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Effect of DDT and MCPA (4-Chloro-2-Methylphenoxyacetic Acid) on Reproduction of the Pond Snail, *Lymnaea stagnalis* L.

Per Woin[1] and Christer Brönmark[2]

Department of Ecology, [1]Ecotoxicology and [2]Animal Ecology, University of Lund, Ecology Building, S-223 62 Lund, Sweden

Reproduction is the single most important function in the life cycle of an organism. Successful reproduction determines fitness of organisms. The inability of an organism to complete any one stage of the reproductive process severely reduces its lifetime reproductive success. Disruptions in the reproduction will ultimately affect the abundance and distribution of the species. Therefore, laboratory tests of long-term impact of sublethal pollutant concentrations on organisms preferably is done on the reproductive success (Sheehan 1984).

Because of the importance of the reproductive process, investigations of reproductive performance in response to stress are essential for a better understanding of the impact of long-term exposure to pollutants on natural ecosystems. To understand the structural and functional changes in polluted ecosystems it is necessary to obtain information on the reproductive success of key species. Freshwater snails are an important constituent of the invertebrate fauna in most eutrophic and mesotrophic lakes. The giant pond snail, *Lymnaea stagnalis* L., has a geographical distribution that extends over large parts of Europe, North America and Asia (Berrie 1965) and the basic features of its biology are well known (Noland and Carricker 1946; Berrie 1965; Joosse 1975; Geraerts 1976).

Pollutants of diverse structure may affect the reproductive system which is sensitive to toxic agents. Certain pollutants, notably the organochlorine compounds, have been shown to affect the male and female reproductive systems (Jernelöv et al. 1978). We have studied the effect of sublethal concentrations of DDT and the herbicide 4-chloro-2-methylphenoxyacetic acid (MCPA) on the reproductive output of the pulmonate snail *Lymnaea stagnalis* under a 2-mon exposure period.

MATERIALS AND METHODS

Snails were collected in a small, eutrophic pond in southern Sweden in late July. The size of the snails used in the experiment ranged from 25 to 35 mm (mean ± SE: 28.16 mm ± 0.30). The experiment was performed using 1.5-L glass jars containing tapwater (1 L) with a defined concentration of the test substance. The

Send reprint requests to Per Woin at the above address.

water temperature were kept at 19±1°C. The water was gently aerated and the light regime was kept at LD 12:12. Two snails were placed in each jar and were fed with lettuce twice a week. There was no significant difference (unpaired students t, $p>0.05$) between treatments in snail size distribution. All snails received the same amount of food, measured as a defined area of lettuce leaves. If a snail died, it was replaced with one of similar size and treated as the replaced. After a 2-wk pre-exposure period, the manipulation was started followed by 2 wk of acclimatization to experimental conditions. Data were then recorded for 45 days.

The substances tested were the insecticide p,p'-DDT and the herbicide MCPA (4-chloro-2-methylphenoxyacetic acid). The substances were dissolved in acetone and the solution was added to water and rigorously shaken. The amount of acetone in the water of the jars was kept at 0.05%(v/v). The nominal concentrations of the substances in the treatments were: 0 (Control 0), 50 and 500 μgL^{-1} DDT, 10 and 100 mgL^{-1} MCPA, and 250 μgL^{-1} DDT + 50 mgL^{-1} MCPA. In addition, one treatment contained 0.05% acetone (Control A) to determine if there was any effect of acetone alone. The combination of MCPA and DDT was used to elucidate the occurrence of additive or synergistic effects. Each treatment contained 6 replicates which were randomly assigned to experimental jars. The water (with test substances) was renewed twice a week.

The analytical procedure was performed by reversed phase extraction (C18-columns: PrepSep™) of 150 mL acidified (pH 1-2) experimental water. The substances were then eluted from the column with dichloromethane. MCPA was depolarized by converting the carboxyl group to a methyl ester derivative, using acethylchloride as reagent. Prior to the GC-analysis, DDT and the derivative of MCPA were solved in hexane. The compounds were determinated by capillary gas chromatography (column DB5-30m, ID 0,33mm) with electron capture detection (Varian 3700) according to Okla and Wesén (1984).

Lymnaeid snails attach egg masses, containing large number of eggs, to solid substrates when reproducing. The egg masses produced by the snails in the experiment were removed twice a week and the number of eggs in each eggmass counted under a dissecting microscope.

One factor ANOVA for repeated measures was used to determine differences in mortality between the treatments. A nonparametric Wilcoxon Signed-rank test was used to determine the differences in eggproduction between non-exposed and exposed animals. The tests were performed on Macintosh with the program Statview SE+.

RESULTS AND DISCUSSION

The mortality (Table 1) was high in the two control treatments (7 dead snails out of 12 in Control 0 and 9 dead snails in Control A) while no mortality occurred in snails exposed to DDT. However, the mortality in the DDT treatments were significantly different ($p<0.05$, ANOVA) only to the Control A. There were no significant differences in mortality between DDT and Control 0, between DDT and

Table 1. Exposure levels and mortality in snails treated with DDT and MCPA. Control 0 is the nontreated group. Control A is treated with 0.05% acetone added to the water. Mortality= total no. of dead animals during the exposure period (dead animals were continuously replaced with new ones).

