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Oxygen Uptake of *Pseudosuccinea columella* and *Fossaria cubensis* Treated with Sublethal Concentrations of CUTRINE-PLUS®, an Algicide

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It has been previously reported that low concentrations of some herbicides have a significant influence on the mortality of the intermediate snail hosts of Fasciola hepatica (Christian and Tate 1983). Schober and Lampert (1977) indicated that sublethal responses of aquatic animals to herbicides is inadequately known, although Klekowski and Zvirgzds (1971) had observed variability in oxygen consumption resulting from exposure to 2, 4-D Amine.

CUTRINE-PLUS, commonly known as copper chelate (copper II alkanolamine complex) is registered for use as an aquatic algicide in potable water reservoirs, irrigation conveyance systems, farm, fish and shrimp ponds and lakes and fish hatcheries. It is infinitely soluble in water at 20°C. The mechanism of action of CUTRINE-PLUS appears to be inhibition of photosynthesis in plants (Herbicide Handbook 1983) and inhibition of DNA synthesis in animals (Bhuyan and Betz 1968). Review of the literature indicates that no toxicity work on snails has been reported for CUTRINE-PLUS. Since CUTRINE-PLUS is sprayed in and around aquatic ecosystems, it may have some biological effect on the survival and respiration of non-target organisms in the environment. Oxygen consumption is a useful measure of sublethal effects because energy processes serve as an indicator of overall physiological state (Klekowski and Zvirgzds 1971), but it is not a technique for the identification of specific toxic mechanisms (Cheng and Rodrick 1974). The current study compares the influence of sublethal concentrations of CUTRINE-PLUS on the oxygen uptake of uninfected (free from trematode larvae) Pseudosuccinea columella and Fossaria cubensis, intermediate host snails of Fasciola hepatica and Fasciola gigantica in

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Louisiana and world wide.

MATERIALS AND METHODS

In this study, 5-6 week old (10th generation), uninfected laboratory-reared Pseudosuccinea columella and Fossaria cubensis were used. P. columella were maintained in the laboratory in either ten gallon glass aquaria or in 28x12x10 cm plastic containers containing mud and artificial spring water. The snails were fed (endive) lettuce ad libitum. The containers were aerated constantly. Mature snails deposited gelatinous egg masses almost daily on the sides of the containers and on the mud. The egg masses were carefully removed from the glass aquaria using a penbrush or spatula and placed in separate containers with freshly conditioned spring water and mud. Hatching of the young snails occurred within 10-12 days and the young snails matured in 5-6 weeks. F. cubensis were maintained similarly to P. columella, except that F. cubensis were fed algae and their buckets were not aerated.

The concentrations of CUTRINE PLUS which were sublethal to the snails were initially determined. Groups of 10 snails were then randomly selected and each group was initially exposed to 0.3, 0.6, 0.9 and 1.5 ppm of CUTRINE-PLUS for 96 hours before the oxygen consumption test began. This was done to ascertain the lowest level at which the normal oxygen consumption value was significantly altered, without killing the snails. The preparation of the test solution and exposure methods were done according to our earlier reports (Christian and Tate 1983). Oxygen consumption by the snails was then measured manometrically with a Warburg Apparatus at 25±0.5°C as described by Umbriet et al. (1972) using a Gilson Differential Respirometer. CUTRINE-PLUS treated snails as above were then tested at 0.3ppm, 0.6ppm, 0.9ppm, 1.5ppm, and 0.0ppm (control), for oxygen consumption. The reaction vessels containing CUTRINE PLUS and snails were shaken for 30 minutes prior to the start of the measuring period to provide enough time for complete temperature equilibration. At the end of the three hour test period, the pH remained constant at 6.8-7.2 as it was at the beginning of the experiment, hence no change of pH occurred. Oxygen consumption measurements were taken at 15-minute intervals for a period of 3 hours. Each treatment was replicated 10 times.

