The Effects of p,p'-DDCN on Tadpoles of the Frog *Rana temporaria*

by A. S. COOKE
Monks Wood Experimental Station, Abbots Ripton Huntingdon, England
and
J. A. ZORO
Organic Geochemistry Unit, School of Chemistry University of Bristol, BS8 1TS, England

ALBONE et al. (1972) and JENSEN et al. (1972) recently reported the formation of bis(p-chlorophenyl)acetonitrile (p,p'-DDCN) when 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (p,p'-DDT) was incubated with biologically active sewage sludge. JENSEN et al. (1972) detected p,p'-DDCN in the sediment of a Swedish lake and intimated that this cyanide derivative of p,p'-DDT might be found to be relatively widespread in mud and sediment in the field. Because of lack of information on the toxicity of this compound, its effects have been studied in the laboratory on tadpoles of the frog Rana temporaria.

Materials and Methods

The p,p'-DDCN was prepared from the corresponding acid (Ralph N. Emmanuel Ltd.) as previously reported (SKERRETT and WOODCOCK, 1950). Use of oxalyl chloride in place of thionyl chloride to prepare the acid chloride, allowed cleaner formation of the intermediate amide. The p,p'-DDCN was recrystallized from ethanol to m.p. 87-89°C (uncorrected) and showed one peak on analysis by gas-liquid chromatography (g.l.c.) using a flame ionization detector with an OV-17 (1.5%) + QF-1 (1.95%) mixed stationary phase. Analysis: $C_{14}H_9NCl_2$ requires C 64.12%, H 3.44%, N 5.34%, Cl 27.10%; found, C 63.99%, H 3.61%, N 5.46%, Cl 27.19%.

The proton nuclear magnetic resonance (n.m.r.) spectra of DDT-type compounds have been discussed by KEITH et al. (1969), and BIROS (1970) has used n.m.r. for the confirmation of DDT and DDE residues. The n.m.r. spectrum of p,p'-DDCN (100 MHz, $CDCl_3$ solution) showed a one proton singlet at 508 Hz and an aromatic AA'BB' system. The two most intense bands of the AA' portion were centred at 732 Hz and 741 Hz, and the two most intense bands of the BB' portion were centred at 720 Hz and 729 Hz.

Techniques for dosing and maintaining the tadpoles were as previously described (COOKE, 1970, 1972). Initially in each treatment group there were 40 tadpoles, each having hind limb paddles or small hind legs (Stages V - XIII; TAYLOR and KOLLROS, 1946). The mean weight of these tadpoles was 230 mg, and they were within the size range, snout - anus length 8.5 - 11 mm.

Bulletin of Environmental Contamination & Toxicology, Vol. 13, No. 2 ©1975 by Springer-Verlag New York Inc.

To prepare a treatment medium, p,p'-DDCN was dissolved in acetone, and 1 ml of the solution was added to 1 litre of aged tap water (Huntingdon, June 1973). One group of tadpoles was exposed to each of the following concentrations: 0 (Control), 0.001, 0.01, 0.1, 1.0 ppm p,p'-DDCN. Treatment media were renewed 24 hours after the experiment began. After a further 24 hours, treatment media were replaced with aged tap water, which was then renewed daily. The experiment lasted a total of 8 days. Tadpoles were fed on chopped spinach during the post-treatment period. Ambient temperature varied between 21° and 32°C.

Tadpole samples were taken for analysis after 24, 48, 120 and 192 hours. For this, 10 tadpoles were taken at random from each group, weighed and extracted with hexane and acetone. The extract was subjected to clean-up on an alumina column, the eluting solvent being hexane : diethyl ether (95 : 5 by volume). The purified extract was analysed by g.l.c. using an electron capture detector. Apiezon L was the stationary phase. In addition to the peak identified as p,p'-DDCN, another peak was noted on g.l.c. chart recordings of the tadpole extracts. With Apiezon L, QF-1, DC-200 or Silicone SE-30 as the stationary phase, the retention time of this second compound was always identical to that of 4,4'-dichloro benzophenone (DBP). Recoveries of p,p'-DDCN and DBP in the analytical procedure were >95%. Detection limits were: p,p'-DDCN, 0.1 µg/sample; DBP, 0.01 µg/sample.

Results and Observations

Residues were detected in tadpoles from the 0.1 and 1.0 ppm p,p'-DDCN treatment groups (Table 1) but not in those from the 0.001 or 0.01 ppm p,p'-DDCN groups. After 24 hours, 34% and 78% of the p,p'-DDCN initially added to the water could be accounted for by residues in the tadpoles in the 0.1 and 1.0 ppm treatment groups respectively. Residues were rapidly lost when tadpoles were kept in clean water (Table 1).

None of the tadpoles died, but tadpoles in the 1.0 ppm DDCN group behaved abnormally. At first they dashed about in the tank, but then became lethargic. By the end of the treatment period they were apparently very weak, often lying on their sides rather than in the normal position, on their bellies. At this time, 10 of the 30 tadpoles had upcurved tails, an abnormality similar to that noted for toad (Bufo bufo) tadpoles treated with DDT or dieldrin (COOKE, 1972). None of the tadpoles in the other groups either behaved in an unusual manner or displayed any morphological abnormality.

