

SETAC – Brazil

Comparative Studies of Delthametrin Acute Toxicity in Exotic and Brazilian Fish

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ABSTRACT

Deltamethrin (2,2-dimetil-3-(2,2-dibromovinil)-ciclopropilcarboxilato alpha-cianofenoxibenzilo), a type II pyrethroid, is used in agricultural applications such as fruits and vegetables plantations, and as maidservants in the combat to fleas, ticks and mange on pets. In the present study, tests of acute toxicity were performed with this pesticide in commercial formulation for the species *Danio rerio*, *Hyphessobrycon bifasciatus*, *Geophagus brasiliensis* and *Oreochromis niloticus* with the objective of determining the values of lethal concentration 50% (LC 50), 48h. The tests demonstrated similar toxicity to the pesticide for *D. rerio* and *H. bifasciatus*. The values of LC50;48h is 0.078 $\mu\text{g L}^{-1}$ to *D. rerio* and 0.082 $\mu\text{g L}^{-1}$ for *H. bifasciatus* with 95% confidence limits 0.05-0.11 μL^{-1} for both. The tests performed with the species *G. brasiliensis* presented values of LC 50;48h of 0.594 $\mu\text{g L}^{-1}$ with 95% confidence limits 0.33-0.94 $\mu\text{g L}^{-1}$. The species *O. niloticus* presented in the tests the largest values of LC50;48h among the used species, 0.954 $\mu\text{g L}^{-1}$ with 95% confidence limits 0.71-1.22 $\mu\text{g L}^{-1}$. The tests demonstrated a great toxicity of the pesticide for all tested species of the fishes, demonstrating that the contamination of aquatic environmental can cause great fish slaughter in a short period of time, indicating the require of special attention in relation to its use.

Key word: fish, deltamethrin, *Danio rerio*, *H. bifasciatus*, *G. brasiliensis*, *O. niloticus* acute toxicity.

RESUMO

Comparação da toxicidade aguda da deltametrina em peixes autóctones e exóticos

Deltametrina (2,2-dimetil-3-(2,2-dibromovinil)-ciclopropilcarboxilato alpha-cianofenoxibenzil) é um praguicida piretróide do tipo II empregado no combate a insetos na agricultura e pecuária no controle de pulgas, carrapatos e outros insetos. No presente trabalho foi estudada a toxicidade aguda de uma formulação comercial deste praguicida nas espécies de peixes *Danio rerio*, *Hyphessobrycon bifasciatus*, *Geophagus brasiliensis* and *Oreochromis niloticus* com o objetivo de determinar os valores da concentração letal 50% (CL50) em 48h. Os testes mostraram toxicidade similar ao pesticida em *D. rerio* e *H. bifasciatus*. Os valores da CL;48h são 0,078 $\mu\text{g L}^{-1}$ para o *D. rerio* e 0,082 $\mu\text{g L}^{-1}$ para o *H. bifasciatus* com limites de 95% de probabilidade 0,05-0,11 μL^{-1} para ambos. Os testes feitos com a espécie *G. brasiliensis* apresentaram valores de CL50;48h de 0,594 $\mu\text{g L}^{-1}$ com limites de 95% de probabilidade de 0,33-0,94 $\mu\text{g L}^{-1}$. A espécie *O. niloticus* apresentou os maiores valores de CL50 entre as espécies estudadas, ou seja, 0,954 $\mu\text{g L}^{-1}$ com limites de confiança de 95% de probabilidade de 0,71-1,22 $\mu\text{g L}^{-1}$. Os resultados indicam que o praguicida é altamente tóxico para as espécies de peixes em estudo e alertam que a contaminação do ambiente aquático pode causar rapidamente a morte destes animais, requerendo atenção especial quando do seu emprego.

Palavras-chave: peixe, deltametrina, *Danio rerio*, *H. bifasciatus*, *G. brasiliensis*, *O. niloticus*, toxicidade aguda.

