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Toxicity of Chlorantraniliprole and it's Formulated Product, Altacor®, to Individuals and Populations of *Ceriodaphnia Dubia* Richard

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Abstract

The toxicity of the insecticide chlorantraniliprole and its formulated product Altacor® was determined for the Cladoceran, *Ceriodaphnia dubia* Richard. Acute toxicity (48 h) and 21 d population studies were conducted. The hypothesis of this study was that these two compounds would have different toxicities. We conducted acute and chronic toxicity studies for each compound and compared the results to test this hypothesis. 48 h LC50s (95% CL) for chlorantraniliprole and Altacor® were 8.5 (6.6–11.5) and 6.0 (3.7-9.0) µg chlorantraniliprole/L water, respectively. Therefore, chlorantraniliprole and Altacor® were equitoxic to *C. dubia* at LC50 based on overlap of the 95% CL. In the population study, chlorantraniliprole and Altacor® concentrations equivalent to the acute LC5, 10, 25, and 50 for each product were evaluated on populations of *C. dubia*. Number of individuals after 21 d was the endpoint evaluated. T-tests conducted at each LC value indicated that there was no significant difference in population size between these two products at each LC value evaluated. Previous studies show that toxicity can vary greatly between formulated and technical grade pesticides. However, our results show that chlorantraniliprole and its formulated product, Altacor® were equally toxic to *C. dubia*. Therefore, making assumptions about the toxicity of formulated and unformulated products is ill advised.

Keywords Chlorantraniliprole Altacor® acute toxicity population effects *Ceriodaphnia dubia*

Despite breakthroughs in cultural and biological controls over the past decades, insecticides are still the major tool for control of insect pests in agriculture, human and animal health (Pimental 2019). Although insecticides have been beneficial in terms of increasing food production and improving animal and human health, a number of them have caused damage to the environment,. e.g., DDT); new insecticide chemistries have been and are being introduced for pest control that purport to be much less damaging to non-target organisms (Goff and Giraudo 2019). One of these newer chemistries is chlorantraniliprole (3-Bromo-N-[4-chloro-2-methyl-6-(methylcarbamoyl) phenyl]-1-(3-chloro-2-pyridine-2-yl)-1 H-pyrazole-5-carboxamide). Chlorantraniliprole is an anthranilic diamide insecticide that activates insect ryanodine receptors (RyRs), which play a critical role in muscle function (Lahm et al. 2009). Chlorantraniliprole binds to insect RyRs in muscle cells causing the channel to open and release calcium ions (Ca^{2+}) from internal stores into the cytoplasm (Lahm et al. 2009). Depletion of internal Ca^{2+} results in immediate paralysis preventing further muscle contraction and ultimately leading to death (USEPA Pesticide Fact Sheet: Chlorantraniliprole 2008). As of 2015, chlorantraniliprole was the fourth most commonly used insecticide in the world, accounting for 8% of total global insecticides sales (Sparks and Nauen 2015). Two Dupont insecticides, Coragen and Altacor, containing chlorantraniliprole are now registered and are being used for control of insect pests on a range of crops, including pome fruits, stone fruits, leafy vegetables, brassicas, cucurbits, cotton, potatoes, grapes, rice, turf and ornamentals.

The toxicity of formulated and technical chlorantraniliprole has been evaluated in pest insect species (Guo et al.

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2013; Han et al. 2012; Huang et al. 2016; Sial and Brunner 2010; Su et al. 2017) and beneficial insects and mites (Biondi et al. 2012; Lefebvre et al. 2011; Moscardini et al. 2013; Nawaz et al. 2017) as well as crayfish (Barbee et al. 2010). Although chlorantraniliprole is commonly used for control of specific insect pests in agriculture, other than the study by Barbee et al. (2010), there is a dearth of information on its potential toxicity to aquatic invertebrates.

