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硫丹对罗非鱼(*Oreochromis aureus* × *O. niloticus*)和草鱼(*Ctenopharyngodon idellus*)的急性毒性研究

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摘要: 为控制虫害农业上大量使用硫丹, 因此硫丹可通过多种途径进入水环境中。养殖环境和水产品中已发现硫丹残留, 这给养殖环境和食品安全造成潜在威胁。通过半静态试验方法测定了罗非鱼和草鱼的半致死浓度, 并估算了其在水环境中的安全浓度。结果表明, 罗非鱼和草鱼的半致死浓度分别为 1.97 (1.26—2.87) $\mu g \cdot L^{-1}$和 2.33 (1.66—3.32) $\mu g \cdot L^{-1}$, 安全浓度分别为 0.20和 0.23 $\mu g \cdot L^{-1}$。为进一步验证这 2种鱼对硫丹的抗性, 将罗非鱼和草鱼在最低剂量组 (0.7 $\mu g \cdot L^{-1}$)中暴露 60 d, 发现这 2种鱼个体状态良好。因此, 初步证明这两种鱼在水中的安全浓度比目前国家规定的标准值要高。但是关于硫丹对这 2种鱼的内分泌干扰、生殖和生长等方面的影响还需作进一步的研究。

关键词: 急性毒性; 硫丹; 罗非鱼; 草鱼
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Study on Acute Toxicity of Endosulfan to Tilapia (*Oreochromis aureus* × *O. niloticus*) and Grass Carp (*Ctenopharyngodon idellus*)

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Abstract: Endosulfan has been discovered in aquaculture environment and aquatic products, which was massively used in agriculture. It was a potential hazard to environment quality and food safety. The acute toxicity of endosulfan to tilapia (*Oreochromis aureus* × *O. niloticus*) and grass carp (*Ctenopharyngodon idellus*) were measured using a semi-static bioassay. The 96 h LC50 values were 1.97 (1.26—2.87) $\mu g \cdot L^{-1}$ and 2.33 (1.66—3.32) $\mu g \cdot L^{-1}$ for *Oreochromis aureus* × *O. niloticus* and *Ctenopharyngodon idellus*, respectively. Correspondingly, the safe concentrations of endosulfan for the two fish were 0.20 and 0.23 $\mu g \cdot L^{-1}$. Furthermore, the fish exposed to 0.7 $\mu g \cdot L^{-1}$ dose for 60 days were always in good physical condition. Therefore, the level of endosulfan in water which the two fish can be tolerant was higher than the level reported in water environment in China.

Keywords: acute toxicity; endosulfan; tilapia; grass carp

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Endosulfan is a member of the cyclodiene subgroup of organochlorines, which is widely used as an insecticide and acaricide. However, it has a number of side effects in non-target organisms, including mortality and endocrine disruption. In 2008, there was a formal proposal to list endosulfan as a persistent organic pollutant under the Stockholm Convention[1]. Despite the side effects and the potential listing, endosulfan is still registered to be used in most regions, including the United States, South America, Africa, most of Asia (excluding Thailand), and Australia (http://www.foodsmart.govt.nz/whats-in-our-food/chemicals-nutrients-additives-toxins/agricultural-production/endosulfan/). Although endosulfan is forbidden to be used in aquaculture, it sometimes enters water bodies as a result of runoff from agricultural application[2-4]. Therefore, the survival, growth, and reproduction of aquatic organisms, including fish, may be threatened.

Endosulfan has been registered for use in China since 1990s. Since then, the annual production has averaged ~2 400 t[5]. Endosulfan distributes throughout in China and its total usage between 1994 and 2004 is estimated to be 25 700 t[6]. Aquatic environment and aquatic products are under potential hazard. In 2006, endosulfan was detected in eels exported to Japan, which was a dangerous signal for environment and food safety. For aquatic product safety, metabolic process of pesticide in fish from early cultivated stage to marketable stage is very important. Our objective was to evaluate the acute toxicity of endosulfan to *Oreochromis aureus*, *O. niloticus* and *Ctenopharyngodon idellus* in early cultivated stage which can provide base for the further research.

1 Materials and Methods

1.1 Chemicals

We purchased technical-grade endosulfan (35% EC) from the Hangzhou Dragon Chemical Co. (Hangzhou, China). The compound was a mixture of two stereo isomers, α- and β-endosulfan (2:1). Stock solution of 35 g·L^{-1} was prepared by dissolving the compound in acetone.

