# THE EFFECTS OF DDT, DIELDRIN AND 2,4-D ON AMPHIBIAN SPAWN AND TADPOLES

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*ABSTRACT*

*Spawn of the common frog* (Rana temporaria) *or tadpoles of the common frog, common toad* (Bufo bufo) *or smooth newt* (Triturus vulgaris) *were maintained for* 24 *or* 48 *h in amphibian saline to which pesticide had been added. After treatment they were transferred to fresh saline. Tadpoles that hatched from treated spawn and tadpoles that had been treated were observed for up to three weeks.*

*DDT did not penetrate well-developed spawn and was only detected in hatching tadpoles after freshly laid spawn had been treated. These tadpoles became hyperactive after their external gills were lost. In the experiments in which frog and toad tadpoles were exposed to DDT, tadpoles were most susceptible either just before or just after developing hind limb buds and at these and later stages they became hyperactive when tissue concentrations reached* 2–3 *and* 3–4 *ppm respectively. During tail resorption small frogs, unlike small toads, were susceptible to tissue residues of DDT that had been acquired during larval development. At every stage of development, toads were more resistant to DDT than frogs, some toad tadpoles surviving despite tissue concentrations of* >300 *ppm. After treatment with DDT, DDE was often detected in newt tadpoles and in frog and toad tadpoles with hind limbs.*

*Dieldrin caused lower mortality amongst tadpoles than DDT and had less effect on behaviour, although, like DDT, it produced distinct behavioural changes and morphological abnormalities. Only frog tadpoles were treated with* 2,4-*D. It had no visible effect and no tissue residues could be detected even after treatment in* 50 *ppm for* 48 *h.*

## INTRODUCTION

During the 1960s several herpetologists suggested that pesticides may have contributed to the decline of the common frog *Rana temporaria* in Britain (Frazer, 1964;



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Perring, 1966; Simms, 1969) and, more recently, there was a report of the reproductive success of a frog population being reduced after atrazine, a herbicide, had been sprayed nearby (Hazelwood, 1970). In 1970 I organised an enquiry into the recent changes in status in Britain of the common frog and common toad *Bufo bufo*, partly in order to investigate the possibility of pesticides causing widespread declines. Details of this survey, to which over 1000 people contributed, will be published later. Results indicated that the toad, which was thought to be holding its own (Perring, 1966), had, like the frog, declined over much of Britain during the last decade. Declines for both species tended to be associated with high human population density. Although certain chronological and geographical relationships between declines and pesticide usage were noted, these were probably largely circumstantial, destruction or modification of suitable habitat being forwarded by contributors as the major reason for the declines. Pollution of breeding sites, mainly by pesticides, oil, petrol or rubbish, was cited as the second most important factor. Many of the reasons given for local declines were based entirely on observations, *e.g.* loss of breeding sites when ponds were filled in prior to housing development. Other reasons, however, such as pollution of the aquatic environment, were often only opinions and should be treated with caution, although observations of oil on the water surface or pesticide containers littering a pond added weight to these opinions.

Whilst it is unlikely that pesticides have been responsible for the national population declines, it is quite probable that there have been many instances of pesticides having harmful effects on frog or toad populations at a local level. However, only one such incident has been reported in the literature (Hazelwood, 1970) and, like the others, this would probably have been overlooked or remained unrecognised or uninvestigated, had this particular frog population not been the subject of a long-term ecological survey (Hazelwood, 1969). In order to be able to recognise and evaluate possible pesticide effects in the field, it is necessary to carry out toxicological experiments in the laboratory. For instance, tissue residue levels of animals lethally poisoned with pesticides need to be determined so that these can be compared with levels found in field samples. To help the field worker know what to look for in an instance of suspected sublethal poisoning, morphological abnormalities and obvious changes in behaviour should be elucidated, as should the most susceptible stages of the life cycle. Other information required includes interspecific differences and the effects of different pesticides. The effects of pp'-DDT on frog tadpoles with hind limb buds and with hind legs have already been reported (Cooke, 1970) and the present paper describes further experiments testing frog spawn or frog, toad or smooth newt (*Triturus vulgaris*) tadpoles with DDT; frog or toad tadpoles with dieldrin; and frog tadpoles with 2,4-D.

## METHODS

Frog spawn was collected from Felmersham Nature Reserve, Bedfordshire, toad spawn from Castor Hanglands National Nature Reserve near Peterborough, and newt tadpoles from a disused claypit near Woodhurst, Huntingdon.

*Treatment of spawn*

Under laboratory conditions frog spawn hatched about six days after being laid, and spawn was treated with DDT at three different stages of development. In each experiment two small clumps of spawn were removed from a larger clump. One was immersed for 24 h in 1 litre of treatment medium with a nominal concentration of 0·5 ppm DDT. All media were prepared by adding DDT in ethanol to Holtfreter's amphibian saline, using a mosquito larvae test kit supplied by the World Health Organisation. The second small clump of spawn, which was to act as control, was immersed for 24 h in saline to which a corresponding volume of ethanol had been added. After treatment both clumps were transferred to fresh saline and allowed to hatch. Tadpoles were maintained in saline for up to three weeks after hatching.

*Treatment of tadpoles*

The morphological stages described by Taylor & Kollros (1946) for *Rana pipiens* during development from a tadpole with very small hind limb buds (stage I) to a perfect small frog (stage XXV) were applied to both *R. temporaria* and *Bufo bufo*.

For both the frog and the toad, a series of experiments was carried out in which tadpoles at different stages of development were treated with DDT. Tadpoles were treated in groups of 40 by immersion in 1 litre of media with nominal concentrations of 0·0008, 0·005, 0·02, 0·05 or 0·5 ppm, prepared as described above. A single experiment was carried out with newt tadpoles (all with front and hind limbs), in which they were treated with nominal concentrations of 0·005 or 0·5 ppm DDT in 1 litre dishes as used for frog and toad tadpoles, but the number in each group was reduced to ten to eliminate cannibalism. The effects of nominal concentrations of 0·0008, 0·02 or 0·5 ppm dieldrin (prepared as for DDT) on groups of 40 frog or toad tadpoles with hind limb paddles or hind legs (stages IV-XVI) were determined. Frog tadpoles at the same stages of development were similarly treated with 0·0008, 0·02, 0·5, 10 or 50 ppm 2,4-D (prepared from a solution of free acid in ethanol).