The overall objective of this study was to evaluate the toxicity of chlorantraniliprole and a formulated product containing chlorantraniliprole, Altacor® on the Cladoceran, *Ceriodaphnia dubia.* Both forms of these insecticides were evaluated because differences in toxicity between technical and formulated pesticides have been found in previous studies (Basopo and Naik 2015; Puglis and Boone 2011; Stark 2001). For each of the compounds, we evaluated both individual and population-level toxicities, as previous studies have shown there may be differences in outcomes between short-term measures of effect (e.g., LC50) and population-endpoint outcomes (Forbes and Calow 1999; Stark and Banks 2003; Stark et al. 2015).

Materials and Methods

Test Organism

Ceriodaphnia dubia were obtained from cultures maintained at the Washington State University Research and Extension Center (Puyallup, WA). All *C. dubia* used in this study were reared in reconstituted dilution water (RDW) prepared according to the United States Environmental Protection Agency (USEPA 2002). The RDW satisfied the requirements with pH 7.4–7.8, dissolved oxygen (DO) > 8.0 mg/l, conductivity 260–320 µS, alkalinity of 60–70 mg/l, and a hardness of 80–100 mg/L. *C. dubia* were fed a solution consisting of a 1:1 mixture of yeast-cereal leaves-trout chow (YCT) and the algal species *Pseudokirchneriella subcapitata* (previously *Selenastrum capricornutum*). *C. dubia* cultures were maintained inside a freestanding environmental chamber set at 25 + 0.1 C, and a 16:8-h light:dark regimen.

Insecticides Evaluated

Technical-grade chlorantraniliprole (99.1%) was purchased from Chem Service, Inc (West Chester, PA, USA). Altacor® (350 g chlorantraniliprole/kg water-dispersible granules) was obtained from DuPont™.

Chemical Analysis

Chlorantraniliprole concentrations used in this study were verified by liquid chromatography using an Agilent 1260 series binary pump with tandem mass spectroscopy (Agilent 6460 triple quad mass spectrometer in electro-spray ionization mode - LC/MS/MS). Prepared dilutions of chlorantraniliprole and Altacor® used at the start of each study (acute and chronic) were injected in duplicate and verification of aqueous concentrations was determined using the external standard technique with a minimum of four linearity reference standards. Integration of chromatographic data was performed using Agilent MassHunter software.

48 h static acute mortality tests.

Chlorantraniliprole concentrations were prepared by serial dilution from fresh stock solutions in 20 L RDW at 1,000 µg ai/L. An initial bracketing was done for chlorantraniliprole and Altacor® with seven concentrations (0, 0.01, 0.1, 1.0, 10.0, 100, 1,000 µg chlorantraniliprole/L). Thereafter, the following concentrations were evaluated for chlorantraniliprole: 0, 0.01, 0.1, 1, 2, 4, 6, 8, 10, 50 µg chlorantraniliprole /L. For Altacor®, the following concentrations were evaluated: 0, 0.01, 0.1, 0.5, 1, 2, 4, 6, 8, 10, 50 µg chlorantraniliprole/L. Four batches of 10 neonate (< 24 h) *C. dubia* from the third-filial generation (F_3) were placed in 50 ml glass beakers containing 30 ml of chemical solution or RDW (control) for each concentration tested. A plexiglass lid was placed over the top of each beaker to reduce evaporation. The *C. dubia* were fed with a mixture of yeast-cereal leaves-trout chow (YCT) and the algal species *Pseudokirchneriella subcapitata* in a ratio of 1:1.5. The *C. dubia* were kept in an environmental chamber with a 16 h/8 h day/night cycle, 25.0 ± 0.1°C. This was a non-renewal test and therefore water was not changed over the course of 48 h. Daphniids were considered dead when the heart was not beating. This study was replicated a minimum of three times on different days for a total of twelve replications each for chlorantraniliprole and twelve replications for Altacor.

