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Toxicity of Endosulfan to the Red Hybrid *Tilapia*

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Abstract: Exposure of mature red hybrid *Tilapia* sp. to a range of sub-lethal concentrations of endosulfan for 21 days induced a spectrum of often not clearly distinguishable Behavioural Toxic Symptoms (BTS), ranging from reversible to eventually fatal responses. The No Observed Effect Concentration (NOEC: Below the threshold of BTS) and the Lowest Observed Effect Concentration (LOEC: Above the threshold of low intensity and reversible BTS and below the threshold of any mortality) were <0.005 and <0.0075 mg L^{-1}, respectively. Two other categories of BTS were also distinguishable. Medium Observed Effect Concentration (MOEC: Reversible, medium-intensity BTS in 75% and pronounced, lethal BTS in 25% individuals) and Pronounced Observed Effect Concentration (POEC: Leading to mortality of >25% to 50% of the fish) were 0.01 and 0.02 mg L^{-1}, respectively. The 3-day and 21-day LC_{50} values were 0.031 and 0.016 mg L^{-1}, respectively.

Keywords: Endosulfan, Toxicity, *Tilapia*

Introduction

Hillside farming, rainfall pattern, poor agronomic practices, which can all contribute to soil erosion, have rendered small Caribbean islands agro-ecosystems vulnerable to run-off of pesticide residues (Mansingh, 1993; Robinson *et al.*, 1997; Mansingh *et al.*, 2003). Pesticide residues have contaminated several Jamaican rivers, which drain the agricultural valleys and coastal waters (Mansingh, 1993; Mansingh *et al.*, 1997; 2003). Residues of endosulfan (which was used extensively against the Coffee Berry Borer, *Hypothenemus hampei* Ferrari, in the Blue Mountains and highlands up until 2011) were detected in many wells and springs and in almost all the rivers, coastal waters and aquatic fauna in Jamaica during the 1990s (Robinson and Mansingh, 1999; Witter *et al.*, 1999).

Toxic chemicals can affect non-target organisms by causing hormone imbalances and disruption and can lead to a variety of physiological effects such as abnormal growth and reproduction (Oberdörster and Cheek, 2001). In order to monitor possible impacts of pesticides on the aquatic ecosystem it is necessary to determine the ecotoxicity potential of pesticide residues to the fauna inhabiting these ecosystems. With a history of varying levels of endosulfan residues being detected in Jamaican water, the present study was designed to investigate the reversible and non-reversible toxic effects of sub-lethal concentrations and acute toxicity levels of endosulfan on an indicator species, the hybrid Red *Tilapia* fish. The objective was to attain an insight into the ecotoxicity potential of the insecticide in Jamaican river and coastal waters.

Materials and Methods

Sexually mature individuals of the Red Hybrid *tilapia* (8-9 cm long) were obtained from a commercial farm and held in aerated glass aquaria for at least two weeks for acclimatization. Fish meal was provided daily and water was changed every three days.

Six 20 L glass aquaria with 18 L water were aerated for 24 h before the required amount of Thiodan 35EC (supplied by Hoescht, Germany; purity, checked by Gas Chromatograph (GC); 33.75% active ingredient) was added to the experimental aquaria and the water agitated manually for 5 min. About 30 min later, 18 fishes were introduced into each of the experimental and control aquaria. The fish were not fed for the first three days, but food pellets were provided everyday thereafter, in small amounts, to ensure as little left-over as possible. Each experiment was conducted in triplicate.

The fishes were exposed to different concentrations of endosulfan, ranging from 0.0025 to 0.025 µg L^{-1}. Toxic symptoms (darting, shuddering, side swimming and mortality) were recorded every 8 h for the first 3 days, every 12 h for the next 4 days and every 24 h for the next 2 weeks. The concentration of endosulfan in the water was checked by GC. For determining the 3-day LC_{10-95} of endosulfan, the fishes were exposed to six concentrations of endosulfan, ranging from 0.02 to 0.04 mg L^{-1} of its active ingredient for 24 h. Fish mortality was recorded and the data subjected to Probit analysis.

Concentration of endosulfan in the aquarium water was checked at 0 and 3 days after introduction of the insecticide, by extracting the residues from 100 mL aliquots of water, according to the UNEP methodology described by Robinson and Mansingh (1999).

