Effects of Sublethal Concentrations of the Herbicide Atrazin® on Growth and Reproduction of *Daphnia Pulex*

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Data on sublethal effects of pesticides on fresh water fauna are rare. This is possibly due to there being up till now no generally accepted standard methods for the measurement of such effects. Although generally accepted methods are used to measure acute toxicity, comparisons of studies carried out on concentrations where mortality cannot be applied as a criterion are not very meaningful, because widely differing parameters together with a whole variety of methods are in use. In view of this obvious need to establish the ecologically relevant effects of substances which are capable of contaminating the environment, it has become urgently necessary to standardize the criteria and methods used for this purpose.

Particularly relevant ecologically are the effects on growth and reproduction as even small changes in these parameters can disturb the balance in a biocoenosis quite drastically. In this paper we present results of investigations into the possibilities of measuring such effects on Daphnia pulex - an animal commonly used for toxicological testing. As test substance a herbicide was chosen because little is known either about the distribution of these pesticides throughout the environment or the effects on organisms other than plants, although the application of herbicides plays an ever increasing role.

Material and Methods

The herbicide tested was the chlorinated triazine Atrazin (Ciba-Geigy AG, Basel), an inhibitor of photosynthesis. The substance, most kindly donated by the producer, was 99,2 % pure. The solubility was given as 33 mg/l (MAIER-BODE 1971), but it dissolved only quite

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slowly in water. As no organic solvent was to remain in the aqaeous Atrazin solution, the required amount of Atrazin dissolved in ethanol was pipetted into a graduated flask. The ethanol was evaporated off by blowing air at room temperature into the flask, thus leaving a fine film of Atrazin crystals on the base of it. The flask was made up to volume with sterile filtered lake water and shaken overnight. Tests with 14-C-labelled Atrazin showed that the full amount could be brought into solution by this method. The experiments were carried out with concentrations of 1, 2, 3, 4, 5, 10 and 20 mg Atrazin/l. To test a possible influence of the organic solvent a few tests were also conducted with addition of 0.1 and 0.5 % ethanol.

As experimental organism Daphnia pulex from a continuous culture already established for some years (LAMPERT 1975), were kept singly during the experiment in 100 ml jars capped with a black lid preventing them from being attached to the water surface. Each experiment was started with new born Daphnia never older than 15 h. 10 duplicates were set up for each concentration. The jars stood in a waterbath at 20°C ±0.5°C under weak continuous illumination from a fluorescent lamp. Their position in the bath was altered randomly every second day to avoid irregular lighting effects.

The Daphnia were transferred every other day to fresh medium made up of the previously prepared Atrazin in sterile filtered lake water solution containing a definite amount of food. This food consisted of Scenedesmus acutus from a chemostat culture mixed with 10 % yeast at a concentration of exactly 4 mg C/l. As the food concentration in the medium changed during the two days due to grazing and sedimentation and as growth and reproduction depend on the actual food available it had to be ensured that any changes in the different Atrazin concentrations and in the controls were always the same. Therefore samples were carefully removed with a pipette from a parallel series for each Atrazin concentration and the controls after 24 and 48 hours, filtered over diatomaceous earth and analysed for particulate carbon. The concentration of particulate carbon decreased by 25 % after 24 h and a further 17 % after 48 h. Between the different Atrazin concentrations and the controls, however, no difference was found thus establishing that the food situation in the series with and without the herbicide was the same.

One experimental series was run for 28 days, another for the whole life span of the Daphnids (approximately 70 days). After the transfer of the adult Daphnia to fresh medium every other day the young produced during the two days were counted.

At the end of the experiment the adults were anaesthetized in CO_2 saturated water and their length measured under the microscope from the top of the head to the base of the tail spine. Finally their carbon content was determined. These data were used as a measurement for growth.

Results

28-day experiments

a) Reproduction: The herbicide had a marked effect on the production of young. The result can be expressed either as production per individual if the number of young at each transfer is divided by the number of surviving adults, or as total young production in the population. The latter differs from the former as the effect of mortality is included. The data for both are presented in Table 1.

