Effects of the pesticide dieldrin on incubation of the earthworm *Eisenia fetida* (Oligochaeta)

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The effects of a long-term exposure of newly hatched earthworms to a dieldrin-treated culture medium were examined with regard to the determination of incubation period and hatching success of cocoons ultimately produced by the exposed worms. Dieldrin concentrations in exposed worms and in cocoons produced by these worms were also examined. *Eisenia fetida* (Savigny 1826) were exposed for 90 days to various concentrations of dieldrin ranging from 10 to 100 mg kg^{-1} in an organic substrate. The study was undertaken to determine possible sub-lethal and accumulative effects of dieldrin. This chemical was still widely used in southern Africa when this study commenced and is generally considered to be non-toxic to earthworms, based on acute toxicity tests (Edwards 1981). The presence of dieldrin significantly prolonged the incubation period of cocoons at relatively high concentrations and the hatching success was similarly influenced. The amount of dieldrin in the worms and cocoons was determined by gas chromatograph and was correlated with that in the substrate. Sufficient evidence was obtained to conclude that dieldrin has a detrimental influence on the life cycle of *Eisenia fetida*.

Die uitwerking van 'n teelmedium wat met dieldrin behandel is, is op pasuitgebroeide erdwurms ondersoek met betrekking tot die inkubasieperiode en uitbroeisukses van kokonne wat geproduseer is deur die blootgestelde wurms. Die konsentrasie dieldrin in blootgestelde wurms en kokonne is ook ondersoek. *Eisenia fetida* (Savigny 1826) is vir 90 dae aan verskillende dieldrinkonsentrasies in die omvang 10 tot 100 mg kg^{-1} in 'n organiese substraat blootgestel. Die studie is ondemeem om moontlike subletale en akkumulatiewe effekte van dieldrin te bepaal. Die chemiese verbinding was met die aanvang van hierdie studie nog algemeen in gebruik in suidelike Afrika en word algemeen as nie-toksies vir erdwurms beskou, gebaseer op akute toksisiteitstoetse (Edwards 1981). Die teenwoordigheid van dieldrin het die inkubasieperiode betekenisvol verleng terwyl die uitbroeisukses ook beïnvloed is. Die hoeveelheid dieldrin in die wurms en kokonne is gaschromatografies bepaal en met dié in die substraat gekorreleer. Genoegsame bewyse is gevind om af te lei dat dieldrin 'n nadelige invloed op die lewensloop van *Eisenia fetida* het.

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The widespread use of agricultural chemicals for purposes of pest management has effects extending to the non-target biological components of an ecological system. Earthworms are an example. These soil organisms are in close contact with the soil environment and are considered to be beneficial ever since the classical work of Darwin (1881).

Heretofore, dieldrin has been considered non-toxic to earthworms. Recently, however, Drewes, Vining & Callahan (1984) reported sub-lethal neurotoxicity for dieldrin by using an ingenious non-invasive electrophysiological monitoring procedure. The lack of suitable testing procedures has discouraged studies on sub-lethal effects of pesticides in spite of the need for more extensive studies (Lebrun 1980). Ecotoxicological studies to determine the possible employment of earthworms as bioindicators are under way at various centres. Kühle (1983) discusses risk assessment by studying the impact of chemicals on earthworms. Various authors have already indicated that earthworms are concentrators of organochlorine pesticides (Edwards 1970; Davis 1971; Beyer & Gish 1980; Reinecke & Nash 1984).

The present study was undertaken in an attempt to quantify sub-lethal effects caused by chemicals in the environment. The influence of dieldrin on growth and development of *Eisenia fetida* (Venter & Reinecke 1985) and reproduction (Reinecke & Venter 1985) is documented but information on incubation periods and hatching success of cocoons is still needed.

Material and Methods

Earthworms belonging to the species *Eisenia fetida* (Savigny 1826) were collected from compost heaps and reared at 20°C in the laboratory as described by Reinecke & Kriel (1981) and Reinecke & Venter (1985). This species was chosen because it can be reared easily, the biology is being studied intensively and a scientific committee of the EEC chose it as bioindicator in future ecotoxicological studies. Extensive ring tests have been conducted with this species (Edwards 1983).

