Toxicity Studies on One-, Thirty-, Sixty Day Old and Adult Stagnicola Reflexa

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Precise toxicity studies on gastropods have been few. Those of practical importance (Chandler, 1920; Krull, 1939; Brackett, 1939) were of a field nature and/or lacked precise methods for the accurate comparison of toxicants used. Although Chandler (1920) also did laboratory experiments with different compounds involving different species of snails, he used percentages instead of molarities in preparing his solutions. His criterion of death was solely a lack of response to tactile stimulation and this together with the fact that some of his apparently dead specimens revived after removal to fresh water made his results rather questionable. Krull (1939) also failed to use molarities and in addition gave neither exposure periods nor information on the possible revival of treated snails. Brackett's work was primarily designed to test the toxicity of copper compounds on aquatic life, principally fishes. He found copper sulphate too toxic to fishes to be of practical value for the destruction of snails. He did observe copper carbonate to be of practical value for the destruction of snails in that it was effective against ten specimens of *Stagnicola emarginata* which comprised all of his experimental gastropod material. His studies included no observations on the revival of the treated snails. It is interesting to note that McMullen, Ishii, and Mitoma (1948) observed copper phosphate and tribasic copper sulphate to be ineffective on *Oncomelania nosophora* in Japan.

Tomcek (1951) working with one-day *Stagnicola reflexa* was the first to utilize definite periods of exposure to various test solutions, followed by consistent observations of the specimens after being returned to fresh water. Her solutions, however, were made on a percentage basis so that comparison of the toxicants is difficult. The present study was undertaken in order to make a comparative analysis of the toxicity of some chemical agents both common and uncommon as molluscacides.

This is the first work to utilize laboratory experiments that test the response of *Stagnicola reflexa* of several ages, to definite exposures of prospective toxicants expressed in moles per liter, accompanied by observations on lethal effects after removal of the exposed specimens to fresh water.

All the experimental animals were obtained from a common progenitor and raised in the aquaria at the University of Notre Dame. A total of 42 individual experiments were conducted utilizing 25,200 snails.

One-thirty- and sixty-day-old *Stagnicola reflexa* (Say, 1821) in individual lots of twenty each (same age) were exposed separately for 15, 30, 45, 60 and 120 minute periods to 1.5×10^{-4}, 1.9×10^{-4}, 4.8×10^{-4}, 2.44×10^{-3} and 4.88×10^{-3} molar solutions of copper sulphate, copper carbonate, magnesium sulphate, magnesium carbonate, sodium carbonate and sodium chloride. Breeding-stock adults in lots as given above were utilized terminally with only copper sulphate and copper carbonate because of insufficient numbers.

In preparation, a copper sulphate-to-water ratio of 1-500,000, expressed in moles per liter, was used as a point of departure since this concentration of copper sulphate was found most effective for elimination of snails by Chandler (1920), Brackett (1939), and McMullen (1941). The snails were placed in test tubes, closed with gauze to prevent escape; immersed in the various solutions, washed twice in tap water, placed in containers, provided with food and then observed 24 and 36 hours later for recovery.

Effects of copper sulphate on one-, thirty-, and sixty-day-old snails were similar. There was one hundred per cent survival after the maximum exposure period of 120 minutes in the 1.5×10^{-4} and 1.9×10^{-4} molar solutions, of this compound in all three age groups. Only in the 4.88×10^{-3} molar solution after 120 minutes was 100% lethality achieved for the one-day-old snails, while it only went as high as 85% in the other two age groups. In the case of exposure of the breeding-stock adults all were dead at the 36-hour observation in all of the above concentrations after removal to tap water.

Results with copper carbonate were markedly different. The adult snails were not as susceptible to this compound as they were to copper sulphate. One hundred per cent lethality was achieved only at the maximum exposure time (120 minutes) in the 2.44×10^{-3} and 4.88×10^{-3} molar solutions. In all the other groups the kill was directly opposite to that found in copper sulphate. Maximum lethality in these young snails was obtained in solutions as low as the 1.9×10^{-4} concentration. Therefore a far greater toxic effect is indicated for copper carbonate than in the case of copper sulphate. The fact that copper carbonate is practically insoluble in water makes this an interesting phenomenon.

The data from experiments with sodium chloride revealed a low toxicity as compared with that for the copper compounds. Lethality for the three age groups was spotty and did not vary directly as the exposure periods for a given age group, except in a general way in the highest concentration, for the one-day-old snails. Neither was there consistent increased lethality in a direct manner for ascending concentrations. Average lethality for all concentrations, however, varied directly with the age of the snails.

