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The impacts of some plant protection chemical OCPs on two crustacean species *Daphnia magna* and *Moina macrocopa*

Tran Thi Thu Huong[1,*], Nguyen Xuan Tong[2], Le Hung Anh[2], Le Van Hau[3]

[1]*Faculty of Environment, Hanoi University of Mining and Geology, Hanoi, Vietnam*
[2]*Institute of Environmental Science, Engineering and Management, Industrial University of Ho Chi Minh city, Ho Chi Minh city, Vietnam*
[3]*Department of Fisheries Biotechnology, Biotechnology Center of Ho Chi Minh city, Ho Chi Minh city, Vietnam*
[*]E-mail: huonghumg@gmail.com

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ABSTRACT

DDT (Dichloro-diphenyl-trichloroethane) and chlordane are the organochlorine pesticides (OCPs) dangerous to human health and easily accumulate in biological tissues, used to control insects in crops, livestock and house protection. This study aimed to evaluate the toxicity of DDT, *cis* (*alpha*-chlordane), and *trans* (*gamma*-chlordane) on the growth of two crustaceans species *Daphnia magna* (*D. magna*) and *Moina macrocopa* (*M. macrocopa*) after 24 h and 48 h. Each test concentration selected 9 individuals of each species and repeated 4 experimental times, the study results showed that the 50% lethal concentration (LC50) of *D. magna* and *M. macrocopa* when exposed to DDT for 24 h were 20.8 $\mu g.L^{-1}$ and 13.5 $\mu g.L^{-1}$, respectively; after 48 h the value decreased to only 4.8 $\mu g.L^{-1}$ and 1.7 $\mu g.L^{-1}$. Similarly, LC_{50} values of *cis* (*alpha*-chlordane) on 2 species after 24 h exposure were 12.4 $\mu g.L^{-1}$ and 11.8 $\mu g.L^{-1}$, respectively; after 48 h were 4.6 $\mu g.L^{-1}$ and 4.9 $\mu g.L^{-1}$. The calculation results of LC50 when exposed to *trans* (*gamma*-chlordane) of *D. magna* and *M. macrocopa* after 24 h are 17.6 $\mu g.L^{-1}$ and 12.4 $\mu g.L^{-1}$, respectively; after 48 h, it decreased to 3.8 $\mu g.L^{-1}$ and 3.7 $\mu g.L^{-1}$ ($p < 0.05$). The results of the acute toxicity assessment also indicated that *M. macrocopa* was more sensitive to toxicity than *D. magna* with the same test conditions.

Keywords: *D. magna*, *M. macrocopa*, LC_{50}, DDT, chlordane, toxicity.

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INTRODUCTION

Organochlorine pesticides (OCPs) are persistent organic pollutants (POPs) that have obtained a significant concern worldwide due to their persistence, long-distance transport, and bioaccumulation [1], negative impacts on aquatic organisms, humans, the environment, and ecosystems. *Daphnia magna* (*D. magna*) and *Moina macrocopa* (*M. macrocopa*) are two invertebrate crustaceans found in the most freshwater ecosystems over the world, directly affected by OCPs such as Dichloro-diphenyl-trichloroethane (DDT), *cis* (*alpha*-chlordane) and *trans* (*gamma*-chlordane). According to the ECOTOX database, the most common species used in the standardized toxicity assessment include *Ceriodaphnia dubia*, *D. magna*, *D. pulex*, and other daphnids such as *M. macrocopa* and *M. microra* [2]. These model organisms are susceptible to toxic chemicals and occupy a central position in the food chain [3]. In addition, *D. magna* and *M. macrocopa* are indicator organisms with high environmental sensitivity and easy to identify and control toxic substances, so they are usually used as standard organisms for toxicity testing on a pilot scale. Therefore, many previous studies have used *D. magna* and *M. macrocopa* to evaluate water quality and ecological toxicity [4–6]. However, there is little information on the acute toxicity assessment of OCPs on two crustaceans *D. magna* and *M. macrocopa*, in Vietnam. Several relevant published studies, such as those evaluating the acute toxicity of *o,p'*-DDT on fish *Oryzias curvinotus* embryos, had recorded lethal concentrations of 50% (LC50) of 0.0813 µg/L and 0.0406 µg/L at 24 h and 48 h, respectively, and these concentrations have a substantial impact on heart rate, organ malformations and eye edema [7]. Another study showed that endosulfan - a pesticide OCPs caused morphological changes, affecting the growth and survival of *D. magna* with LC_{50} values at 24 h and 48 h of 0.332 µg/L and 0.129 µg/L, respectively [8]. This study was performed to fill in the lack of data regarding the acute effects of OCPs such as DDT, *cis*, and *trans*-chlordane on freshwater crustaceans.

