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Toxicity of Chiral Pesticide *Rac*-Metalaxyl and *R*-Metalaxyl to *Daphnia magna*

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Abstract Chirality in pesticides has become a challenge because of enantiomers' different toxicities to non-target organisms. Acute and chronic toxicities of *Rac*-metalaxyl and *R*-metalaxyl to *Daphnia magna* were determined and compared. The 48-h LC50 for *Rac*- and *R*-metalaxyl to *Daphnia magna* were 51.5 and 41.9 mg/L. In a 14-day chronic test, the lowest-observed-effective concentration (LOEC) and no-observed-effective concentration (NOEC) of *Rac*-metalaxyl were 2 and 1 mg/L, respectively, whereas those of *R*-metalaxyl were 1 and 0.1 mg/L. Body length, days-to-first-brood and number of broods per female were significantly ($p < 0.05$) affected by *R*-metalaxyl at >1.0 mg/L, but affected by *Rac*-metalaxyl at ≥2.0 mg/L.

Keywords Metalaxyl · Chiral pesticide · *Daphnia magna* · Toxicity

Many modern pesticides contain chiral structures and therefore consist of enantiomers. About 25% of currently used pesticides are chiral, and use is increasing as compounds with more complex structures are introduced into the market (Liu et al. 2005). Enantiomers of the same compound have identical physical and chemical properties, and are treated as a single compound in standard chemical analysis. For economic reasons, chiral pesticides are primarily used as mixtures of enantiomers or racemates. Enantiomers are known to selectively interact with biologic systems that are usually enantioselective, and may behave in a dramatically different way (Liu et al. 2005). The role of enantioselectivity for pesticides in environmental safety is poorly understood. This knowledge gap is reflected in that most chiral pesticides are used and regulated as if they were achiral (Williams 1996).

A chiral pesticide has two or more enantiomers that share identical physiochemical properties. Enantiomers of the same pesticide may behave differently with respect to biologic activities and in biologically mediated environmental processes because biochemical receptors and enzymes distinguish chirality (Liu et al. 2005). Different enantiomers have different bioactivity, but the environmental risks of chiral pesticides are poorly understood and are regulated as if they were achiral (Lewis et al. 1999; Harner et al. 2000). Increasing evidence suggests that enantioselectivity may occur in environmental processes and different enantiomers of a chiral pesticide may possess different ecotoxicological risks (Williams 1996; Kohler et al. 1997). Studies on environmental enantioselectivity of pesticides first appeared in the early 1990s. Most studies focused on residual chiral pesticides (e.g., *a*-HCH, chlordane, *o*, *p*-DDT) (Hegeman and Laane 2002) and little is known about the environmental enantioselectivity of chiral pesticides (e.g., metalaxyl). Many pesticides have high activity against non-target organisms and are also chiral; they are acutely toxic to a wide range of aquatic organisms at trace levels (Liu et al. 2005). *Rac*-metolachlor was more toxic than *S*-metolachlor to *Daphnia magna* (Liu et al. 2006), but the toxicity of metalaxyl has not been reported.

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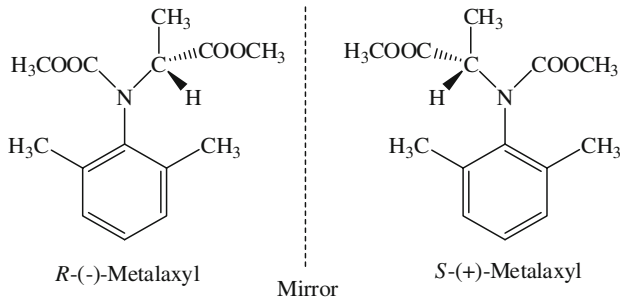

Fig. 1 Chemical structure of *R*-metalaxyl and *S*-metalaxyl

Metalaxyl is an important acetanilide fungicide widely used to control plant disease caused by pathogens of the Oomycota division. Metalaxyl contains an asymmetrically substituted C atom ("C"-chirality) and has two enantiomers; the anti-fungicidal activity mostly originates from the *R*-enantiomer (Fig. 1) (Buerge et al. 2003; Kurihara and Miyamoto 1997). Metalaxyl has a half-life of 7–170 days in soil. A representative half-life in moist soils is about 70 days (Wauchope et al. 1992). The half-life of metalaxyl in water was >4 weeks at pH 5–9 and temperatures of 20–30°C (EPA 1988).

