 96 hours to concentrations of 25 µg L-1 of cypermethrin present post-hatching malformations in the fry, showing evidence of abnormalities. such as curvature of the body axis, pericardial edema and enlargement of the yolk sac. For their part, Lim et al (2017), associate the damage produced in the notochord with the loss of the Cavin1b protein, which would prevent the synthesis of caveolae. Once synthesized, caveolae are inserted into the plasma membrane, forming a lipid raft rich in sphingolipids, glycosphingolipids, phosphatidylinositol-4,5-bisphosphate associated with microtubules and actin microfilaments (Bastiani and Parton, 2010), so their absence would result in injury generated by adverse membrane failure and cell collapse.

CONCLUSIONS

Through this study it was possible to verify that exposure to cypermethrin in early phases causes damage to the notochord of Danio rerio, even at concentrations as low as 1 µL/L and with a short period of exposure. This damage to the notochord can cause alterations or difficulties in their locomotion, which would mean a handicap for their survival.

The process by which cypermethrin causes these malformations is still not clear, so it is important to carry out more studies that provide us with a better understanding of how these alterations occur.

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