Results

No toxic symptoms were observed in the control fish or those exposed to 0.0025 mg L^{-1} of endosulfan for 21 days (Table 1). However, exposure to 0.005 mg L^{-1} induced slight toxic symptoms, such as darting and shuddering, in 14% of the fish, from which they recovered 12-24 h later. At 0.0075 mg L^{-1}, most of the 29% fish affected displayed pronounced toxic symptoms of side-swimming, from which 14% could not recover. Almost all the fish exposed to residues ranging from 0.01 to 0.025 mg L^{-1} were visibly affected and 30 to 90% of them succumbed. Pronounced toxic symptoms were visible in most fish within 30-60 min of exposure to 0.02 and 0.025 mg L^{-1}, which caused 12 to 45% mortality within 2 days. At the end of the 21-day exposure to these concentrations, 73 to 90% of the fish had died, 5 to 10% were still alive but appeared sick, while 5 to 17% had recovered.

The 21-day and 3-day $LC_{10–95}$ data are presented in Table 2; lethal concentrations ranged from 0.008-0.049 mg L^{-1}. In the 3-day acute toxicity experiments, mortality at higher concentrations occurred within 24 h. Some of the individuals which did not die displayed pronounced toxicity symptoms even after 3 days of exposure.

Discussion

Ecotoxicity of insecticides depends upon the concentration and physico-chemical properties of the chemical, the physiological and developmental state of the species and the extent of exposure to the toxicant (Lyman, 1995). It induces invisible, reversible or irreversible physiological lesions, which are manifested through a spectrum of behavioural responses, some of which may not be qualitatively identifiable, others more pronounced and deadly. Though it is almost impossible to categorize a fluid and often reversible physiological phenomenon of toxicity, quantification of visible toxic symptoms is essential for rapid assessment of ecotoxicity of a pesticide.

Based upon the acute lethal toxicity of a compound to a test organism over a period of time, Strik (1990) suggested the terms Non-Observed Effect Concentration (NOEC) and Lowest Observed Effect Concentration (LOEC) for the visible acute toxicity to the least number of individuals. Ellersiek *at al.* (1994) and Lee *et al.* (1995) recommended extrapolation of acute toxicity data for the calculation of NOEC and LOEC. A two step linear extrapolation of data to a dose at which toxic effects are arbitrarily small and acceptable and the other at which no more than 25% are affected was suggested by Hoekstra and van Ewijk (1993)

Such criteria are valid for small test organisms such as *Daphnia* spp. However, the present results suggest that data on the reversibility or irreversibility of visible toxic effects may be more applicable in the assessment of toxicity of a compound than the acute toxicity data. Robinson *et al.* (2002) provided data on the reversibility of visible toxic symptoms in the shrimp *Macrobrachium faustinum* De Sassure, which were exposed to sub-lethal concentrations of dieldrin. They suggested that NOEC be defined as the concentration just below the threshold of any observable behavioural toxic symptom and LOEC, just above the threshold of mild- and low-intensity, reversible toxic symptoms and below the threshold of mortality. Two new terms were proposed for medium and pronounced toxic symptoms, which may or may not be reversible. Medium Observed Effect Concentration (MOEC) was defined for visible toxic symptoms in most individuals, leading to 50% mortality and Pronounced Observed Effect Concentration (POEC) for pronounced toxic symptoms in most individuals, leading to over 50% mortality.

The present data support the suggestion of Hoekstra and van Ewijk (1993) of extrapolating acute toxicity data for the calculation of LOEC. The data on the reversible toxicity to the fish (Table 1) and the 21-day LC_{10-95} data (Table 2) are comparable. Both sets of data indicate that NOEC, when no toxic symptoms were visible, may be established at <0.005 mg L^{-1}; LOEC, which is just below or at the threshold of toxicity, may be $LC_{5,}$ which is <0.0075 mg L^{-1}; MOEC at LC_{30}, which is <0.015 mg L^{-1} and POEC at LC_{75}.

Most of the test organisms which recover from manifested toxic symptoms, depending on the concentration of the chemical and extent and exposure, may suffer adverse physiological effect, which could influence their reproduction and survival (Robinson *et al.*, 2002; van Leeuwen and Hermens, 1995). Indeed, NOEC and LOEC levels of dieldrin caused the resting Oxygen Consumption Rate (VrO_2) of *Macrobrachium faustinum* to increase by 48%, while the active Oxygen Consumption Rate (VaO_2) decreased by 13%. The VrO_2 and VaO_2 in *M. amazonicum* also decreased by 43 and 70%, respectively (Lawrence *et al*., 1986a; 1986b).