Newly hatched worms were exposed to various concentrations of dieldrin in an organic culture medium consisting of cow manure. A moisture content of 65% was maintained in the culture medium during this study. Five groups consisting of 25 – 30 newly hatched worms in each group were used at a constant temperature of 20°C in an environmental control chamber. One group served as a control while the other four groups were exposed to dieldrin concentrations of 10, 30, 50 and 100 mg kg^{-1} (dry weight of the substrate) respectively. The culture medium was treated as described by Venter & Reinecke (1985). The worms attained maturity at various rates in the differently treated mediums. Newly formed cocoons were collected and removed from the culture medium every second day and placed into numbered compartments of repli dishes containing distilled water (Reinecke & Kriel 1981). Distilled water was used in order to see and remove the tiny hatchlings more easily. To determine the incubation time of individual cocoons, the production date as well as the date when the first hatchling emerged, were noted. The dishes were kept in the dark to prevent bacterial growth and the distilled water was replaced regularly. Monitoring of the hatchlings from a cocoon was terminated if no more hatchlings emerged for ten consecutive days. In a few cases monitoring continued and worms were observed emerging for up to 22 days after the first hatchling had appeared from the same cocoon.

To determine bioaccumulation of dieldrin in the earthworms, 15 hatchlings were placed in each of three flasks containing dieldrin. Groups of three flasks were set up for dieldrin concentrations of 0, 10, 30, 50 and 100 mg kg^{-1} of medium (dry) weight. The flasks were kept at 20°C. Worms

and samples of the medium were collected on days 30, 60 and 90 and steam distilled as described by Venter (1983) and Nash (1984). Analysis of dieldrin was performed by gas chromatograph using a Carlo Erba Strumentazione Fractovap model 2150. Various column sizes and packing materials were used during the course of the study. Column 1: 1,5 m × 4 mm, 3% OV-17 on 80–100 Chromosorb WAW-DMCS; Column 2: 2 m × 4 mm, 1,5% SP-2250 plus 1,95% SP-2401 on 100–120 US Supelcoport; Column 3: 2,5 m × 4 mm 1,5% SP-2250 plus 1,95% SP-2401 on 100–120 Supelcoport. The oven temperature was 245°C. Nitrogen served as carrier gas at a flow rate of 60 cm^3 per minute. A Ni^{63} detector was used at a temperature of 300°C.

Cocoons produced at the various exposure groups were sampled every second day and steam distilled as soon as sufficient numbers of cocoons were obtained. The cocoons were briefly dipped into hexane as a possible means of removing dieldrin from the external surface before homogenizing and extraction.

Results and Discussion

Incubation of cocoons

The mean incubation period of 41 days for cocoons produced by control worms (Figure 1) was much longer than that obtained by Tsukamoto & Watanabe (1977) and Reinecke & Kriel (1981). They obtained incubation periods of 25,2 and 23,3 days respectively. Little importance could, however, be attached to these differences since the difference in particle size and organic matter content (and therefore water availability) in these authors' mediums are unknown. Apart from the expected difference in moisture availability in the culture mediums, the breeding stock was essentially the same as that of Reinecke & Kriel 1981. Earthworm growth is also affected by soil moisture availability since feeding behaviour is affected (Mitchell, Mulligan, Hartenstein & Neuhauser 1977; Reinecke & Venter 1985). These authors indicated that the rate of digestion is directly related to the moisture content of the medium as long as a threshold value of 50% moisture is maintained. Once the cocoons were produced in this study, however, they were treated identically during the incubation period.