1.2 Animals

Healthy *Oreochromis aureus*, *O. niloticus* (about one months old, mean weight 64.3±6.9 g) and *Ctenopharyngodon idellus* (about one months old, mean weight 128.4±12.1 g) were purchased from a local fish breeding farm in Panyu District, Guangzhou. The fishes were transported to the laboratory within 1 h, and placed in tanks containing aerated, dechlorinated tap water which dissolved oxygen was (6.8±0.5) mg·L^{-1}, pH (7.2±0.3) at (22±1) ℃. Water was changed in every 24 h to prevent buildup of waste products. Before use, the water was aerated using an air compressor for at least 48 h to remove the chlorine. To prevent dermal infection, the fishes were treated with 0.05% potassium permanganate solution. The fishes were acclimated to the experimental tanks for 7 d. During acclimation, the fishes were fed once daily with dry pellet food. We were unable to detect measurable levels of endosulfan in the feed. Every effort was made to provide optimal conditions for the fishes during the acclimation period.

1.3 Exposure

Following acclimation, we randomly selected 84 fishes of each species and divided them into 7 treatment groups (N=12 individuals/treatment). Each group was stocked into a single tank. Six of the groups were exposed to endosulfan at a concentration of 0.7, 1.4, 2.8, 5.6, 11.2 and 22.4 μg·L^{-1} for 96 h. The range of concentrations was based on the results of a preliminary test. The remaining group served as control. Prior to exposure, the animals had not be fed for 24 h. During the exposure period, the water was changed on alternate day. During the exposure period, we recorded the behavior, breath, color of eyes and skins of the fish periodically and the number of mortalities in each tank. Fishes with no respiratory movements and no response to tactile stimuli were considered dead and removed immediately. After the exposure experiment, the 0.7 μg·L^{-1} dose group and the control group remained for 60 days to observe behavior change[25-26].

1.4 Endosulfan determination

The dry pellet food samples were homogenized, ultrasonic extracted in ethyl acetate for twenty minutes and centrifuged at 4 000 rpm for 10 min. The supernatant were purified through a glass chromatography column (filled orderly with florsil and anhydrous sodium sulfate from bottom to top), concentrated and determined in HP 6890N Gas Chromatography which has a electron capture detector equipped with autosampler and HP 19091－413 max 325℃ HP capillary (30 m ×0.25 mm × 0.25 μm). Operating conditions were as follows: the injector temperature was set at 280℃ and the detector temperature was 300℃. The oven was programmed to increase from 150℃ (hold for 1 min) to 220℃ (hold of 7 min) at a rate of 10℃·min^{-1} and to 280℃ (hold for 1 min) at a rate of 30℃/min. Nitrogen was used as carrier gas at a flow rate of 60 mL·min^{-1}. Under the conditions, retention times for α and β endosulfan were 8.76 min and 9.25 min, respectively.

1.5 Data analysis

We used the percentage mortality data from each treatment group to calculate the 96 h LC50 value. The 95% fiducial limits were obtained using an unweighted regression method of probit analysis. Analytical soft was SPSS 12.0 (SPSS, Chicago, IL, USA).

2 Results

The dose-mortality relationship for the fish exposed to endosulfan is illustrated in Fig. 1. 96 h LC50 values and the 95% fiducial limits (in parentheses) were 1.97 (1.26－2.87) μg·L^{-1} and 2.33 (1.66－3.32) μg·L^{-1} for Oreochromis aureus× O. niloticus and Ctenopharyngodon idellus, respectively.

Behavior of the fish in different doses was recorded. Oreochromis aureus× O. niloticus and Ctenopharyngodon idellus shared a similar symptom. Responses of the two to endosulfan were fierce with the increase of concentration. Fishes exposed to the highest dose were visibly affected within the first 3 h of exposure period. During the first 3 h in the highest dose, the symptoms appeared in the fishes including excitation, swimming rapidly, however, near the water surface, an increase symptoms occurred such as respiration, convulsions, and death. No behavior abnormity appeared in the fish of the lowest dose group during the 60 days.