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INTRODUCTION

Synthetic pyrethroids, a new generation of high bioefficacy insecticides, are gaining wide acceptance for insect control all over the world. These insecticides are pyrethrins analogs, extracts from the *Chrysanthemum cinerariaefolium*, developed to circumvent the rapid photo degradation problem encountered with the insecticidal natural pyrethrins. The pyrethroids are widely used in field pest control and household usage, as veterinary and human pediculicides, and are among the most potent insecticides known (Smith & Stratton, 1986). The widespread use of these pesticides consequently leads to its exposure (and to its possible toxic effects) to manufacturing workers, to field applicators, to the ecosystem and finally to the public. These insecticides are commonly divided into Type I compounds (or T-syndrome pyrethroids), which lack an alpha-cyano substituent, and Type II compounds (or CS-syndrome pyrethroids), which contain an alpha-cyanophenoxybenzyl substituent (Verchoyle & Aldridge, 1980).

In Brazil, deltamethrin (2,2-dimetil-3-(2,2-dibromovinil)-ciclopropilcarboxilate alpha-cianofenoxibenzilo), a type II pyrethroid, is used in agricultural applications, in the fruits and vegetables plantations, as maidservants, in the combat to fleas, ticks and mange on pets. Deltamethrin has been classified as an "immobile" by the US EPA (URL 2). Thence, in the field, most of the affected organisms show rapid recovery.

Pyrethroids are specially advantageous for use in cold climate zones since exhibit a negative temperature coefficient of toxicity. They are also considered relatively non-persistent and, therefore, are not expected to be biomagnified through the food chain. Maximum bioconcentration factors ranged from 698X for whole fish (deltamethrin) to 6090X (bifenthrin) (URL 2). However, pyrethroids have been reported to be extremely toxic to fish and to some beneficial aquatic arthropods, such as lobster and shrimp (Bradbury & Coats, 1989; URL1; Srivastav *et al.*, 1997).

The environmental fate and effects of synthetic pyrethroid insecticides have been summarized by Hill (1989). Due to their lipophilicity, pyrethroids have a high rate of gill absorption, which in turn would be a contributing factor in the fish sensitivity to aqueous pyrethroid exposures. Pyrethroid insecticides are part of ester chemical category. The main reaction involved in the metabolism of these substances in mice and rats is the ester cleavage mainly due to the action of carboxyesterase. Metabolism in fish is largely oxidative (Demoute, 1989). Especially in low temperatures, ectothermic animals, like fish, metabolize less pyrethroids because of the activity of its reduced enzymatic system. Fish make intimate contact with the surrounding water through the gills. After short-term deltamethrin exposure, adult *Heteropneustes fossilis* (freshwater catfish) showed hypocalcemia and the researchers attribute this condition to the possible impairment of either net electrolyte influx at the gill or renal function.

Deltamethrin exposure also caused hypophosphatemia and was linked to the possible redistribution of electrolytes

between intracellular or extracellular compartments and/or impairment of renal function. Deltamethrin may disturb the calcium and phosphate homeostasis and may lead to an effect on the reproductive state of the fish (Srivastav *et al.*, 1997). Synthetic pyrethroids have been shown to be toxic for fish, aquatic arthropods, and honeybees in laboratory tests. Acute toxicity data for deltamethrin in fish have been summarized in a report of the World Health Organization (WHO, 1990) and classified as highly toxic to fish, being in the LC50 1.0 ppb. The potential hazard to fish is due to its heavy use in many aquatic larvicidal programs. Synergistic interactions between the active ingredient and other components of the formulation should be taken into consideration when evaluating toxicity.

The increasing use of pyrethroids interested the society in reducing the damages caused for this class of pesticides in the environment, particularly in relation to the aquatic ecosystems of fundamental importance to the life preservation in the planet. These ecosystems present a series of physical, chemical and biological mechanism of assimilation of the effects of poisonous substances. However, when those reach levels above the assimilative capacity of the environmental, they degrade the quality of the waters, affecting the survival, the growth and the reproduction of aquatic organisms. These facts in turn made the toxicity tests indispensable to the control of pollution sources. This approach allows monitoring the integrated effects of environmental and pollution conditions, besides being efficient in the detection of the pollutant effects present in levels below the capacity of analytical chemical methods.

The fish have been included among the organisms used in toxicity tests, but a small healthy number of species were used in Brazil. Because of its easy obtaining, maintenance and reproduction in laboratory – decisive characteristics in the choice of a species to be used for tests (ISO, 1982) – the specie *Danio rerio* is the most used one. Also, it has a great amount of data in the scientific literature, since it has been used quite often in several countries.

