Bull Environ Contam Toxicol (2007) 79:557–561
DOI 10.1007/s00128-007-9234-3

Acute and Chronic Effects of the Insecticide-Endosulfan on Freshwater Cladoceran, *Moina macrocopa* Straus

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Received: 26 February 2007 / Accepted: 11 June 2007 / Published online: 18 July 2007

Abstract Acute and chronic effects of insecticide-endosulfan on the survival and reproduction performance of *Moina macrocopa* were determined in a laboratory study. Endosulfan concentrations that cause 50% mortality (LC50) after exposure for 24 and 48 h were 3.34 and 0.16 mg L^{-1}, respectively. Average longevity, initial age of reproduction and intrinsic rate of natural increase were reduced at 0.002 mg L^{-1}. Fecundity was greatly reduced by about 70% at 0.0004 mg L^{-1} and approximately 97% at 0.002 mg L^{-1} as compared to control organisms throughout the whole life span of 15 days. If environmental concentration of endosulfan do not exceed 0.0004 mg L^{-1}, application of this insecticide is unlikely to induce detrimental effects on these cladoceran populations in agro-ecosystem.

Keywords Endosulfan · *Moina macrocopa* · reproduction · survivorship

Pesticides including insecticides, herbicides and fungicides have been intensively used for pest control in rice fields. These pesticides are accidentally discharged into the water bodies such as canal, river, lake and pond during operation season in rice fields and thus create some adverse impacts to non-targeted organisms in the aquatic ecosystem. Endosulfan is one of the most commonly used organochlorine insecticides in rice fields (Cheah and Lum 1998). It has been identified as a pesticide of concern due to health and environmental problems associated with its use in Ecuador, Mauritius and Paraguay (Seethi and Reghunathan 2002). Today, endosulfan is banned or severely restricted in over 30 countries, but it is still widely used in some of the developing countries like Indonesia and Thailand (EJF 2002). It has a water half-life of more than 14 days but it persists longer in soil with half life approximately 60–800 days (EJF 2002).

Moina macrocopa, one of zooplanktons, is widely distributed in ponds and rice fields of Southeast Asia. It is sensitive to pollutants, such as heavy metals (Wong 1993) and organophosphate insecticides (Wong et al. 1995). Existence of endosulfan in the aquatic environment may affect its survival, reproduction and physical changes. Since zooplanktons are important links in the aquatic food chain, toxicants affecting the populations would have indirect effects on their predators and preys, thereby causing changes at the community and ecosystem levels eventually (Chu et al. 1997). Thus, the objectives of this study were to determine acute and chronic effects of endosulfan on survival and reproduction of *M. macrocopa*.

Materials and Methods

Moina macrocopa were obtained from the freshwater hatchery, Universiti Malaysia Terengganu (UMT). Matured and healthy *M. macrocopa* were selected to be cultured as stock animals. *Moina macrocopa* cultivation was carried out under glasshouse conditions with light intensities ranging from 800 to 1200 $\mu Em^{-2}\ s^{-1}$ and a 12-h photoperiod. *Moina macrocopa* were cultured using

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Table 1 The retention time and endosulfan concentration remaining in the test solution at 0 and 48 h

Endosulfan concentration (mg L^{-1})	Endosulfan isomers	Retention time (min)	Endosulfan concentration (mg L^{-1})	
			0 h	48 h
Control	α-endosulfan	ND	ND	ND
	β-endosulfan	ND	ND	ND
2.0×10^{-3}	α-endosulfan	11.30	13.85×10^{-4}	9.26×10^{-4}
	β-endosulfan	16.90	7.21×10^{-4}	6.73×10^{-4}
0.5	α-endosulfan	11.30	0.351	0.235
	β-endosulfan	16.90	0.180	0.157
5.0	α-endosulfan	11.30	3.257	2.112
	β-endosulfan	16.90	1.674	1.386

ND not detected

dechlorinized tap water under optimal water parameters as follows: temperature at 25 ± 1°C; pH at 6–8; dissolved oxygen at 6 mg L^{-1} (APHA 1985). Freshwater microalgae such as *Chlorella* sp., *Scenedesmus* sp. and *Coelastrum* sp. were cultured as *M. macrocopa* food sources. Commercial fish meal (Cargill®) was fermented and used as fertilizer to accelerate growth of microalgae during the culture period.