Treatment of tadpoles in each experiment was continued for 24 or 48 h, after which time they were transferred to fresh amphibian saline for an observation period of 1–15 days. During 48 h treatment, pesticide media were renewed after 24 h, and during the observation period the saline was changed daily. Methods of feeding, weighing live tadpoles and calculating mortality were as previously described (Cooke, 1970). The temperature range during the experiments was 17–29°C.

*Analysis*

At 25°C the solubilities of the pesticides in water are pp'-DDT, 0·01–0·1 ppm; dieldrin, 0·25 ppm; and 2,4-D, 50–900 ppm (Gunther *et al.*, 1968), so for DDT and dieldrin the nominal concentrations sometimes exceeded the reported solubilities. Pesticide in the body of the liquid will have been reduced by adsorption on the glass surface, by deposition at the air–liquid interface, by co-distillation into the air and particularly by uptake by the tadpoles. Therefore it is important that pesticide effects should be related to tissue residue levels rather than to the nominal concentrations of the treatment media. Tadpoles that had hatched from treated spawn were analysed in groups of 10, 20 or 30. During the experiments in which tadpoles were treated with DDT or dieldrin, samples of 10 frog or toad tadpoles or 5 newt tadpoles were analysed 24 and 48 h after the beginning of treatment. Each sample was extracted with acetone and hexane, subjected to clean-up with an alumina column and analysed by gas–liquid chromatography using an electron capture detector. The detection limit was 0·01 μg. DDT analysis was carried out on only one extract from the first two frog tadpole experiments, the remaining extracts being accidently destroyed while in storage. Bulked samples of tadpoles exposed to 2,4-D were analysed for residues of this herbicide. Samples were extracted with chloroform as described by Yip (1964) and 2,4-D was converted to its methyl ester by the method of Metcalfe & Schmitz (1961), before analysis by gas–liquid chromatography. The detection limit was 0·1 μg and the recovery of 10 μg 2,4-D added to a bulked sample of pesticide-free tadpoles was 80%.

TABLE 1

THE EFFECTS OF DDT ON FROG (*Rana temporaria*) SPAWN

| *Description of spawn* | *Time before hatching when treated (days)* | *Volume of spawn on each treatment (ml)* | *Approximate number of ova in each group* | *Treatment medium (nominal ppm)* | *DDT content of subsequent tadpoles at external gill stage (ppm)* | *Effects on tadpoles* |
|---|---|---|---|---|---|---|
| Fresh | 5 | 11 | 180 | 0 | ND | 0·5 ppm group were hyperactive 8–13 days after hatching |
| | | | | 0·5 | 19·4 | Development and gain in weight were retarded |
| Ova beginning to elongate | 3 | 35–40 | 300 | 0 | ND | None |
| | | | | 0·5 | ND | |
| Head and body of each tadpole distinguishable | 2 | 20–25 | 170 | 0 | ND | None |
| | | | | 0·5 | ND | |

ND = not detected, <0·01 μg/sample.
Percentage hatch and larval mortality were unaffected by DDT treatment.
DDE was not detected in any sample.

## RESULTS AND OBSERVATIONS

*Treatment of Spawn*

Details of the experiments in which frog spawn was treated are given in Table 1. When spawn was within three days of hatching when treatment began, the subsequent tadpoles did not contain detectable DDT residues. When freshly laid spawn was treated, the tadpoles that hatched from it behaved normally during the external gill stage, but a sample of these tadpoles contained 19·4 ppm DDT (0·083 μg/tadpole) one day after hatching. Four days later the external gills had been lost and tadpoles in another sample from this group contained a mean of 0·080 μg DDT/tadpole, but because they had increased in weight, their tissue content dropped to 7·6 ppm. Surviving tadpoles became hyperactive eight days after hatching, but five days later they were behaving normally again and no DDT residues could be detected in their tissues. They did, however, weigh less than control tadpoles that had hatched from untreated spawn. Weights of samples of 10 treatment and control tadpoles were 202 and 185 mg respectively when hyperactivity began and 350 and 568 mg respectively when hyperactivity ceased. One week after hyperactivity ceased, numbers at each development stage were:

Treatment tadpoles: IV, 1; V, 23; VI, 1

Control tadpoles: V, 18; VI, 17

The difference in development was highly significant ($x^2 = 13·7$, $P < 0·001$).

*Treatment of Tadpoles*

*Behaviour and Mortality:* (i) *DDT.* Results of experiments with DDT on frog tadpoles are presented in Table 2, those on toad tadpoles in Table 3 and those on newt tadpoles in Table 4. Prolonged exposure to DDT induced series of distinct behavioural changes in tadpoles (Table 5). Frog tadpoles up to stage XIV (medium hind legs) first became 'frantic' with tail lashing, body twisting and rapid swimming, then, on further exposure to DDT, entered a 'resigned' phase during which they persistently swam in a slow, twisting manner. Tadpoles in both phases were markedly hyperactive, but after the resigned phase they became moribund and died. From stage XIV to stage XX (appearance of front legs) behavioural changes were the same except that the moribund phase was absent, and during the frantic and resigned phases these tadpoles allowed their hind legs to trail limply instead of holding them out away from the tail. When toad tadpoles were treated with DDT, frantic and resigned phases were observed, but the moribund phase was replaced by a period of tail shuddering. Newt tadpoles first became frantic, then, instead of entering a resigned phase, they began a period of body and tail shuddering. This was followed by a moribund phase in which movement was limited to a slight flicking of the gills.

