ASSESSMENT OF THE EFFECTS OF TOXIC CHEMICALS UPON EARTHWORMS

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ABSTRACT

The beneficial effects that earthworms have upon soil fertility, the toxicity of chemicals to earthworms, the theory of testing for toxicity, the analysis of toxicity data, and the methods for testing toxicity to earthworms are discussed.

Several methods for testing the toxicity of chemicals to earthworms in the laboratory were evaluated experimentally using E. fetida. Earthworms were exposed to chemicals in water, sand, silica paste or natural soil, on glass, glass granule or filter paper surfaces, or in an agar-agar gel which was fed forcibly.

New techniques for testing toxicity to earthworms were developed, namely the filter paper contact and artificial soil tests. These tests were used subsequently to assess the toxicity to earthworms of 14 chemicals. Independent collaborators were invited to experiment with these methods, and were circulated with test protocols and three unidentified chemicals. The reproducibility of the data that were returned was satisfactory.

The effects of chemicals upon earthworms in the field was investigated in experiments on different types of soil. After one month, chlordane and carbaryl were very toxic to earthworms, thiophanate-methyl and triazophos were moderately toxic and pentachlorophenol had little effect. Only the effects of carbaryl did not persist for six months. The artificial soil, silica paste and sand tests predicted the toxicity of chemicals to earthworms in the field most accurately.

The susceptibility of several species of earthworm to chemicals in the laboratory was dissimilar. A. caliginosa was very susceptible to chemicals, followed in a decreasing order of susceptibility by L. terrestris, A. longa, E. fetida andrei and E. fetida fetida. The ability to accumulate and detoxify chlordane and iodoacetamide, studied using chromatographic and radiolabelling techniques, differed between the subspecies of E. fetida.

Gel electrophoresis of esterases indicated that resistance to pesticides that inhibit cholinesterase may have evolved in a population of A. longa exposed to such compounds frequently.

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TO

My mother and father, Pauline and Peter Geoffrey Goats

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Summary

1. Earthworms are beneficial soil organisms which are affected adversely by many chemicals including the salts of heavy metals, certain organochlorine, organophosphorus and carbamate insecticides, most nematicides and the benzimidazole fungicides. Other fungicides and the herbicides are usually of low toxicity to earthworms.

2. Methods for testing the toxicity of chemicals to earthworms in the laboratory and the field were reviewed. Several methods were evaluated experimentally to select those that were inexpensive to use, could test the toxicity to earthworms of any chemical sensitively and reproducibly, were able to predict the toxicity of chemicals to earthworms in the field and seemed suitable for a statutory programme of ecotoxicologial testing. These tests included methods in which the earthworms were exposed to the test chemicals in water, sand, natural soil or a mixture of silica paste and glass balls; on a glass, glass bead or filter paper surface, or in an agar-agar gel with which the earthworms were force fed.

3. The period for which the earthworms had to be exposed to the chemicals to obtain a reproducible estimate of the LC_{50} differed between the testing methods, and was probably related to the intimacy of the contact achieved between the earthworm and the chemical. The tests gave results of similar reproducibility, although the LC_{50}'s that were estimated for the same chemicals in different tests varied considerably and were expressed necessarily in diverse units of concentration that were related to the conditions within each test. A comparison of the results obtained from these tests was made using the order in which the LC_{50}'s of the test chemicals were ranked. The ranking seen with the short duration tests using non-adsorptive or chemically inert media was similar, and was dissimilar to that seen with the longer duration tests using adsorptive or chemically active media. Several methods had practical disadvantages that precluded further development.

4) Two new tests for the toxicity of chemicals to earthworms were developed to overcome the inadequacies of the foregoing methods. The filter paper contact test was refined from the prototype method studied previously with few modifications. The artificial soil test combined the advantages inherent in the silica paste-glass ball and natural soil tests. Both of the new tests were used subsequently to test the toxicity of a wide range of chemicals to earthworms.

5) The final version of the filter paper contact test consisted of a transparent glass vial (8 cm x 3 cm diameter) fitted with an airtight plastic cap. The side walls of the vial were lined internally with 75 cm^2 moist cellulose filter paper that was treated with the test chemical. Each vial contained one E. fetida andrei (Andre) weighing 0.4-0.6 g (with an empty gut), and ten replicates were used at each of six concentrations of the test chemical. The period of exposure was 48 hours at 20° ± 2°C in darkness.

6) The toxicity of chemicals to earthworms in the filter paper contact test correlated negatively with the body weight and sexual maturity of the earthworms, and the absorbant capacity of the filter paper; but was correlated positively with the test temperature, the period of exposure, ventilation of the vials and testing in darkness. Some formulating agents increased the toxicity of chemicals to earthworms. The area of the filter paper and the number of replicates that were used did not affect the magnitude of the LC_{50}'s, although the reproducibility of the LC_{50}'s was correlated positively with the number of replicates used when that number was less than ten.

7) The artificial soil consisted of 71% fine quartz sand, 20% kaolinitic clay, 8% air dried and finely milled sphagnum peat, and 1% calcium carbonate (dry weight). At a moisture content of 48% the soil had a pH of 6.8 and an adsorptive capacity of 152 $meq.kg^{-1}$, and thus resembled a natural sandy loam. Four replicates consisting of 750 g (wet weight) soil in a one litre plastic flask with a ventilated lid, each containing ten E. fetida andrei weighing 0.4-0.6 g (with an empty gut), were used at six concentrations of the test chemical applied to the soil previously as a spray and incorporated manually. The period of exposure was 14 days at 20° ± 2°C under illumination.

