# Assessment of Mercury Bioaccumulation in Zebra Cichlid (*Cichlasoma Nigrofasciatum*) Exposed to Sublethal Concentrations of Permethrin

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## ABSTRACT

**Background:** Aquatic ecosystems are frequently subjected to contamination by toxic heavy metals and pesticides, yet very little is known about the influence of pesticides on bioaccumulation of heavy metals in aquatic organisms. Mercury is a toxic metal with no known biological benefit to organisms. Bioavailability of mercury in aquatic environments depends on biological and non-biological parameters including other pollutants. Therefore, the objectives of this research were to determine the effects of permethrin on bioaccumulation of mercury in zebra cichlid.

**Methods:** Acute toxicity ($LC_{50}$) of permethrin and mercury chloride was evaluated by estimating mortality in Probit Model in SPSS (version 19.0 IBM). In sub-lethal toxicity, zebra cichlid (*Cichlasoma nigrofasciatum*) was exposed to various concentrations of permethrin (0.0, 0.40, 0.80, 1.20 and 1.60 $\mu g.L^{-1}$) combined with 20 $\mu g.L^{-1}$ mercury chloride for 15 days. At the end of the experiment, mercury concentrations were measured using ICP-OES-Perkin elmer (optima 7300-DV).

**Results:** 96 h $LC_{50}$ values of permethrin and mercury for *C. nigrofasciatum* were calculated to be 17.55 $\mu g.L^{-1}$ and 140.38 $\mu g.L^{-1}$, respectively. Our results clearly showed that the bioaccumulation of mercury in the specimens increased with increasing concentrations of permethrin to 1.20 and 1.60 $\mu g.L^{-1}$.

**Conclusion:** Increasing the concentration of permethrin had synergistic effects on the bioaccumulation of mercury in fish.

**Keywords:** Bioaccumulation, Mercury, Permethrin, Zebra cichlid.

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## INTRODUCTION

Mercury is a highly toxic element that is found both naturally and as an introduced contaminant in the environment. Mercury contamination of fish is a natural occurrence exacerbated by human activities [1-3]. In freshwater, there are many biological and non-biological factors including individual characteristics of each species, tropic interactions, source of Hg, physicochemical quality of water, and types and levels of pollutants that have significant effects on the bioavailability and bio-accumulation of mercury in fish [2, 4-7]. Since complete detoxification of mercury in fish is not allowed by inducing metallothioneins [8], the bioaccumulation of mercury is a real threat to the survival of fish. Moreover, mercury bio-concentration increases through food chain [3, 6]. The potential bioaccumulation of mercury in gills and liver of freshwater fish was higher than that in white muscle and heart [9]. Monteiro et al. [9] suggested that the oxidative stress occurred in response to mercury chloride exposure could be the main pathway of toxicity induced by mercury in fish.

Permethrin, 3-phenoxybenzyl-(1Rs) *cis-trans*-3(2, 2-dichlorovinyl)-2, 2 dimethylcyclopropane- carboxylate, is a synthetic pyrethroid insecticide. Exposure to pyrethroid pesticides in aquatic systems may occur through surface and groundwater contamination [10]. Permethrin is a pyrethroid (I) that is relatively persistent in the environment and stable to hydrolysis and photolysis. After the fish are exposed to sub-lethal concentrations of permethrin, it is rapidly absorbed and distributed

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into various tissues [11], and is slowly metabolized and excreted later on, though it may also be accumulated in fat tissues [11]. Glickman et al. [12] found that the concentration of permethrin was approximately 400 times greater in the fish fat than in the ambient water following a 24 hour permethrin exposure.

Studies on animals have indicated that the major steps in the metabolic pathways include hydrolytic and oxidative cleavage of the ester bonds and the formation of 4'-HO-Permethrin [12, 13], which may be conjugated with glucuronide and excrete from the body. The 4′-HO-permethrin has been well documented as a primary metabolite of permethrin in various animals [13]. Final metabolites are often excreted from the body through the bile, urine (as glucuronidated metabolites), and some other metabolites are eliminated by skin, gills or excreted in the feces as glutathione conjugated metabolites [12]. Pyrethroids can block Na channel and affect the function of GABA-receptors in the nervous system. Oxidative stress is another mechanism for the toxicity of insecticides resulting in cellular necrosis and apoptosis and dysfunction in cellular physiology [14-16].

