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Molluscicide Acute and Sub-Chronic Toxicity to the Three Non-Target Organisms *Hexagenia limbata, Ceriodaphnia dubia,* and *Pimephelas promelas,* and Neutralization of That Toxicity by Bentonite Clay

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Zebra mussels *(Dreissina polymorpha)* have caused problems for raw water users along the shores of the Great Lakes by clogging pipes and decreasing water flow within water systems of raw water users. The molluscicide H-130 (didecyl-dimethyl-ammonium chloride) has been proposed to both remove and prevent colonization of water systems by zebra mussels. Briefly, the water in the systems would first be treated with H-130. Then, just prior to releasing it into receiving waters, the water would be treated again with bentonite clay to neutralize H-130. Other pesticides have been used in a similar manner against related mussels such as the asiatic clam *(Corbicula fluminea).*

Three non-target organisms were used in this study to investigate H-130 toxicity; *Hexagenia limbata* (larval mayfly nymphs), *Ceriodaphnia dubia,* and larval *Pimephelas promelas. Hexagenia limbata* was chosen as a representative benthic organism likely to come into contact with and ingest adsorbed molluscicide. *Ceriodaphnia dubia* and *Pimephelas promelas* were chosen as representative pelagic organisms. Both the toxicity of H-130 and the effectiveness of bentonite clay in neutralizing H-130 toxicity were investigated in acute and sub-chronic toxicity tests (Horning and Weber 1985).

MATERIALS AND METHODS

Both *C. dubia* and *P. promelas* were taken from cultures maintained at the Ohio Environmental Protection Agency (EPA) bioassay laboratory. Less than 24H old *P. promelas* and *C. dubia* neonates were used in both acute and sub-chronic tests. Reproductive performance of the *C. dubia* population was assessed by subjecting them to quality assurance tests (Horning and Weber 1985) prior to use of neonates in the toxicity tests. *H. limbata* nymphs were collected from wild populations. The length of the nymphs used in the acute tests was between 12 and 33 mm.

The components used in these tests were the molluscicide H-130 and bentonite clay. The quaternary ammonium compound H-130 is positively charged. When in the presence of bentonite clay, the cationic molluscicide is attracted to the anionic charge on the surface of the clay which is the basis for use of the clay as a sorbent medium. Most

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of the test solutions were dosed with both H-130 and bentonite clay. Water was dosed directly with H-130 while bentonite clay was first activated prior to dosing. Activated bentonite clay was prepared by adding dry clay to hard reconstituted water and mixing for 2-3 hours on a magnetic stirring plate to ensure complete clay hydration. After adding H-130 and bentonite clay, the solution was then stirred for ten minutes followed by vacuum filtration to remove suspended clay along with adsorbed molluscicide. The only test solutions not dosed with bentonite clay were those used for LC_{50} determination.

Only *C. dubia* and *P. promelas* were subjected to static renewal, sub-chronic toxicity tests since established methods for those two species have been developed (Horning and Weber 1985; Norberg and Mount 1985). Endpoints were the mean number of neonates produced per adult for *C. dubia* and final mean replicate weight for *P. promelas*. All three organisms were subjected to two sets of 24H acute toxicity tests. In the first set of acute tests, the dose response relationship (LC_{50}) between each organism and H-130 was determined. Then in the second set of acute tests, the effectiveness of the clay in neutralizing the acute toxicity of H-130 was determined.

Sub-chronic test solutions were prepared to give bentonite clay to H-130 concentration ratios of 5:1. Five replicates of five individuals were exposed to each of the following concentrations, in mg/L H-130/mg/L bentonite clay, 0.5/2.5, 5/25, and 15/75. Two controls were used, one of undosed water and one of water dosed with clay at a concentration of 75 mg/L.

Two dosing scenarios were used in the sub-chronic toxicity tests to represent two possible exposure situations in the field. In scenario one (bulk preparation method), the exposure solution used to start the test and for daily water changes was prepared in bulk at the beginning of the test. This method represented potential exposure of the organism to degraded H-130 and clay. In scenario two (daily preparation method), the exposure solution used for water changes was prepared daily. This represented daily exposure of the organism to undegraded H-130 and clay.

Dissolved oxygen, conductivity, temperature, and pH were recorded for each concentration at the beginning and conclusion of each sub-chronic test. The same water parameters were measured daily for the highest test concentration and controls.

Test solution concentrations for the first set of acute tests were determined by range finding assays. The solution concentrations of H-130 used in the second set of acute tests were arbitrarily chosen to considerably exceed previously determined LC_{50} values. However, the proportion of clay to H-130 was the same as in the sub-chronic tests (i.e. 5 parts clay to 1 part H-130). Due to the potential for toxicant sorption to food in the acute tests, the organisms were not fed during the acute tests. The ability of the organisms to survive 24H without being fed was evaluated prior to both sets of acute tests.