8) E. fetida andrei did not survive in artificial soils that contained less than 10% water or 5% organic matter, but was unaffected by the amount of clay in the soil or the pH within the range studied. Chemicals were usually less toxic to earthworms in soils that contained a large amount of organic matter or a small amount of water, but the clay content of the soil and the pH did not influence the effect of the chemicals. The toxicity of chemicals to earthworms was correlated positively with the test temperature and the period of exposure, but was unaffected by the quantity of soil in each replicate and the method that was used to apply the chemical to the

soil. The magnitude of the LC_{50}'s was not affected by the number of replicates that were used, although the reproducibility of the LC_{50}'s correlated positively with the number of replicates when that number was less than four.

9) The filter paper contact, artificial soil and silica paste-glass ball tests were evaluated by independent laboratories for the efficiency with which a sensitive and reproducible assessment of the toxicity of chemicals to earthworms could be obtained. These collaborators were circulated with instructions for the tests, three unknown chemicals and one reference compound. They were asked to criticise the tests and to report an LC_{50} for each chemical using E. fetida (Savigny). Two such exercises were done. The criticism that was received of the filter paper contact and artificial soil tests after the first exercise allowed the methods to be improved and resulted in an enhanced reproducibility of the LC_{50}'s that were obtained. The standard errors of the LC_{50}'s that were determined using the filter paper contact test were smaller than those of the artificial soil and silica paste-glass ball tests.

10) Two field experiments were done on pastures in different types of soil during 1981 and 1982 at Rothamsted and Sittingbourne respectively. Five chemicals were applied at two rates to the plots in early spring, and the numbers and weights of the earthworms in the populations of L. terrestris (Linnaeus), A. longa (Ude), A. caliginosa (Savigny) and of the remaining species were sampled using the formalin extraction method after one and six months post-treatment. The data were processed by analyses of variance allowing for covariates. L. terrestris was collected at both sites on both sampling dates, although A. longa, A. caliginosa and the other species of earthworm were found infrequently in the samples collected during the spring, particularly at the Sittingbourne site.

11) The numbers and weights of the earthworms found in the plots usually responded to the chemical treatments in a similar manner. Occasionally the numbers of the earthworms were reduced more than the weights or vice versa, and sometimes the earthworms apparently became more numerous in the plots that had been treated with a toxic chemical. These observations were explained in terms of the relative susceptibility to chemicals of adult and juvenile earthworms, the migration of earthworms into and from the treated areas, the recruitment of earthworms into the population from cocoons and the half-life of the chemicals in the soil.

12) Chlordane and carbaryl were very toxic to earthworms in the field, thiophanate-methyl and triazophos were moderately toxic to earthworms, and pentachlorophenol apparently had little effect. A. caliginosa and A. longa seemed to be more susceptible to these chemicals than L. terrestris and the other species. The toxicity of chlordane to earthworms was lower in the soil that contained a large amount of organic matter at Sittingbourne. Chlordane, thiophanate-methyl, triazophos and pentachlorophenol remained toxic to earthworms in the soil for at least six months, whilst the effects of carbaryl were seen only in the post-treatment samples taken after one month.

13) The results of the field experiments were compared with assessments of the toxicity of the same chemicals to earthworms made using seven tests in the laboratory. The artificial soil, silica paste-glass ball and sand tests identified correctly several aspects of the toxicity of chlordane, carbaryl, thiophanate-methyl, triazophos and pentachlorophenol to earthworms in the field, although the laboratory tests seemed to underestimate the differences that occurred in the field between the toxicities of these chemicals to earthworms. The LC_{50}'s of the chemicals tested in artificial soil in the laboratory were much higher than the concentrations that were needed to reduce the numbers of earthworms in the field. This was probably caused by the short duration of the laboratory tests relative to that used in the field. The results from the filter paper contact test correlated poorly with those from the artificial soil and silica paste-glass ball tests, although the results from the latter tests were correlated highly.

14) E. fetida was usually less susceptible to toxic chemicals than L. terrestris, A. longa and A. caliginosa when tested using the filter paper contact and artificial soil tests. These tests showed that A. caliginosa was usually more sensitive to chemicals over a narrower range of concentrations than the other species of earthworm. E. fetida andrei was more susceptible to iodoacetamide and, to a lesser extent, to chlordane than E. fetida fetida (Andre).

15) Extracts of the tissues of E. fetida andrei and E. fetida fetida were analysed by gas-liquid chromatography and found to contain the same lipids in amounts that were similar. Further experiments using GLC and radiolabelled chemicals showed that earthworms of both subspecies of E. fetida accumulated alpha-chlordane readily from aqueous solution, but released none subsequently into distilled water. E. fetida fetida

accumulated alpha-chlordane slightly more quickly than E. fetida andrei and the earthworm tissue:water partition coefficient for alpha-chlordane was, as expected, high. Alpha-chlordane was not metabolised significantly by earthworms of either subspecies of E. fetida, although small amounts of oxychlordane and chlordane chlorohydrin were detected in the tissues of both subspecies by thin-layer chromatography.

16) The rate at which alpha-chlordane entered E. fetida fetida immersed in an aqueous solution of this chemical, and the rate of efflux of alpha-chlordane from earthworms in distilled water proceeded at a much faster rate as the earthworms began to die. This suggested that the distribution of such chemicals across the body wall of a healthy earthworm was maintained actively.

17) Iodoacetamide was accumulated and released in very small amounts by E. fetida fetida, although E. fetida andrei took up and released even less. Earthworms of both subspecies metabolised this compound readily, and the earthworm tissue:water partition coefficient for iodoacetamide was, as expected, low.

18) A. longa collected from an experimental orchard that had been treated regularly with a large amount of a pesticide that blocked the action of cholinesterase, apparently showed an increase in the rate of polymorphism of carboxylesterases that were separated using gel electrophoresis. L. terrestris collected from the same site did not show this response. This preliminary investigation suggested tentatively that A. longa exposed frequently to such pesticides may develop a resistance to them.

"Earthworms, though in appearance a small and despicable link in the chain of Nature, yet, if lost, would make a lamentable chasm."

