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Dieldrin and Growth and Development of the Earthworm, *Eisenia fetida* (Oligochaeta)

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Although the use of pesticides has never been as intense in southern Africa as in Europe and North America, concern about their environmental effects remains. The effects of agricultural chemicals on non-target organisms are seldom studied.

Sperlingh & McLaughlin (1976) and Venter & Reinecke (1984) stress that environmentally "acceptable" concentrations of a chemical under conditions of chronic exposure cannot be determined on the basis of the results of acute lethal tests. Sub-lethal effects on behaviour, growth and development are not revealed by conventional LD_{50} tests (Brown 1982). Very little is known about the influence of pesticides on the growth and development of sexual characteristics in earthworms. Edwards (1978) and Lofs-Holmin (1980) have suggested earthworms for LD_{50} and sub-lethal testing.

Sub-acute testing with a fairly wide variety of species representing various trophic levels and habitats is needed to establish a more satisfactory basis for decision-making. The purpose of the present study was to contribute towards this goal. Invertebrates have, until recently, for traditional and other practical reasons, been neglected as experimental animals. The aim of this study was to identify readily measurable quantitative effects resulting from the presence of a chemical contaminant in the environment.

Pesticides are normally non-specific in their action and the amount used may be far in excess of that required to control the pest. In the environment where the forces of stress and natural selection are operative a very slight loss in growth rate and the retardation of the reproductive cycle as well as small changes in behaviour may have important consequences for the whole population. The sub-lethal effects of environmental contaminants could in many cases be of great ecological importance.

Dieldrin was banned for domestic and agricultural use in the U.K. in 1964. Most developed countries have followed suit. In the Republic of South Africa the use of dieldrin for domestic and agricultural purposes was restricted in 1981, shortly after the commencement of this study. Special permission is now required to use it for certain industrial purposes. A conservative esti-

mation of chlorinated hydrocarbons sold since 1976 shows a decline of 2 000 tons in 1976 to 200 tons of active ingredient per annum in 1981.

The present study was undertaken to determine the influence of the chlorinated pesticide dieldrin on the earthworm Eisenia fetida as a typical non-target organism.

MATERIALS AND METHODS

Eisenia fetida was collected from compost heaps and reared in the laboratory in environmental control chambers at a temperature of 20°C on an organic substrate (Venter, 1983). According to Lofs-Holmin (1980) sub-lethal effects of pesticides on the life cycle of earthworms are more readily demonstrated by the growth rate of juveniles, the development of sexual characteristics such as the tuberculae pubertates and the clitellum. Newly-hatched worms were selected and exposed to various concentrations of dieldrin. The pesticide was mixed with the organic culture medium consisting of washed cow manure. The culture medium was pretreated with dieldrin dissolved in acetone and thoroughly mixed to ensure a uniform distribution of the pesticide. Acetone was allowed to evaporate over a period of 24 hours at 22°C before the medium was moistened to attain a moisture content of approximately 60%. The actual moisture content of the different flasks was confirmed by analysis with an infrared moisture detector. Five groups consisting of 25-30 newly hatched worms in each group were used. One group served as control. The other four groups were exposed to concentrations of 10 mg kg^{-1}, 30 mg kg^{-1}, 50 mg kg^{-1} and 100 mg kg^{-1} respectively. Environmental samples from areas where dieldrin had been used for many years at rates of 5-15 kg ha^{-1} had varying concentrations of 3 and 50 mg kg^{-1}.

The newly hatched worms were weighed at the start of the experiment. The biomass of every individual worm was subsequently determined every 15 days over a period of 90 days. The development of the clitellum was used as one parameter to determine the influence of dieldrin on the sexual development of Eisenia fetida. Worms were closely observed every second day starting four weeks after they had hatched. Worms were classified as juvenile, preclitellate and clitellate, using the rather subjective criterion of clitellum absence, partial development of the clitellum and the presence of a fully developed clitellum.

Dieldrin was extracted from the culture medium by steam distillation. This extraction procedure was successfully used by Nash (personal communication) and is fully described, together with the clean-up procedure by Venter (1983) and also by Nash (1984). Analysis of dieldrin was performed gaschromatographically using a Carlo Erba Strumentazione Fractovap model 2150 GC. Various column sizes and packing material were used during the course of the study. Column 1: 1,5 m x 4 mm, 3% OV-17 on 80-100 chromosorb WAW-DMCS; column 2: 2 m x 4 mm, 1,5% SP-2250 plus 1,95% SP-2401 on 100-120 US Supelcoport; column 3: 2,5 m x 4 mm 1,5% SP-2250 plus 1,95%,

SP2401 on 100-120 US Supelcoport. The columns performed satisfactorily at 245°C. Nitrogen served as a carrier gas at a flow rate of 60 cm^3 per minute. A Ni^{63} detector was used at a temperature of 300°C. Nash & Harris (1973) and Suzuki, Ishikawa, Sato & Sakai (1979) used very similar conditions for the GC analysis of organochlorines.