The use of species adapted to autochthonous environmental characteristics certainly could provide much closer results of the reality, when compared to the results obtained with exotic species. This study was conducted to compare the acute toxicity to deltamethrin, a synthetic pyrethroid, in exotic fish species, the *Danio rerio* and *Oreochromis niloticus*, with the brazilian native species *Hyphessobrycon bifasciatus* and *Geophagus brasiliensis*, using the static test system. Therefore, it will be possible to determine the most interesting specie to perform fish toxicity tests in brazilian natural conditions.

MATERIAL AND METHODS

The organisms, with maximum size of 5 cm, obtained directly from the commercial raiser (Izael Ba Hi, city of Indaiatuba, São Paulo State, Brazil), were brought to the laboratory within 30 min in plastic bags with enough air. The

plastic bags were placed into the maintenance aquarium (80 L) for about 30-35 min for acclimatization. Then, the bags were cut open and the fish were allowed to swim into the aquarium water. The animals were maintained by 15 days in laboratory for acclimatization. As water maintenance was used the dechlorinated supply water of São Paulo, maintained in temperature at 23°C (\pm 2°C) by heaters. The water hardness was 42 mg L⁻¹CaCO₃ and pH 7.0 (0.2). The luminous intensity was of 600 lux and the photoperiod of 12h light. Except for dosing instance, all aquaria were aerated, through compressors of air with connected outputs to filtration systems with acrylic wool and active coal, to improve the water quality. Every 7 days, 25% of the total water volume was changed. To fish feeding, the ration Tetramin® (supplied as recommended by the manufacturer and in according to the guidelines of CETESB – 1990) was used. The methodology adopted for the toxicity tests with fish in static system was based in the EPA guidelines (1994) and CETESB (1997).

The solutions for the tests were made with a commercial product (K-Othrine® CE 25-25 g i.a. L⁻¹ of deltamethrin). To determine the interval among the deltamethrin smallest concentration that causes 100% fish immobility and those that did not cause fish immobility, preliminary tests for 48h toxicity were performed.

The test chambers were filled with 3 L of tap water. The aquarium conditions were the same of the maintenance aquarium. The tests had duration of 48h and were held at 23°C (2). During this period the organisms were not fed and the pH variables, dissolved oxygen and conductivity were analyzed at the beginning and at the end of each test. For each species, five tests with five animals each were performed. In each test, five deltamethrin sample concentrations were used and one aquarium with five

animals containing only tap water served as a control. Lethality was controlled 3, 6, 24 and 48h after the start of the tests. Dead was considered when the fish did not demonstrate reaction after being touched in the caudal peduncle. All dead animals were removed immediately from the aquarium. Behavioral and some physiological changes were also observed.

To calculate the toxicity curves as well as to determine the deltamethrin lethal concentration (LC₅₀) in 48h and the 95% confidence limits, the “LC₅₀ Programs JSPear test” was used (Hamilton *et al.*, 1977). To compare the LC 50% of different species, a non parametric ANOVA followed by a multiple comparative test were employed. In all cases results were considered significant for P < 0.05.

RESULTS

The calculated LC₅₀ value for 48h (95% confidence limits) of the commercial deltamethrin product, using a static bioassay, was 0.078 µg L⁻¹ to *D. rerio* (Table 1) and 0.082 µg L⁻¹ to *H. bifasciatus* (Table 2), with 95% confidence limits 0.05-0.11 µg L⁻¹ for both. The specie *G. brasiliensis* presented values of CL₅₀;48h of 0.594 µg L⁻¹ with 95% confidence limits 0.33-0.94 µg L⁻¹(Table 3). The specie *O. niloticus* presented in the tests the largest values of CL₅₀;48h among the used species, 0.954 µg L⁻¹ with 95% confidence limits 0.71-1.22 µg L⁻¹ (Table 4).

The ANOVA showed significant differences between the LC₅₀ of the fish species [F(3/19) = 269.85, P < 0.0001]. The Tukey-Kramer comparison test showed that LC₅₀ of *D. rerio* and *H. bifasciatus* were similar while in the other groups LC₅₀ were different (Figure 1). No mortality was observed in the control group during the experiments.