Increased contamination of aquatic ecosystems through industrial and municipal wastewater discharge, surface runoffs and agricultural drainage may affect the bioavailability of heavy metals such as mercury in fishes. Moreover, alterations in cellular membranes and their physiological functions may affect the bioaccumulation of mercury in various tissues. However, there is little information on the effects of other pollutants such as pesticides on heavy metals bioaccumulation in fish. So, this study was conducted to investigate the effects of sub-lethal concentrations of permethrin on the bioaccumulation of mercury in Zebra cichlid (*Cichlasoma nigrofasciatum*).

## MATERIALS AND METHODS

### *Chemicals*

Permethrin, [3-(Phenoxyphenyl) methyl (I)-cis, trans-3-(2,2-dichloroethenyl)-2,2-dimethylcyclo-propanecarboxylate] (emulsified 20%) was purchased from Moshkfam Fars Company, Iran. Mercuric chloride ($HgCl_2$) was obtained from Merk Co. Germany.

### *Fish Treatments*

Zebra cichlid (*Cichlasoma nigrofasciatum*) weighing 0.33 ± 0.02 g were used in the present study and we complied with the instructions of National Ethical Framework for Animal Research in Iran [17].

### *Acute Toxicity*

The preliminary acute toxicity test was performed in accordance with OECD guidelines to estimate the lethal concentration that cause mortality (50%) at 96 h (96-h $LC_{50}$). The fish were exposed to different nominal concentrations of permethrin (0.00, 7.5, 15, 30, 60 and 120 $\mu g.L^{-1}$), and $HgCl_2$ (0.00, 60, 120, 240, 480 and 960 $\mu g.L^{-1}$) at 27±2 °C in a static-renewal system, where water and pesticides were completely replaced every 24 h in 40-liter glass aquaria. During the 96 h acute toxicity experiment, water was aerated and had the same conditions as the acclimation period. The static-renewal tests exposed the specimens to the test solution for 96 h. Test solution was exchanged every 24 hours. Water was changed daily to reduce the buildup of metabolic wastes and to keep concentrations of pesticides or heavy metals close to the nominal level. Fish mortality was recorded 0, 24, 48, 72, and 96 h after exposure to pesticides or heavy metals. $LC_{50}$ values were calculated using Probit Analysis, SPSS (IBM, 19).

### *Sub-Lethal Toxicity*

The specimens were housed as 18 fish per aquaria at 27±2 °C under a daily photoperiod of 16 h light/ 8 h dark and were fed with pellet diet (BioMar Co.). The fish were randomly assigned to nine groups. Group I: specimens were maintained in tap water, and were considered the control group. Group II: specimens were exposed to 20 $\mu g.L^{-1}$ mercury chloride. Group III, IV, V and VI: specimens were exposed to 0.40 $\mu g.L^{-1}$ permethrin & 20 $\mu g.L^{-1}$ $HgCl_2$, 0.80 $\mu g.L^{-1}$ permethrin & 20 $\mu g.L^{-1}$ $HgCl_2$, 1.2 $\mu g.L^{-1}$ permethrin & 20 $\mu g.L^{-1}$ $HgCl_2$, and 1.6 $\mu g.L^{-1}$ permethrin & 20 $\mu g.L^{-1}$ $HgCl_2$, respectively for 15 days. Test solutions were renewed every 24 h. On the other hand, fifty percent of water was

exchanged daily to reduce the buildup of metabolic wastes and to keep concentrations of permethrin and mercury chloride near the nominal level. During the experiment, the fish were fed twice a day with commercial food, and their mortality was recorded.

### *Analysis of Mercury Levels in Different Tissues*

At the end of the experiment, specimens were euthanized by decapitation and the samples were weighed and placed in an electrical furnace to obtain the ashes for 8 hours at 550°C. Then 1 g of ash was mixed with HNO3 and HCl (1:1). In order to separate ash particles, the solution was filtered, mixed with deionized water and diluted to 25 ml.