The insecticide used was commercial endosulfan (CH endosulfan®, purity 33%, 64%–67% α; 29%–32% β). Endosulfan concentration in the testing solution was assessed at 0 and 48 h. The method involving chemical extraction and gas chromatograph detection was modified from Tan and Mustapha (2003) as follow: A total of 500 mL test solutions were extracted twice by using 25 mL DCM:*n*-hexane (50% v/v). Samples were then filtered through 10 g of sodium sulfate anhydrous (Merck) to remove excess water in the extract. After that, the extracts were concentrated by using a rotary evaporator (Buchii). The concentrated samples were then collected and blown down to dryness under a generous stream of purified nitrogen. Lastly, 200 µL of DCM:*n*-hexane (50% v/v) was added into the vial and the samples were analyzed by using gas chromatography-mass spectrometer (QP2010, Shimadzu). Results revealed that, concentration of the test solutions were well prepared with a recovery of 103% ± 4%. It was found that the endosulfan concentration of test solutions remained within 70% after 48 h (Table 1).

The acute toxicity test was performed based on the standard methods of APHA (1985). In the 24-h study, 0, 0.05, 0.5, 2.0, 4.0, 8.0 and 16.0 mg L^{-1} of endosulfan were used for examination, whereas, another seven concentrations: 0, 2×10^{-4}, 2×10^{-3}, 5×10^{-2}, 5×10^{-1}, 3.5 and 5.0 mg L^{-1} of endosulfan were used in the 48-h study. No food was provided during the testing period. A total of number 20 neonates of *M. macrocopa* (<24 h) were placed in each 200 mL beaker containing 100 mL of test solution plus one blank control. Test solutions were not renewed during the 2-day static toxicity experiment. Three replicates were run in the experiment. Observation was made at every 24 h interval until all *M. macrocopa* were dead at the end of experiment. Dead neonates were recorded and discarded daily. Concentration that causes 50% mortality (LC50) was estimated by probit method. Non-observed effective concentration (NOEC) at 48 h was determined.

For chronic toxicity test, the effects of endosulfan on the survivorship and reproductive performance were assessed in a semi-static test based on the standard protocol for *Daphnia magna* (APHA 1985). A total number of 20 neonates (<24 h) were used for each concentration with each Petri dish containing 40 mL test solution and a single test organism. During the experiment period, each *M. macrocopa* was fed on commercial bakers yeast (Gold pakmaya®) at 1 mg mL^{-1} and commercial spirulina (Algae 100®) at 1 mg mL^{-1} daily. Three concentrations at 0 (control), 4×10^{-4} and 2×10^{-3} mg L^{-1} of endosulfan were used in this experiment. All test solutions were renewed every 2 days. Survival and reproduction (fecundity) of *M. macrocopa* were monitored for each Petri dish daily. Time to the first reproduction and total number of neonates produced by a female were used to evaluate the fecundity. Newborn neonates were recorded daily and discarded. The experiment was terminated in 15 days until all individuals were dead. Life table parameters were determined. According to Lotka (1913), the intrinsic rate of natural increase (r) can be calculated by using the following formula:

$$\sum I_\chi m_\chi e^{-r\chi} = 1$$

Where;

I_χ = the proportion of individuals surviving to age$_\chi$ (survivorship),
m_χ = the age specific fecundity (number of neonates produced per surviving female at age χ),
χ = days.