Reverend Gilbert White (1853)
Selborne 1777

ABBREVIATIONS

Organisations

EEC	European Economic Community
OECD	Organisation for European Cooperation and Development
PSPS	(United Kingdom) Pesticides Safety Precautions Scheme

Chemical names

2,4-D	2,4-dichlorophenoxyacetic acid
DBCP	1,2-dibromo-3-chloropropane
D-D	1,2-dichloropropane/1,3-dichloropropene mixture
DDE	2,2-(4-chlorophenyl)-1,1-dichloroethene
DDT	1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane
DNOC	4,6-dinitro-o-cresol
HCB	1,2,3,4,5,6-hexachlorobenzene
HCH	1,2,3,4,5,6-hexachlorocyclohexane
MCPA	4-chloro-2-methylphenoxyacetic acid
MCPB	4-(4-chloro-2-methylphenoxy)butyric acid
PCP	pentachlorophenol
2,4,5-T	2,4,5-trichlorophenoxyacetic acid
2,3,6-TBA	2,3,6-trichlorobenzoic acid
TCA	trichloroacetic acid
TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin

CHAPTER 1

GENERAL INTRODUCTION

1.1 Earthworms and environmental pollution

Earthworms are beneficial soil organisms. White (1853) observed that "the earth without worms would soon become cold, hard-bound and void of fermentation; and, consequently, sterile". This observation was confirmed by Darwin (1881) in his book 'The Formation of Vegetable Mould Through the Action of Worms'. Earthworms increase soil fertility by incorporating and mineralising plant nutrients, and by altering the carbon:nitrogen ratio, allowing nitrogen to be taken up by plants more easily (Edwards, Reichle and Crossley, 1970). Earthworms aerate and drain the soil, improve its structure by breaking down large particles and assist the production of mineral aggregates that are resistant to erosion and compaction (Edwards and Lofty, 1977). This activity can speed the reclamation of open cast mining land (Laird and Kroger, 1981; Stewart and Scullion, 1985) and polder soils (Hoogerkamp, Rogaar and Eijsackers, 1983). In an arable soil the beneficial activities of earthworms may increase crop yields (Edwards and Lofty, 1977), particularly when the soil is direct drilled or minimally cultivated (Edwards, 1975b). By incorporating leaf litter, earthworm activity can reduce the persistence of foliar plant pathogens such as the apple scab Venturia inaequalis (Cooke) on fallen leaves (Hirst, Storey, Ward and Wilcox, 1955).

Occasionally the activity of earthworms is undesirable and methods of control are required. Sports turf (Escritt, 1955; Shread, 1952; Stuurman and Kamp, 1971; Walton, 1928) and lawns (Dawson-Bingley, 1949) may be spoiled by worm casts. Large flocks of birds can congregate on the closely mown grass surrounding airports and may, in their search for

earthworms, endanger aircraft (Tomlin, 1981; Tomlin, Tolman and Thorn, 1981).

In recent years, the possibility that pesticides and industrial chemicals were having an adverse effect upon non-target organisms such as earthworms has become a cause for concern. Many governments have responded to this concern by introducing registration requirements for pesticides. Countries within the jurisdiction of the European Economic Community and Organisation for European Cooperation and Development are subject also to increasingly stringent environmental protection legislation concerned with the use of industrial chemicals. This legislation (Anon., 1979) reflected the views of European exotoxicologists and required that any chemical, marketed in an amount greater than 10 tonnes per annum, be tested for acute toxicity to five indicator species. These indicator species include an alga, a higher plant, a freshwater crustacean, a fish and an earthworm.

1.2. Earthworms as indicators of pollution

An indicator species is one considered to be capable of indicating the toxicity of a chemical to other organisms in the same type of habitat. Several species have been suggested as suitable for indicating the toxicity of chemicals to the soil fauna including springtails (Berankova, 1978; Edwards and Thompson, 1973), millipedes, wireworms (Berankova, 1978), pauropods (Edwards and Thompson, 1973), carabid beetles, staphilinid beetles and earthworms (Edwards, 1978).

Springtails, millipedes, wireworms, slugs and earthworms consume living or decayed vegetable matter. Tests for the toxicity of chemicals using these organisms can estimate the toxicity of a chemical to the rest of the soil fauna in the field more accurately than those using predatory species. This is because organisms that feed upon plant material do not

accumulate high concentrations of chemicals from their prey and therefore indicate the direct effects of a given concentration of the test chemical in the soil or on vegetation.

Pesticides and industrial chemicals can threaten the populations of beneficial invertebrates in the soil and so it is important that the toxicity of chemicals to these organisms is assessed accurately. Annelids have been used successfully to monitor marine pollution (Barnett, 1983; Coates and Ellis, 1980) and the earthworm seemed to be well suited to the assessment of terrestrial contamination. The earthworm fulfilled several of the criteria for a good indicator organism. These criteria included the ability to respond in a sensitive way to the effects of chemicals, to have an important role in the maintenance of soil fertility (Anon., 1975) and to be cultured easily in the laboratory. Earthworms also seemed to be capable of indicating the toxicity of chemicals to the soil fauna in general and were therefore selected to represent these organisms in toxicity tests.

1.3 The taxonomy of earthworms

Terrestrial Annelids belong to the Order Oligochaeta, of which the largest European family is the Lumbricidae containing approximately 220 species. Ten lumbricid species are commonly found in British arable soils and a further three species live in dung or compost heaps.

Earthworm taxonomy is under constant revision. The present study follows the Linnean Society classification (Gerard, 1964) and a recent revision of the valid names of the Lumbricidae (Easton, 1983). The earthworms that were used in the experimental work included *Lumbricus terrestris* (Linnaeus), *Aporrectodea longa* (Ude), *Aporrectodea caliginosa* (Savigny) and *Eisenia fetida* (Savigny) and, with the exception of *E. fetida*, have been described adequately by these authors.