The mean incubation period of 41,3 days for cocoons produced at a dieldrin concentration of 10 mg kg^{-1} did not differ statistically significantly from the controls. Considerable variation occurred and some cocoons took more than 60 days to hatch while the cocoons in the control group had all hatched after 49 days. The observed variation (Table 1) could reflect the detrimental effect of dieldrin on the development of at least some of the cocoons. Statistical analysis to compare the incubation periods of cocoons of the exposure groups of 30 mg kg^{-1} and 50 mg kg^{-1} showed no significant differences but both values differed significantly ($p < 0,01$) from the control group and from the group exposed to 10 mg kg^{-1}. Table 1 contains a summary of the computed results of the incubation periods for the various groups. Although the incubation period of a cocoon is defined as the time lapse since cocoon formation and the emergence of the first hatchling, Vail (1974) has shown that the time between emergence of the first and the last hatchling could vary considerably, independently of the number of hatchlings involved. During the present study 52% of all the worms emerged within four days from 34% of cocoons containing two or more hatchlings (Figure 1). Seventy five per cent of the worms emerged within nine days while in one instance the first worm emerged 22 days

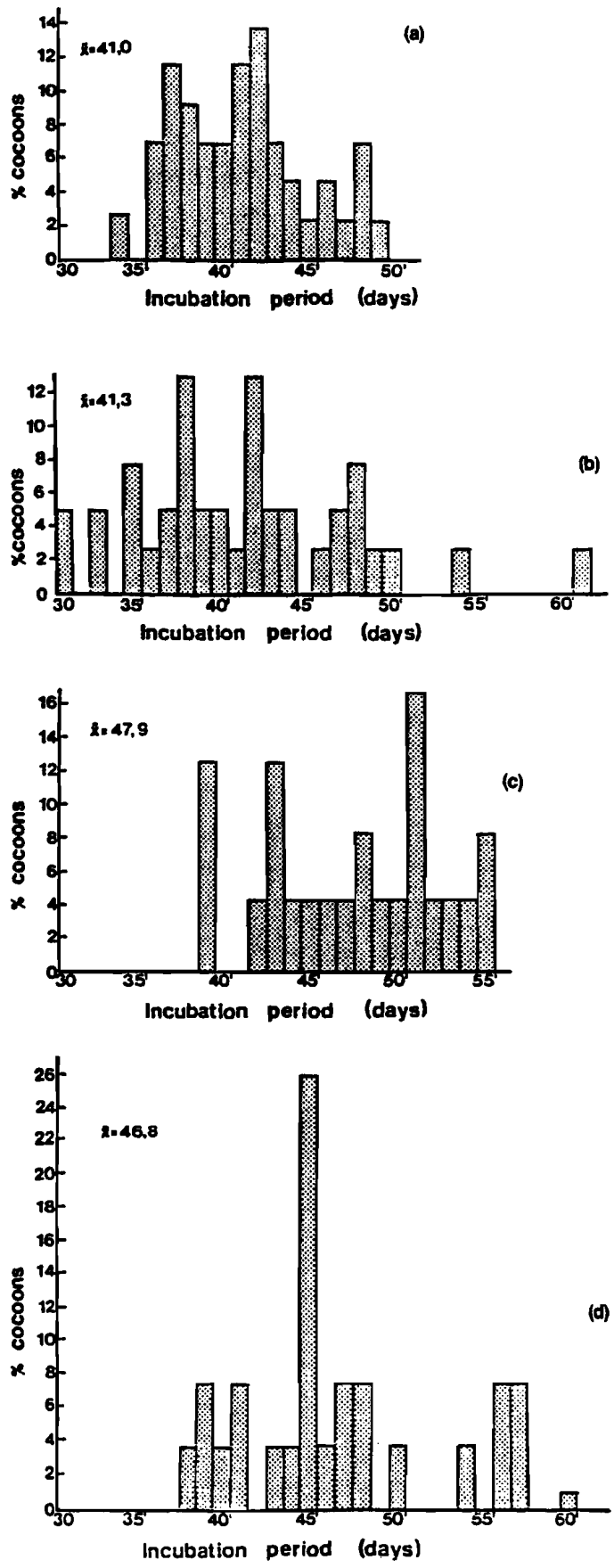

Figure 1 The incubation period of cocoons produced by *Eisenia fetida* cultured on cow manure containing 50% moisture at 20°C. 1a: control; 1b: 10 mg kg^{-1} dieldrin; 1c: 30 mg kg^{-1} dieldrin; 1d: 50 mg kg^{-1} dieldrin.

before the last from the same cocoon. This observed variation could influence the determination of mean incubation periods