The method of solution preparation in the acute tests for *C. dubia* and *P. promelas* was the same as described previously. However, solution preparation in the acute tests for *H. limbata* differed. Bentonite clay was not filtered from the test solution since nymphs in the field would likely come into contact with settled clay. Therefore, activated bentonite clay and H-130 were added directly to the test aquaria and were both part of the test solution.

Since *H. limbata* nymphs were often motionless in or on the artificial substrates, H-130 affects upon the nymphs were determined by gently prodding them with a glass stirring rod. Either no attempt or a weak attempt to escape indicated adverse affects. They were considered dead if there was no gill movement in response to prodding.

Toxicity data obtained from both chronic and acute tests were subjected to chi-square analyses to determine significant mortality. LC_{50} values were determined by probit analysis (Finney 1973). Single factor ANOVA was used to determine significant differences between treatments for mean larval weight of *P. promelas* and mean number of young for adu1t *C. dubia.*

RESULTS AND DISCUSSION

Considering the LC_{50} values for all three organisms (Table 1), the presence of bentonite clay appeared to effectively decrease the acute lethality of H-130 (Table 2).

Table 1. 24 Hr. LC_{50} Values for *C. dubia, P. promelas,* and *H. limbata*

	LC_{50} (mg/L)	95% Confidence Limits
Ceriodaphnia dubia	0.076	0.069 - 0.082
Pimephelas promelas	0.47	0.45 - 0.50
Hexagenia limbata	5.7	4.5 - 6.9

Lethality was not the only endpoint of note in the acute tests. A behavioral effect was observed for *H. limbata*. Nymphs have been shown to leave their burrows and crawl or swim around when stressed (Henry et al. 1986). This behavior was observed for nymphs following 15 to 30 minutes of exposure to higher H-130 concentrations when they were observed crawling around outside of the substrates. A 24H EC_{50} of 5.2 mg/L (95% C. I. of 4.2 to 6.2 mg/L) was determined for this behavior.

However, bentonite clay's effectiveness in neutralizing H-130 lethality in the sub-chronic tests was unclear. The clay appeared to reduce lethality in the bulk preparation method (top graph of Figs. 1 and 2), but was less effective in the daily preparation method (bottom graph of Figs. 1 and 2). H-130 concentrations in the chronic tests were all at least the same as the LC_{50}. Perhaps H-130 was not effectively removed by

Table 2. Percent mortality for *C. dubia, P. promelas,* and *H. limbata* following 24H of exposure to solutions treated with H-130 and bentonite clay. An asterisk (*) next to the % mortality value indicates the organisms were exposed to an H-130 concentration greater than its LC_{50}.

H-130 (mg/L) : clay (mg/L)	%Mortality		
	C. dubia	*P. promelas*	*H. limbata*
0 : 0	0	0	0
0 : 500	0	0	0
0.01 : 0.05	0	3	0
0.1 : 0.5	5*	0	0
1 : 5	20*	0*	0
10 : 50	20*	0*	15*
100 : 500	5*	3*	40*

bentonite clay in the daily preparation method thereby resulting in the observed toxicity. However, lethality was observed in only the daily preparation method, not in the bulk preparation method. This inconsistency is difficult to explain considering the clay's apparent effectiveness in reducing H-130 lethality in the acute tests.

It was not clear whether larval *P. promelas* growth was affected by chronic exposure to H-130. Mean larval weight did not vary significantly between treatments in the bulk preparation method, nor did it vary between treatments and controls (Fig. 3). This indicated solutions treated with both H-130 and bentonite clay had no affect upon larval growth. Complete mortality in the daily preparation method did not allow comparison of larval weights in that method.

Bentonite clay may adversely affect *C. dubia* reproduction. First, no significant difference in fecundity was evident between the low concentration of either solution preparation method, nor was it evident between the low concentrations of either method and the water contro1 of the daily preparation method (Fig. 3). However, fecundity in the clay controls was significantly reduced relative to both the low concentrations and water control (Fig. 3). Both the clay control and the low concentration solutions were treated with clay, however, the clay control was treated with clay at a concentration 200 fold greater relative to the low concentration solution. Even though it was not significantly toxic to *C. dubia* (Table 2), water dosed with high clay concentrations decreased fecundity.