Treatment (n=6) Nominal Concentration	Measured Concentration in water (μgL^{-1})	SD	Mortality
Control 0	-		7
Control A (Acetone)	0.01	0.01	9
50 μgL^{-1} DDT	0.90	0.33	0
500 μgL^{-1} DDT	3.91	0.23	0
10 mgL^{-1} MCPA	6308	2245	5
100 mgL^{-1} MCPA	$200*10^3$	$48.8*10^3$	3
MCPA(50 mgL^{-1})+DDT(250μgL^{-1})	3.17(DDT)	1.15	0

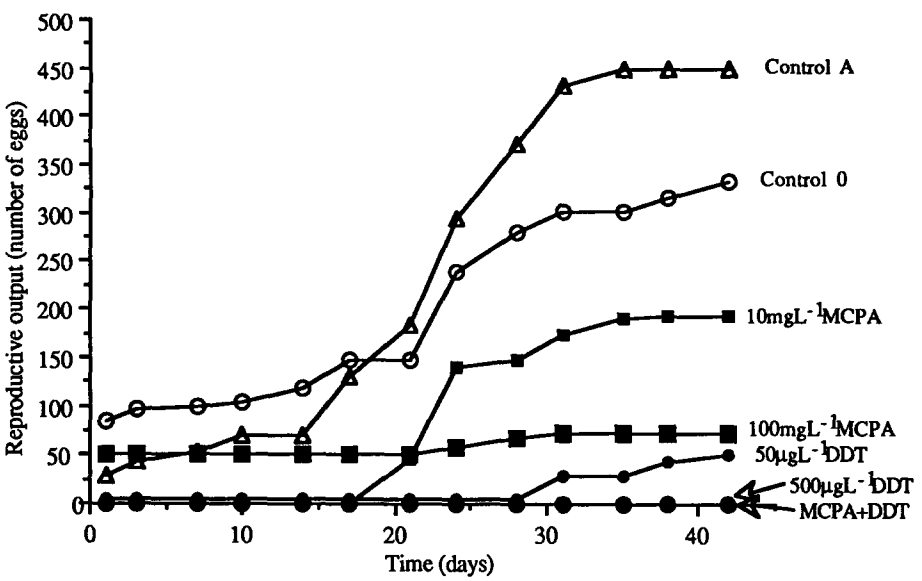

Figure 1. The cumulative production of eggs by the pond snail (*Lymnaea stagnalis*) exposed to 0, 50 and 500 μgL^{-1} DDT, 10 and 100 mgL^{-1} MCPA. Control 0 depicts nonexposed animals, Control A animals exposed to 0.05% acetone. When MCPA and DDT were added together (MCPA+DDT) a concentration of 50 mgL^{-1} MCPA and 250 μgL^{-1} DDT were used. The egg production between nonexposed snails (Control 0 and A) is not significantly different. Each control group, or both together, is significantly different from the polluted groups ($p<0.01$, Wilcoxon signed-rank).

MCPA treated snails or between the MCPA treated snails and controls.

The cumulative reproductive output (Figure 1) was highest in the two control treatments (Control 0: 334 eggs and Control A: 449 eggs). Snails exposed to DDT showed the lowest fecundity (50 μgL^{-1}: 51 eggs and 500 μgL^{-1}: 0 eggs), while snails exposed to MCPA had a fecundity that was intermediate between that of controls and DDT (10 mgL^{-1} MCPA: 194 eggs and 100 mgL^{-1} MCPA: 73 eggs). In the combined treatment with DDT and MCPA no reproduction was recorded. There was no significant difference between the two control groups as regards egg production. However, there was a significant reduction in egg production in exposed snails compared to the controls (Wilcoxon signed-rank, $p<0.01$). At 50 μgL^{-1} DDT the egg production were significantly higher ($p<0.05$) than at 250 μgL^{-1} DDT (in combination with MCPA) and at 500 μgL^{-1} DDT. The 10 mgL^{-1} MCPA treated snails produced significantly ($p<0.05$) more eggs than the snails exposed to the two highest DDT concentrations. Other treatments (50 μgL^{-1} DDT versus 10 and 100 mgL^{-1} MCPA, 500 μgL^{-1} DDT versus 100 mgL^{-1} MCPA and 250 μgL^{-1} DDT, 10 mgL^{-1} MCPA versus 100 mgL^{-1} MCPA, and 100 mgL^{-1} MCPA versus 250 μgL^{-1} DDT) were not significantly different.

Measured MCPA concentrations are in agreement with the calculated (Table 1). However, the actual concentrations of DDT in water are up to more than hundred times lower than the calculated concentrations (500 μgL^{-1} DDT estimated = 3.91 μgL^{-1} DDT measured). The measured exposure concentrations of the treatment solutions differs from the estimated, since evaporation, degradation and adsorption to surfaces occur.