TABLE 2

THE EFFECTS OF DDT ON FROG (*Rana temporaria*) TADPOLES AT DIFFERENT STAGES OF DEVELOPMENT

| *At start of treatment*<br>*(a) Description of tadpoles*<br>*(b) Snout–anus length (mm)*<br>*(c) Total length (mm)* | *Treatment medium (nominal ppm)* | *Treatment time (h)* | *Mortality (%)* | *Type of behaviour* | *Mean wt of tadpoles (mg)* | *Mean pesticide content DDT (ppm)* | *DDE (ppm)* | *Observation period (days)* | **Abnormalities and changes in rate of development during and after treatment* | **Mortality and behaviour during observation period* |
|---|---|---|---|---|---|---|---|---|---|---|
| (a) External gills<br>(b) 3·5<br>(c) 11 | 0 | 24 | 0 | normal | 15·9 | — | — | 2 | Several in 0·02 or 0·5 ppm groups with laterally deflected tails, kinks at the base of the tail or with down-curved bodies and tails. 0·5 ppm DDT group retained gills longer | Day 2: 0 mortality |
| | 0·0008 | 24 | 0 | normal | 14·8 | — | — | | | |
| | 0·02 | 24 | 0 | normal/frantic | 15·9 | — | — | | | |
| | 0·5 | 24 | 0 | resigned | 14·6 | 75·5 | ND | | | |
| (a) Traces of left external gills<br>(b) 4–4·5<br>(c) 12–14 | 0 | 24 | 0 | normal | 21·4 | — | — | 1 | None | Day 1: 90% mortality in 0·5 ppm group, others still 0% |
| | | 48 | 0 | normal | 27·9 | — | — | | | |
| | 0·0008 | 24 | 0 | normal | 23·1 | — | — | | | |
| | | 48 | 0 | normal | 26·9 | | — | | | |
| | 0·02 | 24 | 0 | normal/frantic | 22·0 | — | — | | | |
| | | 48 | 0 | frantic/resigned | 16·8 | — | — | | | |
| | 0·5 | 24 | 0 | resigned/moribund | 19·5 | — | — | | | |
| | | 48 | 80 | moribund | 17·8 | — | — | | | |
| (a) Internal gills, no hind limbs<br>(b) 5·5–7<br>(c) 15–20 | 0 | 24 | 0 | normal | 47·4 | ND | ND | 1 | None | All survivors in 0·05 ppm group taken for analysis at end of treatment |
| | | 48 | 0 | normal | 44·7 | ND | ND | | | |
| | 0·005 | 24 | 0 | normal/frantic | 42·4 | 2·4 | ND | | | |
| | | 48 | 6 | resigned | 31·2 | 3·9 | ND | | | |
| | 0·05 | 24 | 15 | resigned | 38·1 | 13·1 | ND | | | |
| | | 48 | 80 | moribund | 29·4 | 19·4 | ND | | | |
| | 0·5 | 24 | 25 | moribund | 37·6 | 53·2 | ND | | | |
| | | 48 | 100 | — | — | — | — | | | |
| (a) Hind limb paddles or hind legs (stages IV–XIV)<br>(b) 8–11<br>(c) 24–34 | 0 | 24 | 0 | normal | 160 | 0·23 | ND | 5 | 87% had abnormal snouts in 0·02 ppm group. Condition persisted and some developed jowled appearance | Day 2: Normal behaviour in 0·02 ppm group<br>Day 5: 29% mortality in 0·02 ppm group, others still 0% |
| | | 48 | 0 | normal | 152 | ND | ND | | | |
| | 0·0008 | 24 | 0 | normal | 177 | 0·17 | 0·01 | | | |
| | | 48 | 0 | normal | 154 | 0·29 | 0·03 | | | |
| | 0·02 | 24 | 0 | frantic/resigned | 127 | 2·4 | 0·12 | | | |
| | | 48 | 0 | resigned | 134 | 5·6 | 0·19 | | | |
| | 0·5 | 24 | 13 | resigned/moribund | 150 | 36·6 | 0·67 | | | |
| | | 48 | 100 | — | — | — | — | | | |
| (a) Large hind legs (stages XIV–XVII)<br>(b) 12–14<br>(c) 37–40 | 0 | 24 | 0 | normal | 347 | ND | ND | 8 | Most treated tadpoles had abnormal snouts. Delay in mouth formation plus mouth abnormalities in small frogs | Group 0·5 ppm (b)† became normal/frantic, then frantic again and all died on days 5–8 at stages XXII–XXIV |
| | 0·5 | 24 | 5 | frantic/resigned | 347 | 20·2 | ND | | | |
| | | 48(a)† | 94 | frantic | — | — | — | | | |

* Days relate to time during observation period.

† 0·5 ppm group divided into 2 equal sub-groups after being treated for 24 h: (a) treated for further 24 h, (b) no further treatment.

ND = not detected, <0·01 μg/sample.