As r calculated in *Moina macrocopa* after 15 days was indistinguishable from r estimated for the entire life span, due to the great importance of early reproduction (Van Leeuwen et al. 1985), all calculation would be based on

Table 2 Cumulative mortality of *Moina macrocopa* ($n = 60$ in 3 replicates)

Concentration (mg L^{-1})	24 h Mortality (%)	Concentration (mg L^{-1})	48 h Mortality (%)
Control	0	Control	0
0.05	0	0.0002	0
0.5	0	0.002	0
2	10	0.05	10
4	65	0.5	90
8	100	3.5	100
16	100	5.0	100

15-day experiment. The following variables related to survival and reproduction were derived based on the collected data by using standard procedures (Krebs 1985).

$$\text{Gross reproductive rate} = \sum_{0}^{\infty} m_{\chi}$$

$$\text{Net reproductive rate } (R_0) = \sum_{0}^{\infty} I_{\chi} m_{\chi}$$

$$\text{Generation time } (T) = \sum I_{\chi} m_{\chi} \chi / R_0$$

Results and Discussion

Cumulative mortality of *M. macrocopa* exposed to different concentrations of endosulfan for 24 and 48 h is presented in Table 2. Mortality of *M. macrocopa* increased with endosulfan concentration. No mortality was observed at 0, 0.05 and 0.5 mg L^{-1} at 24 h; 0, 2×10^{-4} and 2×10^{-3} mg L^{-1} at 48 h, but complete mortality occurred at 3.5 and 5.0 mg L^{-1} at 24 h while 8.0 and 16.0 mg L^{-1} at 48 h. Percentages of mortality at 2.0 and 0.5 mg L^{-1} was 10% in both 24- and 48- studies, respectively. Immediate mortality was observed on *M. macrocopa* after being exposed to high concentrations of 2.0, 4.0, 8.0 and 16.0 mg L^{-1} endosulfan in the 24-h study. The organisms appeared to be weak and lethargic even after being exposed to lower concentrations of 0.05 and 0.5 mg L^{-1} for 24 h. Probit analyses revealed that LC50 values of 24, 48 h and NOEC value of 48 h were 3.34 (3.03–3.69), 1.6×10^{-1} (1.1×10^{-1}–2.1×10^{-1}) and 2×10^{-3} mg L^{-1}, respectively. A previous study conducted by Ferrando et al. (1992) has demonstrated that the respective 24- and 48-h LC50 values for endosulfan on *Daphnia magna* were 6.2×10^{-1} and 2.2×10^{-1} mg L^{-1}. While 24-and 48-h LC50 values of endosulfan on *Moinadaphnia macleayi* were 5.2×10^{-1} and 2.2×10^{-1} mg L^{-1}, respectively (Sunderam et al. 1994). The results of present study suggest that *M. macrocopa* was relatively less sensitive at the first 24-h exposure of endosulfan as compared to other cladocerans (Ferrando et al. 1992; Sunderam et al. 1994).

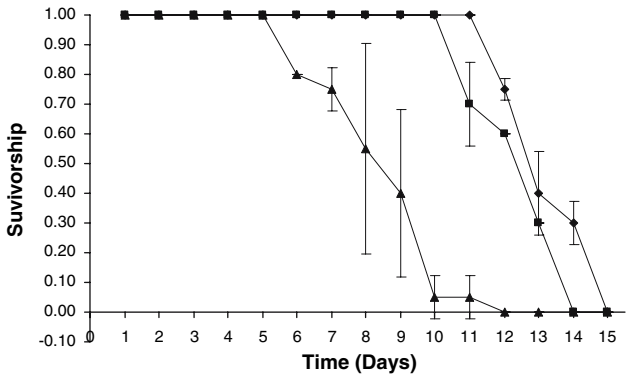

Fig. 1 Survivorship of *Moina macrocopa* ($n = 60$ in0 3 replicates) at 0 (*filled diamond*), 4×10^{-4} (*filled square*) and 2×10^{-3} mg L^{-1} (*filled triangle*) of endosulfan. Error bars indicate standard deviation of mean