RESULTS AND DISCUSSION

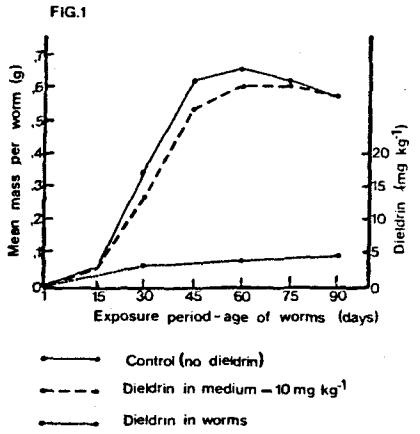
The worms grew fairly normally in all the dieldrin concentrations. Growth rate was measured in terms of change in biomass. The growth pattern was identical to the classical growth curve of Verhulst. The growth pattern was also similar to the results obtained by Neuhauser et al (1980) for the same species in different culture mediums.

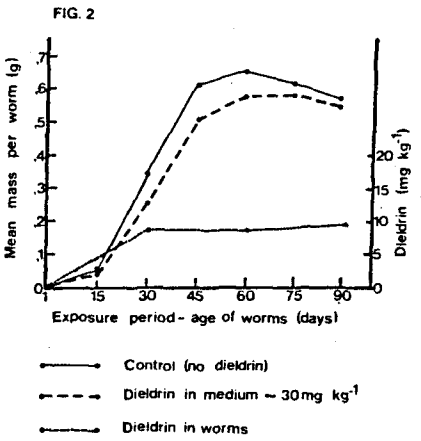
The changes in the biomass of the various groups are summarized in Table 1. Figures 1-4 illustrate the growth curves.

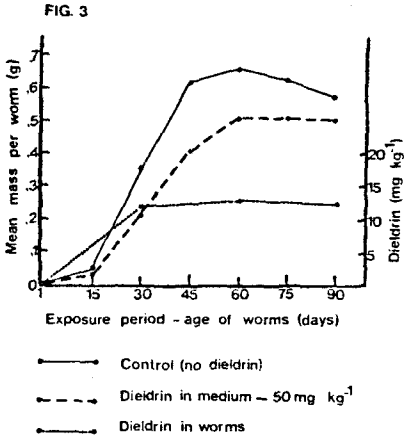
Table 1. Changes in total biomass(g) of five groups of 30 hatchlings of Eisenia fetida at various dieldrin concentrations.

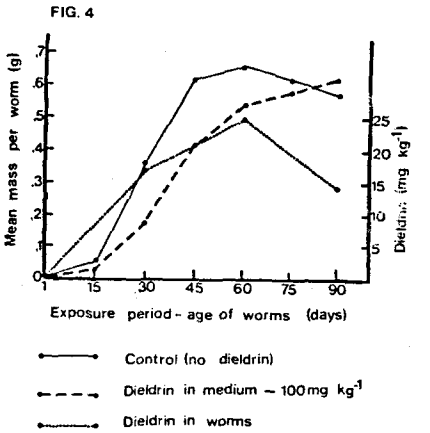
Period (days)	1	15	30	45	60	75	90
Control	0,148	1,585	10,341	18,524	19,515	18,537	17,176
$\bar{x}$	0,005	0,053	0,345	0,617	0,651	0,618	0,573
SE	-	0,002	0,014	0,022	0,023	0,023	0,022
10mg kg^{-1}	0,155	1,294	8,141	15,867	17,986	18,185	17,203
$\bar{x}$	0,005	0,043	0,271	0,529	0,599	0,606	0,573
SE	-	0,002	0,012	0,024	0,028	0,028	0,028
30mg kg^{-1}	0,150	1,184	7,618	15,215	17,372	17,634	16,406
$\bar{x}$	0,005	0,039	0,254	0,507	0,579	0,588	0,547
SE	_	0,002	0,009	0,024	0,026	0,027	0,027
50mg kg^{-1}	0,145	1,025	6,350	12,197	15,350	15,122	15,015
$\bar{x}$	0,005	0,034	0,212	0,407	0,512	0,504	0,501
SE	-	0,002	0,017	0,034	0,042	0,042	0,042
10mg kg^{-1}	0,151	0,750	5,273	12,565	15,966	17,287	18,504
$\bar{x}$	0,005	0,025	0,173	0,419	0,532	0,576	0,617
SE	-	0,003	0,014	0,028	0,033	0,036	0,038

($\bar{x}$ = mean, SE = Standard Error)









Figures 1-4. Change in biomass of newly-hatched specimens of E. fetida over a period of 90 days in the controls and in culture mediums containing various concentrations of dieldrin.