This study aims to determine the effects of OCPs, including DDT, *cis*, and *trans*-chlordane, on two crustaceans *D. magna* and *M. macrocopa*. The experiments were conducted in a medium containing the toxin to estimate the concentration of LC_{50}.

MATERIAL AND METHOD

Experimental organisms

D. magna was developed at the laboratory, Institute of Science, Technology, and Environmental Management Industrial University of Ho Chi Minh at 21 ± 2°C, and the light-dark cycle is 16 h:8 h with intensity lighting varied from 500–800 lux.

M. macrocopa was obtained from the Center for Biotechnology in Ho Chi Minh and developed at 25 ± 2°C, and the light-dark cycle of 16 h:8 h in medium-hard water (medium) EPA [9].

The food for the experimental organism was the unicellular algae *Chlorella vulgaris*, once every 2–3 days and cleaned weekly. The experimental organisms (24 h of age) were not fed for 24 h before being collected for testing.

The medium used for the organism and the experiments was tap water filtered through a 0.45 µm polymembrane filter. The dissolved oxygen concentration was between 5 mg/L and 7 mg/L with pH 7–8. The culture period for one generation was two weeks before testing.

Chemicals

The OCPs selected for experiments include DDT, *cis*, and *trans*-chlordane derived from Sigma-Aldrich with high purity (≥ 97%). The chemicals were diluted into concentration ranges according to the Mexican Norm NMX-AA-087-SCFI guidelines [10].

Stock solutions were stored in dark glass with rough stoppers at 4°C to minimize the possibility of photodegradation.

Methodology

Experimental setup

Experiments were repeated four times in a 6-wells SPL tray (Korea). Each experimental well contains 9 mL of OCPs, and the control sample well is absent of chemicals. Nine individuals of *D. magna* and *M. macrocopa* (24 h of age) were exposed to

the chemical DDT at concentrations 0, 5, 10, 24, 30, 35 µg/L, and *cis*, *trans*-chlordane are 0, 4.5, 6.3, 9.2, 13.5, 19.8, 26 µg/L [11]. The mortality rate at 24 h and 48 h exposure to the chemical were recorded and calculated. The experimental setup steps are shown in Figure 1 below:

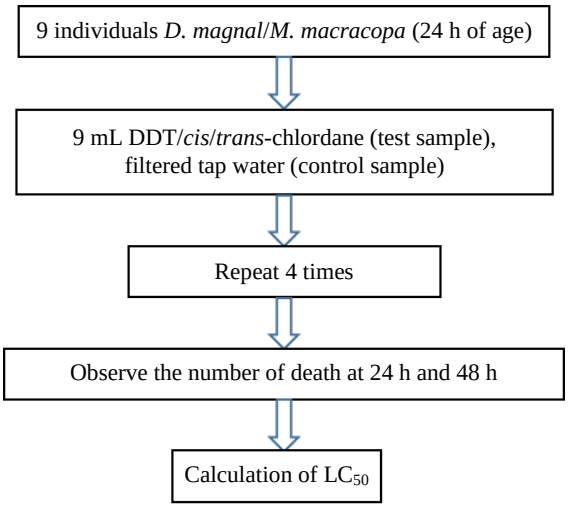

Figure 1. Experimental setup steps

All experiments were done four times, and the data were calculated as mean ± SE (standard error) by the JMP 13 with a significance level of $p < 0.05$. LC_{50} values at 24 h, 48 h, and p were calculated using the statistical probity with SPSS 20 software [12].