The subdivision of the species E. fetida was described first by Avel (1937), who reported two types; a uniformly red form and one possessing yellow bands. The two forms were subsequently named E. fetida unicolour and E. fetida typica by Andre (1963), and then renamed by Bouche (1972) as the subspecies E. fetida andrei (Andre) and E. fetida fetida (Andre) respectively. The latter classification is adopted here.

The lumbricid genus Dendrobaena and non-lumbricid genus, Alma, Diplocardia, Eudrilus, Lampito, Pheretima, Pontoscolex and Helodrilus will also be mentioned. With the exception of Dendrobaena and Helodrilus these are tropical or semi-tropical earthworms. Further description of these non-temperate genus lies outside the scope of this thesis, although they are described fully by Edwards and Lofty (1977).

1.4 The morphology and physiology of earthworms

The principal systematic features of the earthworm include the internal and external segmentation of the body, a coelomic hydrostatic skeleton bounded by muscle layers and a thin setae-bearing cuticle that contains mucous glands and sensory cells. The cuticle is permeable to water and gases as well as many other chemicals. The vascular system consists of dorsal and ventral longitudinal vessels from which the segmental vessels arise. In the anterior segments these segmental vessels may be contractile and act as a heart. The body wall is well supplied with blood vessels, and gas exchange takes place over the cuticle. Nitrogenous excretion occurs through the nephridia, which are tubular structures that regulate the composition of the coelomic fluid in which they lie. The digestive tract is a simple tube running the length of the body, and shows some differentiation into muscular grinding and pumping structures. The nervous system consists of a concentration of ganglia and sensory structures in the anterior segments from which runs a ventral nerve cord.

This contains segmental ganglia that give rise to segmental nerves which innervate the rest of the body. Earthworms are hermaphrodite, though rarely self-fertilizing. The clitellum or 'saddle' is an area of glandular tissue that secretes the cocoon, and is only present in sexually mature individuals. The literature concerning earthworm morphology and physiology is extensive and beyond the scope of this thesis, although several excellent comprehensive reviews exist (Barnes, 1968; Edwards and Lofty, 1977; Laird and Kroger, 1981; Laverack, 1963).

1.5 The effects of chemicals upon earthworms

The reviews that exist on the toxicity of chemicals to earthworms are out of date because much more data are now available. Early surveys of the literature were limited to those chemicals used to eradicate earthworms in sports turf (Escritt and Arthur, 1948) or to the few organochlorine and organophosphorus insecticides then in existence (Bauer, 1964; Davey, 1963; Gough, 1945; Satchell, 1955a).

Edwards and Thompson (1973) and Thompson and Edwards (1974) evaluated the diverse reports with respect to the application rate of the chemical, the route by which the earthworms were exposed to the chemical and the effect that it had upon the populations of earthworms. Dean-Ross (1983) reviewed the action of benomyl and certain organophosphorus and carbamate insecticides in an attempt to define the efficiency with which laboratory tests for toxicity predicted the toxicity of chemicals to earthworms in the field. The studies of Blackshaw (1980) and Clutterbuck (1973) considered the respective effects of the benzimidazole fungicides and of various herbicides upon earthworms.

The data obtained from previous studies of toxicity using earthworms are presented in a form which corrects the deficiencies of existing reviews (Table A1.1). The chemicals that have been tested for toxicity to

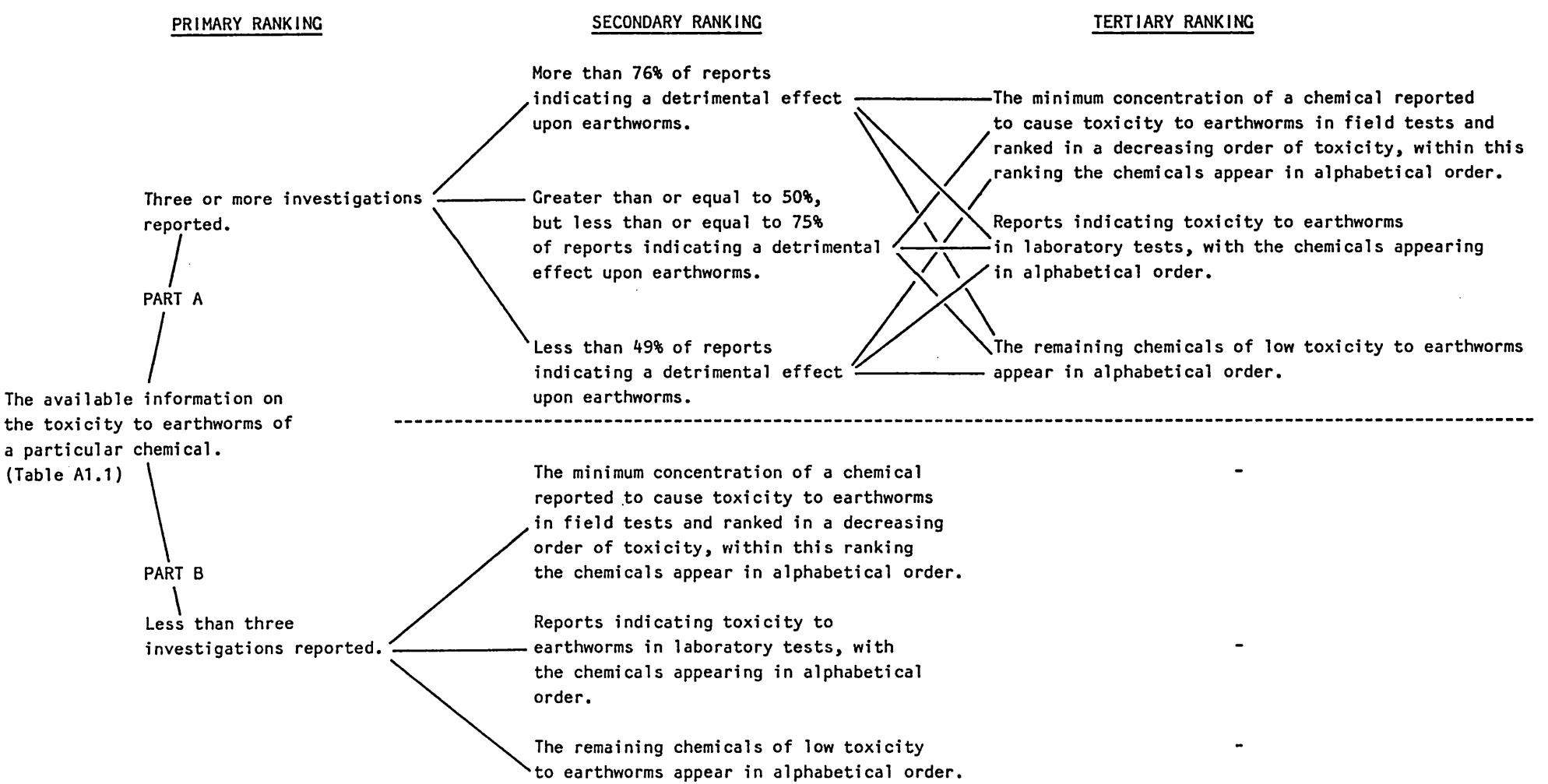
earthworms are listed alphabetically within the following sections: a) inorganic chemicals, b) pesticides of biological origin, c) aromatic and chlorinated hydrocarbon insecticides, d) organophosphorus insecticides, e) carbamate insecticides, f) synthetic pyrethroid insecticides and inhibitors of chitin formation, g) soil fumigants and nematicides, h) fungicides, i) herbicides and j) miscellaneous chemicals. The reports of these studies include details of the rate at which the chemical was applied in the field or the concentration that was used in a laboratory test, the period of exposure, the method of exposure and the species of earthworm used, together with the effect of the chemical upon earthworms and the literature reference. The majority of this information was collected from the original publications, but where important data could not be verified, they are included with an acknowledgement of the secondary source.