Table 1. Mean oxygen uptake (ul/h/g wet weight) of Pseudosuccinea columella exposed to sublethal concentrations of CUTRINE-PLUS

No. of Tests	Concentrations in ppm 0.0	0.3	0.6	0.9	1.5	R-Value
1	179.20	130.60	103.36	100.56	73.92	0.93
2	177.48	124.92	117.96	102.76	74.12	0.94
3	153.12	132.72	102.76	99.32	74.08	0.97
4	177.96	118.52	99.88	99.44	85.04	0.84
5	152.64	129.12	104.16	100.76	79.00	0.96
M	168.08	127.18	105.62	100.57	77.23	0.95
S	±6.21	±2.51	±3.17	±0.62	±2.18	
SD	13.89	5.62	7.08	1.38	4.87	
ANOVA		p<0.01	p<0.01	p<0.01	p<0.01	

Table 2. Mean oxygen uptake (ul/h/g wet weight) of Fossaria cubensis exposed to sublethal concentrations of CUTRINE-PLUS

No. of Tests	Concentrations in ppm 0.0	0.3	0.6	0.9	1.5	R-Value
1	214.32	128.48	129.80	120.64	104.08	0.79
2	178.64	109.68	123.44	113.88	89.44	0.81
3	176.08	123.28	126.28	130.04	98.32	0.84
4	139.04	141.92	124.60	124.32	104.04	0.95
5	179.48	128.92	108.60	119.52	87.60	0.88
M	177.51	126.46	122.54	121.68	96.70	0.87
S	±11.92	±5.20	±3.65	±2.68	±3.51	
SD	26.66	11.63	8.16	5.99	7.85	
ANOVA		p<0.01	p<0.01	p<0.01	p<0.01	

Statistical analyses of the data from these experiments include two-way analysis of variance and correlation and regression using a NIH Prophet Computer.

RESULTS AND DISCUSSION

Table 1 shows the average oxygen consumption rates (expressed in O_2 ul/h/g wet weight) of P. columella exposed to sublethal concentrations of 0.3ppm, 0.6ppm,

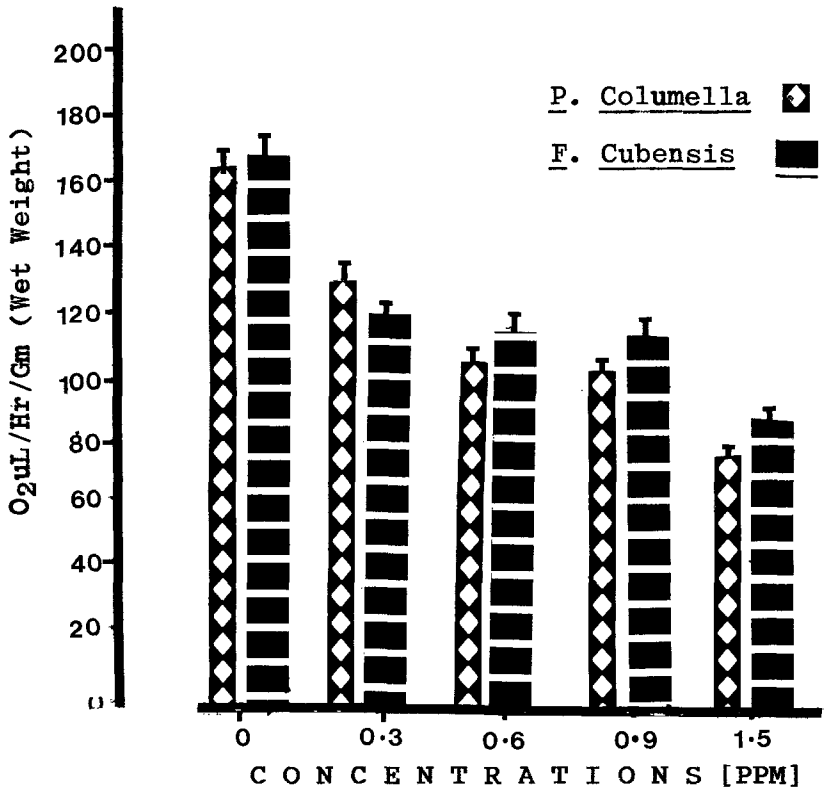

Figure. 1 Comparative study of the oxygen consumption rates of P. columella and F. cubensis treated with sublethal concentrations of CUTRINE-PLUS. (T) ± standard error of the mean.

0.9ppm, and 1.5ppm of CUTRINE-PLUS. The average oxygen consumption of the control snails was 168.08 O_2 ul/h/g wet weight including shell, whereas snails treated with 0.3ppm, 0.6ppm, 0.9ppm, and 1.5ppm of CUTRINE-PLUS consumed an average of 127.18, 105.62, 100.57 and 77.23 O_2 ul/h/g wet weight, respectively.