RESULTS AND DISCUSSION

Effects of DDT

The results of the DDT toxicity assessment on *D. magna* and *M. macrocopa* after 24 h and 48 h are shown in Figure 2 and Table 1.

Table 1 showed that the LC_{50} values on *D. magna* and *M. macrocopa* when exposure to DDT was 20.8 µg/L and 13.5 µg/L at 24 h, respectively; After 48 h, the value decreased to only 4.8 µg/L and 1.7 µg/L ($p < 0.05$). The results showed that *M. macrocopa* is more sensitive to DDT chemicals than *D. magna* when exposed to the same concentration and time of testing.

After 24 h exposure to DDT concentrations of 0, 5, 10, 24, 30, 35 µg/L, the average mortality rate changed of 0, 22, 33, 33, 39, 50% for *D. magna* and 0, 25, 36, 44, 58, 61% for *M. macrocopa*, respectively (Figure 2a). These results proved that DDT caused the death of *M. macrocopa* individuals higher than *D. magna* from concentrations > 10 µg/L.

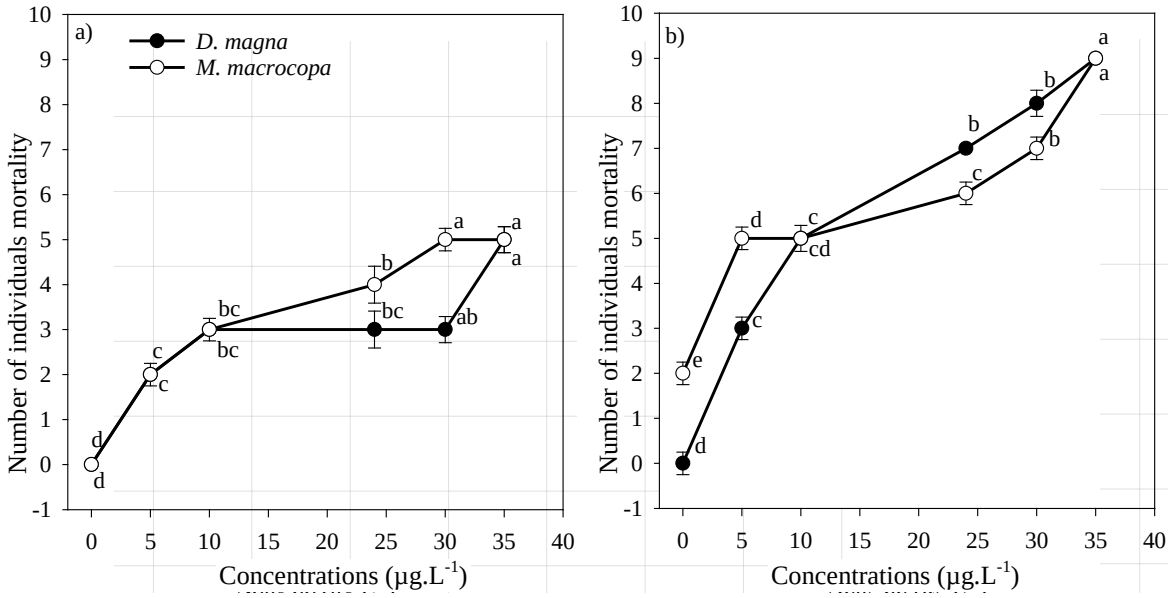

Figure 2. Variation in individual mortality of *D. magna* and *M. macrocopa* after 24 h (a) and 48 h (b) exposure to 0, 5, 10, 24, 30, and 35 g/L DDT

Although there was a change in mortality between the two studied species, the value of 100% was recorded at the highest experimental concentration (35 µg/L) after 48 h, including 0, 3, 42, 50, 78, 83, 100% for *D. magna* and 0, 25, 47, 56, 64, 75, 100% for *M. macrocopa* ($p < 0.05$) (Figure 2b).