The data in this tabular form are still difficult to interpret due to the variety of species of earthworm and methods of assessment that have been used. I have therefore summarised this information by sorting the available data systematically according to a) the number of experiments conducted with a particular chemical and b), whether the toxicity of a chemical to earthworms was seen under field or laboratory conditions (Figure 1.1). This allowed the chemicals to be ranked according to their toxicity to earthworms (Table 1.1).

The sequence in which the chemicals appear should be used only as an indication of the relative toxicity to earthworms, as the sorting process introduced a slight bias into the ranking. Chemicals known to be toxic to earthworms in the field were often used subsequently at high concentrations in laboratory tests, and vice versa, in an attempt to elicit a positive result or prove a particular point. This increased the number of experiments that were done using chemicals that showed a toxic effect.

Figure 1.1

The method by which the data on the toxicity of chemicals to earthworms was ranked



NB. The data for chemicals that are well represented in the literature are given greater weight than those for poorly represented chemicals. Furthermore, the results of field studies are given greater weight than those of laboratory studies.

Table 1.1. Ranked toxicity of chemicals to earthworms

	Ranking	Inorganic chemicals and pesticides of biological origin	Aromatic and chlorinated hydrocarbon insecticides	Organophos-phorus insecticides	Carbamate insecticides	Pyrethroid insecticides, nematicides, soil fumigants and miscellaneous pesticides	Fungicides	Herbicides
PART A	More than 76% of reports indicating a detrimental effect upon earthworms (Within this section the tertiary ranking was made using the criteria described in Figure 1.1)	Rotenone Lead arsenate Mowrah meal Sodium chlorate	Endrin Chordane	Phorate Terbufos Ethoprophos Trichlorphon	Carbaryl Bufencarb Carbofuran Propoxur Methomyl Aldicarb	Chloropicrin Formaldehyde Dazomet	Benomyl Carbendazim Thiophanate-methyl	Atrazine Pentachlorophenol Trichloroacetic acid
		-	Endosulphan	Fenamiphos Fonofos Malathion Paraoxon Phosalone	Thiofanox	-	Thiabendazole	3-Aminotriazol Chlorpropham 2,4-D Diuron
		-	-	-	-	-	-	-
	Greater than or equal to 50%, but less than or equal to 75% of reports indicating a detrimental effect upon earthworms. (Within this section the tertiary ranking was made using the criteria described in Figure 1.1)	Copper sulphate	Heptachlor Isobenzan Dieldrin	Chlorpyriphos Diazinon Fensulfothion Chlorfenvinphos Parathion	Methiocarb Oxamyl	DD	-	Simazine Trichloroacetic acid Paraquat
		Enterobacterin	DNOC	-	-	-	Thiram	Bromacil Chloroacetamide Methabenzthiazuron
		-	-	-	-	-	-	-

Table 1.1. Ranked toxicity of chemicals to earthworms - continued

	Ranking	Inorganic chemicals and pesticides of biological origin	Aromatic and chlorinated hydrocarbon insecticides	Organophos-phorus insecticides	Carbamate insecticides	Pyrethroid insecticides, nematicides, soil fumigants and miscellaneous pesticides	Fungicides	Herbicides
PART A	Less than 49% of reports indicating a detrimental effect upon earthworms. (Within this section the tertiary ranking was made using the criteria described in Figure 1.1)	-	Aldrin DDT HCH	Disulfoton	-	'Dioxin'	Chlorthalonil	Monuron Sodium trichloro-acetate
		-	-	Menazon Thionazin Trichloronate	-	Fenvalerate	Captan	Dalapon Glyphosate Linuron Tri-allate
		-	-	-	-	Hexostrol DBCP	Triadimefon	
PART B	The minimum concentration of a chemical reported to cause toxicity to earthworms in field tests and ranked in a decreasing order of toxicity, within this ranking the chemicals appear in alphabetical order	Mercuric chloride Potassium permanganate Calcium arsenate Sulphur	Toxaphene	Leptophos	Aminocarb	Metham sodium Methyl bromide	Aniyaline Mancozeb	Chlorthiamid