Population Toxicity Study

Stock solutions (1,000 µg chlorantraniliprole/L) of chlorantraniliprole and Altacor® were prepared in 20 L RDW. Based on the results of the 48 h acute toxicity study for each compound, a series of concentrations for chlorantraniliprole and a separate series of concentrations for Altacor® were prepared from these stock solutions. Four concentrations of chlorantraniliprole, 1.3, 1.9, 3.2, 6.0 µg chlorantraniprole/L which corresponded to the acute LC5, LC10, LC25, and LC50, respectively, were tested. Four concentrations of Altacor®, 0.9, 1.5, 3.4, 8.5 µg chlorantraniliprole/L, which

Table 1 Acute mortality (48 h) of Altacor® and chlorantraniliprole to *Ceriodaphnia dubia*

Chemical	Number tested (n)	Slope (± SE)	LC50 (µg/L)	95% Confidence Limits
Altacor®®	1,960	1.7 (0.21)	8.5	6.6–11.5
chlorantraniliprole	1,200	2.5 (0.62)	6.0	3.7-9.0

corresponded to the acute LC5, LC10, LC25, and LC50, respectively were also evaluated.

The *C. dubia* used in the population study were obtained from cultures at the third-filial (F_3) generation. Batches of 10 individual neonates (< 24 h) were placed in 150 ml glass beakers containing 100 ml of chemical solution or RDW (control) for each concentration tested for each compound (chlorantraniliprole and Altacor®). Beakers were covered with plexiglass lids and held under the same environmental conditions described above in the acute toxicity study. During the course of the study, 1 ml of the feeding solution described in the acute study was added to each beaker daily. Chemical solutions were not renewed during the study timeframe. Twenty-one days after the start of this study, all *C. dubia* were counted in each container to determine the final population size. This study was replicated three times. Three beakers of C. dubia were tested at the same time.

Statistical Analysis

Acute Mortality Study

Acute concentration-mortality regressions were estimated by probit analysis (Finney 1971) using the SAS probit procedure (SAS 2013).

Population Toxicity Study

The final number of individuals at the end of this study were compared between the two treatments, chlorantraniliprole and Altacor®, using a T-test at each LC value (LC5, 10, 25, and 50).

Results

Chlorantraniliprole Concentrations

The chlorantraniliprole concentrations tested in the acute and chronic studies were determined to to fall within 76 ± 6% of expected concentrations.

Table 2 Number of *Ceriodaphnia dubia* 21 d after exposure to chlorantraniliprole or Altacor®

	Number of *C. dubia* after 21 days (Mean ± standard deviation)	
Treatment	Altacor®	Chlorantraniprole
Control	1,910 ± 123a	1,704 ± 253a
LC5*	1,542 ± 258a	1,154 ± 313a
LC10	1,246 ± 32a	1,056 ± 445a
LC25	1,235 ± 225a	1,131 ± 203a
LC50	812 ± 235a	604 ± 391a

*/Actual concentrations tested that were equivalent to the LC values for each chemical were: Altacor: LC5 = 0.9 µg/L, LC10 = 1.5 µg/L, LC25 = 3.4 µg/L, LC50 = 8.5; cholorantrailiprole: LC5 = 1.3, LC10 = 1.9 µg/L, LC25 = 3.2 µg/L, LC50 = 6.0.

Means within a row followed by the same letter are not significantly different (T-test; $p > 0.05$).

Acute Mortality Study

The 48 h acute LC50s (95% CL) for chlorantraniliprole and Altacor® were 8.5 (6.6–11.5) and 6.0 (3.7-9.0) µg chlorantraniliprole/l water (Table 1). Therefore, chlorantraniliprole and Altacor® were equitoxic to *C. dubia* at LC50 based on overlap of the 95% CL.

Population Toxicity Study

The final numbers of *C. dubia* 21 d after the start of the chronic study are listed in Table 2. For both chlorantraniliprole and Altacor®, exposure to increasing concentrations resulted in fewer individuals by 21 d in a concentration-dependent manner. T-tests were conducted at each LC value and results indicated that there were no significant differences ($p \geq 0.05$) in population number between these two products at each LC value (Table 2).