Table 1. Toxicity of OCPs to *D. magna* and *M. macrocopa*

Chemicals	Exposure time (h)	Values of LC_{50} (µg/L) ($n = 4$)	
		D. magna	*M. macrocopa*
DDT	24 h	20.8 (19.5–22.2)	13.5 (12.4–14.7)
	48 h	4.8 (4.4–5.2)	1.7 (1.2–2.2)
Cis-chlordane	24 h	12.4 (11.8–13.0)	11.8 (11.2–12.6)
	48 h	4.6 (4.4–4.9)	4.9 (4.6–5.2)
Trans-chlordance	24 h	17.6 (16.7–18.5)	12.4 (11.8–13.0)
	48 h	3.8 (3.5–4.1)	3.7 (3.4–4.0)

Note: Values in brackets represent lower and upper bounds with 95% confidence intervals.

Effect of *cis*-chlordane

The LC_{50} results on *D. magna* and *M. macrocopa* after 24 h exposure with *cis*-chlordane were 12.4 µg/L and 11.8 µg/L, respectively; after 48 h, the rates were 4.6 µg/L and 4.9 µg/L ($p < 0.05$) (Table 1). When the exposure time is prolonged, the toxicity of the chemical increases and reduces the survivability of *D. magna* and *M. macrocopa*.

Figure 3 shows the toxicity assessment results of *cis*-chlordane on *D. magna* and *M. macrocopa* at concentrations of 0, 4.5, 6.3, 9.2, 13.5, 19.8, and 26 µg/L. After 24 h exposure with *cis*-chlordanc, the mortality rates of *D. magna* and *M. macrocopa* were 0, 14, 28, 36, 44, 50, 61% and 0, 6, 14, 25, 33, 39, 47%, respectively (Figure 3a). After 48 h, the maximum mortality rate was 100%, the specific values recorded of0, 36, 44, 53, 67, 81, 100% and 0, 36, 50, 50, 61, 69, 97% ($p < 0.05$) (Figure 3b). These results showed that the toxic effect of *cis*-chlordane on *D. magna* was higher than *M. macrocopa* under the same experimental conditions.

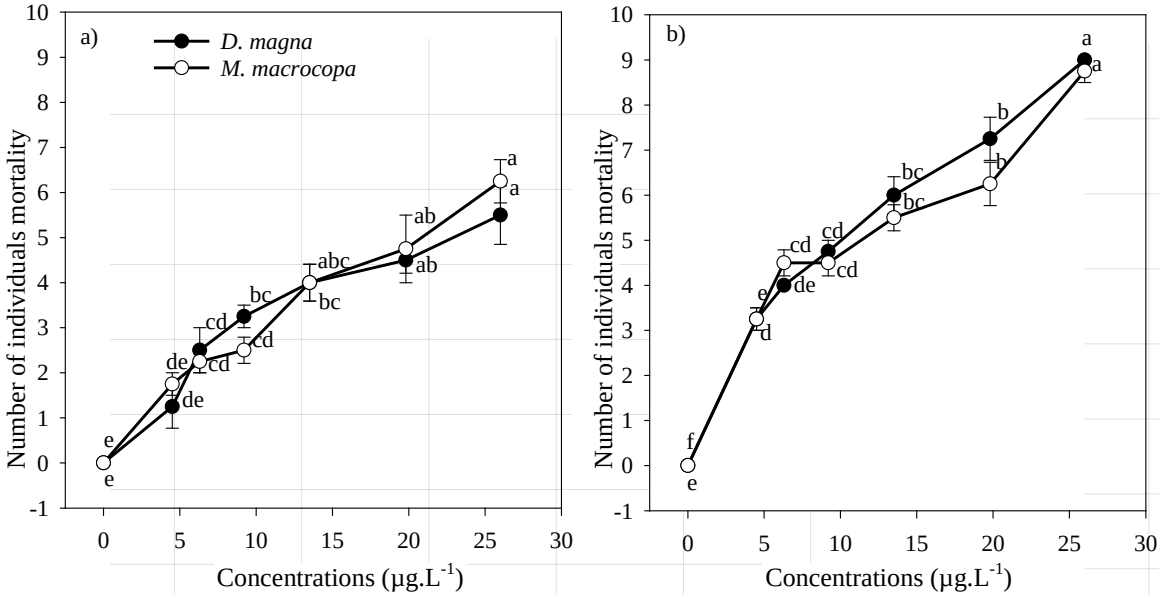

Figure 3. Theindividual mortality of *D. magna* and *M. macrocopa* varied after 24 h (a) and 48 h (b) exposure to 0, 4.5, 6.3, 9.2, 13.5, 19.8, and 26 µg/L pesticides *cis*-chlordane

