Bull Environ Contam Toxicol (2007) 78:510–514
DOI 10.1007/s00128-007-9091-0

Acute and Sub-Lethal Toxicity of Three POEA Surfactant Formulations to *Daphnia magna*

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Received: 4 August 2006 / Accepted: 15 March 2007 / Published online: 22 May 2007

Abstract Polyethoxylated tallowamine (POEA) is a nonionic surfactant used in many herbicide formulations to increase the ability of active ingredients to penetrate leaf cuticles. However, it has also been shown to disrupt respiratory membranes in aquatic organisms. In this study, *Daphnia magna* was used to examine the lethal and sublethal toxicity of three POEA formulations consisting of 5:1, 10:1, and 15:1 average oxide:tallowamine. The formulation consisting of 10:1 was the most acutely toxic with a 48-h LC50 value of 97.0 µg/L and 15:1 was least toxic at 849.4 µg/L. All formulations inhibited growth at concentrations between 100 and 500 µg/L.

Keywords *Daphnia magna* · POEA · Surfactants

Aquatic organisms adapted to ephemeral water bodies are subjected to various environmental stressors of physical, chemical, and biological origin. Those inhabiting temporary water bodies in agricultural areas are often exposed to additional stress due to the application of agrochemicals which include a variety of herbicides.

Herbicides typically consist of an active ingredient, one or more adjuvants, and inert ingredients such as dyes and coloring (Foy, 1987). Adjuvants aid or modify the action of the active ingredient, and are typically surfactants in herbicide formulations (Van Valkenburg, 1982). The herbicide Roundup® is used worldwide, and with the advent of ''Roundup-Ready'' crops the amount released into the environment is growing (United States Department of Agriculture [USDA] 2005). Roundup® contains the surfactant polyethoxylated tallowamine (POEA). POEA is a nonionic surfactant that promotes the penetration of glyphosate into the plant cuticle (Relyea, 2005a). In aquatic organisms, it is believed that POEA disrupts cell membranes on respiratory surfaces. Few studies have examined the toxicity of nonionic surfactants (Lindgren et al., 1996; Sandbacka et al., 2000). Recently, studies have demonstrated that POEA is extremely toxic to aquatic organisms [Brausch and Smith, 2007 (*Thamnocephalus platyurus*); Relyea, 2005b (*Hyla versicolor*, *Bufo americanus*, *Rana pipiens*); Folmer et al., 1979 (*Chironomus plumosus*)].

The objective of this study was to quantify the acute toxicity (48-h LC50) of three formulations of POEA to the aquatic macroinvertebrate *Daphnia magna*. *D. magna* is a common aquatic species used in acute toxicity testing; however, there is only a limited amount of toxicity data for POEA related to *D. magna* (Giesy et al., 2000; Wang et al., 2005).

D. magna exists throughout the northern hemisphere in lakes, ponds, and ephemeral water bodies [United States Environmental Protection Agency (USEPA) 2002]. Therefore, we also examined the growth and growth ratio (growth of *Daphnia* exposed compared to *D. magna* in a control group) of *D. magna* over a 48 h testing period to determine if POEA inhibits growth.

Materials and Methods

D. magna were cultured in moderately hard synthetic freshwater according to the USEPA document EPA-821-

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R02-012 (USEPA 2002). Milli-Q water (18.3 MΩ cm) was supplemented with 96 mg of $NaHCO_3$, 60 mg of $CaSO_4 \cdot 2H_2O$, 60 mg of $MgSO_4$, and 4 mg of KCl per liter giving a pH of 7.4, hardness of approximately 84 $CaCO_3$/L, and an alkalinity of approximately 60 $CaCO_3$/L. Half of the culture media was changed every week and the entire culture media was changed every two weeks.

D. magna were fed every other day with a YCT (yeast, Cerophyll®, and Trout Chow) mixture as described by the USEPA (USEPA 2002); however, they were not fed during testing. Briefly, the food consisted of 5.0 g of flake food (Carnivore pellets, Kamihota Fish, Himeji, Japan), 5.0 g of dry yeast, and 5.0 g of powdered cereal leaves which were all mixed separately in 1 L of milli-Q water and then filtered to remove undissolved material. The three solutions were then mixed in equal volumes and frozen until needed. *D. magna* were maintained under a 16:8 light:dark cycle at a temperature of 25 ± 1°C.