TABLE 1

Residues in frog tadpoles (Rana temporaria) after treatment with p,p'-DDCN

Time after start of experiment (hours)	p,p'-DDCN treatment groups							
	0.1 ppm				1.0 ppm			
	p,p'-DDCN		DBP		p,p'-DDCN		DBP	
	ppm wet weight	ppm in lipid	ppm wet weight	ppm in lipid	ppm wet weight	ppm in lipid	ppm wet weight	ppm in lipid
24	4.4	640	ND	ND	99	14,000	3.2	460
48*	16	2,300	0.13	18	150	20,000	6.4	850
120	ND	ND	ND	ND	7.2	610	0.04	4
192	ND	ND	ND	ND	ND	ND	ND	ND

*end of treatment

ND = not detected: p,p'-DDCN <0.1 ppm wet weight or <20 ppm in lipid
DBP <0.01 ppm wet weight or <2 ppm in lipid
lipid = material extractable with hexane and acetone

When kept in clean water, tadpoles in the 1.0 ppm DDCN treatment group all began to behave normally within 24 hours, and most of those that had developed upturned tails had lost this deformity three days after treatment ended.

The rate of metamorphosis of tadpoles in the 0.001 ppm DDCN group was greater than that of tadpoles in the other groups. After 192 hours, the mean (± S.E.) development stages (TAYLOR and KOLLROS, 1946) of the 10 tadpoles in each of the last analytical samples were: Control, 13.2 ± 0.8; 0.001 ppm, 16.1 ± 0.7; 0.01 ppm, 12.1 ± 0.5; 0.1 ppm, 11.5 ± 0.4; 1.0 ppm, 11.4 ± 0.7. There was a significant difference between the controls and tadpoles in the 0.001 ppm DDCN group ($t_{18} = 2.83$, $P < 0.05$), and between tadpoles in the 0.001 ppm p,p'-DDCN group and those in the three higher dose groups ($P < 0.001$ when compared with each group). Tadpoles in the higher dose groups tended to be less advanced than the controls, but differences were not significant.

Discussion

Compared with p,p'-DDT, p,p'-DDCN has little harmful effect on frog tadpoles. None of the tadpoles died despite tissue levels of up to 150 ppm DDCN (wet weight) at the end of treatment. Tissue levels of DDT of this magnitude are lethal to tadpoles (COOKE, 1972, 1973a). Tadpoles in the 0.1 ppm p,p'-DDCN group behaved normally throughout, although they contained 16 ppm wet weight at the end of the treatment; yet frog tadpoles become hyperactive when tissue residues of p,p'-DDT reach only 2-3 ppm (COOKE, 1972). The extent of the uptake of DDCN from the treatment media was similar to DDT uptake (COOKE, 1972), but the rate of loss of DDCN by these tadpoles was markedly more rapid (>95% in three days) than the rate of loss of DDT in similar experiments (COOKE, 1970).

Low levels of DDT or dieldrin can increase the rate of metamorphosis of tadpoles (COOKE, 1972, 1973b). Since tadpoles in the 0.001 ppm p,p'-DDCN group were significantly more advanced than the others, it is possible that very small amounts of DDCN have a similar effect.

ALBONE *et al.* (1972) and JENSEN *et al.* (1972) pointed out that little is known about the toxicological properties of p,p'-DDCN. This study indicates the compound to have a relatively low short term toxicity to frog tadpoles.

Acknowledgements

Our thanks are due to P. R. Fryer and N. A. Woodland for preparing the p,p'-DDCN, to Drs. N. W. Moore and F. Moriarty for commenting on the manuscript and to M. C. French for analysing the tadpole samples. One of us (J. A. Z.) thanks the Natural Environment Research Council for support under Research Grant GR/3/1257.

References

ALBONE, E.S., G. EGLINTON, N.C. EVANS and M.M. RHEAD: Nature 240, 420 (1972).
BIROS, F.J: Jnl Ass. Off. analyt. Chem. 53, 733 (1970).
COOKE, A.S: Environ. Pollut. 1, 57 (1970).
COOKE, A.S: Environ. Pollut. 3, 51 (1972).
COOKE, A.S: Environ. Pollut. 5, 259 (1973a).
COOKE, A.S: Copeia 1973, 647 (1973b).
JENSEN, S., R. GOTHE and M.O. KINDSTEDT: Nature 240, 421 (1972).
KEITH, L.H., A.L. ALFORD and A.W. GARRISON: Jnl Ass. Off. analyt. Chem. 52, 1074 (1969).
SKERRETT, E.J. and D. WOODCOCK: J. chem. Soc. 2718 (1950).
TAYLOR, A.C. and J.J. KOLLROS: Anat. Rec. 94, 7 (1946).