Table 1 Summary of the data for the mean incubation periods of cocoons of *E. fetida* produced by worms exposed to various concentrations of dieldrin ($\bar{x}$ = mean, *SD* = Standard Deviation, *SE* = Standard Error)

Dieldrin concentration mg kg^{-1}	No. of cocoons	Incubation period min.	max.	$\bar{x}$	*SD*	*SE*
0 (Control)	43	34	49	41,09	3,791	0,578
10	39	31	61	41,38	6,348	1,017
30	24	39	58	47,96	5,760	1,176
50	27	38	60	46,89	6,123	1,178

in the laboratory and had to be taken into account in the statistical analysis since this phenomenon is not necessarily dependent on or influenced by exposure to dieldrin.

The hatching success of cocoons is influenced by temperature (Watanabe & Tsukamoto 1976; Reinecke & Kriel 1981). The present study at a favourable temperature of 20°C was a follow-up study (Table 2) of one performed by Reinecke & Venter (1985). A hatching success of 76% was obtained for the control group and the value decreased with an increase in the dieldrin concentration in the environment. The values in Table 2 are based on replicates. Chi-square analysis indicated that the presence of dieldrin in the culture medium significantly influenced the length of the incubation period as well as the hatching success at least in the 50 mg kg^{-1} group when compared to the control group. A linear regression of the hatching success at the various concentrations provided a fit of 85% over the whole spectrum.

Table 2 The hatching success of cocoons of *E. fetida* produced by worms exposed to various concentrations of dieldrin: Cocoons were produced over a period of seven days

Dieldrin concentration mg kg^{-1}	No. of cocoons	No. of cocoons hatched	% hatching success
0 (Control)	25	19	76
10	25	17	68
30	25	17	68
50	25	13	52

The influence of dieldrin on the hatching success could either be direct or indirect. If the presence of dieldrin affects the feeding behaviour of mature worms as suggested by Venter & Reinecke (1985) who showed that growth is affected adversely, then cocoons produced in such an environment could be of a poorer quality. On the other hand the presence of dieldrin in the cocoons could have a direct influence on development. It therefore seemed logical to analyse the cocoons as well to determine whether any amount of dieldrin ended up in the cocoons where it could have a direct effect.

Accumulation of dieldrin

The results of the analysis of earthworms exposed for 90 days to various concentrations of dieldrin, are illustrated in Figure 2. From Figure 2 it can be seen that the worms concentrated relatively less dieldrin at the higher concentrations than at the lower concentrations. Expressed as a percentage of the concentration in the culture medium the uptake of dieldrin was

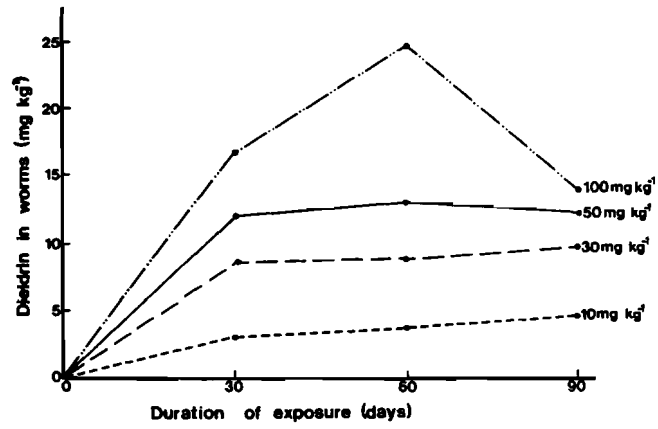

Figure 2 The dieldrin concentration in *Eisenia fetida* at various stages during an exposure period of 90 days.

30%, 28%, 24% and 16% for the 10, 30, 50 and 100 mg kg^{-1} exposure groups respectively. The concentration of dieldrin in the bodies of the earthworms remained fairly constant after 30 days of exposure and in the case of the group exposed to 100 mg kg^{-1} the high level reached at day 60 decreased at day 90, possibly suggesting an excretory control mechanism (see Figure 2). Comparison of the dieldrin in the culture medium with that in the worms, is presented in Figure 3 where the concentration is expressed in terms of worm biomass as well as dry weight.