TABLE 3

THE EFFECTS OF DDT ON TOAD (*Bufo bufo*) TADPOLES AT DIFFERENT STAGES OF DEVELOPMENT

| *At start of treatment* (a) *Description of tadpoles* (b) *Snout–anus length (mm)* (c) *Total length (mm)* | *Treatment medium (nominal ppm)* | *Treatment time (h)* | *Mortality (%)* | *Type of behaviour* | *Mean wt of tadpoles (mg)* | *Mean pesticide content DDT (ppm)* | *DDE (ppm)* | *Observation period (days)* | **Abnormalities and changes in rate of development during and after treatment* | **Mortality and behaviour during observation period* |
|---|---|---|---|---|---|---|---|---|---|---|
| (a) External gills | 0 | 24 | 3 | normal | 9·3 | ND | ND | 2 | No abnormalities. | Day 2: 15% mortality in 0·5 ppm group, others still 0%. No change in behaviour |
| (b) 3·5–4 | | 48 | 6 | normal | 13·1 | ND | ND | | Several in 0·5 ppm | |
| (c) 9–10 | 0·005 | 24 | 0 | normal | 9·8 | ND | ND | | group late losing gills | |
| | | 48 | 0 | normal | 12·5 | ND | ND | | | |
| | 0·05 | 24 | 0 | normal | 10·0 | 30·0 | ND | | | |
| | | 48 | 0 | normal/frantic | 12·7 | ND | ND | | | |
| | 0·5 | 24 | 0 | normal | 9·8 | 82·0 | ND | | | |
| | | 48 | 0 | frantic | 13·1 | 306 | ND | | | |
| (a) Internal gills, no hind limbs | 0 | 24 | 0 | normal | 26·3 | ND | ND | 7 | None | Day 5: 5% mortality in 0·005 ppm group, 90% in 0·5 ppm, 0% in others. Day 7: still resigned in 0·05 ppm group |
| (b) 4·5–5·5 | | 48 | 0 | normal | 24·1 | ND | ND | | | |
| (c) 12·5–14 | 0·005 | 24 | 0 | frantic | 24·8 | 6·9 | ND | | | |
| | | 48 | 0 | frantic | 25·2 | 13·5 | 0·40 | | | |
| | 0·05 | 24 | 0 | frantic | 24·6 | 65·2 | 1·0 | | | |
| | | 48 | 0 | resigned | 23·0 | 139 | ND | | | |
| | 0·5 | 24 | 0 | resigned/shuddering | 21·5 | 326 | ND | | | |
| | | 48 | 0 | shuddering | 20·1 | 478 | ND | | | |
| (a) Hind limb buds (stages 0–V) | 0 | 24 | 0 | normal | 72·0 | ND | ND | 5 | None | Day 5: 5% mortality in 0·02 ppm group, others still 0% |
| (b) 6–8·5 | | 48 | 0 | normal | 72·2 | ND | ND | | | |
| (c) 16–20·5 | 0·0008 | 24 | 0 | normal | 68·0 | 0·30 | ND | | | |
| | | 48 | 0 | normal | 68·4 | 0·37 | ND | | | |
| | 0·02 | 24 | 0 | frantic | 71·6 | 4·8 | ND | | | |
| | | 48 | 3 | normal/frantic | 63·7 | 4·3 | 0·24 | | | |
| | 0·5 | 24 | 0 | resigned/shuddering | 64·6 | 96·0 | ND | | | |
| | | 48 | 100 | — | — | — | — | | | |
| (a) Hind limb paddles or hind legs (stages VI–XVI) | 0 | 24 | 0 | normal | 105 | ND | ND | 15 | 1 in 0·02 ppm group with abnormal snout. All in 0·5 ppm group turned pale brown, but reverted to black by day 2. Some up-curved in same group. Day 11: 13% of control group with front legs; 0·0008 ppm 37%; 0·02 ppm 15% | Day 5: 100% mortality in 0·5 ppm group. Normal behaviour in 0·02 ppm group |
| | | 48 | 0 | normal | 88 | ND | ND | | | |
| (b) 7·5–9·5 | 0·0008 | 24 | 0 | normal | 111 | 0·09 | ND | | | |
| (c) 19–23 | | 48 | 0 | normal | 93 | 0·54 | ND | | | |
| | 0·02 | 24 | 0 | normal/frantic | 106 | 3·5 | 0·24 | | | |
| | | 48 | 0 | frantic/resigned | 92 | 7·6 | 0·22 | | | |
| | 0·5 | 24 | 0 | resigned/shuddering | 101 | 73·2 | 3·0 | | | |
| | | 48 | 20 | shuddering | 79 | 134 | 5·1 | | | |

* Days relate to time during observation period.
ND = not detected, <0·01 μg/sample.

TABLE 4

THE EFFECTS OF DDT ON NEWT (*Triturus vulgaris*) TADPOLES*

| *Treatment medium (nominal ppm)* | *Treatment time (h)* | *Mortality (%)* | *Type of behaviour* | *Mean wt of one tadpole (mg)* | *Mean pesticide content DDT (ppm)* | *DDE (ppm)* |
|---|---|---|---|---|---|---|
| 0 | 24 | 0 | normal | 79·5 | ND | ND |
| | 48 | 0 | normal | 98·4 | ND | ND |
| 0·005 | 24 | 0 | normal/frantic | 75·7 | 1·5 | ND |
| | 48 | 0 | frantic | 90·4 | 3·0 | 0·13 |
| 0·5 | 24 | 10 | moribund | 74·2 | 86·3 | 2·7 |
| | 48 | 33 | moribund | 74·7 | 116 | 2·2 |

* All tadpoles had 4 legs and external gills, and, at the start of treatment, were within the length ranges, snout–anus 10–12 mm, total 19–26 mm.
There was no observation period, all surviving tadpoles being sacrificed at the end of treatment.
ND = not detected, <0·01 μg/sample.

TABLE 5

SUCCESSIVE BEHAVIOURAL PHASES OF FROG (*Rana temporaria*), TOAD (*Bufo bufo*) AND NEWT (*Triturus vulgaris*) TADPOLES DURING TREATMENT WITH DDT OR DIELDRIN. WITH CONTINUOUS EXPOSURE TADPOLES PROGRESSED THROUGH THE PHASES MARKED √ AND MISSED OUT THOSE MARKED ×

| | | *Frantic* | *Resigned* | *Shuddering* | *Moribund* | *Death* |
|---|---|---|---|---|---|---|
| Frog | DDT (up to stage XIV) | √ | √ | × | √ | √ |
| | DDT (after stage XIV) | √ | √ | × | × | √ |
| | Dieldrin | √ | √ | × | √ | √ |
| Toad | DDT | √ | √ | Tail | × | √ |
| | Dieldrin | √ | √ | × | Not distinct | √* |
| Newt | DDT | √ | × | Body and tail | Gill movements only | √ |

* Assumed—no deaths under experimental conditions described.

For the frog the type of behaviour shown under fixed DDT treatment conditions depended on the developmental stage of the tadpoles. For instance, for tadpoles maintained in the 0·5 ppm DDT medium, behaviour after 24 h was:

(1) for tadpoles with external gills, normal/frantic (some normal, some frantic);
(2) left external gills, resigned/moribund;
(3) internal gills but no hind limbs, moribund;
(4) hind limb paddles or hind legs, resigned/moribund;
(5) large hind legs, frantic/resigned.

Behaviour indicative of the most advanced poisoning was shown by frog tadpoles with internal gills but no hind limbs. For the toad, immersion in the 0·5 ppm DDT medium for 24 h had no effect on the behaviour of tadpoles with external gills, but produced resigned behaviour or tail shuddering in larger tadpoles. If the tail shuddering stage of toad tadpoles is regarded as being equivalent to the moribund stage of frog tadpoles, then identical DDT conditions produced behavioural changes in frog tadpoles that were often suggestive of more advanced poisoning

than those in toad tadpoles, this being particularly noticeable in the earlier stages of development.

Percentage mortalities are given in Tables 2, 3 and 4, and the acute mortalities determined after 48 h treatment show that frog tadpoles were most susceptible when they had internal gills but no hind limbs, and toad tadpoles when they had hind limb buds. Tadpoles of both species seemed most resistant when they had external gills, then became more susceptible as they developed and finally became more resistant again as they approached the metamorphic climax (stage XX). During the observation period, there was high mortality among tadpoles which had been in the resigned, moribund or tail-shuddering phases at the end of treatment (Tables 2 and 3). Latent mortality was noted after frog tadpoles with large hind legs (stages XIV–XVII) were subjected to the 0·5 ppm DDT medium. Treatment was discontinued after 24 h (Table 2, group b) when tadpoles were in the frantic or resigned phase with legs trailing limply, but 4 h later the behaviour of many tadpoles had returned to normal and their legs were held normally. However, by 18 h after this, all tadpoles had become frantic and the legs trailed limply again. Tadpoles then developed front legs and began to behave in the same uncoordinated manner as described by Cooke (1970). All died during tail resorption (stages XXII–XXIV) 4–9 days after treatment ended. Similar latent mortality was not observed amongst small toads that had been treated as tadpoles in the hind limb paddles or hind leg stages (VI–XVI).