Studies of the toxicity of chiral pesticides on non-target organisms set important models for the evaluation of the effect of pesticides on the aquatic environment. *Daphnia magna* has been widely used to conduct acute and chronic toxicity assays (Liu et al. 2004). Little information is available about the toxicity of Rac-metalaxyl and *R*-metalaxyl to *Daphnia magna*.

The present study determined the acute and chronic toxicities of *Rac*-metalaxyl and *R*-metalaxyl to a freshwater cladoceran *Daphnia magna*. Differences in toxicity of *Rac*-metalaxyl and *R*-metalaxyl were also illustrated.

Materials and Methods

Daphnia magna was obtained from continuous cultures maintained in the laboratory at 20 ± 1°C in OECD M4 culture medium (OECD 1995). A light:dark period of 16:8 h was maintained. Animal density was <50 individuals/L.

The medium was renewed twice a week. Daphnids were fed daily with the alga *Scenedesmus obliquus*, which were cultivated using a nutrient medium (Ma 2002). Test animals used in the experiment were neonates aged ≤24 h.

Rac-metalaxyl and *R*-metalaxyl (purity ≥98.0%) were purchased from Zhejiang Heben Pesticide and Chemicals Company Limited (Wenzhou, China). Stock solution was prepared by dissolving each toxicant in diluted water (ISO 1996) and the concentration was 1000 mg/L. Stock solution was stored in darkness in a refrigerator at 4°C.

The standard protocol of the acute toxicity test for *Daphnia magna* was used (ISO 1996). Twenty neonates were transferred into glass beakers filled with 20 mL of test solution at concentrations of 0.01–5.0 mg/L. Four replicates for each treatment were done. Test animals were incubated at 20 ± 1°C for 24 h. Daphnids were not fed during the test. Mortality of daphnids was observed after incubation for 48 h.

We knew that metalaxyl at 0.01 mg/L had no effect on *Daphnia magna* because of a preliminary test. In the chronic toxicity test, daphnids (age, ≤24 h) were exposed to the reagent for 14 days (Tong and Huailan 1996) at five concentrations of *Rac*-metalaxyl or *R*-metalaxyl following the guidelines set by (OECD 1995). Daphnids were raised individually in 50-mL glass beakers containing 20 mL of test solution, which comprised OECD M4 culture medium with food and pesticide. *Scenedesmus obliquus* was added at a density of 5×10^5 cells/mL. Ten replicates for each treatment were done. Incubation temperature was controlled at 20 ± 1°C, and the 16:8 h light:dark period maintained.

Data from the chronic test were analyzed using one-way ANOVA to detect differences ($p < 0.05$). Analyses were done not only between treated groups and blank control, but also between *Rac*-metalaxyl and *R*-metalaxyl, followed by Duncan's test ($p = 0.05$) with the SPSS computer program (Nie and Hull 1981). The confidence interval (CI) was set at 95%.

Results and Discussion

The LC50 of *Rac*-metalaxyl and *R*-metalaxyl for *Daphnia magna* at 48-h were 51.5 mg/L (45.5–58.3 mg/L;) (Fig. 2) and 41.9 mg/L (35.2–49.8 mg/L) (Fig. 3), respectively. Both agents had low toxicity based on the classification standard of toxicity (Xiong 2001). The acute toxicity of enantiomers was consistently different. The acute toxicity of other enantiomeric chemicals to *Daphnia magna* has been reported (Yen et al. 2003). The LC50 value of (−)-leptophos is much higher than those of (+)- and (±)-leptophos, indicating that (−)-leptophos is much less toxic to *Daphnia magna* than its enantiomer. The 48-h LC50 value in our test showed that the acute toxicity of *R*-metalaxyl was slightly higher than that of *Rac*-metalaxyl.

