Bull. Environ. Contam. Toxicol. (1990) 45:343–349
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Environmental Contamination and Toxicology

Sublethal Effects of 2,2-Dichloropropionic Acid (Dalapon) on *Fossaria cubensis*, Intermediate Host of the Liver Fluke, *Fasciola hepatica*

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Dalapon (2,2-dichloropropionic acid) is one of several herbicides used in Louisiana and the Gulf Coast areas to control annual and perennial grasses in sugarcane, corn, potatoes, citrus and non-crop lands. Application rate ranges from 0.75 lb/A in flax to about 20 lb/A in non-crop lands (LSU Extension Service 1985). Its mode of action has been suggested to be through the modification of protein structure and allowance of accumulation of toxic levels of ammonia in plants (Ware 1983). But microorganisms in soil have been found to degrade the herbicide into pyruvate, a useful organic compound (Allison et al. 1983). Dalapon is highly soluble in water at 20°C. There is a general concern that certain levels of this herbicide could reach the aquatic ecosystem and possibly alter the physiological state of aquatic life.

Fossaria cubensis, the intermediate host of the sheep liver fluke, Fasciola hepatica, is usually found on sparsely vegetated mudbanks in shallow water (less than 5 cm deep), hence the susceptibility of the snail to encountering certain levels of herbicides in its environment. Previous studies on the influence of pesticides on snails discussed the extent of toxicity of the toxicants to snails (Christian and Tate 1983; Khangarot and Ray 1987). However, the purpose of this study was to investigate the chronic effects of dalapon on the hatching success of F. cubensis snails after several generations of exposure at sub-lethal levels.

MATERIALS AND METHODS

Ninth-generation F. cubensis snails were randomly selected from laboratory culture and exposed to the following sub-lethal concentrations (0, 0.1, 1.0 and

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10.0 mg/L) of dalapon in plastic trays measuring (28 x) 18 x 12 cm). Dalapon (95% technical grade) was obtained from Chemical Services Inc., West Chester, Pennsylvania. Dalapon was dissolved in distilled water to obtain the concentrations used, while distilled water also served as the control. Previous studies in our laboratory showed that the 24 hour LC_{50} of dalapon to F. cubensis was 166.67 mg/L (unpublished data). The concentrations of dalapon chosen for this study were levels at which there were no mortalities of test organisms for the duration of the study (23 days).

Snails were fed with cerophyll (ad libitum) obtained from Ward's Natural Sciences Establishment, Rochester, New York. After a 72 hr acclimatization period, all egg masses subsequently laid were removed and 4 egg masses each containing an average of 28 eggs were placed in 20mL finger bowls containing 0, 0.1, 1.0 and 10.0 mg/L dalapon respectively. There were 10 replicates of each concentration. Solutions were renewed every 4 days and maintained at temperatures between 25-26°C and a pH of 7.2-7.8. Eggs were observed under a Bausch and Lomb dissecting microscope (7 x objective) and counted with a laboratory counter (Clay Adams). Daily mean percentage of eggs hatching were recorded.

The hatched snails from the above study were then allowed to undergo three successive generations of rearing in 0, 0.1, 1.0 and 10.0 mg/L dalapon. Daily observations and recordings were made of days taken by young snails to hatch. Daily mean percentage of snails hatching were calculated and recorded. The number of snails hatched were analyzed by Analysis of Variance and Multiple Comparison test (Tukey-Test) among treatments.

RESULTS AND DISCUSSION

Presented in Tables 1 and 2 are the mean percentages of snails hatched. In the 1st generation there was 100% embryonation and hatching of F. cubensis snails in the control by the 14th day while only 5.55%, 8.47% and 66.6% hatched in the 0.1, 1.0 and 10.0 mg/L dalapon, respectively (Table 1, Figure 1). Analysis of the data indicated that there were significant differences ($p < 0.05$) between the number of snails hatched in the various concentrations (Tables 1 and 3). There was also a significant delay in hatching of the young snails in the dalapon-treated groups ($p < 0.05$). However, 10.0 mg/L dalapon showed a slight stimulation of hatching compared with 0.1 and 1.0 mg/L.

Table 1. Percent embryonation and hatching of 1st generation **F. cubensis** exposed to dalapon (mg/L)

	Dalapon concentration (mg/L)			
Day	0.0	0.1	1.0	10.0
9	5.41	0.00	0.00	9.3
10	51.35	0.00	0.00	16.67
11	81.08	0.00	0.00	27.78
12	91.89	0.00	0.00	42.59
13	94.59	0.00	0.00	53.7
14	100.00	5.55	8.47	66.67
15	100.00	8.33	20.34	83.33
16	100.00	19.44	28.81	94.44
17	100.00	36.11	38.98	98.15
18	100.00	38.89	45.76	100.00
19	100.00	41.67	55.93	100.00
20	100.00	61.11	74.58	100.00
21	100.00	69.44	93.22	100.00
22	100.00	88.89	96.00	100.00
23	100.00	94.44	96.00	100.00