*Behaviour and Mortality:* (ii) *Dieldrin.* The effects of dieldrin on frog or toad tadpoles with hind limb paddles or hind legs are shown in Table 6. Both species exhibited the same series of behavioural changes in dieldrin as in DDT, except that toad tadpoles had a somewhat indistinct moribund phase in place of tail shuddering (Table 5). Tadpoles of both species returned to normal behaviour more quickly than tadpoles at the same stages treated with DDT. For instance, the 0·5 ppm dieldrin group of frog tadpoles recovered from the moribund phase in a day, but the 0·02 ppm DDT group needed two days to recover after being resigned. Dieldrin had less effect on behaviour and mortality than corresponding tissue or nominal media concentrations of DDT. The frog tadpoles treated with dieldrin were not kept until after the front legs appeared, but the toad tadpoles were, and again latent mortality was not observed during tail resorption.

*Behaviour and Mortality:* (iii) 2,4-*D.* Frog tadpoles with hind limb paddles or hind legs were treated with up to 50 ppm 2,4-D for two days and were then observed for a further five days, but this herbicide caused no change in behaviour, and none of the tadpoles died.

*Weight loss:* Mean weights of tadpoles in the analytical samples taken after 24 and 48 h treatment are given in Tables 2, 3, 4 and 6. Loss in weight or failure to gain weight at a normal rate was associated with hyperactivity, as previously observed (Cooke, 1970).

*Abnormalities:* Tadpoles suffered morphological abnormalities, changes in skin

TABLE 6

THE EFFECTS OF DIELDRIN ON FROG (*Rana temporaria*) AND TOAD (*Bufo bufo*) TADPOLES WITH HIND LIMB PADDLES OR HIND LEGS

| *At start of treatment*<br>*(a) Development stages*<br>*(b) Snout–anus length (mm)*<br>*(c) Total length (mm)* | *Treatment medium (nominal ppm)* | *Treatment time (h)* | *Mortality (%)* | *Type of behaviour* | *Mean wt of tadpoles (mg)* | *Mean dieldrin content (ppm)* | *Observation period (days)* | **Abnormalities and changes in rate of development during and after treatment* | **Mortality and behaviour during observation period* |
|---|---|---|---|---|---|---|---|---|---|
| Frog | 0 | 24 | 0 | normal | 160 | ND | 5 | Many in 0·5 ppm group with abnormal snouts, but most recovered before appearance of front legs | Day 1: Normal behaviour in 0·5 ppm group<br>Day 5: No further mortality |
| (a) IV–XIV | | 48 | 0 | normal | 152 | ND | | | |
| (b) 8–11 | 0·0008 | 24 | 0 | normal | 161 | 0·17 | | | |
| (c) 24–34 | | 48 | 0 | normal | 145 | 0·31 | | | |
| | 0·02 | 24 | 0 | normal | 131 | 1·8 | | | |
| | | 48 | 0 | normal | 132 | 6·1 | | | |
| | 0·5 | 24 | 5 | frantic/resigned | 163 | 31·3 | | | |
| | | 48 | 47 | moribund | 147 | 42·9 | | | |
| Toad | 0 | 24 | 0 | normal | 105 | ND | 15 | Majority in 0·5 ppm group had abnormal snouts, but most soon recovered. Several in same group were up-curved. Colour changed to pale brown for all in 0·5 ppm group, but returned to normal by day 2.<br>Day 8: mean wts control 151 mg, 0·02 ppm 155 mg, 0·5 ppm 98 mg.<br>Day 11: 15% in control group with front legs, 60% in 0·02 ppm and 0% in 0·5 ppm | Day 2: normal behaviour in 0·5 ppm group |
| (a) VI–XVI | | 48 | 0 | normal | 88 | ND | | | |
| (b) 7·5–9·5 | 0·02 | 24 | 0 | normal | 100 | 3·4 | | | |
| (c) 19–23 | | 48 | 0 | normal | 90 | 5·6 | | | |
| | 0·5 | 24 | 0 | resigned | 78 | 88·5 | | | |
| | | 48 | 0 | resigned | 80 | 138 | | | |

* Days relate to time during observation period.
ND = not detected, <0·01 μg/sample.

colour and delays in development after exposure to DDT or dieldrin, but 2,4-D had no such effects.

(*a*) *Abnormal snouts:* When treated with the 0·02 or 0·5 ppm DDT media for two days, many frog tadpoles in the hind limb paddles or hind legs stages developed abnormal snouts, a condition that has been noted previously (Cooke, 1970). The first symptom was ragged tissue around a hole in the snout (Fig. 1(a)) and this abnormality persisted throughout larval development. After appearance of the front legs (stage XX) some small frogs that had had abnormal snouts as tadpoles developed abnormal mouths (*e.g.* as in Fig. 1(b)), while in others normal mouth development was retarded relative to tail resorption. Small frogs classified as being at a certain development stage on the basis of tail resorption were one or two stages behind with mouth formation as the criterion. It is not known whether normal mouth development could be completed under these conditions since all the small frogs died before completion of metamorphosis because of latent poisoning by DDT.

In the experiment in which toad tadpoles with hind limb paddles or hind legs were treated with DDT, one tadpole in the 0·5 ppm group developed an abnormal snout, the only toad tadpole to do so after DDT treatment.

Dieldrin caused abnormal snouts in both frog and toad tadpoles, and, as with DDT, only tadpoles with hind limb paddles or hind legs were affected. Again the first symptom was ragged tissue above the upper mandible (Fig. 1(c)), but unlike abnormal snouts caused by DDT, those due to dieldrin quickly healed after treatment ended, leaving scar tissue where the hole had been. For instance, of the frog tadpoles in the 0·02 ppm dieldrin group, 9 out of 20 had abnormal snouts one day after dieldrin treatment finished, but this was reduced to only 2 out of 20 four days later.