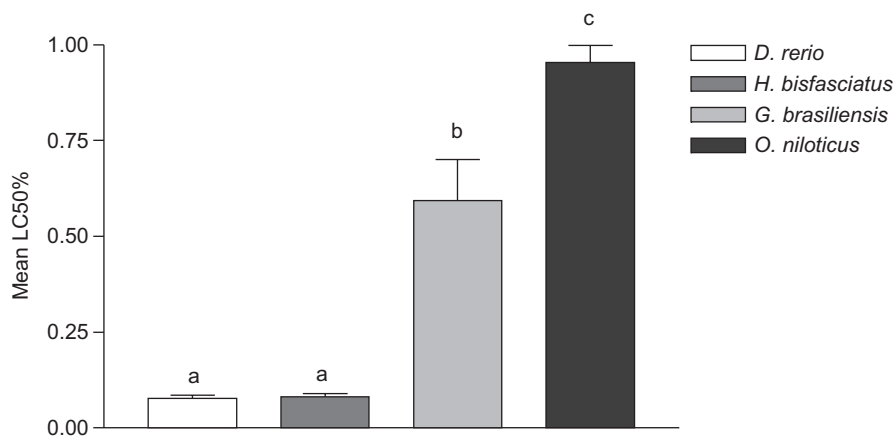


Figure 1 – LC₅₀% of deltamethrin in different species of fishes. Data are presented as means \pm SD. N = 5. Equal letters – without significant differences. P < 0.05 Tukey-Kramer comparative test.

At low deltamethrin doses it was observed behavioral changes, loss of balance, respiratory difficulties, attempt to breathe from the surface and increased opercular frequency.

DISCUSSION

The present results suggested that the pesticide in study is highly poisonous for all the fish species tested. The low toxicity of deltamethrin to mammals may be misleading at this point in ecotoxicological terms and lead to extrapolation problems to aquatic species. The use in agriculture as well as in mosquito control programs, which need frequent sprinkles, emphasize the potential risks to the aquatic system organisms.

USDA National Agricultural Pesticides Impact Assessment Program's EXTOTNET, reported the LC50 deltamethrin for fish between 1.0 and 10 $\mu\text{g L}^{-1}$, but these values refer to the substance in technical degree with 99.9% of active principle. The commercial products composition, as the ones used in this investigation, present in its formulation between other chemical substances, the 2-(2-butoxi-etoxi)-4, 5-metilenodioxo-2-propiltolueno, known as piperonyl butoxide (PPB). This chemical inhibits the pyrethroid degradation, increasing its half-life in the animal organism (Larini, 1999). Thus, the technical and commercial products present a great difference in the acute toxicity level. This fact emphasize the importance of performing the toxicity tests with the commercial form of the product when the objective is to know the possible impact that can be caused to the environment, for the reason that this is the form found in the environmental contamination.

In the literature, the values of toxicity to deltamethrin for several fish species present great differences of values of LC50. Rukiye et al. (2003) showed that the CL50;48h for *Poecilia reticulata* is 5.13 $\mu\text{g L}^{-1}$. For larvae and embryos of *Cyprinus carpio* the values of CL50;48h were 0.213 $\mu\text{g L}^{-1}$ and 0.074 $\mu\text{g L}^{-1}$, respectively, demonstrating the adverse effects of the deltamethrin in the reproduction and in the growth of the species (Köprücü, 2004). An insecticide with deltamethrin

as the active ingredient, used in the control of mosquitoes, is one of the responsible by the great mortality of eels (*Anguilla anguilla*) in the Lake Balaton, Hungary, between 1991 and 1995 (Balint et al., 1997). The presence of this pesticide in tissues of other species as *Abramis bellows*, *Stizostedion lucioperca* and *Larus canus* (Balint et al., 1997) were also found.

