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Effects of the Triazine Herbicide Cyanatryn on Aquatic Animals

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Aquatic herbicides may affect non-target organisms directly, through toxicity, or indirectly, by changing the environment (BROOKER and EDWARDS 1975, NEWBOLD 1975). They are selected, on the basis of short-term toxicity tests (LC_{50} type) and field trials, for their high phytotoxicity and low toxicity to non-target organisms, thus minimising the risk of direct effects (BROOKER and EDWARDS 1975). Triazine compounds meet these requirements and of these, cyanatryn (2-(4-ethylamino-6-methylthio-s-triazin-2-ylamino)-2-methylpropionitrile) has the highest inherent phytotoxic activity (PAYNE 1974).

The long-term ecological effects of cyanatryn have been studied in drainage ditches (SCORGIE 1978). All submerged macrophytes and filamentous algae were destroyed in the treated areas (0.2 ppm nominal concentration) and several species of invertebrate subsequently suffered a decline in numbers. Snails, Lymnaea peregra, the young of which were particularly common initially, had virtually disappeared several weeks after treatment. Laboratory experiments described in this paper aimed to establish whether cyanatryn might act via direct toxic effects on Lymnaea and thus explain the observed declines. In addition, experiments were carried out on Daphnia pulex and tadpoles of the frog Rana temporaria (species rare or absent from the experimental sites). These organisms are known to be sensitive to chemical pollution and are frequently used in toxicity tests in the laboratory (SANDERS 1970, COOKE 1972, COOKE and ZORO 1975, HADDOW et al. 1974).

MATERIALS AND METHODS

Test solutions were made up using either pure compound or slow-release pellets containing 10% active ingredient (a.i.). Concentrations quoted in this paper assume total release of the a.i. and should be regarded as nominal.

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Lymnaea peregra

Adult snails were treated in groups of 5 in glass jars containing 500 ml filtered ditch water. There were 2 groups in each treatment. Treatments used were 0.2, 2 and 20 ppm cyanatryn (nominal concentrations), made up using slow-release pellets. Survival and egg production were monitored for 48 hours.

Egg masses containing 5 to 10 eggs per mass were exposed to 0.2, 2 and 20 ppm cyanatryn (pure compound) in glass vials of 40 ml capacity. There were 6 replicates of each treatment with one egg mass in each. The test solution was again made up in filtered ditch water, which was renewed daily. Observations on embryonic development and survival of juvenile snails were made at frequent intervals for a period of 20 days.

Daphnia pulex

Adult Daphnia were treated in groups of 10 in glass vials containing 40 ml filtered ditch water. There were 2 groups in each treatment: 0.2, 2 and 20 ppm cyanatryn (pure compound). Numbers swimming were recorded at frequent intervals during the first 24 hours of the experiment and survival was monitored over a 3-day period.

Rana temporaria

Initially, tadpoles weighed 40 ± 5 mg and had hind limb buds (stage 25, WITSCHI, 1956). In both experiments, tap water, which had been allowed to stand for about one week, was used as the basis of the test medium. Tadpoles were fed on washed canned spinach.

i) Acute dosing experiment Tadpoles were treated in groups of 10 in 250 ml test medium with 2 groups in each treatment. Treatments used were:

0.2 ppm)
2 ppm) pure cyanatryn
20 ppm)

20 ppm) cyanatryn slow-release pellets
200 ppm) (nominal concentrations)

Observations were made during 3 periods to monitor 1) the initial effects of treatment on activity (0-5 hours), 2) the numbers feeding on spinach added at the end of the first period (5-7 hours) and 3) the persistence of any effects observed in the early stages

(21-24 hours). After 24 hours the tadpoles in each group were weighed collectively.

ii) Prolonged dosing experiment Tadpoles were treated in groups of 20 in 4 litres test medium. Treatments used were 0.2, 2 and 20 ppm cyanatryn (nominal concentrations made up from slow-release pellets as above). Behaviour was monitored and samples of tadpoles were weighed after 3, 7 and 11 days. The experiment was ended after 11 days and water samples were analysed by gas-liquid chromatography for cyanatryn residues.