% Means after day 14	Tukey Grouping
0.0mg/L - 16.66	A
0.1mg/L - 1.00	B
1.0mg/L - 1.33	B
10.0mg/L - 11.16	C

Means with the same letter are not significantly different. Alpha level = 0.05

Table 2. Percent embryonation and hatching of 3rd generation **F. cubensis** exposed to dalapon (mg/L)

	Dalapon concentration (mg/L)			
Day	0.0	0.1	1.0	10.0
9	5.45	0.00	0.00	10.67
10	51.35	0.00	0.00	20.39
11	81.08	3.00	5.88	36.89
12	91.89	11.00	13.73	50.
13	94.59	21.00	25.49	63.11
14	100.00	29.00	38.24	74.67
15	100.00	42.00	57.84	88.35
16	100.00	58.00	72.55	100.00
17	100.00	78.00	88.24	100.00
18	100.00	89.00	100.00	100.00
19	100.00	98.00	100.00	100.00
20	100.00	100.00	100.00	100.00

% Means after day 14	Tukey Grouping
0.0mg/L - 16.66	A
0.1mg/L - 4.50	B
1.0mg/L - 6.33	B
10.0mg/L - 12.3	C

Means with the same letter are not significantly different.Alpha Level = 0.05

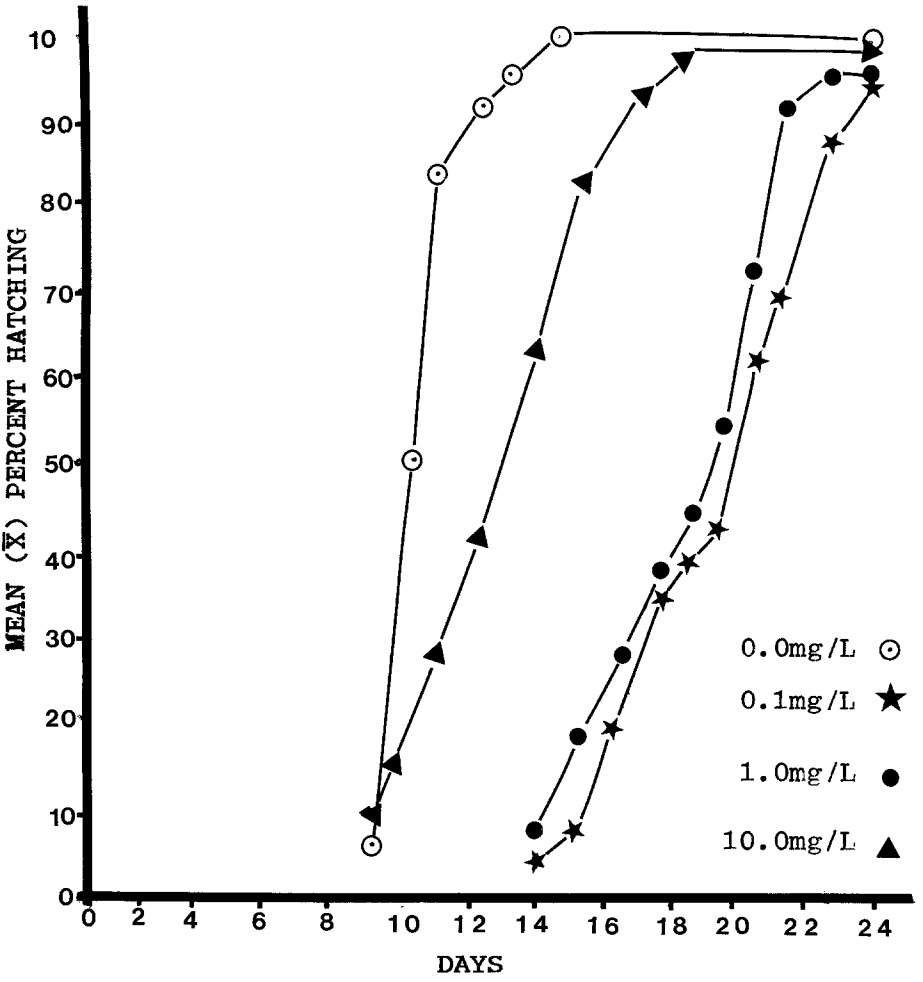

Figure 1. Percent embryonation and hatching of 1st generation F. cubensis exposed to dalapon

Table 3. Analysis of variance

Source	DF	SS	F value	PR>F
Conc.	3	4278.0	36.23	0.0001***
Delay	6	1206.3	4.28	0.0011**
Generation	1	678.8	13.20	0.0004***

P<0.05 * significant
P<0.01 ** very significant
P<0.001*** highly significant

In the 3rd generation, embryonation and hatching in 0.1, 1.0 and 10.0 mg/L dalapon improved to 29%, 38.24% and 74.67% respectively by the 14th day (Table 2, Figure 2). Moreover, there was a 100% hatching in all