Populations of *C. dubia* exposed to the LC50s for each chemical in the population study were greatly reduced compared to the control. Populations exposed to the Altacor LC50 were reduced by 57% compared to the control. Populations exposed to the chlorantraniliprole LC50 were 65% lower than the control population.

Discussion

Results of this study showed that there were no significant differences in neither acute nor population-level toxicity between chlorantraniliprole and Altacor® to *C. dubia*.

To our knowledge, there are no published studies documenting the toxicity of these products to Daphniids. However, acute 48 h EC50 for *D. magna* for technical

chlorantraniliprole and a formulated product with the same amount of active ingredient as Altacor® (35%) are listed in the USEPA Fact sheet for chlorantraniliprole (USEPA Pesticide Fact Sheet: Chlorantraniliprole 2008). The *D. magna* 48 h acute EC50s for chlorantraniliprole and the formulated product with the same chlorantraniliprole concentration as Altacor® are 11.6 and 11.0 µg chlorantraniliprole/L water, respectively. The chlorantraniliprole EC50 for *D. magna* (11.6 µg chlorantraniliprole/L) falls just outside the 95% CL developed in our study (6.6–11.5 µg chlorantraniliprole/L) for *C. dubia* indicating that *D. magna* and *C. dubia* have similar susceptibility to chlorantraniliprole. The *D. magna* EC50 for the formulated product (11.0 µg chlorantraniliprole/L) falls within the 95% CL developed for *C. dubia* in our study, indicating that *C. dubia* and *D. magna* have similar susceptibility to a formulated product of chlorantraniliprole.

We found that *C. dubia* populations were significantly reduced compared to the control populations after chronic exposure to the LC50 of Altacor and chlorantraniliprole. According to the USEPA Pesticide Fact Sheet for chlorantraniliprole (USEPA Pesticide Fact Sheet: Chlorantraniliprole 2008) the 21 d range of expected environmental concentrations (EEC) in freshwater systems is 0.37–9.1 µg chlorantraniliprole/l water. The concentrations we tested in our population studies fell within this EEC range. Our highest concentrations tested in the population study were 8.5 µg chlorantraniliprole/l for Altacor and 6.0 µg/l for chlorantraniliprole. Both of these concentrations were lower than the high range of the EPA EECs for this insecticide. This indicates that this insecticide may pose a risk to *C. dubia*. However, future studies should be conducted to evaluate risk of this species to chlorantraniliprole.

Barbee et al. (2010) conducted a 96 acute toxicity study and two longer-term (144 h and 36 d) chronic toxicity studies of technical chlorantraniliprole to the red swamp crayfish *Procambarus clarkii* Girard. This crayfish species is reared on a rotational basis with rice in Louisiana, USA. Chlorantraniliprole is applied to rice paddies to control rice water weevil (*Lissorhoptrus oryzophilus* Kuschel). Barbee et al. (2010) found that chlorantraniliprole was highly toxic to crayfish according to a USEPA category with an LC50 (95% Cl) of 951 µg/L (741–1118 µg/L). They estimated a no observed effect concentration (NOEC) of 480 µg/L. However, they concluded that chlorantraniliprole was three orders of magnitude less acutely toxic to *P. clarkii* than pyrethroid insecticides used for weevil control and therefore should be more compatible with rice–crayfish crop rotations than these pyrethroids. A comparison of the chlorantraniliprole LC50 that Barbee et al. (2010) developed for *P. clarkii* and LC50 developed for *C. dubia* (951/8.5) in this study show that *C. dubia* is 112 times more susceptible to this insecticide than *P. clarkii.*

This large difference in susceptibility indicates that more toxicity studies with chlorantraniliprole need to be conducted with a wider range of aquatic invertebrates to determine whether or not this insecticide poses a risk to aquatic ecosystems.

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