Evaluating the toxicity of low concentrations of *Rac*-metalaxyl and *R*-metalaxyl in the environment is important. Results of the chronic test explained the toxicity of the pesticides to *Daphnia magna*. The lowest-observed-effect concentration (LOEC), no-observed-effect concentration (NOEC), days-to-first-brood, body length, longevity, number of broods per female, and number of young per female were determined. Chronic effects of *Rac*-metalaxyl and *R*-metalaxyl on the survival and reproduction of

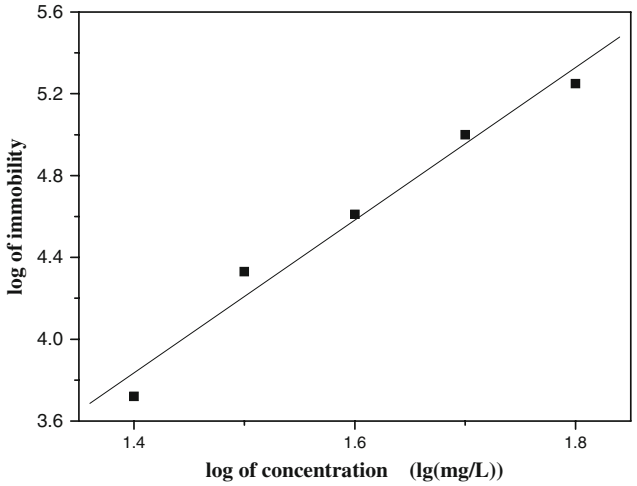

Fig. 2 LC50 of *Rac*-metalaxyl at 48 h

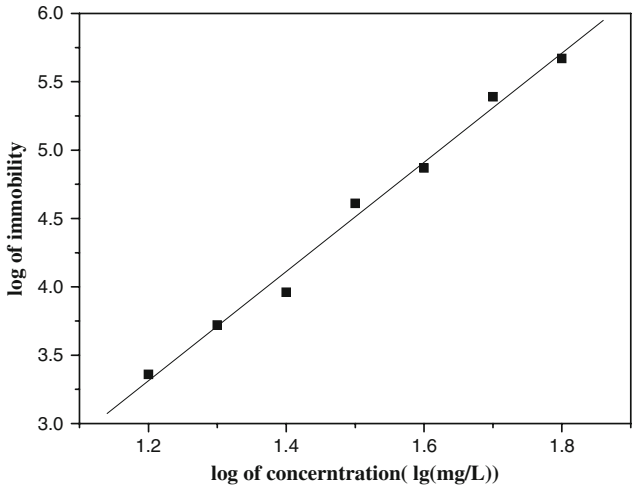

Fig. 3 LC50 of *R*-metalaxyl at 48 h

Daphnia magna are shown in Tables 1 and 2, respectively. Statistical variation of chronic toxicity in *Rac*-metalaxyl and *R*-metalaxyl are shown in Table 3. Compared with the blank control, the longevity of *Daphnia magna* was not affected by *Rac*-metalaxyl and *R*-metalaxyl.

Compared with the blank control, the body length of 14-day-old *Daphnia magna* was significantly reduced for *Rac*-metalaxyl at 2.0 mg/L, whereas the concentration was 1.0 mg/L for *R*-metalaxyl. Compared with those exposed to *Rac*-metalaxyl, the body length of *Daphnia magna* was reduced more for *R*-metalaxyl at the same concentration. Compared with the blank control, significant differences were observed at 1.0, 2.0, and 5.0 mg/L.

The parameter of days-to-first-brood was significantly different from the control ($p < 0.05$) when *R*-metalaxyl was ≥1.0 mg/L. For *Rac*-metalaxyl, the parameter of days-to-first-brood was significantly different from the control at 2.0 mg/L. No significant difference was found between *Rac*-metalaxyl and *R*-metalaxyl when comparing days-to-first-brood.

Compared with the blank control, number of broods per female of *Daphnia magna* decreased significantly ($p < 0.05$) when *Rac*-metalaxyl and *R*-metalaxyl were ≥1.0 mg/L. A significant difference ($p < 0.05$) in the number of young per female was observed between *Rac*-metalaxyl and *R*-metalaxyl at 2 and 5 mg/L. Compared with the blank control, the number of young per female of *Daphnia magna* decreased significantly ($p < 0.05$) when *Rac*-metalaxyl was ≥2.0 mg/L, but a significant effect was found in *R*-metalaxyl at ≥1.0 mg/L.