Sodium carbonate was observed to be relatively non-toxic. This was especially true with regard to the one-day-old snails in which there was no more than five per cent lethality for any concentration at any of the exposures. In the case of the thirty-day- and sixty-day-old snails the kill was slightly higher. It was found that most of the experimental organisms had migrated out of their containers. This experiment, repeated many times, produced the same effect and therefore suggests the presence of an irritant.

Results with magnesium sulphate show a comparatively strong tolerance for the magnesium ion by the thirty-day-old snails. Lethality was greatest in the one-day-old snails in which the kill rose to 85% in the sixty-minute exposure to 4.88×10^{-3} molar solution. The sixty-day-old specimens exhibited lethal effect less than those for the other two age

groups. A similar migrating effect was observed with the use of this chemical as was exhibited in the case of sodium carbonate.

Magnesium carbonate is relatively insoluble, which is indicated by its low toxicity to *Stagnicola reflexa*. The snails in each age group survived most of the concentrations for all exposure periods. The 4.88×10^{-3} molar solution had the greatest effect on the thirty-day-old snails in which the maximum lethality reached 80% for the 120-minute exposure. The one-day-old snails were the least susceptible to this compound and the lethality in the sixty-day-old specimens was intermediate between that of the other two age groups.

The order of descending toxicity, based on the average lethality in all the concentrations of the compounds used for the one-day-old snails is as follows: copper carbonate (89.6%), copper sulphate (43.8%), magnesium sulphate (32%), sodium chloride (15%), magnesium carbonate (1.8%), and sodium carbonate (1%).

The order of descending toxicity, based on the average lethality in all concentrations of the compounds used for the thirty- and sixty-day-old snails is identical and as follows: copper carbonate (98.8%; 90.6%), copper sulphate (42.6%; 40.6%), magnesium sulphate (19.6%; 22.2%), sodium chloride (15.8%; 14.4%), sodium carbonate (13.8%; 5.4%), magnesium carbonate (13.6%; 4.6%). The descending order of toxicity for these two age groups is identical with that of the one-day-old snails except the places of sodium carbonate and magnesium carbonate are reversed. With regard to adult specimens, copper sulphate (100%) was much more toxic than copper carbonate (83.4%).

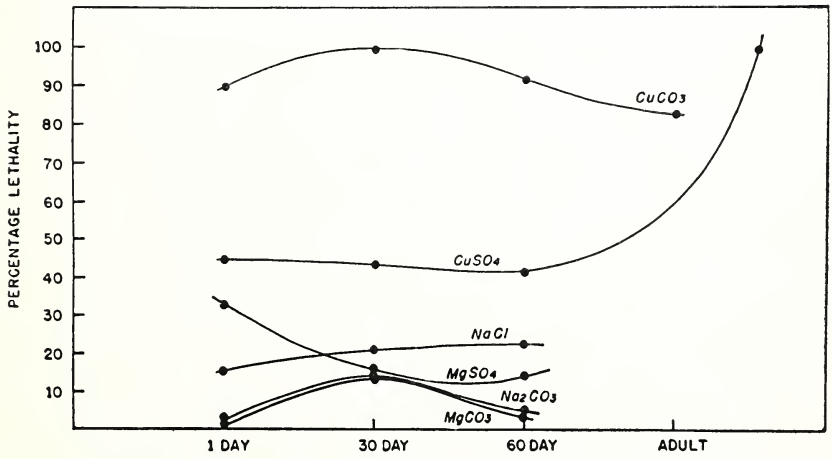

FIGURE 1.- AVERAGE LETHALITY FOR ALL CONCENTRATIONS AND EXPOSURES

The toxic action of the compounds used in this study is problematical. The solubilities and ionization properties of the salts indicate that the carbonate ion is relatively nontoxic. This is strikingly so for the one-day-old snails. On this basis, the high percentage of lethality which copper

carbonate produced was attributable principally to the copper ion which does not follow in the case of copper sulfate which is a more soluble salt and also one which ionizes to a greater degree than copper carbonate. A plausible explanation would involve a difference in pH and the fact that the carbonate ion may act as a conditioner which would make the uptake of the absorption of the copper ion proceed at a much faster rate than in the presence of the sulphate ion. This is supported by the similarity of the toxicity curves (Fig. 1) and the marked discrepancy in the lethalities in copper carbonate and copper sulphate solutions.

With regard to the age susceptibility, the thirty-day-old snails exhibited a greater lethality than any of the other age groups exposed to copper carbonate and magnesium carbonate. Adult snails were more readily killed than were the younger ones in the copper sulphate solutions. The resistance of all subadult *Stagnicola reflexa* varied directly as the age with reference to magnesium sulphate concentrations and indirectly with age in the case of sodium chloride.

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