Activated bentonite clay is likely to be most effective in neutralizing H-130 toxicity. Activation increases the likelihood of contact between the clay and toxicant by increasing the clay's effective surface, thereby increasing available sites for toxicant adsorption. Adding dry inactivated clay to water is not likely to be as effective. In this study, dry clay clumped together and either floated on the surface or sank to

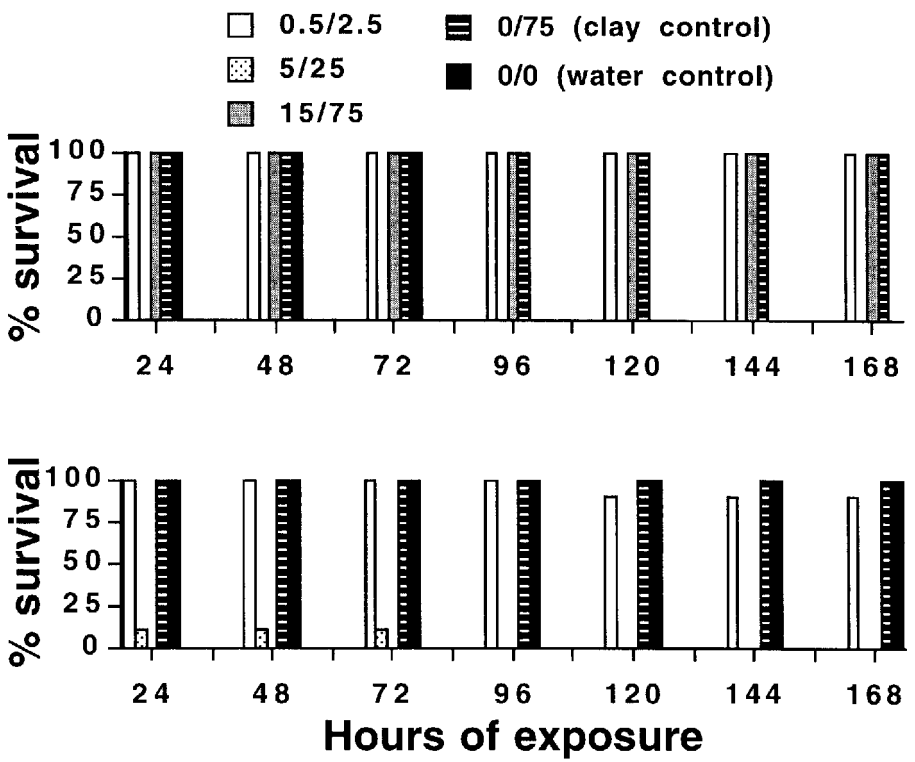

Figure 1. Sub-chronic lethality of the test solutions to *C. dubia*. The top figure is % survival in solutions prepared by the bulk preparation method, while the bottom figure is % survival in solutions prepared by the daily preparation method. Legend values are solution concentrations (mg/L H-130/mg/L bentonite clay).

the bottom of the beaker. The clay would likely do the same in the field, either settling into eddies or floating on the surface.

For the purposes of these tests, the clay was activated to ensure maximum effective surface area. The activated clay was thoroughly mixed with H-130 to maximize contact time between the clay and H-130. Utilizing this method, the clay appeared to be effective in neutralizing acute lethality of H-130, but it appeared to inconsistently reduce toxicity in chronic tests.

Some of the variability may be explained by cationic exchange of the molluscicide by other dissolved cations. Clay is characterized by cation exchange capacity (Menzer and Nelson 1986). Several cations in the solutions used for these tests were Na^{+}, K^{+}, Ca^{2+}, and Mg^{2+} from $NaHCO_3$, KCl, $CaSO_4$, and $MgSO_4$ respectively used to prepare water for these tests.