Effects of *trans*-chlordane

The LC_{50} values on *D. magna* and *M. macrocopa* after 24 h exposure to *trans*-chlordane reached 17.6 µg/L and 12.4 µg/L, respectively; however, after 48 h, the values decreased to only 3.8 µg/L and 3.7 µg/L ($p < 0.05$) (Table 1). The LC_{50} values in this study indicated that *M. macrocopa* is more sensitive to *trans*-chlordane than *D. magna* when the exposure time is prolonged.

After 24 h exposure to 0, 4.5, 6.3, 9.2, 13.5, 19.8, and 26 µg/L *trans*-chlordane, the results showed that the mortality rate of *D. magna* and *M. macrocopa* increased 0, 11, 19, 22, 28, 36, 42% and 0, 17, 22, 28, 33, 39, 50%, respectively(Figure 4a). After 48 h, the control sample still did not detect dead individuals, although, in the experimental sample, the mortality rate gradually increased with the results of 0, 50, 53, 75, 75, 83, 97% for *D. magna* and 0, 47, 53, 64, 75, 83, 97% for *M. macrocopa* ($p < 0.05$) (Figure 4b). The study results on 2 chlordane isomers showed that *cis*-chlordane's toxicity to *D. magna* and *M. macrocopa* is higher than *trans*-chlordane.

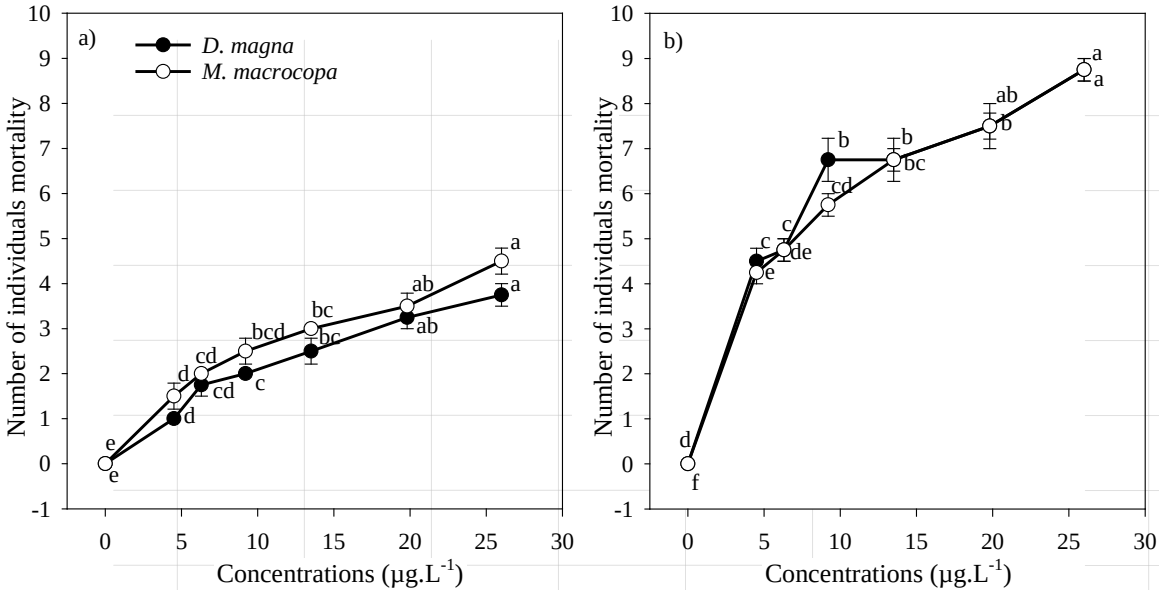

Figure 4. The individual mortality of *D. magna* and *M. macrocopa* varied after 24 h (a) and 48 h (b) exposure to 0, 4.5, 6.3, 9.2, 13.5, 19.8, and 26 µg/L pesticides *trans*-chlordane