Figure 1 shows the survivorship of *Moina macrocopa* at 0, 4×10^{-4} and 2×10^{-3} (NOEC) mg L^{-1} of endosulfan. Percentages of survival at these three concentrations were compared within 15 days of observation. No mortality was found at the first 11 days of observation at control. Survival rate started to decrease after day 5 at 2×10^{-3} mg L^{-1} while survival rate at 4×10^{-4} mg L^{-1} was reduced after day 10. All neonates of *M. macrocopa* at control were dead after 15 days. All individuals exposed to endosulfan at 4×10^{-4} mg L^{-1} died after 14 days. At 2×10^{-3} mg L^{-1}, mortality was increased rapidly from day 7 to day 10 and reached 100% of mortality at day 12. Cheah and Lum (1998) reported that the endosulfan residues at 4×10^{-5}, 3.2×10^{-4}, 3.7×10^{-4} and 2.55×10^{-2} mg L^{-1} were detected at around water resources of Muda rice field area located at the north of Peninsular Malaysia. Based on the result of chronic study, it is likely that 3.7×10^{-4} mg L^{-1} endosulfan detected at rice fields have less impact on survivorship of *M. macrocopa* but endosulfan residue at 2.55×10^{-2} mg L^{-1} would probably decrease the survivorship of *M. macrocopa* in rice field areas.

After exposure to endosulfan; even at μg L^{-1} level, cumulative birth of *Moina macrocopa* decreased (Fig. 2). A *M. macrocopa* brooder produced 78 neonates during her whole life span of 15 days. When *M. macrocopa* exposed to 4×10^{-4} mg L^{-1} endosulfan, the brooder produced only 25 neonates throughout its life span. Cumulative birth of the cladoceran reduced drastically at NOEC; 2×10 mg L^{-1} endosulfan, *M. macrocopa* brooder produced only 2 neonates throughout its life span. Table 3 reveals life table parameters of *M. macrocopa* exposed to endosulfan. Endosulfan caused negative shift of average longevity, initial age of reproduction and intrinsic rate of natural increase for *M. macrocopa*. When *M. macrocopa* were exposed to 4×10^{-4} and 2×10^{-3} mg L^{-1} endosulfan, average longevity of the organisms reduced from 12.5 days

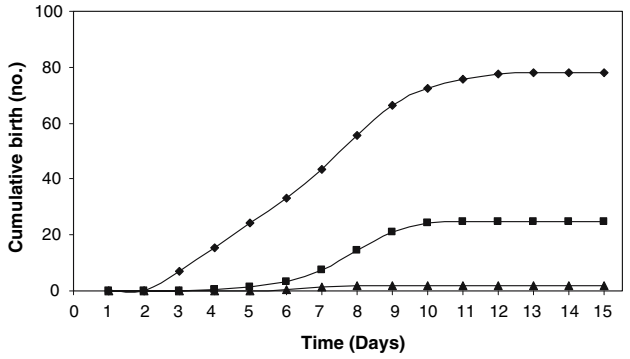

Fig. 2 Cumulative numbers of young produced by a single *Moina macrocopa* after being exposed to endosulfan at 0 (*filled diamond*), 4×10^{-4} (*filled square*) and 2×10^{-3} mg L^{-1} (*filled triangle*)

under control conditions down to 11.6 and 7.6 days, respectively. The intrinsic rate of natural increase (r) for the cladoceron decreased from 0.63 in the control experiment down to 0.39 and 0.13 respectively. Exposure time to endosulfan that causes 50% mortality in total population of organisms is defined as LT50 (LT = Lethal Time) while time budgeted for reproduction of one to other generations by an organism is represented by T. Both LT50 and T values of 4×10^{-4} mg L^{-1} did not differ greatly from their respective controls. However, a marked reduction in LT50 and T of *M. macrocopa* occurred after being exposed to 2×10^{-3} mg L^{-1} endosulfan. Net reproduction rate (Ro) is defined as the total number of young that produced by a single female in her entire lifetime. The value of Ro was reduced drastically as endosulfan concentration was increased even at concentration as low as 4×10^{-4} mg L^{-1}.

