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Attention: 8(e) Coordinator
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency, ICC Building
1201 Constitution Ave., NW
Washington, DC 20004



Dear 8(e) Coordinator:

8EHQ-09-17763

This letter is a supplement to our letter of December 10, 2009, in which we informed the Agency of the results of a chronic toxicity study in *Daphnia magna* with the above referenced test substance. The study was repeated because of an unacceptably high coefficient of variation for control reproduction. In this repeat study, adult survival was the most sensitive endpoint. The NOEC for survival was 4.7 mg active substance/L.

The objective of this study was to determine the long term effects of the test substance on the survival, reproduction and growth of the cladoceran, *Daphnia magna*, under semi-static test conditions for a period of 21 days. The study was conducted according to the procedures outlined in the U.S. Environmental Protection Agency Series 850 – Ecological Effects Test Guidelines (draft), OPPTS Number 850.1300: *Daphnid Chronic Toxicity Test* and OECD Guideline for Testing of Chemicals 211: *Daphnia magna Reproduction Test*.

Daphnids were exposed to five test concentrations and a negative (dilution water) control. Mean measured test concentrations were 0.59, 1.2, 2.4, 4.7 and 9.6 mg active substance (a.s.)/L. Test solutions were renewed three times per week (i.e., Monday, Wednesday and Friday) during the first two weeks of the test and twice during the last week of the test.

Observations of each first-generation daphnid were made daily during the test. At these times, the number of immobile daphnids was recorded along with any clinical signs of toxicity (e.g., inability to maintain position in the water column, uncoordinated swimming, lethargy, opaque color or cessation of feeding). Immobility was defined as a lack of movement, except for minor spontaneous movement of the appendages, following application of a gentle stimulus. The presence of eggs in the brood pouch, aborted eggs, males or ephippia also was recorded daily. With the onset of reproduction, neonates produced by the first-generation daphnids were counted and then discarded every Monday, Wednesday, Friday, or Saturday during the test and at test termination. The body length and dry weight of each surviving first-generation daphnid were measured at the end of the test.

Adult daphnids in the negative control group produced an average of 298 live young per surviving adult (CV = 10.2%). Daphnids in the 0.59, 1.2, 2.4, 4.7 and 9.6 mg a.s./L treatment groups produced an average of 297, 312, 297, 311 and 291 live young per surviving adult, respectively. Dunnett's test indicated there were no statistically significant decreases in mean neonate production in any treatment groups in comparison to the negative control ($p > 0.05$). The NOEC for reproduction was 9.6 mg a.s./L, and the LOEC was greater than 9.6 mg a.s./L. Based on the reproduction observed in the treatment groups, the 21-day EC50 value was greater than 9.6 mg a.s./L, the highest concentration tested.

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Daphnids in the negative control group averaged 5.7 mm in length and 1.35 mg in dry weight. Daphnids in the 0.59, 1.2, 2.4, 4.7 and 9.6 mg a.s./L treatment groups had mean lengths of 5.7, 5.7, 5.7, 5.6 and 5.6 mm, respectively, and mean dry weights of 1.26, 1.32, 1.32, 1.14 and 1.32 mg, respectively. Dunnett's test indicated that mean lengths in all treatment groups were not significantly different from the negative control ($p > 0.05$). A significant difference in dry weight was noted in the 4.7 mg a.s./L treatment group in comparison to the control (Dunnett's test, $p \leq 0.05$). However, it was not considered to be treatment related since no significant difference in dry weight was noted in the 9.6 mg a.s./L, the highest treatment level. Therefore, the NOEC for growth (length and dry weight) was 9.6 mg a.s./L treatment group; the LOEC was greater than 9.6 mg a.s./L.

Adult survival was the most sensitive endpoint for the study. Survival in the 0.59, 1.2, 2.4, 4.7 and 9.6 mg a.s./L treatment groups was 70, 80, 70, 90 and 60%, respectively, at test termination. Daphnids in the test substance treatment groups that survived to test termination generally appeared normal. One surviving adult daphnid in the 4.7 mg a.s./L treatment appeared pale. When compared to the negative control group, statistically significant differences ($p \leq 0.05$) in survival at the 9.6 mg a.s./L treatment level were noted (Fisher's Exact test). The NOEC for survival was 4.7 mg a.s./L and the LOEC was 9.6 mg a.s./L. The MATC was calculated to be 6.7 mg a.s./L. Based on the immobility observed in the treatment groups, the 21-day EC50 value was >9.6 mg a.s./L, the highest mean, measured test concentration.

This information is submitted in accordance with current guidance issued by EPA indicating EPA's interpretation of Section 8(e) of the Toxic Substances Control Act or, where it is not clear that reporting criteria have been met, it is submitted as a precautionary measure and because it is information in which EPA may have an interest.

Sincerely,



A. Michael Kaplan, Ph.D.
Director - Regulatory Affairs

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