TABLE 1

Cumulative production of young by D. pulex within 28 days

Concentration of Atrazin (mg/l)	Number of survivors	Young/animal (% of contr.)	Young/population (% of contr.)
Control	9.4	100.0	100.0
1	8.3	90.4	71.8
2	9.0	73.8	73.2
3	7.5	70.4	54.5
4	8.5	63.0	51.7
5	9.5	66.8	58.0
10	8.0	40.2	46.1
20	0.5	4.2	3.5

Cumulative curves for production of young per individual are presented in Figure 1. The curves are nearly linear as are those for offspring produced per population. Therefore regression lines were fitted for each curve and all regression coefficients were tested for significant difference. The results are presented in Table 2.

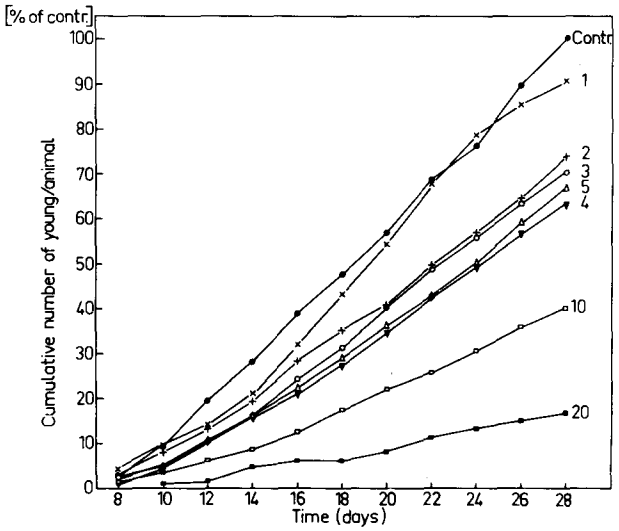

Fig. 1. Effect of Atrazin on the reproduction of D. pulex (28-day experiments). Number on each curve indicates concentration of Atrazin in ppm.

TABLE 2

Test of significance for the slopes of the regression lines fitted for the curves of cumulative production of young in 28-day experiments.
(+ = significant; - = not significant; $p < 0.05$)

	1	2	3	4	5	10	20	C
1		-	+	+	+	+	+	+
2	+		+	+	+	+	+	+
3	+	-		+	+	+	+	+
4	+	+	+		+	-	+	+
5	+	-	-	-		+	+	+
10	+	+	+	-	+		+	+
20	+	+	+	+	+	+		+
C	-	+	+	+	+	+	+	

Cum. number of young/animal

Cum. number of young/populat.

Most differences prove to be significant, i. e. differences exist between the slopes of the curves and therefore between the rates of reproduction. The effect of Atrazin at the population level is evident even at 1 ppm.

b) Growth: With increasing Atrazin concentrations growth is reduced. The mean length after 28 days shows a linear dependence on Atrazin concentration (Fig. 2). This becomes even clearer when the carbon content is taken into consideration (Fig. 3). The greatest reduction (53.7 %) occurs between 1 and 5 mg Atrazin/l.