Growth was slow over the first few days, after which a sharp linear increase in biomass was registered. A mean increase in biomass of 570 mg per worm occurred until day 45. Following this the growth rate slowed down considerably and after day 60 the growth curve showed a downward trend until day 90 when the experiment was aborted. The group exposed to 100 mg kg^{-1} was the only one differing significantly from the others. The highest biomass in this group was registered on day 90. The mean biomass of these worms was also higher on day 90 than those for the control group and other exposed groups. These worms attained their maximum biomass at a slightly lower rate. Dieldrin only seemed to retard growth slightly at the relatively high concentration of 100 mg kg^{-1}.

Slight changes in soil moisture occurred in the flasks during the study. Since this species has a wide tolerance range for moisture, it is doubtful whether the decrease in available moisture measured over the period of 90 days could have influenced the form of the growth curves. Nevertheless, a comparison of the moisture conditions at the various exposure concentrations of days one, thirty, sixty and ninety as well as in the control flasks, clearly shows the uniformity.

Table 2. Mean concentration of dieldrin in E. fetida after exposure to dieldrin in the culture medium.

Dieldrin concentration (mg kg^{-1}) in medium	Exposure period (days) 30	60	90
10	3,00 ± 0,12	3,72 ± 0,24	4,62 ± 0,25
30	8,53 ± 0,06	8,70 ± 0,24	9,66 ± 0,22
50	12,08 ± 0,40	12,89 ± 1,32	12,38 ± 0,37
100	16,75 ± 1,05	24,76 ± 0,99	13,92 ± 0,33

Table 2 shows the results of the gaschromatographic analysis of dieldrin in the worms at various time intervals. The concentrations are also illustrated in figures 1-4. In all cases the dieldrin content of the worms increased with time and showed a definite relation to the particular exposure concentration in the culture medium. These results seem to confirm the findings of Wheatley & Hardman (1968) that the amount of pesticides in the environment and that in the worms are not linearly related, and there is proportionately less concentration of pesticide in the tissue of worms in media containing large quantities of dieldrin, than in those with small amounts. This is at least true for the short term.

Clitellums were present in worms 30 days of age collected from the control group and the exposure groups of 10 mg kg^{-1} and 30 mg kg^{-1}.

Table 3. The rate of development of clitellums in E. fetida at various dieldrin concentrations.

Dieldrin (mg kg^{-1})	No of worms	% clitellates at age (days): 41	51	61
Control (0)	30	60,0	90,0	90,0
10	30	56,6	86,6	90,0
30	30	56,6	80,0	90,0
50	30	50,0	76,0	83,0
100	30	30,0	70,0	80,0

In the case of the 50 and 100 mg kg^{-1} groups the first clitellums appeared only three days later. Table 3 compares the clitellum

FIG. 5

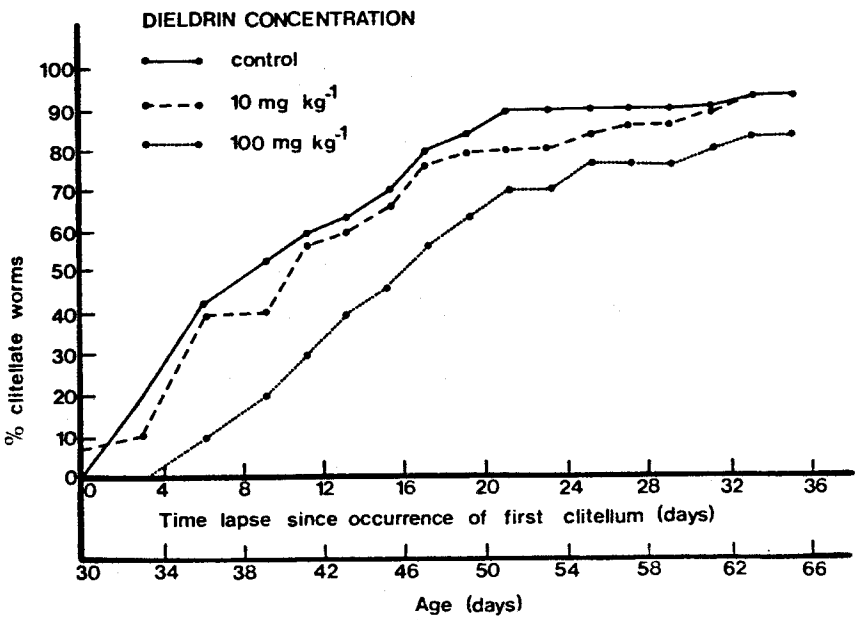


Figure 5. The rate of development of clitellums in E. fetida exposed to various dieldrin concentrations.

development of various age groups exposed to different concentrations of dieldrin. Figure 5 illustrates the results for the control group and the groups exposed to 10 and 100 mg kg^{-1}. The histogram in figure 6 gives a visual presentation of the results.