(*b*) *Other morphological abnormalities:* After frog tadpoles with external gills had been kept in the 0·02 or 0·5 ppm DDT media for 24 h, many were deformed. Out of 40 in the 0·5 ppm group, 13 had a permanent kink at the base of the tail (Fig. 1(d)) and 3 of these were also down-curved (Fig. 1(e)). These abnormalities were also observed in 4 tadpoles in the 0·02 ppm group, where 2 other tadpoles had laterally deflected tails (Fig. 1(f)). All deformities were still present when the observation period was terminated after two days. Up-curving of the body and tail (Fig. 1(g)) was observed in several toad tadpoles with hind limb paddles or hind legs after treatment in the 0·5 ppm DDT or dieldrin media. Tadpoles treated in the former media had all died by the third day of the observation period and some of the corpses had laterally curled tails (Fig. 1(h)), as had dead toad tadpoles in the preceding experiment (stages 0–V).

(*c*) *Colour change:* A distinct colour change was observed amongst toad tadpoles at the hind limb paddles or hind legs stages during treatment in 0·5 ppm DDT or dieldrin media. After 20 h treatment the 'head' of every tadpole in these two groups changed from black to pale brown (Fig. 1(i)), but reverted to normal two days after treatment ended.

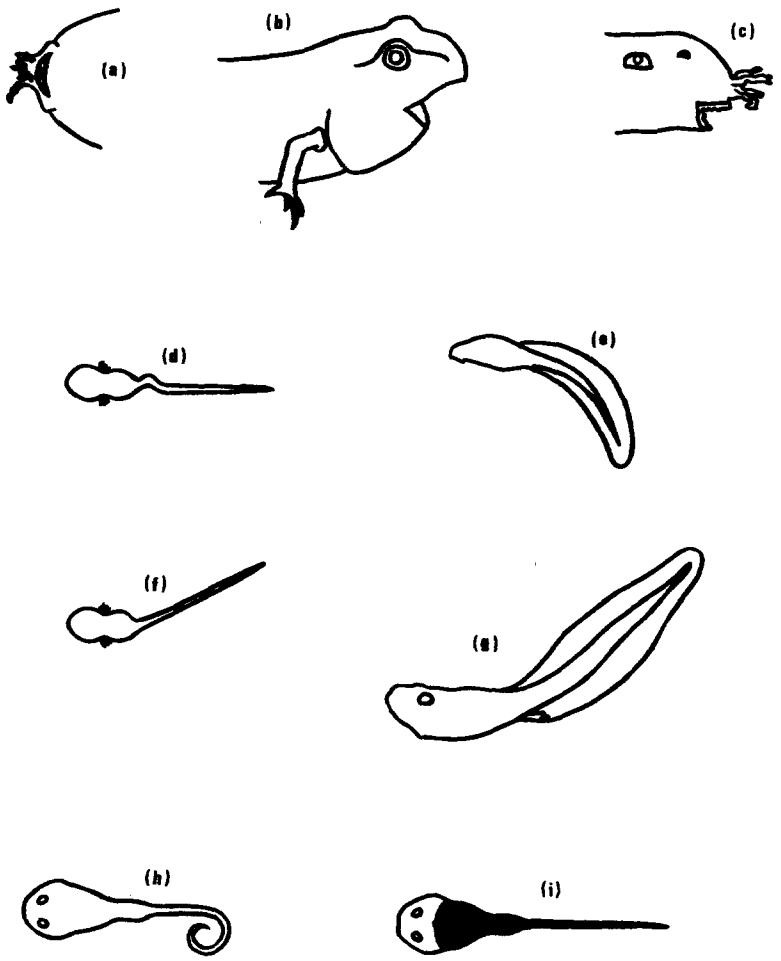


FIG. 1. Abnormalities after frog and toad tadpoles were treated with DDT or dieldrin. Sketches not to same scale.

(a)–(c) Initial stages of abnormal snout formation in tadpoles and the malformed mouth of a small frog that had an abnormal snout as a tadpole.

(a) Ventral aspect of the head of a frog tadpole, stage X, with an abnormal snout after 46 h in the 0·2 ppm DDT medium. (b) Lateral view of a frog, stage XXIII, with an undershot lower jaw seven days after being treated in the 0·5 ppm DDT medium for 24 h. (c) Lateral view of the head of a toad tadpole, stage VII, with an abnormal snout after 21 h in the 0·5 ppm dieldrin medium. (d)–(f) Abnormalities when frog tadpoles with external gills were treated in the 0·5 ppm DDT medium for 12–24 h.

(d) Lateral kink at base of tail. (e) Down-curved tadpole. (f) Laterally deflected tail.

(g)–(i) Abnormalities after toad tadpoles, stages VI–XVI, were treated in the 0·5 ppm DDT or dieldrin media.

(g) Up-curved tadpole after dieldrin treatment. (h) Dead tadpole with laterally curled tail after DDT treatment. (i) Colour change after treatment with DDT or dieldrin. Shaded area was the normal black colour, while the unshaded area was pale brown instead of black.

(*e*) *Delays in development:* The external gills of frog tadpoles in the 0·5 ppm DDT group were still visible at the end of the treatment period, although by this time most tadpoles in the other groups had lost the set of gills on the right side. A count made after 22 h treatment showed that in the control group, one tadpole had lost both sets of gills, 38 had lost one set, and only one retained both sets; while in the 0·0008 ppm group corresponding numbers were 0, 40, 0; in the 0·02 ppm group, 0, 38, 2; and in the 0·5 ppm group 0, 0, 40. Several toad tadpoles in the 0·5 ppm DDT group tended to retain their external gills, but the tendency was not so marked as for the frog tadpoles.

Treatment with DDT or dieldrin sufficient to cause prolonged hyperactivity and a reduction in body weight tended to slow down the rate of metamorphosis. In some instances, however, particularly when toad tadpoles were treated with dieldrin, groups receiving low doses metamorphosed more rapidly. By day 11 of the observation period in this experiment, 3 out of 20 toads in the control group had front legs (reached stage XX), while in the 0·02 ppm dieldrin group, a significantly higher proportion, 12 out of 20, had front legs ($x^2 = 8{\cdot}6$, $P < 0{\cdot}01$). None of the 12 surviving tadpoles in the 0·5 ppm group had reached stage XX by this time.

