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The Influence of Rearing Density on the Subsequent Response to DDT Dosing for Tadpoles of the Frog *Rana temporaria*

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The need is now generally recognised for more information on how variations in the physiological state or fitness of an organism affect its response to a pollutant. This applies to both laboratory and field situations. Anuran embyros and tadpoles are being used with increasing frequency in toxicological tests in the laboratory (e.g. see COOKE and ZORO 1975, DIAL 1976, GREENHOUSE 1976, JOHNSON 1976, MARCHAL-SEGAULT 1976, JORDAN et al. 1977). Some authors have advocated including these organisms in routine screening procedures. Anuran tadpoles are convenient animals for use in experiments designed to examine the change in response to a pollutant according to variations in physiological state, such as might be brought about by other forms of stress. As well as providing information on stress/pollutant interactions, such experiments are of relevance to any toxicological tests in which tadpoles are employed. In the experiment described here, tadpoles of the frog Rana temporaria were reared at two different densities and then exposed to pp'-DDT.

METHODS

Tadpoles with external gills (stage 23 : WITSCHI 1956) were selected from a single stock culture. They were maintained outside for seven weeks in tanks containing 16 litres aged tap water at a density of either 10 tadpoles/litre or 50/litre. Spinach was given daily and the water was replaced regularly. At the end of this preliminary period there was a marked difference in size. For the remainder of this paper, tadpoles that had been kept at a density of 10/litre are referred to as 'large', whereas those reared at the higher density are termed 'small'. Mean weights of ten tadpoles taken at random were: large 689 ± 44 mg (± S.E.), small 363 ± 11 mg ($t_{18} = 7.18$, $P < 0.001$). Most tadpoles then had small hind legs (stage 28).

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Treatment with pp'-DDT followed the static bioassay technique outlined by COOKE (1972). Groups of 40 tadpoles were exposed to nominal concentrations of 0.1 ppm pp'-DDT for two days. For both the small and large tadpoles, four groups were treated with DDT and two groups were maintained as controls. At the end of DDT treatment, a sample of seven small tadpoles or three large tadpoles was taken from each group for analysis of pp'-DDT and its metabolites by gas-liquid chromatography and for determination of dry matter and crude lipid content. Survivors were observed for a further two days, behaviour and development of abnormalities being monitored (see COOKE 1972).

RESULTS

No residues of DDT were detected in the control tadpoles of either size. DDT treatment had no noticeable toxic effect on the large tadpoles; they all survived to the end of the experiment, none developed abnormalities and behaviour was normal relative to their controls. Observations on the DDT treatment groups of small tadpoles are compared directly with those for the corresponding large tadpoles in Tables 1 and 2.

TABLE 1

Analytical details for samples taken after DDT treatment

(Mean ± S.E.)

		large tadpoles	small tadpoles
No. of samples		4	4
Wet weight:	mg/tadpole	708 ± 15	300 ± 12 ***
Dry matter:	mg/tadpole	62 ± 2	26 ± 2 ***
	mg/g	98 ± 1	87 ± 2 **
Crude lipid:	mg/tadpole	17 ± 1	5.4 ± 0.2***
	mg/g	23 ± 1	18 ± 1 **
pp'-DDT:	µg/tadpole	1.8 ± 0.1	2.3 ± 0.1*
	ppm wet weight	2.5 ± 0.2	7.5 ± 0.6***
	ppm lipid	110 ± 10	420 ± 10 ***

*Significantly different from mean for large tadpoles, $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

After two days, when samples were taken for analysis, small tadpoles not only contained lower absolute amounts of dry matter and lipid, but they also had lower amounts per g wet weight than large tadpoles (Table 1). pp'-DDT was found in all samples, together with much lower levels of pp'-DDE and pp'-TDE. Despite their size, small tadpoles had a higher body burden of pp'-DDT, and in terms of ppm wet weight or ppm in lipid, their residue levels were relatively higher still (Table 1).

These residues of DDT had no significant lethal effect. In two of the treatment groups of small tadpoles there was some mortality (3% and 9% after four days), but none occurred in any of the other groups. There were, however, marked sublethal effects on the small tadpoles (Table 2). For instance, they soon became hyperactive displaying 'frantic' or 'resigned' behaviour (see COOKE 1970, 1972), and by the third day there was a significant tendency to float or swim near the surface. By this time many had developed a hole in the snout, a deformity characteristic of DDT poisoning (COOKE 1970).

TABLE 2

Details of behaviour and deformities for the DDT treatment groups from the time at which differences became particularly apparent (Mean percentage with range in brackets)

	Time after start of experiment (d)	Large tadpoles	Small tadpoles
No. of treatment groups		4	4
% active at any one time:	2	4 (2-5)	34 (33-34)*
	3	3 (1-9)	18 (14-21)*
	4	6 (1-15)	27 (17-32)*
% hanging or swimming near surface:	3	1 (0-3)	28 (21-41)*
	4	6 (1-15)	27 (17-32)*
% with snout deformity:	3	0	25 (15-32)*
	4	0	29 (19-36)*

*Using non-parametric Mann-Whitney test, $U_{4,4} = 0$, $P < 0.05$.

DISCUSSION AND CONCLUSIONS

By rearing at two densities that differed five-fold, tadpoles were produced that differed in weight, by a factor of about two-fold. When treated with DDT, the large tadpoles showed no poisoning symptoms under conditions that caused appreciable sublethal effects amongst the small tadpoles. Relative to tadpoles in the field or in stock captive cultures, neither the small nor the large tadpoles were particularly unusual as regards size, although both tended towards the extremes of the normal size range.

Small tadpoles accumulated higher body burdens of DDT. Of the DDT added initially, on average, 40% and 50% could be accounted for by residues in the large and small tadpoles respectively. Uptake may have been limited somewhat by the diminished availability of DDT, although tadpoles are capable of removing all the DDT initially added (COOKE 1972). In series of acute dosing experiments performed previously, hyperactivity always occurred when residue levels exceeded 2.2 ppm pp'-DDT wet weight (COOKE 1973). The large tadpoles in the present investigation behaved normally despite average residues of 2.5 ppm pp'-DDT. These tadpoles were larger than any dosed previously. These observations demonstrate how important physiological state may be in modifying the response of an organism. In experiments with tadpoles, any comparisons in response to dosing, whether they are between different cultures of the same species or between different species, should be made with caution. Within any culture there is inevitably some 'natural' variation in size between individual tadpoles, and it remains to be determined whether large tadpoles are more resistant when dosed than smaller individuals.

This experiment raises a further point of interest. In many toxicological tests involving tadpoles, it may be advantageous to have especially resistant or susceptible animals, and the degree of sensitivity of tadpoles in any culture can be manipulated by adjusting the rearing regime.

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