Table 1. Toxic symptoms of endosulfan to the Red Hybrid *Tilapia*, after 21-day exposure to different concentrations, under static conditions

	% fish with specific symptoms[1] at different days					
Conc. (mg L^{-1})	3	7	10	14	21	Total % displaying toxic symptoms in 21 days
Control	0	0	0	0	0	0; all normal
0.0025±0.0003	0	0	0	0	0	0; all normal
0.005±0.0003	0	0	0	0	7d; 7s	14; but all recovered
0.0075±0.0005	0	5d	7ss	17ss; 5m	7m	29; 14 died, 15 recovered
0.01±0.0008	10d; 20s	10s, 15ss; 10m	10ss; 10m	5ss; 10m	0	85; 30 died, 55 recovered
0.015±0.0008	50s; 10ss; 15m	40ss, 10m	20ss; 5m	25ss; 15m	10ss	100; 45 died, 55 recovered
0.02±0.001	25d; 55ss; 12m	25ss; 15m	10d; 15m	10ss; 20m	10ss	100; 73 died, 17 recovered
0.025±0.001	25d; 10s, 20ss; 45m	25ss; 30m	5 s; 10ss; 10m	5ss; 5m	5ss	100; 90 died, 5 recovered

1. d- darting; m- mortality; s- shuddering; ss- side swimming

Table 2. Twenty-one day and three-day LC_{10}, LC_{50}, LC_{95} (mg L^{-1}) values of endosulfan for 8-9 cm long *Tilapia*, determined under static conditions

Toxicity level	Duration	Concentration (mg L^{-1})	Fiducial limit	Slope
LC_{10}	21-day	0.008	0.006-0.009	4.137±0.308
LC_{50}	21-day	0.016	0.014-0.018	
LC_{95}	21-day	0.039	0.031-0.056	
LC_{10}	3-day	0.021	0.012-0.025	8.185±1.598
LC_{50}	3-day	0.031	0.027-0.035	
LC_{95}	3-day	0.049	0.040-0.100	

Fenvalerate and chlorpyrifos also increased O_2 consumption in the shrimp, *Palaemnetes clara* and *P. pucio*, respectively (McKenney and Hamaker, 1984). When exposed to 0.006 and 0.01 µg/L endosulfan for 96 h, the fresh water shrimp *Channa punctatus* had reduced AchE activity and displayed behavioural toxic symptoms (Gopal *et al*., 1985). Sub-lethal dose of endosulfan also affected reproduction, fecundity and survival of fish hatchlings (Chakravorty *et al*., 1992). Larvae of the American lobster, *Homarus americanus*, had significantly higher levels of CYP45 (detoxification enzyme) and HSP70 (stress protein) for several days following exposure to the cyclodiene pesticide heptachlor (Snyder and Mulder, 2001). Delays in moultings were correlated with changes in ecdysteroid levels, which implied that the pesticide may have caused endocrine disruption in these crustaceans. Prolonged elevation in HSP70, due to pesticide exposure, was directly related to increased mortality. Additionally, Stueckle (2008) found that male fiddler crabs, *Uca pugnax*, had a higher incidence of abnormal limbs than female crabs at high permethrin concentrations and stressful salinity levels. Females also exhibited heightened respiration rates, which suggested increased metabolic rates. Hence, while recovery from toxic symptoms was observed in fish exposed to the lower concentrations (LOEC and MOEC), long term physiological impact may exist but not be evident.

Conclusion

Exposure of *Tilapia* fish to different sub-lethal doses of endosulfan induced a variety of toxic symptoms such as darting, shuddering and side-swimming. The data suggests a very high toxicity dose response gradient with the NOEC being below 0.005 mg L^{-1} and the LOEC being between 0.005 and 0.0075 mgL^{-1} of endosulfan. At LOEC concentrations, the fish were able to recover; however, prolonged exposure resulted in POEC symptoms and increased levels of mortality.

Author's Contributions

All authors contributed equally to this work.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that the other author has read and approved the manuscript and no ethical issues are involved.

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