Newman Keuls multiple range analysis of variance on the oxygen consumption by P. columella indicates significant differences when the control group is compared with the 0.3, 0.6, 0.9 and 1.5ppm CUTRINE-PLUS-treated groups, respectively, at $p<0.01$. When the experimental groups (0.3 vs 0.6ppm; 0.3 vs 0.9ppm; 0.3 vs 1.5ppm; 0.6 vs 1.5ppm; and 0.9 vs 1.5ppm) are compared among themselves, the differences in their oxygen consumption were also significant at $p<0.05$. There were no significant differences between the oxygen consumption of the snails treated with 0.6 vs 0.9 ppm of CUTRINE-PLUS at $p>0.05$. Correlation

coefficient (R-value) for the group was 0.95, which indicates that the values were closely related.

Table 2 shows the average oxygen uptake values of F. cubensis exposed to sublethal concentrations of 0.3, 0.6, 0.9 and 1.5ppm of CUTRINE-PLUS. The average value of the control snails was 177.51, whereas snails treated with 0.3, 0.6, 0.9 and 1.5ppm of CUTRINE-PLUS consumed an average of 126.46, 122.54, 121.68 and 96.70 O_2 ul/h/g wet weight, respectively.

Newman Keuls multiple range test on the oxygen consumption of F. cubensis indicated that there were significant differences in oxygen consumption when the control group was compared against the test groups at $p<0.01$.

The test also indicated that there was a significant difference ($p<0.01$) between the snails treated with 0.3 and 1.5ppm of CUTRINE-PLUS with no other differences noted among other groups. The correlation coefficient (R-value) for the group was 0.87, which indicates the values were closely related. Analysis of variance (Newman Keuls multiple range test) was also performed to test if there were any significant differences in the amount of oxygen consumption levels between F. cubensis and P. columella snails.

ANOVA indicated that there were significant differences when the control (0.0ppm) group, 0.6ppm and 0.9ppm of CUTRINE-PLUS treated P. columella snails were compared with the control (0.0ppm) group, 0.9ppm and 1.5ppm of CUTRINE-PLUS treated F. cubensis snails respectively at $p>0.05$. As shown in figure 1 the results clearly demonstrate that F. cubensis consumed more oxygen than P. columella with the exception of 0.3ppm of CUTRINE-PLUS treated P. columella which consumed more oxygen than F. cubensis.

This study clearly demonstrated that F. cubensis were less affected by the treatment than P. columella. Despite their weight and size differences, the control snails of F. cubensis, with an average size of 0.65 cm, and 0.045 g in weight, consumed more oxygen than P. columella with an average size of 1.5 cm and 0.085 g in weight. This finding correlates with that of MacInnes and Thurberg (1973) who reported that snails treated with low concentrations of copper sulfate showed no weight or size-related differences in their oxygen consumption rates.

Comparison of oxygen consumption rates of F. cubensis and P. columella during the first, second and third hours indicated that both species of snails consumed less oxygen than the control snails. For the first hour, the average oxygen consumption of P. columella snails treated with 0.9 ppm of CUTRINE-PLUS was greater than those treated with 0.6 ppm, whereas F. cubensis consistently showed a dose dependent response throughout the experimentation.

According to Bhuyan and Betz (1968), copper II chelate is an inhibitor of DNA synthesis. It could be, therefore, that DNA synthesis was inhibited. Wolmarans and Yssel (1988) and Reddy and Rao (1987) reported that lymnaeid snails contain hemocyanin (a protein containing copper) which acts as a respiration pigment. Since CUTRINE-PLUS's active ingredient is copper, it could have bounded with the hemocyanin of P. columella and F. cubensis and increased their copper contents in the hemolymph and adversely affected their oxygen carrying capacity.

Babu and Rao (1985) and Cheng and Sullivan (1977) explained that decreases in respiration rates of snails in the endogenous digestive gland could be due to the disruption of membrane permeability and uptake and osmoregulatory function of the epithelium. Copper has also been ascribed to inhibit respiratory enzymes (Cheng and Sullivan 1977).

Walshe (1966) suggested that the ATPase activity necessary for active membrane transport is inhibited by copper. This, in turn, could clarify why the oxygen consumption rates of P. columella and F. cubensis decreased significantly when exposed to sublethal concentrations of CUTRINE-PLUS.

At the end of the experimental period of three hours, no sign of distress or retraction by the experimental snails was observed. This observation did not agree with MacInnes and Thurberg (1973) who reported that lymnaeid snails treated with concentrations of elemental copper showed signs of distress, such as discharge of whitish mucus retraction and/or extension of their body tissue from their shells and death or both depending on the concentrations used.

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