Table 1.1. Ranked toxicity of chemicals to earthworms - continued

	Ranking	Inorganic chemicals and pesticides of biological origin	Aromatic and chlorinated hydrocarbon insecticides	Organophos-phorus insecticides	Carbamate insecticides	Pyrethroid insecticides, nematicides, soil fumigants and miscellaneous pesticides	Fungicides	Herbicides
PART B	Reports indicating toxicity to earthworms in laboratory tests, with the chemicals appearing in alphabetical order	Calcium cyanamide Copper chloride Copper oxychloride Potassium bromide	Naphthalene 1-Naphthol	Acephate Azinphos-methyl Bromophos Chlormephos Chlorpyriphos-ethyl Dialifos Dimethoate Ethyl-parathion Formothion Isazophos Methamidophos Methaphenamiphos Methidathion Phosphamidon Triazophos	Ethiofencarb Promecarb	Chlormequat -chloride Cypermethrin Dibromo-ethene 2,4-dichloro -phenol 1,3-dichloro -propene P-Nitrophenol Pyrethrins 2,4,5-Trichloro -phenol	Bupirimate Captafol Ethazole Fenaminosulf Folpet Fuberidazole Ziram	Asulam Aziprotryne Chlortoluron Dinoseb Diphenamid Diquat Endothal Hexazinone MCPB Metribuzin Monolinuron Oxadiazon Prometryn Propazine Propham Pyrazone Sesone 2,4,5-T 2,3,6-TBA Terbacil Trifluralin
	The remaining chemicals of low toxicity to earthworms appear in alphabetical order	Mustard	Aramite Tetradifon	Carbophenothion Demeton-S-methyl Fenitrothion Isofenphos Methyl-parathion Monocrotophos Tetrachlorvinphos	-	Alphamethrin Diflubenzuron Maleic hydrazide	2-Aminobutane Dichloran Dinocap Maneb Quintozene Triforine	Cyanazine Cycloate Di-allate Lenacil MCPA Mecoprop Nitrofen Phenmedipham

The ranking in Part A (Table 1.1) includes chemicals that had been tested in three or more independent experiments and is thus more reliable than that for chemicals that appear in Part B, which have been studied less thoroughly. Chemicals that appear in Part B may prove to be very toxic to earthworms, but this would have to be confirmed by further studies. Field results provide an accurate estimate of the toxicity of chemicals to earthworms and are thus given greater weight in the data sorting than results obtained in the laboratory.

Where this exercise was comparable with previous reviews, the findings have generally supported the consensus of opinion (Davey, 1963; Dean-Ross, 1983; Edwards, 1980; Edwards and Thompson, 1973; Haque and Ebing, 1983a; Roberts and Dorough, 1984), although the present study is much more extensive. Future studies of the toxicity of chemicals using earthworms should be more standardised to assist the comparison of results obtained independently (Dean-Ross, 1983; Ebing and Haque, 1979; Lofs-Holmin and Bostrom, 1985).

Copper sulphate, lead arsenate and calcium arsenate are used to eradicate earthworms occasionally, whilst other old fashioned pesticides, such as sulphur, sodium chlorate, mercuric chloride or Mowrah meal are only toxic to earthworms at high application rates. The exception to this is rotenone, the isolated active ingredient of Mowrah meal, which is particularly toxic to earthworms. The aromatic or organochlorine insecticides (endrin, chlordane, endosulphan, heptachlor, isobenzan, dieldrin and DNOC), the organophosphorus compounds (phorate, turbufos, ethoprophos, trichlorphon, fenamiphos, fonofos, malathion, paraoxon, phosalone, chlorpyriphos, diazinon, fensulfothion, chlorfenvinphos and parathion) and the carbamate insecticides (carbaryl, bufencarb, carbofuran, propoxur, methomyl, aldicarb, thiophanox, methiocarb and oxamyl), kill earthworms at a concentration equivalent to that achieved by an application to the field at the rate recommended by the manufacturer.

Nematicidal compounds such as chloropicrin, formaldehyde, dazomet and D-D, and the benzimidazole fungicides including benomyl, carbendazim, thiophanate-methyl and thiabendazole, together with the fungicide thiram are also very toxic to earthworms. Furthermore, there is growing evidence to suggest that the herbicides atrazine, 3-aminotriazol, chlorpropham, 2,4-D, diuron, simazine, trichloroacetic acid, paraquat, bromacil, chloroacetamide and methabenzthiazuron may also reduce the size of populations of earthworms.

Some pesticides may cause an increase in the number of earthworms indirectly. This is, perhaps, a predictable effect of chemicals that increase the amount of leaf litter available, such as paraquat or cyanazine. But reports that fenitrothion, isofenphos, thionazine, HCH and aldrin also favour earthworms are explained less easily. The population of earthworms possibly becomes larger than that found in the soil before the application of a non-persistent toxic compound, due to the immigration of adults and by the recruitment into the population of earthworms that hatch from cocoons (Edwards and Brown, 1982).

Efficient methods for testing the toxicity of chemicals to earthworms, together with improved techniques for the application of pesticides should help to preserve natural populations of earthworms. With a greater understanding of the beneficial effects of the activity of earthworms upon the fertility of agricultural soils, it may become possible to calculate the damage that is caused by toxic chemicals to earthworm populations in economic terms. The cost of maintaining soil fertility by cultivation and the addition of fertilisers in the absence of earthworms could be balanced against the losses anticipated from witholding crop protection practices (Bouche, 1984a). This conflict of interest may be partially reduced by the introduction of new pesticide application techniques. Electrostatically charged or ultra-low volume sprays (Stringer and Lyons, 1977) and band placement of granules (Ruppel and Laughlin, 1977) do less damage to

earthworms, whilst new pesticides such as insect hormones and bacterial products are less toxic to earthworms than older materials. The use of improved techniques for pesticide formulation and integrated pest control programmes should also help to protect earthworms by reducing the risk of applying an overdose of pesticide (Graham-Bryce, 1977; Walker, 1979).