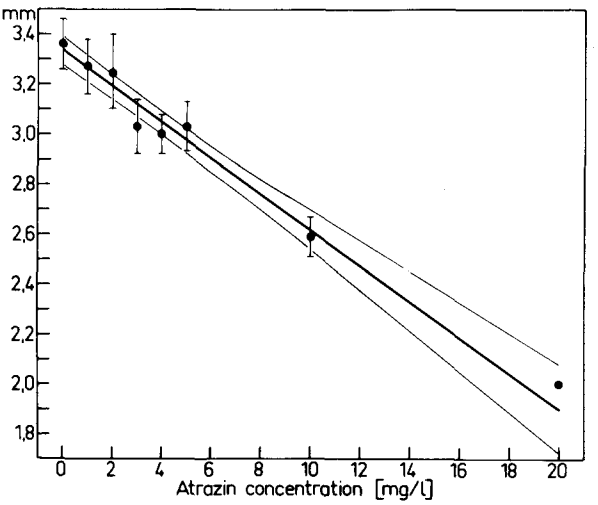

Fig. 2. Length of D. pulex grown for 28 days at different Atrazin concentrations

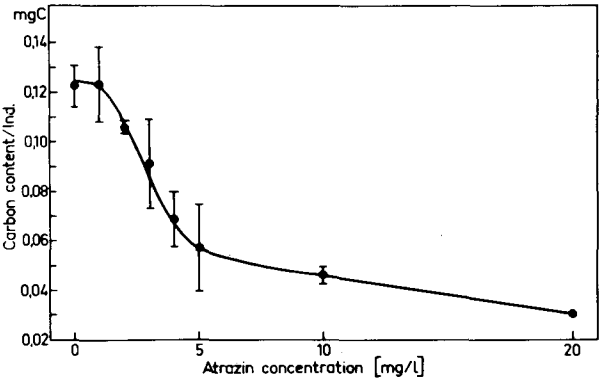

Fig. 3. Carbon content of D. pulex grown for 28 days at different Atrazin concentrations

Experiments lasting a complete life span

a) Longevity: As is evident from the curves of survival, Atrazin up to 10 ppm has no significant effect on longevity (Fig. 4). The high mortality at 1 ppm must be due to some other unknown factor.

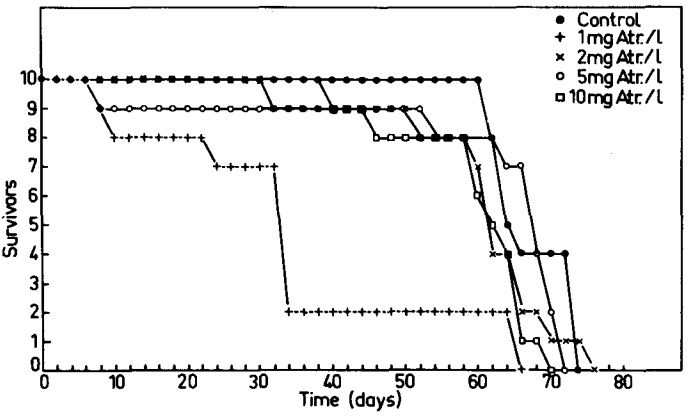

Fig. 4. Survival of D. pulex at different concentrations of Atrazin

b) Production of young: Here again the results can be expressed per individual and per population. They are summarized in Table 3. Offspring produced per population is given in Figure 5. The break in the curve at 1 ppm is caused by the obscure mortality mentioned above.

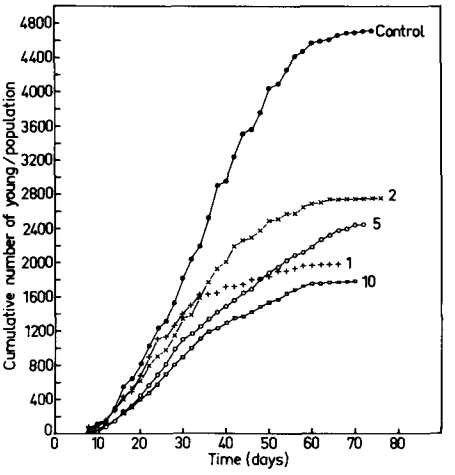

Fig. 5. Effect of Atrazin on the reproduction of D. pulex during the whole life span. Number on each curve indicates concentration of Atrazin in ppm.

TABLE 3

Production of young by D. pulex during the whole life span

Concentration of Atrazin (mg/l)	Mean life span (days)	Young/animal (% of contr.)	Young/ population (% of contr.)
Control	64	100.00	100.00
1	(33)	73.8	(42.0)
2	62	68.6	58.3
5	68	58.2	51.8
10	62	38.9	37.4

Survival of starved individuals

To ascertain whether Atrazin contaminated animals are more sensitive to a worsening of conditions in the medium, a number of individuals were subjected to 0, 1, 5 and 10 mg Atrazin/l for 12 days during which time all of them produced offspring at least once. After 12 days the individuals were transferred to sterile filtered lake water which was changed daily.