DISCUSSION

OCPs enter water bodies through dry and wet deposition from the air, accumulating in rivers, lakes, and global oceans [13], causing negative impacts on aquatic organisms. The LC_{50} value recorded on *D. magna* after 48 h exposure to DDT in this study was 4.8 µg/L, which was higher than the value in the study by Kuo et al., (2012) 1.4 µg/L [14] but lower than the value recorded in the study by Mejía-Saavedra et al., (2005) 5.2 µg/L [15], by Ivorra et al., (2019) 260 µg/L [16]. After 48 h of exposure to DDT, the LC_{50} value in this study with *M. macrocopa* was 1.7 µg/L, much lower than the result recorded by Liu et al., (2008) 324 µg/L [6]. Furthermore, when exposed to DDT, the LC_{50} value on *Hyalella azteca* crustacean was 0.17 µg/L, lower than the value in this study. Due to the exposure time being prolonged up to 96 h, so the toxic effect of the chemical on the organism is also higher[17].

Several studies about acute toxicity have reported that DDT affects the survivability of fish and fish embryos under laboratory conditions [18, 19]. Evaluation of acute toxicity with *o,p'*-DDT on *Oryzias curvinotus* fish embryos recorded LC_{50} values at 24 h and 48 h of 0.0813 µg/L and 0.0406 µg/L, respectively

[7]. The present study results showed that the LC_{50} values on *D. magna* after 24 h and 48 h exposure with *cis*-chlordane, and *trans*-chlordane, respectively, were 12.4 µg/L and 4.6 µg/L; 17.7 µg/L and 3.8 µg/L. These results are lower than the values reported by Manar et al., (2009), 22.6 µg/L and 13.4 µg/L, respectively [20]. Some authors also noted that chlordane affects not only the survival but also the body size and fertility of *D. magna* [20, 21]. In addition, the LC_{50} values on fish with *cis*-chlordane and *trans*-chlordane were recorded at 7.4 µg/L [22] and 70 µg/L, respectively[23].

The results of the toxicological assessment with OCPs showed that they were capable of causing a 100% mortality rate in both crustaceans, including *D. magna* and *M. macrocopa* [24]. The experimental conditions, such as chemicals [7], the exposure time to toxic substances (long or short) [25], were affected and toxic to aquatic organisms. Thus, there is a close relationship between the surviability of organisms with the forms and concentrations of pesticides added to the environment.

CONCLUSIONS

This study investigated the toxicity of DDT, *cis*, and *trans*-chlordane on two crustaceans *D. magna* and *M. macrocopa*. The results showed that chlordane was more toxic on *D. magna* (LC_{50} values at 24 h and 48 h were 12.4 µg/L and 4.6 µg/L for *cis*-chlordane; 17.6 µg/L and 3.8 µg/L for *trans*-chlordane, respectively) compared with DDT (28.8 µg/L and 4.8 µg/L, respectively). Evaluation results on *M. macrocopa* also detected chlordane toxicity (LC_{50} values at 24 h and 48 h were 11.8 µg/L and 4.9 µg/L for *cis*-chlordane; 12.4 µg/L and 3.7 µg/L, for *trans*-chlordane, respectively) was higher than DDT (13.5 µg/L and 1.7 µg/L, respectively) with the same experimental conditions. The results demonstrated that survival of both species decreased after exposure to DDT, *cis*, and *trans*-chlordane chemicals, in which *M. macrocopa* was more sensitive to OCPs than *D. magna*.

This study's findings help improve understanding of the adverse effects of OCPs on freshwater aquatic animals. The results also serve as a reference database for performing toxicity tests against other crustaceans (these groups have optimal growth limits in aquatic ecology or marine environments).

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