Residues of chemicals accumulate in earthworms and may be distributed by them within the "food web". This extensive subject has been reviewed thoroughly elsewhere (Edwards and Lofty, 1977; Moriarty, 1977; Thompson, 1973). The first compounds to be investigated for bioaccumulation were the organochlorine insecticides (Beyer and Gish, 1980; Davis and French, 1969; Edwards and Jeffs, 1974; Gish and Hughes, 1982; Jeffries and Davis, 1968; Wheatley and Hardman, 1964), although more recently there has been concern over the fate of long-lived contaminants such as dioxin (Fanelli et al., 1980a; Fanelli et al., 1980b; Martinucci et al., 1983) and the heavy metals (Van Rhee, 1977). Not all persistent compounds accumulate in the food web or are harmful, whilst some short-lived chemicals can be very toxic (Edwards, 1983b).

Heavy metals accumulate in earthworms and these studies have been reviewed by Beyer (1981) and Ireland (1983). Many of the terrestrial investigations concern heavy metal accumulation from mine spoil tips (Ireland, 1979), land contaminated by the emissions from metal smelting or refining works (Bengtsson, Nordstrom and Rundgren, 1983; Bull, Roberts, Inskip and Goodman, 1977; Ma, Edelman, Van Beersum and Jans, 1983; Wright and Stringer, 1980), motor vehicle emissions (Ash and Lee, 1980), municipal waste sites (Fleckenstein and Graff, 1982) and metals deliberately applied to the soil as pesticides (Ma, 1984), in material dredged from rivers (Marquenie and Simmers, 1985) or in sewage sludge (Beyer, Chaney and Mulhern, 1982).

The ability of earthworms to accumulate chemicals has been exploited for the measurement of the level of contamination in soil due to heavy

metals and long-lived organic chemicals (Ebing et al., 1984; Marquenie and Simmers, 1985; Rhett, Simmers and Lee, 1985), fluoride (Garrec and Plebin, 1984) and radioactive materials including Strontium 90 (Krivolutsky, Turcaninova and Mikhaltsova, 1982; Krivoluckij, Tichomirova and Turcaninova, 1972) and Caesium 137 (Crossley, Reichle and Edwards, 1971). Reliable methods for testing the toxicity of chemicals to earthworms would allow such experiments in which earthworms are used as "biomonitoring tools" to be calibrated more easily, and the amount of a chemical accumulated by the tissues of the earthworm could be related accurately to the concentration present in the soil.

1.6 Toxicity, ecotoxicology, assessments of toxicity and the analysis of the results from tests for toxicity

1.6.1. Toxicity

A toxic compound is one that is capable of inducing detrimental changes in a living system. Toxicity is a function of concentration, period of exposure and, with whole organisms and ecosystems, the rate of transport of the test chemical to the sensitive site. Toxic effects may appear as impaired metabolic function or a behavioural change resulting in an earlier death, reduced growth, decreased reproductive capacity or a lower efficiency in gaining, or avoiding being, a source of food.

1.6.2. Ecotoxicology

Ecotoxicology is "concerned with the toxic effects of chemical and physical agents on living organisms, especially on populations and communities within defined ecosystems, and includes the transfer pathways of those agents and their interactions with the environment" (Butler, 1978).

This description embraces the scope of my studies which, in part, were concerned with the development of methods for assessing the toxicity of chemicals to earthworms in the laboratory that were able to predict the effects of chemicals upon earthworms in the field. By manipulating particular environmental parameters and defining their effect upon the toxicity of chemicals to earthworms in the field, my work attempts to answer some of the criticisms made of earlier studies that "ecotoxicological data will become meaningful only if they can be judged in relation to the type of habitat where the compound will eventually be present" (Koeman, 1982).

1.6.3. Assessments of toxicity

I will describe the theory of those aspects of testing for toxicity that are relevant to this study, although several other authors have recently discussed this subject in detail (e.g. Butler, 1978; Duffus, 1980). A toxicity test is a bioassay and may be used to define a threshold level below which no harmful effect may be expected (Besch, 1976b). Lifespan and the rates of growth or reproduction are often used as indicators of toxicity.

Long-term tests that assess non-lethal effects of chemicals are termed chronic methods. Acute tests measure a rapid, usually lethal, effect of a single exposure to the test chemical. Some of the methods described in this study may be considered sub-acute because they show a slower response than that of an acute test, and in sub-acute tests death results from a continued exposure to the toxic chemical (DuBois and Geiling, 1959). A good testing method will define precisely the conditions within which a harmful effect or end-point occurs. The choice of end-point can have a considerable effect upon the assessment of toxicity and must be defined carefully. Sub-lethal criteria are very varied and behavioural effects, for instance, the ability to work the soil (Stenersen, 1979a) are

often judged subjectively. These criteria may be useful if accompanied by a well defined physical event such as cocoon production, which has been used as a measure of reproductive behaviour (Lofs-Holmin, 1982b). Acute and sub-acute tests generally use death as the end-point (Parkin, 1951). Death can be defined as the failure to respond to a mechanical stimulus, such as prodding the anterior segments of the earthworm with a spatula and observing a twitch reaction (Cathey, 1982; Haque and Ebing, 1983a; Hopkins and Kirk, 1957; Stenersen, 1979a). Some workers have used the physical condition of the body, whether flaccid, moribund or dead, as an indication of acute toxicity (Karnak and Hamelink, 1982), but this is difficult to assess reliably, particularly by inexperienced workers!