Exposure of snails to DDT and MCPA did not result in an increased mortality as compared with the controls. The mortality of *L.stagnalis* when exposed to 2,4,5-T (chlorinated phenoxyacetic acid), a substance structurally similar to MCPA, was found to be dose dependent within 10-40 mgL^{-1}, with the highest mortality at the largest concentrations (Bluzat and Seuge 1983). However, their exposure study was run for a longer period (11 mon) compared with ours, and differences in mortality between the exposure levels were not evident until after 4 mon exposure. Therefore, our experiment may have been run for too short a time to reveal any significant effects on mortality rates. The relatively high mortality in our controls compared to Bluzat and Seuge's study may be due to the fact that their snails were reared in the laboratory. Therefore, their snails may have been more acclimatized to laboratory conditions than our animals which were collected from the field only a short time before the experiment began.

The low mortality by DDT suggests that *L. stagnalis* may be relatively resistant to chlorinated pesticides. Tucker and Leitzke (1984) reported a LC50 of 30 μgL^{-1} DDT for fish (brook trout fingerlings) and 0.67 μgL^{-1} DDT (16 days) for *Daphnia magna*. Woin (1989) recorded fish (minnows) mortality in concentrations below 20 μgL^{-1} DDT, whereas our maximum concentration was as high as 500 μgL^{-1}. The higher mortality in the controls in our experiment could be due to a higher degree of infections compared to the exposed animals. We assume that DDT may act as an

microbial suppressor inhibiting the development of pathogenic parasites, bacteria and fungus in the water.

There was a reduction in reproductive output as a result of the exposure. Bluzat and Seuges (1983) found a 21.8% decrease in reproductive output as a result of exposure to 10 mgL^{-1} 2,4,5-T which can be compared with 50% reduction at a MCPA concentration of 10 mgL^{-1} found in this experiment. At 100 mgL^{-1} MCPA the reduction in reproductive output was 81 %. The 73 eggs produced in this treatment is dominated by the 50 eggs that were laid on the first day of data recording, probably before the pollutant affected the animals. In the remainder of the period only 3 egg capsules with a total of 23 eggs were produced. Since as many as 50 eggs were laid on the first day indicate that MCPA may not have a toxic effect until the concentration in the snails reaches a threshold level for reproductive malfunction. This constraint was considered in the planning of the experiment and therefore the snails were acclimatized for 2 wk prior to the recording of data. This was perhaps not a long time enough for MCPA to have an effect on the reproductive output.

The effect of DDT on snail reproductive output was greater than that of MCPA. In the highest concentration of DDT there was no egg production, which shows that exposure to DDT has a negative effect on the reproduction of this freshwater snail. As in the 500 μgL^{-1} DDT exposure no eggs were produced in the treatment with DDT and MCPA combined. Therefore, it is not possible to elucidate any occurrence of synergism. The effect of DDT alone was probably too high for a critical test of synergistic effects.

The low measured concentrations of DDT as compared to the estimated (Table 1) were probably due to the fact that DDT as other lipophilic substances are adsorbed to surfaces (Södergren 1973, 1982). As the surface in the glass jars was large in relation to the water volume (1 L) a high amount of the substance may have been adsorbed to it. Because of the continuous aeration of the water, stripping and evaporation of DDT may also be an explanation for the low concentrations measured. The snails are probably not only exposed to the free DDT content in the water (the measured concentration, Table 1), but also and most likely at a higher degree, the adsorbed DDT on the glass surfaces. Therefore, the dose to which the snails were exposed is difficult to estimate.

The results of our experiment suggest that although they have no effect on mortality, anthropogenic substances, like DDT and MCPA, may still have profound effects on the distribution and abundance of a species through a reduction in the reproductive potential of the individuals. Snails are an important component of most freshwater systems, making up a significant portion of the diet of many fish and crayfish (Lodge et al. 1987), and also acting as efficient grazers of periphytic algae (Brönmark 1989), which constitutes their main food source. Laboratory and field experiments have shown that snails are able to reduce the biomass and change the species composition of periphytic algae (Brönmark 1989). Further, an excessive cover of periphytic algae is detrimental to submerged macrophytes, through a reduction of incident light and availability of nutrients (Sand-Jensen 1977). Brönmark (1985) showed that snails could have a positive effect on

macrophyte growth by removing the algal cover and thus decreasing competition for light and nutrients. In accordance with this, a reduction of snail density through an increased predation pressure by molluscivorous fish led to an increase in periphyton biomass and a concomitant decrease in growth of the submerged macrophyte *Elodea canadensis* (Brönmark, unpublished manuscript). Thus, the occurrence of DDT and MCPA in freshwater habitats do not only have direct effects on the distribution of snails but may also have indirect effects on both higher (reduction of prey items available to fish and crayfish) and lower (increase of periphyton biomass and reduction of macrophyte growth) trophic levels.

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