POEA surfactant formulations were supplied by Huntsman International LLC (Salt Lake City, UT, USA). The three different POEA formulations used for testing had average oxide:tallowamine ratios of 5:1 (Surfonic® T-5 surfactant, 98.6% purity), 10:1 (Surfonic® T-10 surfactant, 99.8% purity), and 15:1 (Surfonic® T-15 surfactant, 99.4% purity). Analytical grade $MgSO_4$, $NaHCO_3$, KCl, and $CaSO_4 \cdot 2H_2O$ were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

Twenty-four hours prior to testing, adult *D. magna* were placed in aquaria and the young produced overnight were used in toxicity testing. Test containers were 250-mL Pyrex beakers with a solution volume of 200 mL. The test solutions were synthetic freshwater with known concentrations of POEA. Seven concentrations of each POEA formulation as well as one control group were used for each experiment. A serial dilution, based on weight:volume, was used to make all dosing solutions; final nominal concentrations of 0.1, 1, 10, 100, 500, 1,000, 10,000 µg/L were made for all three surfactants. All tests were 48-h LC50 acute toxicity tests with each concentration evaluated in triplicate. Ten less than 24 h old *D. magna* were placed in each beaker using a plastic pipette at test initiation. Mortality was defined as lack of movement and was recorded at 48 h and graphed using logit analysis. The general equation for logistic regression is $\ln(p/[1-p]) = b + m(d)$, where p is the proportion of response (mortality), d is the dose (log units), and b and m are constants. Organisms were not fed and water was not changed during testing.

Surviving *D. magna* were removed for body length measurement after 48 h of exposure. Length was recorded in micrometers and was measured from the eyespot to the posterior end of the carapace (Scheuerell et al., 2002). *D. magna* were measured under a dissection microscope at 6x magnification (Wild Heerbrugg, Switzerland) and a filar eyepiece (Lasico, Los Angeles, California). *D. magna* from the two highest concentrations of Surfonic T-5, the three highest concentrations of Surfonic T-10, and the highest concentration of T-15 were not measured because all individuals had died. At the conclusion of the acute toxicity tests, two live *Daphnia* were removed from each beaker (six individuals from each concentration), and length was determined. Growth ratios were calculated by dividing mean body length for a given treatment by mean body length of the control group. Data analysis was performed using the statistical program R (Version 2.2.1, R Development Core Team, Boston, MA, USA). Length data were analyzed using a one-way ANOVA; where appropriate, the post-hoc Dunnett's test or Tukey's test were used.

Table 1 LC50 values of three POEA formulations to *Daphnia magna* as compared to number of alkyl and oxide carbons

Oxide:tallow ratio	Mean LC50 (µg/L)	Number of alkyl carbons	Number of oxide carbons	Oxide:tallow ratio (by Mass)
5:1	176.4	5	18	4.57
10:1	97.0	3	30	10.51
15:1	849.4	3	38	13.30

Results and Discussion

The dissolved oxygen concentrations were between 7.93 and 8.41 mg/L for the test solution, the pH ranged from 6.4 to 6.7, and the temperature was 25 ± 1°C. These parameters are well within the ranges that are acceptable for *Daphnia* growth and survival (Hutchinson, 1967).

Surfonic® T-10 surfactant was the most toxic POEA formulation with a 48-h LC50 value of 97.0 ± 16.3 µg/L (mean ± standard error [SE]). Surfonic® T-15 surfactant was the least toxic with toxicity values over eight times higher than the T-10 formulation (LC50 = 849.4 ± 26.2 µg/L). LC50 values for Surfonic® T-5 Surfactant were nearly double those for the T-10 formulation with a value of 176.4 ± 12.5 µg/L (Table 1). Mortality was low in control groups, less than 1% throughout the entire experiment, and no more than 10% for any individual test chamber. The LC50 values were determined using the following logistic regression equations: $\ln(p/[1-p]) = -7.0101 + 3.1206(d)$ ($r^2 = 0.985$) for Surfonic® T-5, $\ln(p/[1-p]) = -4.9006 + 2.4665(d)$ ($r^2 = 0.961$) for Surfonic® T-10, and $\ln(p/[1-p]) = -3.1059 + 1.0941(d)$ ($r^2 = 0.920$) for Surfonic® T-15.