For number of broods per female, significant differences between *Rac*-metalaxyl and *R*-metalaxyl were observed at 1, 2, and 5 mg/L. Based on the significant difference of reproduction parameters in different treatments at different concentrations, the LOEC and NOEC of *Rac*-metalaxyl to *Daphnia magna* were 2 and 1 mg/L, respectively, whereas those of *R*-metalaxyl were 1 and 0.1 mg/L, respectively. Differences between *Rac*-metalaxyl and *R*-metalaxyl were in body length; number of broods per female; and number of young per female. It was illustrated that *R*-metalaxyl influences growth and reproduction more than *Rac*-metalaxyl.

R-metalaxyl was much more toxic to *Daphnia magna* than *Rac*-metalaxyl. Parameters of chronic toxicity such as body length, days-to-first brood, and number of broods per female of *Daphnia magna* were significantly ($p < 0.05$) affected by *Rac*-metalaxyl at >1.0 mg/L, but affected by *Rac*-metalaxyl at ≥2.0 mg/L. The NOEC of *Rac*-metalaxyl and *R*-metalaxyl to *Daphnia magna* were 1.0 and 0.1 mg/L, respectively. The LOEC of *Rac*-metalaxyl and *R*-metalaxyl were estimated to be 2.0 and 1 mg/L, respectively.

Table 1 Chronic toxicity test of *Rac*-metalaxyl for 14 days

Rac-metalaxyl (mg/L)	Body length (mm)	Days-to-first-brood (days)	Number of broods per female	Number of young per female
Control	2.53 ± 0.08	7.23 ± 0.25	4.6 ± 0.7	22.9 ± 4.2
0.01	2.52 ± 0.02	7.25 ± 0.17	4.3 ± 0.8	22.9 ± 4.17
0.1	2.50 ± 0.03	7.20 ± 0.16	4.4 ± 0.8	23.3 ± 3.7
1.0	2.48 ± 0.05	7.53 ± 0.22	3.9 ± 0.7*	22.2 ± 2.8
2.0	2.16 ± 0.15*	7.68 ± 0.35*	3.8 ± 0.8*	20.3 ± 3.9*
5.0	1.58 ± 0.05*	7.63 ± 0.27*	3.6 ± 0.5*	10.2 ± 1.6*

* $p < 0.05$

Table 2 Chronic toxicity test of *R*-metalaxyl for 14 days

R-metalaxyl (mg/L)	Body length (mm)	Days-to-first-brood (days)	Number of broods per female	Number of young per female
Control	2.53 ± 0.08	7.23 ± 0.25	4.6 ± 0.7	22.9 ± 4.2
0.01	2.52 ± 0.07	7.28 ± 0.14	4.2 ± 0.8	20.4 ± 1.7
0.1	2.51 ± 0.03	7.25 ± 0.17	4.3 ± 0.8	20.6 ± 1.35
1.0	2.42 ± 0.03*	7.55 ± 0.20*	3.7 ± 0.7*	18.8 ± 2.15*
2.0	1.99 ± 0.07*	7.73 ± 0.30*	3.6 ± 1.0*	15.8 ± 2.5*
5.0	1.48 ± 0.10*	7.68 ± 0.29*	3.3 ± 0.7*	9.6 ± 2.0*

* $p < 0.05$

Table 3 Significant differences between *Rac*-metalaxyl and *R*-metalaxyl with respect to various parameters

Rac-metalaxyl (mg/L)	0.01	0.1	1	2	5
R-metalaxyl (mg/L)	0.01	0.1	1	2	5
Body length			*	*	*
Days-to-first-brood					
Number of broods per female				*	*
Number of young per female			*	*	*

* $p < 0.05$

Racemic metalaxyl is currently being replaced by *R*-metalaxyl worldwide (Hegeman and Laane 2002). This "chiral switch" is expected to reduce the amounts of pesticide applied, as well as potential side effects on non-target organisms. It is believed that much less toxicity will be introduced into the ecosystem with the commercialization of *R*-metalaxyl.

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