3 Discussion

3.1 Acute toxicity test

The median lethal concentrations for tilapia and grass carp are consistent with the values published for other species (Table 1). The published LC50 values ranged from 0.09 to 10.7 μg·L^{-1} endosulfan. In the species tested to date, Mugil cephalus, Chanos chanos, and Menidia menidia are the most sensitive and Channa gachua is the least sensitive. LC50 for most species range from 1.0 to 2.7 μg·L^{-1} endosulfan, suggesting that endosulfan is highly toxic to fish, even it at very low concentrations. Though the mechanism of toxicity is unclear, it clearly shows the effect of endosulfan on neurotransmitters. Exposure to endosulfan alters the brain levels of acetylcholine[16] and serotonin[17]. Both these neurotransmitters are involved in the control of spontaneous motor activity. Additionally, endosulfan can act as a noncompetitive GABA antagonist at the chloride channel within the GABA receptor in brain synaptosomes. It could block the receptors for the GABA neurotransmitter in nerve cells[18]. Binding of GABA to its receptors induced the uptake of chloride ions by neurons. Blockage of this uptake by endosulfan resulted in a state of uncontrolled excitation[19]. That may be the reason why the fishes appeared so fierce in high dose endosulfan.

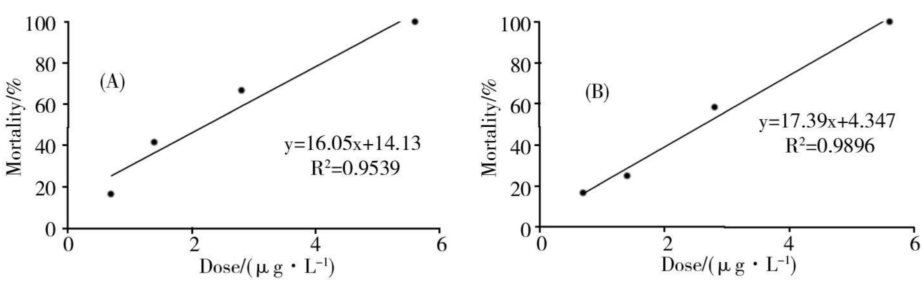

Fig. 1 Fig. 1 Dose and mortality regression for Oreochromis aureus O. niloticus (A) and Ctenopharyngodon idellus (B) exposed to endosulfan in water for 96 h

3.2 Tolerance to endosulfan

The fish in the 0.7 $\mu g \cdot L^{-1}$ group were normal in behavior in the later 60 days, indicating that fish are tolerant to the dose. The concept of safe concentrations was proposed by Sprague[20], which calculated by formula $LC50_{96h} \times 0.1$. The safe concentration for Oreochromis aureus × O. niloticus and Ctenopharyngodon idellus are 0.20 and 0.23 μg endosulfan $\cdot L^{-1}$, respectively. In U.S.A., Environmental Protection Agency (EPA) recommends that levels do not exceed 0.22 $\mu g \cdot L^{-1} \cdot d^{-1}$ (criterion maximum concentration)[21]. The reported concentrations in the Guanting Reservoir (0.00215 $\mu g \cdot L^{-1}$, Beijing)[22], Tonghui River (0.0808 $\mu g \cdot L^{-1}$, Beijing)[23], and Minjiang Estuary (0.108 $\mu g \cdot L^{-1}$, Fujian)[24] are lower than the levels recommended by the EPA and this paper. According to the results in the study, Oreochromis aureus × O. niloticus and Ctenopharyngodon idellus are tolerant to endosulfan greatly. Further researches need to be carried out one evaluate the effect of endosulfan on neurotoxicity, endocrine disruption, growth and reproduction of the two fishes.

Table 1 Acute toxicity values (LC50) for fish species following a 96 h exposure to endosulfan

Species	LC50/($\mu g \cdot L^{-1}$)	Reference
Channa gachua	10.7	[7]
Cyprinodont variegatus	2.70	[8]
Ctenopharyngodon idellus	2.32	This study
Menidia beryllina	1.50	[9]
Oreochromis aureus × O. niloticus	1.97	This study
Gambusia affinis	1.30	[10]
Cirrhinus mrigala	1.30	[11]
Atherinops affinis	1.30	[9]
Fundulus heteroclitus	1.15	[12]
Chanos chanos	0.56	[13]
Menidia menidia	0.38	[14]
Mugil cephalus	0.09	[15]

Biography: Lin Qin (1956—), male, bachelor, professor, research fields: fishery ecological environment and food safety.

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