There was a significant difference among sizes of *D. magna* in the control group and the highest concentrations of all three surfactant formulations. Surfonic® T-10, the most toxic of the three formulations, inhibited growth

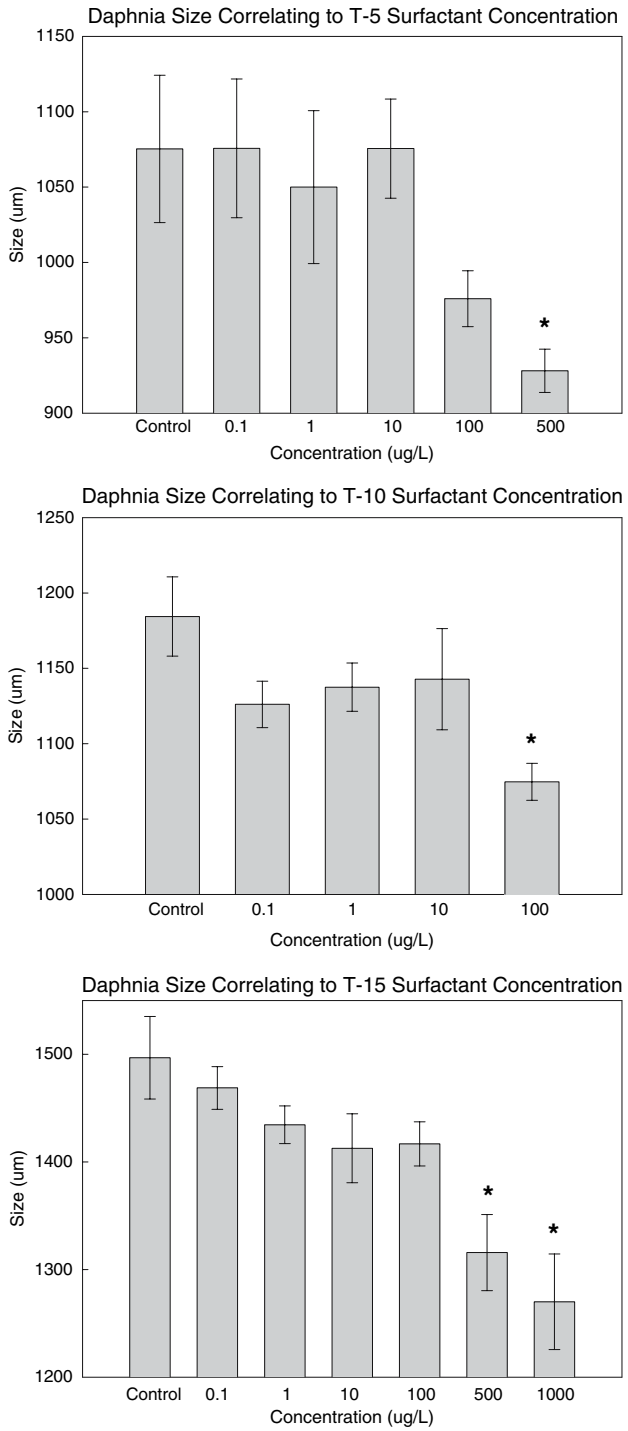

Fig. 1 Length (from eyespot to posterior end of carapace; mean ± SE) of *Daphnia magna* exposed to three different POEA formulations after 48 h of exposure. Sizes of *D. magna* are in micrometers and concentrations in micrograms per liter. Significant differences in size ($p < 0.05$) between test groups and control groups are indicated by *. Error bars indicate standard errors

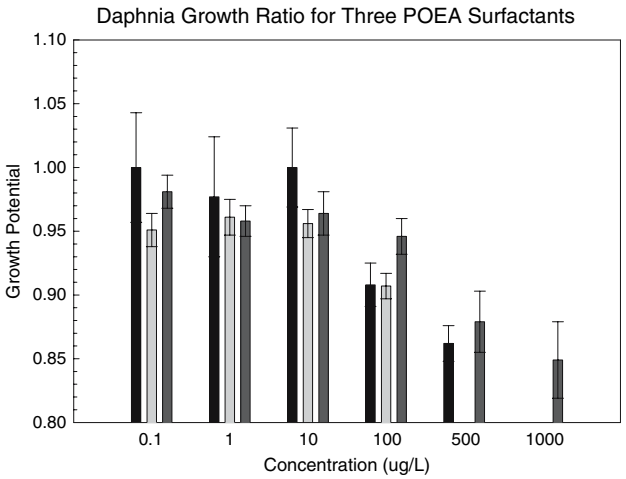

Fig. 2 Growth ratio (mean body length per treatment/ mean body length of control) of *Daphnia magna* exposed to three formulations of a POEA surfactant (5:1 oxide:tallowamine [T-5] is in black, T-10 in light gray, and T-15 in medium gray). Error bars indicate standard error

at the lowest concentrations [100 µg/L at 48 h ($p = 0.001$)]. Surfonic® T-5 also inhibited growth after 48-h at slightly higher concentrations [500 µg/L at 48 h ($p = 0.042$)]. This was also true for Surfonic® T-15 where a significant difference was observed between the control group and 500 µg/L and 1000 µg/L at 48 h ($p = 0.001$ and $p = 0.0001$, respectively; Fig. 1). There were no significant differences between *D. magna* growth ratios for any of the chemicals (Fig. 2) suggesting that the concentration of POEA, not the formulation, is responsible for inhibiting growth.

