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Joint toxicity of permethrin and cypermethrin at sublethal concentrations to the embryo-larval zebrafish

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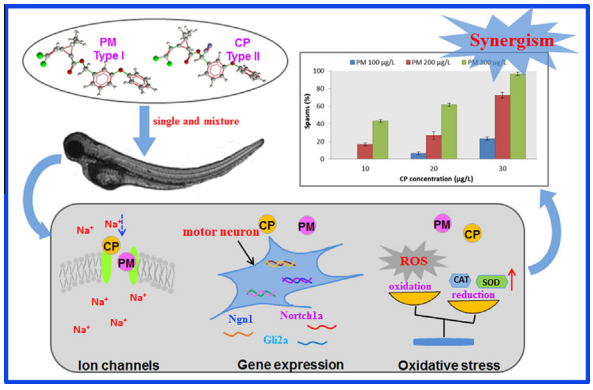
HIGHLIGHTS

- The mixture of PM and CP produced greater sublethal toxicity to zebrafish.
- The developmental toxicity of PM and CP was related to disruption of ion channels.
- The mixture of PM and CP caused greater inhibition in proneural gene expression.
- The mixture of PM and CP increased SOD and CAT activities in embryos.

GRAPHICAL ABSTRACT



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ABSTRACT

Pyrethroids, the widely used pesticides, are highly toxic to aquatic organisms. However, little information is so far available regarding the joint toxicity of type I and type II pyrethroids to fish. Zebrafish is a well-accepted aquatic vertebrate model for toxicity assessment due to small size, easy husbandry, high fecundity and transparent embryos. In this study, we utilized embryo-larval zebrafish to elucidate the combined effects of sublethal concentrations of permethrin (PM) and cypermethrin (CP), which are the most frequently used type I and type II pyrethroids, respectively. Fish were exposed from 3 h postfertilization (hpf) to 144 hpf to binary mixtures of nominal concentrations of 100, 200, 300 μg L^{-1} PM (PM100, PM200, PM300) and 10, 20, 30 μg L^{-1} CP (CP10, CP20, CP30). Analytical data of the real concentrations of the chemicals showed a significant degradation of the pyrethroids but an obvious recovery after the renewal of the exposure solution. Defect rates of embryos exposed to these low concentrations of single PM or CP exhibited no statistically significant difference from the control,while the application of combination of PM and CP resulted in deleterious effects on zebrafish embryonic development. In all PM200 and PM300 exposure groups, increasing CP concentrations acted additively to the action of PM in terms of all sublethal endpoints. Co-treatment of embryos with the specific sodium channel blocker MS-222 and pyrethroids (individuals or the mixture) caused a decline in the incidences of body axis curvature and spasms compared to treatment of animals with pyrethroids alone, suggesting that the developmental toxicity of PM and CP to zebrafish was related to disruption of ion channels. We further revealed that mixture of the two pyrethroids caused greater down-regulation in the mRNA levels of proneural genes. The individual pesticides had no effect on the activity of superoxide dismutase (SOD), while the mixture exposure caused significant induction. Treatment with CP or the mixture increased the activity of catalase (CAT). Taken together, our data indicated that the mixture of PM and CP caused higher incidence of morphological defects, greater inhibition in proneural

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gene expression and more oxidative stress, compared to the single chemical at the corresponding doses. Our findings suggest that the combination of type I and type II pyrethroids poses a greater risk to fish in the water column.

1. Introduction

Pyrethroids, a class of broad-spectrum and high-efficiency pesticides, are becoming increasingly popular in agricultural, veterinary, medical and home use over the past two decades, accounting for about one quarter of the world pesticides market (Oros and Werner, 2005). In recent years, residues of pyrethroids have been extensively detected in soil, urban and agricultural streams, as well as indoor dust, which poses a potential risk to wildlife and humans (Hladik and Kuivila, 2009; Kuivila et al., 2012). Pyrethroids are less toxic to mammals and humans than other most insecticides, such as organochlorine, carbamate and organophosphorus compounds. However, great attention has been paid to pyrethroids residues in the runoff and stream water because of their high toxicity towards aquatic organisms, like fish and invertebrates (Werner and Moran, 2008).

Toxicity of pyrethroids to fish has been shown to be 1000-fold greater than to mammals and birds at comparable concentrations (Bradbury and Coats, 1989). The sensitivity of fish to aqueous pyrethroid exposure is due partly to a high rate of gill absorption and rather slow hydrolytic detoxification, but principally to the hypersensitivity of the piscine nervous system to these pesticides (Aydin et al., 2005; Viran et al., 2003). The primary target sites for pyrethroids are the voltage-gated sodium channels (VGSCs) (Casida et al., 1983). Pyrethroids exert neurotoxicity by binding to and delaying the inactivation (closing) of the sodium channels, resulting in convulsions, prostration and ultimately death (Werner and Moran, 2008). It has been well accepted that zebrafish is a refining vertebrate model for toxicity assessment by virtue of small size, easy husbandry, high fecundity and transparent embryos, which conforms to the 3R principles (Replacement, Reduction, and Refinement) in animal experimentation (Hill et al., 2005).