Mercury concentrations were measured using ICP-OES-Perkin elmer (optima 7300-DV). After drawing a diagram for calibration of Hg, metal levels in these prepared soluble were measured. Samples were filtered using whatman filter (0.22 µm) and finally Hg concentration in each sample was measured with ICP-OES-Perkin elmer. Hg levels were measured in triplicates and measurements were repeated three times.

### *Statistical Analysis*

A significant difference in bioaccumulation of Hg in specimens exposed to different concentrations of permethrin was identified using one-way ANOVA. All the data were examined for normality (Kolmogorov-Smirnov test). The significant means were compared by Duncan's test and a $p < 0.05$ was considered statistically significant. Statistical analyses were performed using SPSS IBM 19. Data are presented as mean ± SD.

## RESULTS

The effects of permethrin and mercury toxicity on *C.nigrofasciatum* were evaluated by using probit analysis and the percentage mortality, LC1-LC99, 95% confidence limits for fish exposed to permethrin and mercury at 24, 48, 72 and 96 hours are presented in Table 1-2. These key data allowed probit lines to be plotted (Figure 1-2). Fish mortality increased with increasing the concentration of permethrin, cadmium and mercury.

**Table 1.** Acute toxicity of Permethrin on *C.nigrofasciatum* at different exposure times.

| Probability | 24h | 48h | 72h | 96h |
|---|---|---|---|---|
| $LC_1$ | 7.03 | 4.20 | 2.97 | 2.25 |
| $LC_{10}$ | 15.01 | 8.94 | 6.98 | 5.66 |
| $LC_{25}$ | 23.32 | 13.86 | 11.48 | 9.67 |
| $LC_{50}$ | 38.07 | 22.57 | 19.96 | 17.55 |
| $LC_{75}$ | 62.15 | 36.75 | 34.68 | 31.84 |
| $LC_{90}$ | 96.59 | 56.99 | 57.02 | 54.44 |
| $LC_{99}$ | 206.33 | 121.27 | 134.20 | 137.02 |

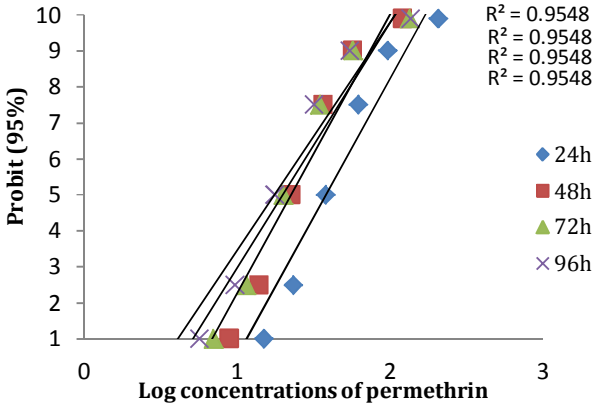


**Figure 1.** Percentage mortality of *C.nigrofasciatum* exposed to various concentrations of permethrin at 24, 48, 72 and 96 hours.

Table 2 summarizes the LC1-LC99 values, (95% confidence limits) for the mercury at 24, 48, 72 and 96 hours of exposure. These key data allowed probit lines to be drawn (Figure 2). Fish mortality increased significantly when the concentration and time of exposure was increased. Mercury chloride is highly toxic to zebra cichlid.

**Table 2.** Acute toxicity of Mercury to *C.nigrofasciatum* at different exposure times.

| Probability | 24h | 48h | 72h | 96h |
|---|---|---|---|---|
| $LC_1$ | 62.74 | 32.23 | 24.95 | 17.98 |
| $LC_{10}$ | 132.85 | 72.91 | 58.03 | 45.25 |
| $LC_{25}$ | 205.44 | 117.15 | 94.75 | 77.36 |
| $LC_{50}$ | 333.45 | 198.43 | 163.37 | 140.38 |
| $LC_{75}$ | 541.22 | 336.08 | 281.68 | 254.73 |
| $LC_{90}$ | 836.94 | 540.01 | 459.95 | 435.51 |
| $LC_{99}$ | 1772.25 | 1221.48 | 1069.56 | 1096.13 |