The results of this chronic study have demonstrated that although low concentration of endosulfan at 4×10^{-4} mg L^{-1} has less impact on survivorship of *M. macrocopa*, the cumulative birth of *M. macrocopa* was found to be greatly decreased after being exposed to the same concentration. Cumulative birth of *M. macrocopa* was brought down by endosulfan drastically with Ro value at 4×10^{-4} and 2×10^{-3} being reduced up to approximately 70% and 97% as compared to control, respectively. Therefore, endosulfan residues detected at 3.70×10^{-4} and 2.55×10^{-2} mg L^{-1} in the rice fields of Muda area could possibly reduce population of *M. macrocopa*.

However, the results of laboratory toxicity test cannot be directly extrapolated to field conditions since differences in survival and reproduction rate may be caused by population density of zooplankton and algae in the environment (Hanazota 1998). As the population density of zooplankton increased, the algae level decreased and the measured concentration of endosulfan in the water increased. In contrast, when the zooplankton population densities decreased, the algae population number increased while the measured concentration of endosulfan in the water decreased (Hanazota 1998). Barry et al. (1995) interpreted that endosulfan absorbed by algae has low toxicity to zooplankton because endosulfan can undergo reversible binding dynamics with the plant material.

Previous studies indicated that cumulative birth of gravid females grass shrimp, *Palaemonetes pugio*, was reduced by 31% at 2×10^{-4} mg L^{-1} and 39% at 4×10^{-4} mg L^{-1} after endosulfan exposure (Wirth et al. 2002). The average number of young produced by one female and number of broods per female of freshwater cladoceran, *Daphnia magna*, after being exposed to 0.20–0.31 mg L^{-1} of endosulfan was reported significantly different from control (Fernandez-Casalderry et al. 1993). In subsequent study of the same researchers, it was found that *D. magna* also decreased feeding rate and filtration at the same concentrations (Fernandez-Casalderry et al. 1993). Our findings imply that *M. macrocopa* is much more sensitive than other cladoceran and crustacean in response to toxicity of endosulfan.

The time of first reproduction was delayed by endosulfan exposure in which average initial age of *M. macrocopa* reproduction at control, 4×10^{-4} and 2×10^{-3} mg L^{-1} was at day 4, 5 and 6, respectively. Similar effect of endosulfan in first reproduction on *D. magna* also has been reported by Fernandez-Casalderry et al. (1993). However, the result of this study was slightly different from some other reports which found the time of first reproduction in *M. macrocopa* (Wong et al. 1995; Wong 1993) was not affected by exposure to malathion and heavy metals. This study has demonstrated that both survivorship and reproduction parameters are very important in term of endosulfan impact to *M. macrocopa*. These parameters should be always taken into account when estimating the toxicity effects of the insecticide on this cladoceran.

In conclusion, it can be stated that endosulfan is highly toxic to *M. macrocopa*. The chronic test has revealed that net reproduction and intrinsic rate of natural increase were

Table 3 Life table parameters of *Moina macrocopa* at different concentrations of endosulfan

Concentration (mg L^{-1})	Average longevity (days)	LT50 (days)	Initial age of reproduction (days)	Ro	r	*T* (days)
0	12.5	~13.0	4	78.4	0.63	7.0
4×10^{-4}	11.6	~12.0	5	24.8	0.39	8.0
2×10^{-3}	7.6	8.0–9.0	6	2.1	0.13	3.9

reduced sharply at 2×10^{-3} mg L^{-1} (NOEC) and even at concentration as low as 4×10^{-4} mg L^{-1}. This fact should be taken into consideration when this insecticide is applied in aquatic agro-ecosystem.

Acknowledgments This research was funded by UMT fundamental research grant 54152. We would like to express our appreciation to Prof Dr Wong Chong Kim from The Chinese University of Hong Kong for his technical assistance.

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