Based on the chemical structure, pyrethroids are divided into type I and type II (US Environmental Protection Agency (USEPA), 2010). Permethrin (PM) is the most frequently used type I pyrethroid. Cypermethrin (CP), a type II pyrethroid, is one of the top-five pyrethroids in use (Oros and Werner, 2005). It is chemically modified via the addition of α-cyano at the phenoxybenzyl alcohol moiety of PM, and as such, its photostability is improved, resulting in a greater toxic potency than that of PM (Solomon et al., 2001). The concentrations of PM in water column have been found to range from 0.05 μg L^{-1} to 811 μg L^{-1} (Shahsavari et al., 2012; Weston et al., 2009; Weston and Lydy, 2010). CP was usually detected at levels of 0.01–9.8 μg L^{-1} in water column, but its concentration can be as high as 194 μg L^{-1} in the runoff of some farmed areas following pesticides applications (Laabs et al., 2002; Marino and Ronco, 2005; Vryzas et al., 2011; Xing et al., 2012). A few studies have examined the toxic effects of individual pyrethroids on zebrafish and shown that developmental exposure of zebrafish to pyrethroids caused morphological lesions and loss of movement coordination behavior, such as crooked body axis, pericardial edema, yolk edema, spasms and the whole body tremors (DeMicco et al., 2010; Jin et al., 2009). To our knowledge, but as yet, no study has been reported to investigate the joint toxicity of type I and type II pyrethroids to zebrafish. Moreover, the mechanisms of joint toxicity of PM and CP to early development of zebrafish remain poorly understood.

The present study was designed to evaluate the individual and combined toxicity of waterborne PM and CP to the early development of zebrafish. Both lethal and sublethal endpoints, including morphological deformations (crooked body, pericardial edema, non-inflation of the swimbladder) and neurobehavioral toxic effects similar to that observed in mammals (spastic response), were employed for the evaluation of single chemical effects. In addition, we determined the real concentrations of PM and CP on day 0 and day 5 during the exposure experiments. In the mixture studies, instead of traditional acute toxicity for LC50 estimates, sublethal toxicity test was performed to estimate the potential interaction of PM and CP at rather low concentrations by using two-way analysis of variance. Furthermore, the potential mechanisms responsible for neurotoxicity were explored. We hypothesized that the neurotoxicity induced by both the single chemical and the binary mixtures was associated with the disruption in VGSCs, the interference of motorneuron development and oxidative damage.

2. Materials and methods

2.1. Zebrafish husbandry and egg collection

Wild-type zebrafish (2-month-old), obtained from a local supplier, were bred in 15 L glass tanks in a semi-static system at 28 ± 1 °C with a 14-h light: 10-h dark photoperiod. Half of the water was renewed with fresh charcoal-dechlorinated tap water twice per week. Fish system water was aerated and analyzed daily to maintain dissolved oxygen concentration at 8-9 mg L^{-1} and pH at 7.0–7.6. The values for ammonia, nitrite and nitrate were less than 0.2 ppm, 0.05 ppm and 0.05 ppm, respectively. The fish were fed with brine shrimp (Artemia nauplii, Tianjin Ocean Pal Carol Biotech Co.) twice daily. The feces and residual food were siphoned from the aquaria periodically.

Male and female adult fish (5-month-old) with an optimal ratio of 2:1 were placed in pairs overnight in a spawning aquarium equipped with grids close to the bottom to keep the eggs from being eaten by the adult fish. Spawning was induced by the light irritation on the next morning. Fertilized eggs were collected, washed with fish system water for several times and incubated in embryo media (Hank's solution, 0.137 M NaCl, 5.4 mM KCl, 0.25 mM Na_2HPO_4, 0.44 mM KH_2PO_4, 1.3 mM $CaCl_2$, 1.0 mM $MgSO_4$ and 4.2 mM $NaHCO_3$). Then the eggs were observed under microscope (Leica Microsystems, Wetzlar, Germany) and normal ones free of infection and disease were maintained in Petri dishes loaded with Hank's solution. Embryos at 3 h postfertilization (hpf) were selected for exposure experiment. All the conditions for zebrafish husbandry and egg collection were by reference to well-established protocols (DeMicco et al., 2010; Jin et al., 2009).

2.2. Chemicals and reagents

Permethrin (PM, 98.3%, mixture of isomers), cypermethrin (CP, 97.0%, mixture of isomers) and MS-222 (tricaine methanesulfonate) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Stock solutions of the pyrethroids (nominal concentrations of 100, 200, 300, 400, 600, 800 mg L^{-1} for PM and 10, 20, 30, 50, 100, 150 mg L^{-1} for CP) were prepared in ethanol and stored at −20 °C. Exposure solutions were diluted from the stock solution in embryo media. MS-222 was dissolved in deionized water and stored at 4 °C.