50 % of the individuals from all concentration levels had died after 8 days. After a further 6 days only 4 remained alive - these had originated from the 10 mg Atrazin/l medium and were extremely small. An influence of the herbicide on longevity under starvation conditions could not be proved.

Effects of an organic solvent

It is common practice in toxicological work to use an organic solvent to bring the test substance into solution; consequently a series of 28-day experiments were conducted with addition of 0.1 and 0.5 % ethanol. As no effect from 0.1 % was evident after 2 weeks this series was dropped. The addition of 0.5 % ethanol, however, caused a considerable increase in the effects of Atrazin. Table 4 shows a comparison of the parameters for growth and reproduction with and without ethanol. All Daphnia in 10 mg Atrazin/l with added 0.5 % ethanol had died by the twelfth day.

The effect of Atrazin and ethanol together is greater than the sum of the individual effects, i.e. a synergistic effect comes into play.

TABLE 4

Comparison of results of a 28-day experiment with and without organic solvent. Upper numbers refer to series without additional solvent, the lower ones to those with 0.5 % ethanol.

Concentration of Atrazin (mg/l)	0	1	3	5	10
Number of survivors	10 8	8 4	9 3	8 3	7 +
Number of young per population					
absolute	1555 726	1264 198	850 162	986 61	812 +
% of contr.	100.0 100.0	81.3 27.3	54.7 22.3	63.4 8.4	52.2 +
Number of young per animal					
absolute	215.6 123.1	178.4 77.6	157.5 87.8	152.3 30.5	97.2 +
% of contr.	100.0 100.0	82.8 63.0	73.1 71.3	70.6 24.8	45.1 +
Mean length (mm)	3.38 2.44	3.27 2.43	3.07 2.25	3.05 1.95	2.59 +
Carbon content (µg/animal)	122.5 35.5	123.5 35.7	91.8 30.4	57.2 15.2	46.3 +

+animals died off till the 12th day

Discussion

The results of this investigation clearly indicate that the determination of the acute toxicity of a substance allows only a rough estimation of its actual poisoning effect. More refined methods need to be applied before the consequences of an environmental contamination become clear. In concentration ranges where no visible effect on survival occurred growth and reproduction were markedly reduced which in nature would have considerable consequences to population dynamics and ecology.

Indeed the concentration of 1 - 2 ppm necessary for a measurable effect is in comparison with other substances relatively high, yet an effect of the herbi-

cide on fauna is evident, although the mechanism for it remains unclear. Thus it is obvious that the influence of a herbicide on the whole environment must also be thoroughly investigated, as it has for example already been shown that the herbicide Diuron, too, can cause a decrease of the reproduction rate of Daphnia (KERSTING 1975).

Daphnia might prove a suitable test organism, should tests on sublethal effects become standardized, as it is relatively easy to keep and breed them in the laboratory and as their reproduction rate is easy to measure. In our case 28 days was a long enough period to establish an effect. Little further information was gained by extending the experiments to the deaths of the animals.

Important is a well defined food concentration. Even if it is difficult to standardize the absolute concentration and quality of the food it is necessary to ensure that intrinsic changes during the experiment in both dosed and control groups are the same. Growth and reproduction are strongly dependent on nutrition and if unsufficient control of the food is maintained it is possible that effects are measured on Daphnia which are actually due to the food organisms and not to the animals themselves. This problem is not always fully taken into account. It is not sufficient to work above the "incipient limiting level" (MC MAHON & RIGLER 1965) because growth and reproduction are also here affected by the food concentration although not so markedly as below it.

The biggest problems, however, result from use of organic solvents. It is usual to bring substances difficult to dissolve in water initially into solution by use of small amounts of ethanol, methanol or acetone (GROSCH 1973; MORGAN 1972; SANDERS et al. 1973). The results of our experiments show that this can cause considerable errors, so their use should be avoided whenever possible.

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