Acute lethality tests on *D. magna* are the foundation of aquatic environmental safety testing (USEPA 2002). The main objective of this study was to determine the toxicity of a rarely studied, yet heavily used surfactant. The results of this experiment demonstrate that POEA can be toxic to *D. magna* in concentrations less than 100 parts per billiom (ppb). However, there is a very large range in toxicity, with Surfonic® T-10 being tenfold more toxic than Surfonic® T-15 and twofold more toxic than Surfonic® T-5. Studies using the POEA formulation found the LC50 values ranged from 2.0 to 4.9 mg/L in Roundup® (Giesy et al., 2000; Wang et al., 2005). Previous studies with nonionic surfactants have demonstrated that toxicity increases with increasing alkyl chain length (Dorn et al., 1993; Wong et al., 1997), but this trend was not observed for the ANEO surfactants tested here (Table 1). The wide range of toxicities of different POEA formulations indicates that by altering the structure of the surfactant toxicity can be greatly reduced.

In previous experiments done with the same three surfactants, Surfonic® T-15 appeared to be most toxic (2.14 µg/L) to T. platyurus while Surfonic® T-5 was least toxic (5.5 µg/L; Brausch and Smith., 2007). As compared to this study, *T. platyurus nauplii* are 400 times more

sensitive to POEA surfactants than *D. magna*. These results are similar to those of Uppgard et al., (2000) and Kaluza and Taeger (1996) in which *T. platyurus* were much more sensitive than *D. magna* to numerous alcohol ethoxylate (AEO) surfactants. Similar to our results, these studies found that *T. platyurus* is up to 100 times more sensitive to AEO surfactants than *D. magna* (Krogh et al., 2003).

Exposure to POEA surfactants reduced growth of *D. magna*. *Daphnia* reached larger body length at the lowest concentrations and in the control group, with smallest body length associated with the highest concentrations of POEA. Figure 1 shows the mean body length of *D. magna* at 48 h during the test period. There appears to be only a small range of concentrations just below the 48-h LC50 that inhibit growth. In studies using phthalate ethers, growth was also reduced in *D. magna* at levels just below the LC50 value (Adams et al., 1995).

Growth inhibition is an important sub-lethal effect for many aquatic invertebrates. *D. magna*, similar to other aquatic invertebrates, become sexually mature based on size and not age. Therefore, external stressors can affect the ability of *D. magna*, and potentially other aquatic invertebrates, to reproduce (Schwartz, 1984; Lynch, 1989).

In normal situations, a balance exists where organisms use energy for maintenance of internal conditions in addition to growth and reproduction. However, anthropogenic stressors can alter the balance of energy usage (Congdon et al., 2001). Exposure to sub-lethal concentrations of chemicals have been shown to slow growth by causing organisms to divert resources to the maintenance of internal homeostatic conditions rather than growth and development (Sibly and Calow, 1989). Many mechanisms exist to maintain homeostatic conditions; however, these mechanisms are energetically costly and divert energy away from growth and reproduction (Forbes and Calow, 1996). In organisms inhabiting short-lived water bodies, stunted or delayed growth can endanger their ability to reproduce before desiccation.

Because tests were run at different times, final body lengths of *D. magna* were not comparable among the three surfactant formulations. However, the use of growth ratios (mean body length relative to the control group) permitted comparisons across chemicals. We acknowledge that initial lengths likely influenced final lengths of *D. magna*, but because *D. magna* were neonates they grew very quickly (Lynch, 1989) and their growth should not have been significantly influenced over a 48 h period.

The results of this study indicate that POEAs are both acutely toxic and can cause non-lethal effects in *D. magna*. POEA inhibits growth of *D. magna* and could potentially cause similar effects in other aquatic organisms. More studies are needed to determine if POEAs cause other non-lethal effects such as decreased fecundity in *D. magna* and other aquatic organisms.

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