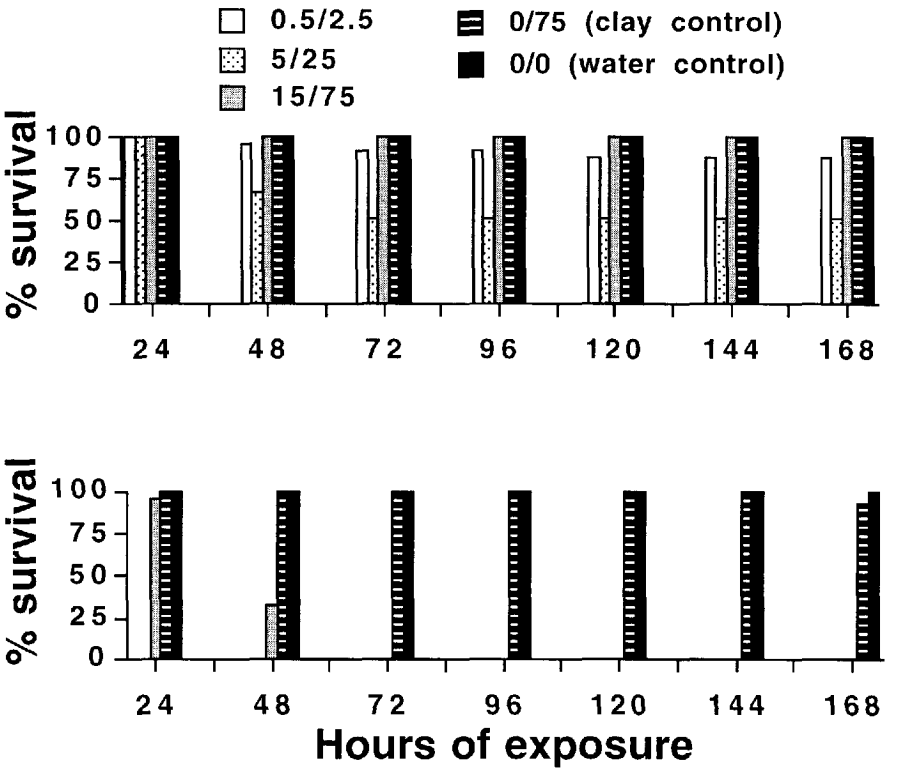

Figure 2. Chronic lethality of the test solutions to *P. promelas*. The top figure is % survival in solutions prepared by the bulk preparation method, while the bottom figure is % survival in solutions prepared by the daily preparation method. Legend values are test solution concentrations (mg/L H-130/mg/L bentonite clay).

This resulted in a large concentration of Na^{+}, Ca^{2+}, and Mg^{2+} in the water which may have competed with the molluscicide for anionic sites on the clay. These cations are also common environmental components. Road salts have large amounts of these cations. Soil and bedrock also contain these as well as other cations (Sposito 1989). Rainstorms and snowmelt would therefore result in flux of cations from roads, soils, and bedrock into lakes and streams where exchange of H-130 by available cations could re-release the molluscicide into solution.

If bentonite clay is proposed to be used as a sorbent medium for H-130, the method of clay administration should be an important consideration. Clay should be activated prior to its use as a sorbent medium. Otherwise, dissolved H-130 in the receiving waters may result in significant toxicity for non-target organisms. Also, further investigation of bentonite clay is recommended to elucidate both its effectiveness as a sorbent medium and its potential toxicity upon planktonic organisms such as *C. dubia*.

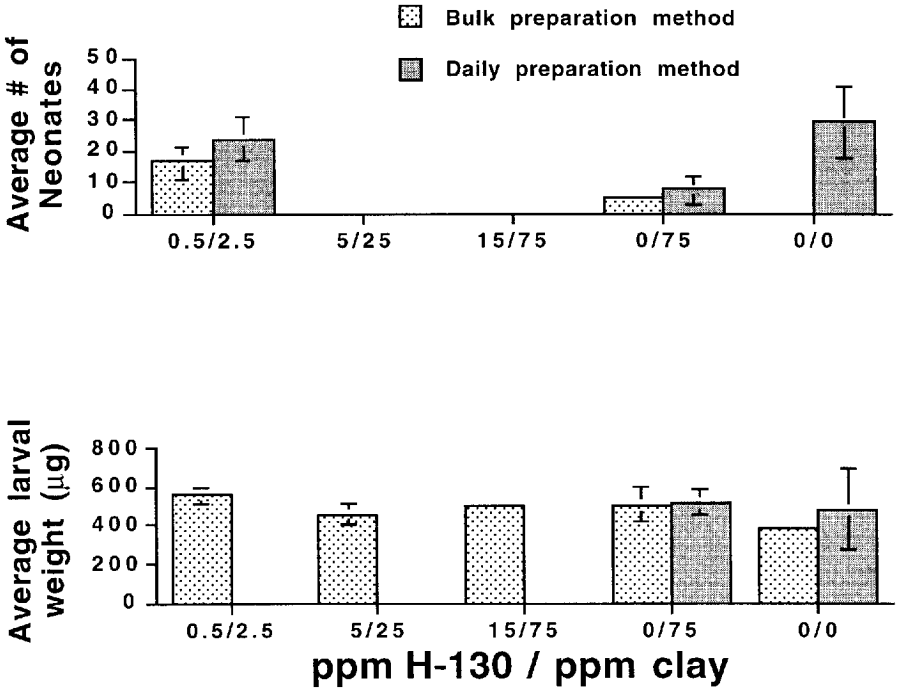

Figure 3. Final average weights (bottom graph) of surviving *P. promelas* larvae, and mean number neonates produced by adult *C. dubia* (top graph) during chronic tests. Error bars are 95% confidence intervals. Mean weights were not significant ($p=0.219$). Reduction in mean number of *C. dubia* young produced in clay controls was significant ($p<0.01$).

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