*Pesticide residues:* (i) *DDT.* Concentrations of pp′-DDT and pp′-DDE found in the bulked samples are given in Tables 2, 3 and 4. Tadpoles removed more DDT from the medium as they increased in size. For instance, after treatment in the 0·5 ppm medium for 24 h, frog tadpoles with external gills had removed 8·8% of the total DDT added to the saline; those with internal gills, 16%; those with hind limb paddles or hind legs, 45%; and those with large hind legs, 56%. At lower concentrations tadpoles removed higher percentages of DDT and for some groups of tadpoles with internal gills, because of sampling and analytical errors, the calculated recovery of DDT after 24 h treatment was >100%. For example, recoveries in toad tadpoles (stages 0–V) were 0·5 ppm group, 50%; 0·02 ppm group, 69%; 0·0008 ppm group, 102%.

After 24 h treatment, bulked samples of frog tadpoles contained DDT concentrations 40 to 480 times greater than the nominal concentrations of the treatment media, while the factor for toad tadpoles varied from 150 to 1400. Rate of uptake of DDT by tadpoles from a medium would be expected to decline as the tadpoles acquired DDT, but under the conditions studied here, the increase in tissue residues during the second day of treatment was usually comparable to that during the first day, although it should be remembered that the number of tadpoles in each dish had decreased.

The metabolite of pp′-DDT most commonly reported in vertebrate tissues, pp′-DDE, was often detected in small concentrations in newt tadpoles and in frog or toad tadpoles with hind limb paddles or hind legs. DDE was, however, detected in only 3 samples of tadpoles (all toad) that had not reached the hind limb paddle stages.

*Pesticide residues: (ii) Dieldrin.* The dieldrin levels in the samples are given in Table 6, and are very similar to the DDT concentrations in tadpoles at the same stages for both the frog (Table 2) and the toad (Table 3).

*Pesticide residues: (iii)* 2,4-*D.* 2,4-D was not detected in any sample, so content was always $<0{\cdot}1$ ppm.

## DISCUSSION

Amphibian breeding sites in Britain most likely to become contaminated with harmful pesticide levels are (1) those in, or bordering, agricultural land that has recently been treated, (2) those in which used pesticide containers are dumped, and (3) those being treated with insecticides to control mosquito larvae. Service (1970) determined the extent of mosquito control in England and Wales by sending questionnaires to about 1800 public health inspectors. Out of 522 replies, 19% reported that they used insecticides against mosquitoes, DDT being the chemical most commonly applied. In America concentrations of DDT and other insecticides used against mosquito larvae have been shown to be toxic to tadpoles of the bullfrog *R. catesbeiana* (Mulla, 1963), so in Britain frog populations in some localities may have been reduced by mosquito control.

Toxicological experiments carried out in the laboratory help to assess effects observed in the field and the experiments described here provide information on when, during development, exposure to pesticide is most harmful and at what stages the animals are most susceptible. For the first few days after being laid, spawn swells by taking in water and during this period DDT was able to penetrate the jelly and reach the ova, but DDT did not penetrate well-developed spawn. Tadpoles that hatched from poisoned spawn behaved normally, but excretion of DDT residues was slow, and although the residues were 'diluted' as the animals grew, hyperactivity began after loss of the external gills. That hyperactivity should be delayed until the internal gill stages, another example of a latent effect, is in accordance with behaviour data from the experiments in which frog tadpoles were treated directly with DDT. Tadpoles were found to be very resistant when they had external gills, but were most susceptible when they had internal gills, but no hind limbs (Table 2). Similarly toad tadpoles were most resistant at the external gill stage and most susceptible when they had hind limb buds (Table 3). Although tadpoles with external gills were most resistant, they contained relatively high tissue concentrations of DDT, and their resistance may be connected with high tissue lipid content. With a high level of body fat, they would probably be able to store more pesticide without adverse effects. Variations in susceptibility to DDT of tadpoles at different stages of development have previously been suggested for the frog *R. temporaria* (Cooke, 1970) and the American species, Fowler's toad, *Bufo woodhousei fowleri* (Sanders, 1970).

Throughout larval development, toads were more resistant than frogs although they always accumulated higher residues relative to the nominal concentrations. Some toad tadpoles survived despite tissue residues of several hundred ppm at the end of treatment, but since the amphibian saline was changed daily during the observation period, rapid excretion of pesticide probably occurred. Higher mortalities might have resulted if the period that these high levels were in the tissues was prolonged such as would occur in the field if the pesticide persisted in the environment. Thus, after spraying a marsh with labelled DDT at a concentration of 0·22 kg/ha, Peterle (1966) reported that tadpoles of the leopard frog *Rana pipiens* contained 24·3 ppm after one day and 2·9 ppm a month later, while, kept in the laboratory in amphibian saline that was replaced daily, *R. temporaria* tadpoles reduced their tissue content of DDT from 29·5 to $<1$ ppm in only ten days (Cooke, 1970). Despite the relatively high rate of excretion observed under laboratory conditions, tadpoles tended to retain 0·01–0·2 μg DDT in their tissues, which was equivalent to about 0·03–0·6 ppm when the front legs appeared (stage XX), and was sufficient to cause high mortality amongst the small frogs during tail resorption (Cooke, 1970). Therefore, if a frog tadpole is exposed to DDT and survives its most susceptible period during larval development, it might die after the metamorphic climax. Toads treated with DDT as tadpoles were not affected during tail resorption, and toad populations appear far less vulnerable than frog populations to reduction by DDT by increased mortality during larval or immediate post-metamorphic climax (stages XX–XXV) development. The relative resistance of toads has also been observed amongst American species, adult Fowler's toads *Bufo woodhousei fowleri* being more resistant to insecticides than adult cricket frogs *Acris crepitans* and *A. gryllus* (Ferguson & Gilbert, 1967).

These experiments give little information on tissue residue levels indicative of death by DDT poisoning because no dead tadpoles were analysed and tadpoles were subjected to short term, rather than chronic, exposure. Under these conditions, toad tadpoles with external gills had tissue levels of $>300$ ppm DDT, but suffered only 15% mortality when kept in saline for two days. Toad tadpoles with hind limbs died when tissue levels reached 100–200 ppm. Frogs were unable to tolerate such high tissue levels, and death occurred at $<50$ ppm when tadpoles had large hind limbs and $<1$ ppm during tail resorption (Cooke, 1970).