RESULTS

Lymnaea peregra

Adult L. peregra were seriously affected by short-term exposure to high concentrations of cyanatryn. After 2 days, 60% of animals in the 20 ppm treatment (nominal concentration) were dead. Some mortality was observed in the other treatment groups. Egg production was also affected by cyanatryn. After 2 days, the control group had produced 106 eggs (in 8 egg masses) compared with 39 (3), 10 (1) and 10 (2) in the 0.2, 2 and 20 ppm treatments respectively.

Eggs of L. peregra exposed to 20 ppm cyanatryn (pure compound) suffered a high incidence of embryonic deformities and all failed to hatch. All eggs in the other treatments hatched after 10 to 14 days. Subsequent survival of young snails in the 2.0 ppm treatment was significantly less than in the control group ($P<0.05$, see Table 1). Survival of the 0.2 ppm group was not affected.

TABLE 1

Percentage mortality of juvenile snails (L. peregra) exposed to cyanatryn (Mean of 6 replicates with range in brackets)

Days	Control	0.2 ppm	2 ppm
12	12 (0-40)	7 (0-20)	37 (11-80)[a]
20	20 (0-40)	12 (0-40)	46 (22-80)[a]

[a]Using Mann-Whitney non-parametric U test, significantly different from control and 0.2 ppm treatment ($P<0.05$)

Daphnia pulex

High concentrations of cyanatryn had an almost immediate effect on Daphnia pulex (Fig. 1). After 15 minutes at 20 ppm (pure compound), several individuals showed uncoordinated swimming movements and sank to the bottom. Within 4 hours, virtually all animals in this treatment group were at rest, only moving their antennae when provoked. Three days later they were all dead. Cyanatryn at 2 ppm produced a similar, though delayed response in most of the animals in this group. Some appeared to be completely unaffected, even after 24 hours exposure. Most of the 0.2 ppm treatment group behaved normally but some showed signs of distress (for example, they tended to swim in tight circles) after 3 hours. After 3 days, 25% of the 2 ppm group and 10% of the 0.2 ppm group were dead. There were no deaths in the control group during this time.

Rana temporaria

i) Acute dosing The results for groups of tadpoles exposed to the pure compound are summarised in Table 2. There were no significant effects on mean numbers active, although the behaviour of tadpoles exposed to 20 ppm cyanatryn was changed. These were generally lethargic, but sometimes displayed spasmodic twitching and shuddering and also showed a significant tendency to rest at the water's surface (Table 2). When spinach was added after 5 hours, the number feeding at any one time was reduced as the level of exposure increased; tadpoles exposed to 20 ppm were not recorded feeding at all. Observations on behaviour were similar at the end of the 24 hour period to those made from 5 to 7 hours. Final wet weight of the tadpoles was negatively related to the concentration of cyanatryn to which they had been exposed (Table 2) and was related positively to the level of feeding activity recorded during the period from 5 to 7 hours (Fig. 2).

Tadpoles exposed to 20 or 200 ppm cyanatryn (pellets) behaved in a similar manner to those treated with 20 ppm pure compound, so observations on these groups are omitted from Table 2. The only difference was that they were significantly more active than the controls during the first 5 hours. Mean numbers active were: 20 ppm, 9.9 out of 20 (range for 7 observations, 8.8-11.0, Mann-Whitney test $U_{7,7} = 0$, $P < 0.05$); 200 ppm, 10.1 (8.4-11.2, $P < 0.05$). Tadpoles appeared to be more active than normal because any contact with the pellets resulted in a rapid avoidance reaction.