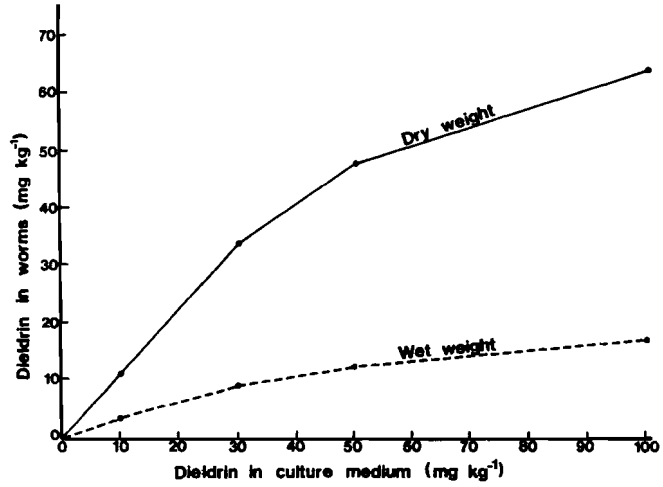

Figure 3 The relation between the dieldrin concentration in the culture medium and the worms after an exposure period of 90 days.

These results are very similar to those obtained by Edwards (1970), Davis (1971) and Lord, Briggs, Neale & Manlove (1980) for the accumulation of organochlorine pesticides in general. The idea of an excretory mechanism for organochlorine substances was forwarded by Reinecke & Nash (1984) who studied accumulation of dioxin in other earthworm species. No final proof of such a mechanism can be presented and the possibility of such a mechanism needs further study.

The amount of dieldrin in the cocoons of *Eisenia fetida* increases with an increase in the exposure concentration. A two-tailed regression of the data in Table 3 showed a highly significant correlation ($p < 0,01$). The relation is presented in Figure 4. The relation between the amount of dieldrin in the

Table 3 Concentration of dieldrin in cocoons of *E. fetida* produced by worms exposed to various concentrations of dieldrin

Dieldrin concentration mg kg^{-1} (substrate)	Dieldrin in cocoons (mg kg^{-1})					
	Replicate 1	Replicate 2	Replicate 3	$\bar{x}$	*SD*	*SE*
10	2,240	2,371	2,249	2,287	0,073	0,042
30	4,989	4,929	5,337	5,087	0,220	0,127
50	7,901	8,230	7,049	7,727	0,610	0,352

$p < 0,01$ (two tailed) $R^2 = 0,981$

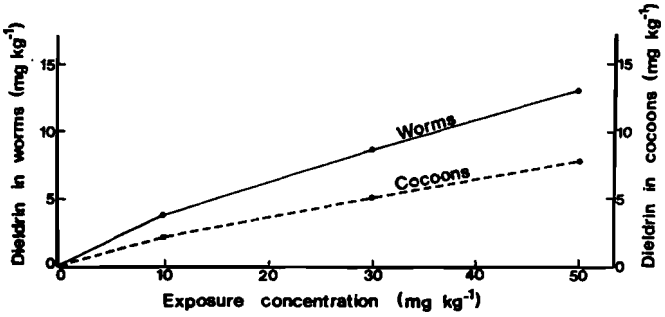

Figure 4 The dieldrin concentration expressed in terms of biomass in worms and cocoons of *Eisenia fetida* after exposure to various concentrations of dieldrin in the culture medium.

culture medium, the worms and the cocoons was compared on the sixtieth day of exposure (Table 3). The presence of the dieldrin in the cocoons also corresponds to a decrease in the hatching success and an increase in the incubation period. We conclude that dieldrin, even at non-lethal levels, may have a marked influence on at least some aspects of the life cycle of this species. The detrimental effect exerted on reproduction should have a substantial effect on earthworm populations especially under conditions of environmental stress. The biological significance of our findings seems self-evident but requires further study of earthworm populations.

Since the present study was undertaken under relatively low moisture conditions, the effect of the non-polar dieldrin under relatively moist conditions could be markedly different. High accumulation rates in the worms and in the cocoons could be expected in the short term owing to increased activity of worms under more favourable moisture conditions.

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