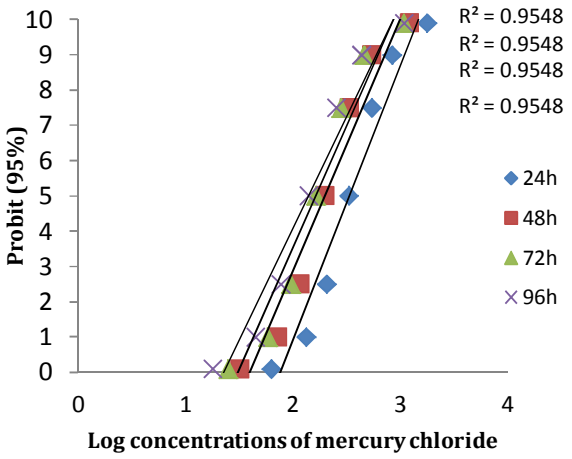


**Figure 2.** Percentage mortality of *C.nigrofasciatum* exposed to various concentrations of mercury chloride at 24, 48, 72 and 96 hours.

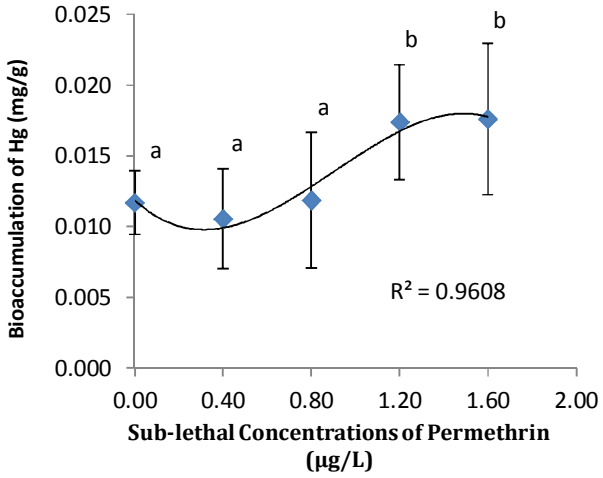


**Figure 3.** Mercury bioaccumulation in the whole body of *C.nigrofasciatum* exposed to sub-lethal concentrations of permethrin.

Throughout the experiment, no behavioral changes and mortality were observed in the control group. During the acute toxicity tests, the fish exposed to toxicants often showed abnormal swimming and lethargy. Mucus secretion increased and the fish swam in a vertical posture and became motionless and died when they could not overcome toxic effects.

Information on mercury bioaccumulation is shown in Table 3 and Figure 3. Although, no significant difference was observed between mercury bioaccumulation in the whole body of fish exposed to 0.40 and 0.80 µg.L$^{-1}$ permethrin mixed with 20 µg.L$^{-1}$ mercury chloride, bioaccumulation of mercury in the specimens increased along with increasing concentrations of permethrin to 1.20 and 1.60 µg.L$^{-1}$.

**Table 3.** Mercury bioaccumulation in the whole body of *C.nigrofasciatum* exposed to sub-lethal concentrations of permethrin.

| Mercury chloride (µg.L$^{-1}$) | Permethrin (µg.L$^{-1}$) | Heavy metal bioaccumulation (mg.g$^{-1}$) |
|---|---|---|
| $HgCL_2$ 20 µg.L$^{-1}$ | 0.00 | 0.012±0.002$^{a}$ |
| | 0.40 | 0.011±0.004$^{a}$ |
| | 0.80 | 0.012±0.005$^{a}$ |
| | 1.20 | 0.017±0.004$^{b}$ |
| | 1.60 | 0.018±0.005$^{b}$ |