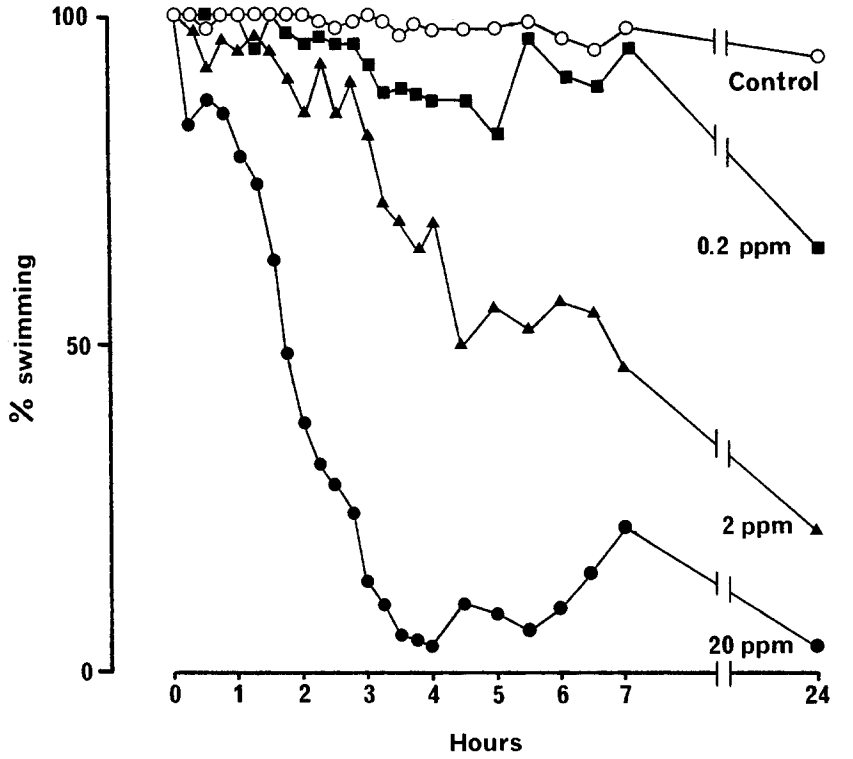

Fig. 1 Effect of cyanatryn on swimming activity of D. pulex.

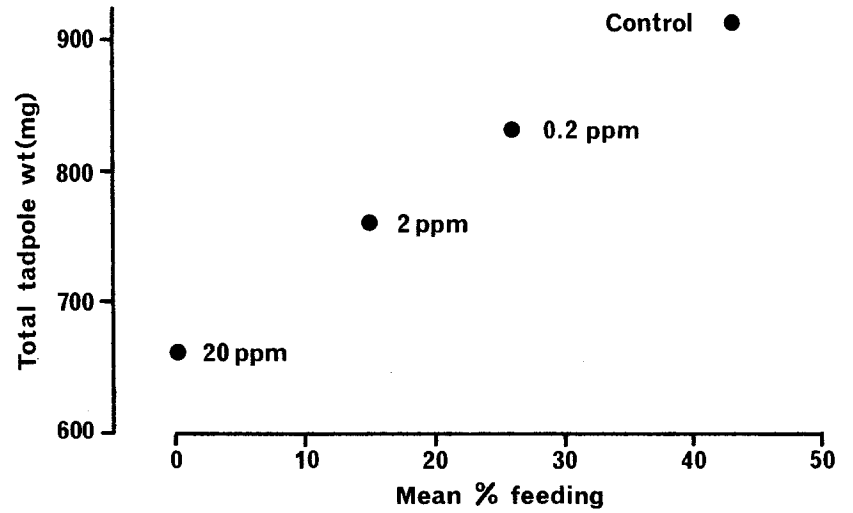

Fig. 2 Relationship between final tadpole weight and numbers feeding, 5-7 hours (see Table 2).

TABLE 2

Observations on behaviour and weight of tadpoles (R. temporaria) exposed to cyanatryn (pure compound) for 24 hours. Replicates have been combined giving 20 tadpoles per treatment. (Mean with range in brackets).