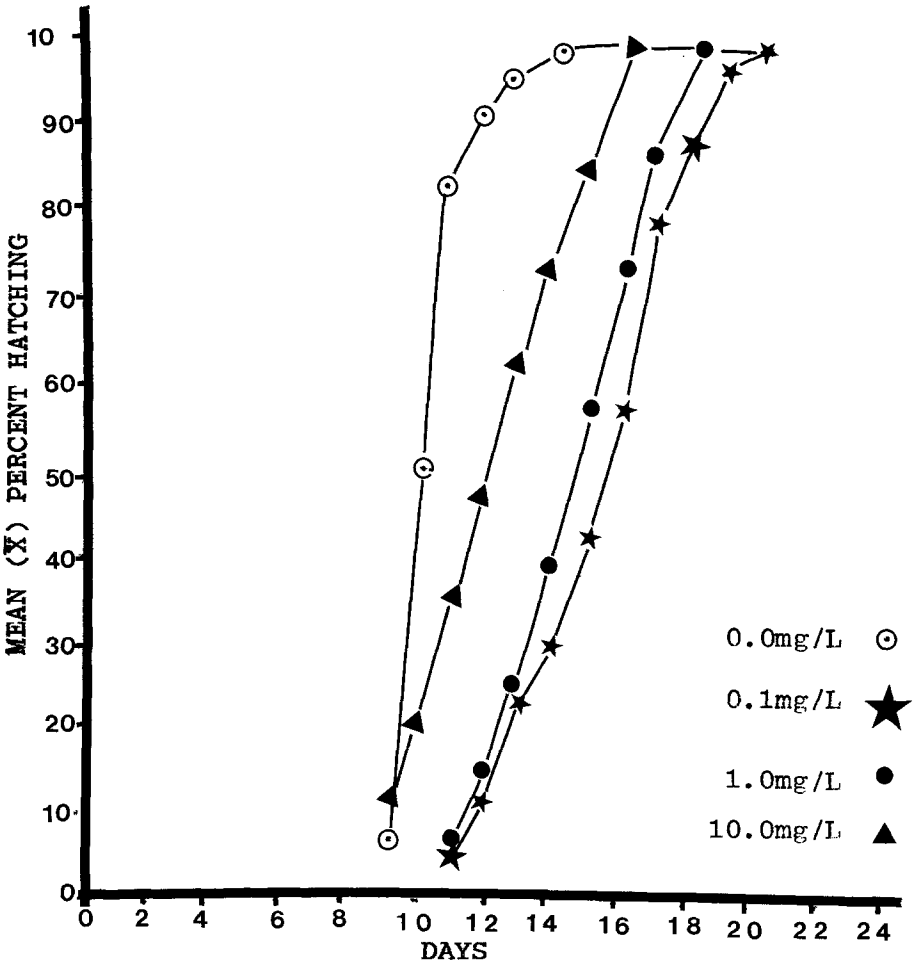

Figure 2. Percent embryonation and hatching of 3rd generation F. cubensis exposed to dalapon

concentrations by the 20th day, whereas in the 1st generation, only the control and 10mg/L dalapon-treated snails attained a 100% hatching. The Analysis of variance and Tukey's test indicated that there was a significant difference ($p < 0.05$) between the number of snails hatching in the first generation and that in the 3rd generation (Table 3). In the 10 mg/L dalapon, there was a 100% hatching of young snails by the 16th day compared to a 100% hatching by the 18th day and 20th day for 1.0 and 0.1 mg/L dalapon, respectively.

The results indicated that there was a marked improvement in the hatching success of young snails in the 3rd generation over the 1st. It was reported by Greaves et al. (1981) that low levels of dalapon was found to

stimulate respiration in soil. The degradation of dalapon into pyruvic acid, a growth supporting organic compound, has also been studied (Magee and Colmer 1959; Kearney et al. (1964). Allison et al. (1983) further reported the involvement of certain dehalogenating enzymes (whose synthesis are induced only in the presence of dalapon) to be responsible for the conversion of the herbicide into pyruvate. There is a possibility that through the decomposition of pyruvate into carbon dioxide and water more ATP's may have been made available to enhance embryonic development and hatching in F. cubensis. The current study indicated that it may be possible that through rearing snails in sub-lethal concentrations of dalapon, there could be a shortening of time taken by snails to embryonate and hatch depending on the concentrations used. This could result in more snails available for the propagation of the liver fluke, F. hepatica, which in turn could further increase the incidence of liver fluke infections.

Acknowledgments. We thank Dr. Abdel Emile Malek, Tulane Medical Center for Tropical Medicine and Parasitology, New Orleans, Louisiana, for personal communications and positive identification of F. cubensis. This study was supported by Grant No. NIH RR 08025.

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Received July 23, 1989; accepted December 18, 1989.