## DISCUSSION

The present results show that permethrin is highly toxic to Zebra cichlid (*Cichlasoma nigrofasciatum*). The toxicity of permethrin on *C. nigrofasciatum* increased with increasing concentration and exposure time. When the fish were exposed to 7.5 µg/L permethrin, only 23.33% died after 96 h, whereas all the fish (100%) died after 48 h when exposed to a concentration of 60 µg/L permethrin. In addition, the 24, 48, 72 and 96 h LC50 values of permethrin on C. nigrofasciatum were calculated as 38.07, 22.57, 19.96 and 17.55 µg.L-1, respectively. Başer et al. [18] found out a 48 h $LC_{50}$ value for permethrin in guppy (*Poecilia reticulata*) of 245.7 µg.L$^{-1}$. The 96 h $LC_{50}$ values of permethrin in *Morone saxatilis*, *Pimephales promelas*, *Salmo salar* and *Gambusia affinis* were 15.6, 16, 12 and 15 µg.L$^{-1}$, respectively [Quoted from 18] which are highly comparable to our results. The inability of fish to rapidly hydrolyze permethrin may result in an overall low rate of detoxification and this could be a factor in the fish sensitivity to permethrin [11, 16].

The calculated 24, 48, 72 and 96 h $LC_{50}$ values of mercury chloride, using a static bioassay system to zebra cichlid, were 333.45, 198.43, 163.37 and 140.38 µg.L$^{-1}$, respectively. Our results demonstrate that mercury is highly toxic to *C. nigrofasciatum*. The $LC_{50}$ values of

$HgSO_4$ for *Capoeta fusca* at 24, 48, 72, and 96 h of exposure were 0.32, 0.28, 0.26, and 0.24 mg $L^{-1}$, respectively [19]. The 96 h $LC_{50}$ values of mercury in *Clarias gariepinus, Cyprinus carpio, Barbus conchonius*, *Clarius batrachus* and *Etroplus maculatus* were 600, 930, 181, 510 and 130 $\mu g.L^{-1}$, respectively [20, 21, Quoted from 22]. The degree of susceptibility of zebra cichlid to lower concentrations of mercury may be attributed to differences in the physiological response of fish to this pollutant.

No mortality was observed in fish exposed to sub-lethal concentrations of permethrin and mercury chloride as well as control group during the experimental period. Increased mucus secretion, dark skin pigmentation, loss of appetite, bleeding at the base of the fins and eye balls, behavioral changes such as tremors, lethargy, unbalanced swimming, swimming in surface water and extreme irritability were important changes observed in fish exposed to permethrin and mercury chloride during experimental periods. These changes were intensified at the end of experimental periods. Behavioral changes in fish exposed to permethrin and mercury chloride could be associated with the neurotoxicity of these pollutants. Neurological disorders in animals are often attributed to changes in neuronal ion channels, and key enzymes involved in neurological functions such as $Na^+/K^+$ ATPase activity, monoamine oxidase and acetylcholinesterase activity after exposure to pyrethroid pesticides and mercury chloride [9, 23-25].

The present study reflected an increasing trend in mercury bioaccumulation with increasing concentrations of permethrin. Interaction between permethrin and mercury chloride may increase bioavailability of mercury to fish. Ji [26] suggested that disposal of detergents, shampoo, pesticides, waste; etc to the natural environment will augment the toxicity of mercury by binding to the metal and enhancing its bioavailability, while some will diminish the toxicity by decreasing the bioavailability. Mercury binds to cysteine, and this facilitates mercury's passage through the cellular membrane [26]. Moreover, the inability of fish to detoxify and excrete mercury is another reason for bioaccumulation of mercury in various tissues [9]. Permethrin poisoning may cause oxidative stress which in turn leads to generation of reactive oxygen species that can damage cellular membranes. Hence, cell membrane dysfunction may have a significant effect on the penetration of mercury into the cells. Our literature reviews revealed that permethrin exhibited significant oxidative stress and cytotoxicity in fishes [11, 16]. In other words, increasing the concentration of permethrin had reinforcing effects on the bioaccumulation of mercury in fish.

## CONCLUSION

Investigating the exposure of zebra cichlid to permethrin and mercury highlighted the high toxicity of these compounds by the mortality recorded in the poisonous fish. Our results showed that the presence of different concentrations of permethrin in aquatic ecosystems could increase bioaccumulation of mercury in fish inhabiting polluted water. This study also demonstrated the necessity to regulate the disposal of municipal and industrial wastewater and agrochemical substances to the surface waters.

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