Dieldrin had less effect on both frog and toad tadpoles than DDT, although uptake from the treatment media was similar. In contrast, dieldrin was found to be more toxic than DDT to tadpoles of the American species *Rana catesbeiana, Bufo woodhousei fowleri* and the chorus frog, *Pseudacris triseriata* (Mulla, 1963; Sanders, 1970).

Sanders (1970) also reported that the amine salt of 2,4-D was about 100 times less toxic than DDT to *Pseudacris triseriata* tadpoles. In the present experiment the free acid was not toxic to *Rana temporaria* tadpoles even at 50 ppm, although at the stages tested there was 100% mortality after two days in the 0·5 ppm DDT

medium. No tissue residues were detected and whether this was due to failure to absorb 2,4-D from the aqueous environment or to very rapid metabolism remains to be determined. This herbicide is unlikely to be a direct hazard to tadpole populations, but it might affect a population by killing aquatic vegetation. Even this, however, need not necessarily be harmful as Krefting & Hansen (1969) demonstrated that aerial application of 2,4-D can benefit a deer population by killing food plants that deer usually avoid, so allowing the species they normally browse to increase.

When trying to assess sublethal effects on tadpoles in the field the easiest changes to observe are morphological abnormalities and aberrant behaviour. Several abnormalities have been noted in the laboratory experiments, and one of these, the kink at the base of the tail (Fig. 1(d)), was reported amongst tadpoles in a field pond believed to be contaminated with atrazine (Hazelwood, 1970). The abnormal snout condition restricted feeding and retarded larval development in the laboratory (Cooke, 1970) and sometimes led to abnormal mouth development in small frogs (Fig. 1(b)). In the field, tadpoles with this or any of the other morphological abnormalities shown would probably be at a disadvantage, due to restriction of feeding or reduction of mobility.

Toad tadpoles changed colour from black to pale brown when treated with DDT or dieldrin, and previously a colour change from green to grey has been reported for adult *R. pipiens* after immersion in solutions of aldrin, dieldrin, endrin, chlordane or BHC (Kaplan & Overpeck, 1964). Exposure of *R. clamitans* larvae to DDT produced an increase in the level of melanocyte stimulating hormone in the pituitary (Peaslee, 1970), but since no colour change was observed it was assumed that circulating levels of hormone had not increased. That the toad tadpoles' skin became paler in the present experiment indicates a decrease in circulating hormone, perhaps due to increased activity of the hormone release inhibiting factor (Kastin *et al.*, 1969).

Tadpoles of all three species displayed distinct types of behaviour when exposed to DDT or dieldrin, and although those that reached moribund or shuddering phases usually developed more slowly, if they survived at all, tadpoles in the frantic phase at the end of treatment soon behaved normally when kept in fresh saline. This contrasts with the observations of Sanders (1970), who reported that the behaviour of *Pseudacris triseriata* and *Bufo woodhousei fowleri* tadpoles was affected irreversibly by DDT and dieldrin. In the laboratory hyperactive tadpoles, *Rana temporaria*, have been shown to be more readily preyed upon than normal, untreated, tadpoles by warty newts, *Triturus cristatus* (Cooke, 1971) and in the field hyperactivity would probably be disadvantageous because of increased predation and decreased food intake (Cooke, 1970). Apart from those with external gills, frog and toad tadpoles became hyperactive when tissue levels reached 2–3 and 3–4 ppm DDT respectively. Studies of pesticide excretion (Cooke, 1970) indicated that hyperactivity amongst frog tadpoles ceased when tissue concentrations

fell to about the same level, 2–3 ppm. So if *Rana temporaria* tadpoles acquire and retain residue levels similar to those of the *R. pipiens* tadpoles described above (Peterle, 1966), treating a marsh with 0·22 kg DDT/ha, a typical concentration used against mosquito larvae in Britain, might be expected to cause hyperactivity amongst the tadpoles for about a month. Hyperactivity or uncoordinated behaviour due to insecticides has never been reported amongst British amphibian populations in the field, but Ferguson & Gilbert (1967) observed northern cricket frogs (*Acris crepitans*) dying in a Mississippi stream adjacent to a cotton field where DDT and other insecticides had been sprayed. These frogs had the same symptoms, muscular spasms with the hind limbs extended, as small frogs dying during tail resorption after being treated with DDT as tadpoles (Cooke, 1970).

Some smooth newt tadpoles (*Triturus vulgaris*) were hyperactive with only 1·5 ppm DDT in their tissues, yet adult warty newts (*T. cristatus*), containing up to 2·21 ppm DDT + 0·63 ppm DDE, behaved normally (Cooke, 1971), a fact which indicates either an age difference or a species difference.

Although toad tadpoles with internal gills but no hind limbs were larger than those with external gills, and so had a lower ratio of surface area: volume, they had higher tissue residue levels after 24 and 48 h treatment (Table 3). Ferguson *et al.* (1966) demonstrated that uptake of endrin from the aqueous environment by mosquitofish (*Gambusia affinis*) was mainly via the gills, and the high uptake by toad tadpoles with internal gills could have been due to the absorption of residues from the large volume of water being pumped through the gill system. That all the DDT initially added to the media could sometimes be accounted for at the end of treatment by the tissue residues of tadpoles with internal gills may illustrate how thoroughly the gills extracted DDT. Only one sample of frog tadpoles was analysed from the first two experiments and further analyses need to be carried out to determine whether frog tadpoles with internal gills also acquire relatively high residues. If they do so, then the delay in the advance of the opercular sheath in tadpoles with external gills which were immersed in the 0·5 ppm DDT medium will have been beneficial by relatively reducing the uptake of DDT.

During the later stages of development both frog and toad tadpoles were able to metabolise pp′-DDT to pp′-DDE, yet previously DDE was not detected in frog tadpoles or small frogs that had been exposed to DDT (Cooke, 1970). Tadpoles in the previous experiments were collected from a suburban site at Uppingham, while those used in the present experiments hatched from spawn collected from a Nature Reserve of 130 ha situated in arable farmland at Felmersham. Since populations of American cricket frogs (*Acris crepitans* and *A. gryllus*) from regions that had been treated with DDT were more resistant to DDT than were frogs from untreated areas (Boyd *et al.*, 1963), it is possible that previous exposure to DDT has produced a frog population at Felmersham capable of metabolising DDT during the later stages of larval development.

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