Present data show significant differences between the species analyzed. In fact, *D. rerio* and *H. bifasciatus* showed similar LC50. *D. rerio* is used broadly in toxicity tests by several advantages such as low maintenance cost, low space requested, short development period and reproduction (Lele & Krone, 2002). However, it is important that the toxicity of pollutant potentials for autochthonous species is determined providing comparison of the values obtained with the species pattern. The values of DL50;48h obtained for the specie *H. bifasciatus* demonstrate to be the appropriate species for toxicity tests with that substance, because it is sensitive as *D. rerio*, revealing similar toxicity for its natural habitat. The toxicity found for *G. brasiliensis* and *O. niloticus* show that the sensitivity to the pesticide is not similar for fish of the same family, even though homogeneous groups of animals with the same size were used. The great sensitivity difference to deltamethrin found among the four used species was expected, because the *O. niloticus* and *G. brasiliensis* are found at places with pollution levels that did not permit the survival of the *H. bifasciatus*, which has a great sensibility to any alteration in the water quality.

During the tests, the fishes submitted to the several concentrations of pesticides, already in the initial instants, presented different behavior from the fishes of the control group, as increase of the opercular frequencies, irregular swim, mucus formation and constant presence in the surface of the aquarium, what would turn the vulnerable surviving fish to diseases and predators in their habitat. Similar behavior is reported in *P. reticulata*, submitted to solutions containing the pesticide beta-cypermethrin, although in very larger concentrations (Polat et al., 2002).

Table 1 – LC50;48h values of deltamethrin to *Danio rerio*. Data were calculated from tests with 5 animals/test.

Tests	LC50;48h ($\mu\text{g L}^{-1}$)	Confidence interval
1	0.08	0.07-0.10
2	0.07	0.06-0.09
3	0.07	0.05-0.09
4	0.08	0.07-0.10
5	0.09	0.07-0.11
LC50;48h mean	0.078	
Range of toxicity ($\pm 2\sigma$)	0.05 a 0.11	
Standart deviation	0.008367	
Variance coefficient	10.73%	

Table 2 – LC50;48h values of deltamethrin to *Hyphessobrycon bifasciatus*. Data were calculated from 5 tests with 5 animals/test.

Tests	LC50;48h ($\mu\text{g L}^{-1}$)	Confidence interval
1	0.09	0.08-0.11
2	0.07	0.05-0.10
3	0.08	0.07-0.10
4	0.08	0.07-0.10
5	0.09	0.08-0.11
LC50;48h mean	0.082	
Range of toxicity ($\pm 2\sigma$)	0.05 a 0.11	
Standard deviation	0.008637	
Variation Coefficient	10.53%	

Table 3 – LC50;48h values of deltamethrin to *Hyphessobrycon bifasciatus*. Data were calculated from 5 tests with 5 animals/test.

Tests	LC50;48h ($\mu\text{g L}^{-1}$)	Confidence interval
1	0.66	0.51-0.85
2	0.45	0.33-0.61
3	0.63	0.46-0.85
4	0.71	0.53-0.94
5	0.52	0.39-0.69
LC50;48h mean	0.594	
Range of toxicity ($\pm 2\sigma$)	0.33 a 0.94	
Standard deviation	0.106442	
Variation Coefficient	17.91%	

Table 4 – LC50;48h values of deltamethrin to *Oreochromis niloticus*. Data were calculated from 5 tests with 5 animals/test.

Tests	LC50;48h ($\mu\text{g L}^{-1}$)	Confidence interval
1	0.97	0.80-1.19
2	0.95	0.81-1.12
3	1.02	0.85-1.22
4	0.90	0.71-1.13
5	0.93	0.79-1.10
LC50;48h mean	0.954	
Range of toxicity ($\pm 2\sigma$)	0.71 a 1.22	
Standart deviation	0.045056	
Variance coefficient	4.72%	

CONCLUSIONS

In conclusion, the present study indicates high toxicity to the commercial deltamethrin formulation for all species tested. The values found LC50;48h demonstrated that the contamination of aquatic habitats for this pesticide can cause great slaughter of fish in a short period of time, indicating that careful attention should be taken with its use. The species *D. rerio* and *H. bifasciatus* showed great sensitivity to the pesticide, being suitable for toxicity tests which have as objective the evaluation of the impact by its contamination. The species *O. niloticus* and *H. bifasciatus* presented values of much larger LC50;48 than the other species employed, although the values obtained are extremely low. These data suggest that more studies are necessary to examine the toxicity for fish of commercial products that contain deltamethrin, not only in relation to the acute toxicity but also in the chronic, physiologic and behavioral sphere.

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