Almost 90% of the worms in the control group had developed clitellums when they were 66 days old (see figure 5). The same pattern occurred in the worms exposed to 10 mg kg^{-1} dieldrin. The thirty hatchlings showed a great variation in the rate of clitellum development as compared to the group exposed to 10 mg kg^{-1} dieldrin, and a slightly lower rate than the control. The worms exposed to 100 mg kg^{-1} dieldrin started to develop clitellums somewhat later and at a slower rate.

At low concentration levels, closely related to what could sometimes be expected under field conditions, the influence of dieldrin was negligible. Earthworm population numbers in the field should normally recover within one or two seasons provided that the pesticide is degraded fast and has no accumulative characteristics. The worms containing the higher concentration of dieldrin (table 2) were the slowest to develop clitellums and a number of the worms exposed to higher concentrations never developed clitellums, even after 100 days of exposure. Lofs-Holmin (1980) is of the opinion that the time earthworms need to attain sexual maturity is of great ecological significance since retardation in clitellum development could affect the population size adversely.

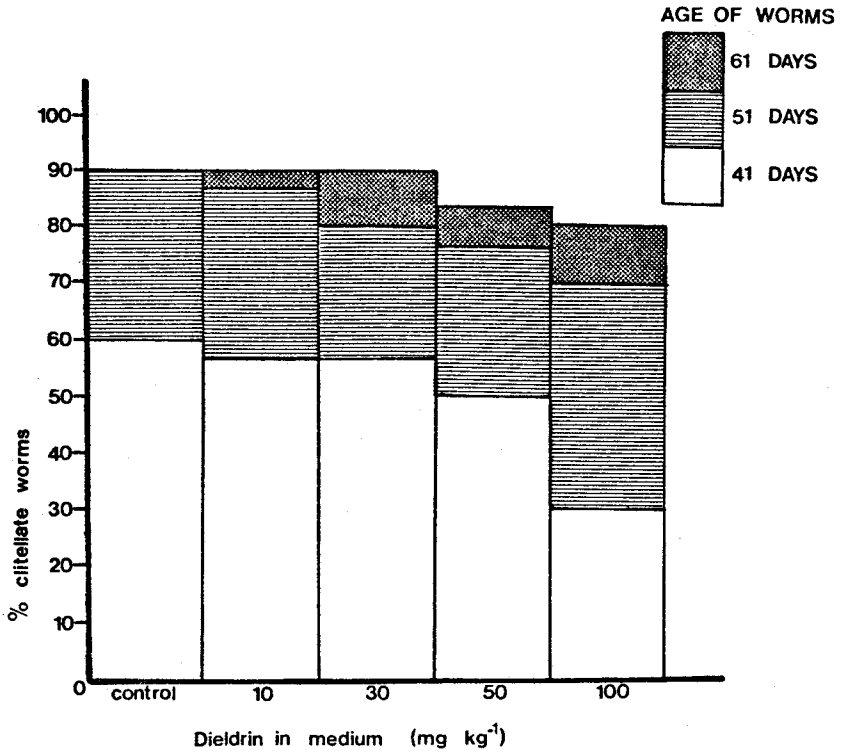


Figure 6. The percentage of clitellate worms (E. fetida) after exposure of the groups to various dieldrin concentrations.

The results of the present study clearly indicates that dieldrin will, even at agricultural doses of 5 kg ha^{-1}, retard growth in E. fetida.

A decrease in growth rate will evidently retard the development of the tuberculae pubertates and the clitellum. This is confirmed by Lofs-Homin (1980). Van Rhee (1977) showed that the presence of DDT caused only 33% of the experimental worms (A. caliginosa) to attain sexual maturity. In the present study dieldrin did not have such a marked effect although clitellum development was retarded. This in turn will influence rate of reproduction.

Since a decrease in the reproductive potential would affect population density, this sub-lethal effect of the pesticide is of prime importance for purposes of eco-toxicological evaluation.

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