	No. of obs	Control	0.2 ppm	2 ppm	20 ppm
Mean number active					
0-5 h	7	7.5(6.8-8.0)	8.5(7.0-10)	9.2(6.6-12)	7.4(4.4-11)
5-7 h	3	4.8(4.4-5.2)	5.0(4.8-5.4)	5.6(5.4-6.0)	6.8(6.2-7.8)
21-24 h	3	5.7(4.2-6.8)	6.4(5.8-6.8)	7.4(7.2-7.8)	7.7(6.8-8.4)
Mean number at surface					
5-7 h	5	1.2(0-3)	2.0(1-4)	1.6(1-3)	11.2(8-15)[a]
21-24 h	3	1.3(0-2)	1.7(0-3)	2.6(1-4)	11.4(9-14)
Mean number feeding					
5-7 h	6	8.5(4-11)	5.2(3-7)	3.0(2-4)[a]	0[a]
21-24 h	3	5.4(2-10)	3.0(1-6)	4.0(3-5)	0
Total wet weight (mg)					
24 h[b]		910	830	760	660

[a]Using Mann-Whitney non-parametric U test, significantly different from control ($P < 0.05$)

[b]Initial wet weight = 800 mg

ii) Prolonged dosing Tadpoles exposed to cyanatryn in pellet form initially showed changes in behaviour similar to those observed in the acute dosing experiment. These changes occurred to about the same extent in the 3 treatment groups, 0.2, 2 and 20 ppm cyanatryn (nominal concentrations). Cyanatryn levels in the water at the end of the experiment were 0.23, 0.87 and 1.61 ppm respectively, so although the range of nominal concentrations was x 100, the real range at this time was only x 7. Results for the 0.2 ppm treatment are referred to in some detail below, since this level is within the range of recommended treatment rates.

Throughout the first day, tadpoles in the 0.2 ppm group were significantly more active than the controls, were recorded at the surface more frequently and fed less often (Mann-Whitney U tests, $P < 0.05$). From 3 days onwards, however, all of these behavioural effects were reversed to a significant degree ($P < 0.05$). Although they gained in weight from day 3 to day 11, treated tadpoles were still only about half the weight of the controls at the end of the experiment: treated tadpoles, 114 ± 9 (mean wet weight in mg ± S.E., n = 10); controls, 240 ± 11 ($t_{18} = 8.8$, $P < 0.001$).

DISCUSSION AND CONCLUSIONS

The recommended treatment rates for cyanatryn are normally between 0.05 and 0.2 ppm (ANON. 1976), but nominal concentrations as high as 0.4 ppm may be used under certain conditions (for example, in highly organic waters). The 96 hour LC_{50} values for Rana temporaria tadpoles and for Daphnia (longispina) are 30 ppm and 15.4 ppm respectively, whilst adult L. peregra are not affected by 8 days exposure to 10 ppm cyanatryn (see HADDOW et al. 1974).

This series of experiments showed that D. pulex and tadpoles of the frog R. temporaria are affected by short-term exposure to levels of cyanatryn as low as those recommended for use in the field. Lymnaea peregra juveniles showed no adverse effects at these levels, but egg production appeared to be affected. Since juveniles survive at field concentrations, it seems unlikely that the population declines of L. peregra observed in field experiments with cyanatryn were due to a direct toxic effect of the chemical (SCORGIE 1978).

Tadpoles were the most sensitive of the animals tested. When, however, groups of tadpoles were caged in drainage channels and exposed to 0.02 ppm cyanatryn, one tenth of the recommended concentration, there were no detectable effects (unpublished observation). In the field, changes in behaviour or reductions in growth rate could be disadvantageous to tadpoles, for example by increasing the risk of predation by newts (Triturus) (COOKE 1971, 1974).

More attention needs to be given to the potential sublethal effects of aquatic herbicides that might occur at normal treatment rates, i.e. at concentrations which are far below the published LC_{50} figures.

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