The results of tests for toxicity are often expressed in terms of the concentration affecting a given percentage of test organisms within a certain period (e.g. x hour LC_{50}), or the period required for a given percentage of the test organisms to die at a single concentration of the test chemical (e.g. x concentration LT_{50}). From a statistical aspect, the most reliable estimate of toxicity is based upon the concentration of test chemical, or period of exposure at which 50% of the test organisms are affected. Such values are used frequently to compare the effects of different chemicals and the various types of value that are used in my work include the LC_{50}, LD_{50}, LT_{50}. These toxicity data were processed statistically by probit analysis.

1.6.4. The analysis of the results from toxicity tests

Probit analysis is a technique that is often used to process the type of 'exposure'-mortality data discussed above. The statistical considerations of the dose-mortality relationship and of probit analysis are beyond the scope of this thesis and are the subject of several detailed works (Healy, 1950; Kuenen, 1957; Finney, 1971, 1978; Wadley and Sullivan, 1943), although an introduction to these techniques is given below.

Assuming that there is a toxic dose of a chemical that will kill any particular organism, then the individual lethal doses within a population of organisms will be normally distributed about a mean. The mean and the standard deviation may be estimated by fitting a line to the observed data. This procedure may be simplified by two transformations of the data. The assymetry present in the usual 'S' shaped dose-response curve is removed by assuming a log-normal, rather than a normal distribution of the individual lethal doses, whilst the mortality data are transformed into Normal Equivalent Deviations (NED's) from the mean. A normal equivalent deviation will contain a predetermined proportion of data that are normally distributed, for instance 16.6% of the test organisms will be killed by a dose within the range of the mean lethal dose minus one standard deviation, which has an NED of -1. To avoid the inconvenience of using negative values, a "probit" value is adopted which is the NED +5. Linear regression may then be used to fit a line to the dose-response relationship obtained and any deviations from linearity that occur may be assumed to arise from sampling errors. The mortality data near the 50% value show least variability and are thus used generally for comparisons between the toxicity of chemicals. Data analysed in this way must include one or more observations of partial mortality, i.e. when some of the test organisms survive in any particular set of exposure conditions (Bernstein and Weatherall, 1952; Finney, 1971).

Fiducial limits and confidence limits, whilst not interchangeable measures of the variability of an LC_{50} or LT_{50} are often approximately equal (Finney, 1971). They indicate the limits within which the true LC_{50} or LT_{50} would be expected to fall for a given proportion of repeated experiments, often 95%, should these investigations be conducted with the same population of test organisms under the same conditions. Therefore, fiducial and confidence limits do not give the best indication of the precision of an acute mortality test, its repeatability or reproducibility,

since they relate to the experiment that has already been conducted (Stephan, 1977).

Differences between replicated estimates of toxicity may be analysed by chi-squared (χ^2) tests for the total heterogeneity, parallelism and Y-intercept position of the replicate probit lines. The chi-squared test for the position of the Y-intercept is only meaningful when that for parallelism is not significant. These comparisons may give an indication of the reproducibility of an experimental result.

1.7 Methods for assessing the toxicity of chemicals to earthworms.

Field experiments were amongst the first techniques to be used to assess the toxicity of chemicals to earthworms (Anon, 1983; Edwards and Lofty, 1971; Edwards and Brown, 1982; Thompson, 1971). These methods remain the only reliable way of estimating toxicity to earthworms in the field, although such methods are difficult to standardise and are both slow and expensive to conduct. I have summarised the details of these methods (Table 1.2), together with those of the techniques for sampling populations of earthworms using formalin (Raw, 1959), baits (Lofs-Holmin, 1979) or an electric current (Rushton and Luff, 1984). This information is presented as a) the type of test and the form in which the results are usually expressed, b) the species of earthworm studied, if reported, c) a description of the experimental method, d) a critical commentary upon the merits of the method for testing the toxicity of chemicals to earthworms and e) the source of reference.

Methods of assessing the toxicity of chemicals to earthworms in the laboratory are presented in a manner similar to that used for the field techniques (Table 1.3). Some of these methods were reported subsequently to the filter paper contact and artificial soil tests that were developed during this study, and benefited from my work by receiving a description of these new methods for testing toxicity to earthworms during

the course of collaborative work. Methods that are derived from my own techniques are indicated by an asterisk.

Several methods for testing toxicity to earthworms in the laboratory have proved to be valuable as research tools, but do not reproduce the conditions under which earthworms are exposed to chemicals in the field and yield results from which it is difficult to extrapolate to the effects of chemicals upon a natural population of earthworms. Such methods include measurements of nervous activity by electrophysiological means (Drewes and Callahan, 1985; Drewes, Vining and Callahan, 1984), topical application of the test chemical (Aspock and An Der Lan 1963; Fisher, 1984), immersion of the earthworm in a solution of chemical (Lebrun, DeMedts and Wauthy, 1981; Martin and Wiggins, 1959) and contact between the earthworm and a deposit of the test chemical on a glass or filter paper surface. The injection of the test chemical into the coelomic cavity of the earthworm (Gilman and Vardanis, 1974; Nakatsugawa and Nelson, 1972; Stenersen, Gilman and Vardanis, 1973) and voluntary feeding (Stringer and Wright, 1973) or forced feeding (Stringer and Wright, 1976) with a treated substrate also failed to reproduce the natural method of exposure to chemicals experienced by most species of earthworm.

Some testing methods used in the laboratory have employed paper waste (Cathey, 1982) or vermiculite (Bharathi and Subba Rao, 1984) to act as a soil substitute; however such materials do not interact with the test chemicals in the same way as a soil and do not produce assessments of toxicity to earthworms comparable with those made in a soil. A test in which the earthworms were exposed to chemicals mixed into a soil (Anon, 1983; Caseley and Eno, 1966: Edwards and Jeffs, 1974; Fayolle, 1979; Haque and Ebing, 1983a; Martin, 1982; Ruppel and Laughlin, 1977) or a medium that behaved like a soil, e.g. fine silica (Ferriere, Fayolle and Bouche, 1981) seemed to offer the best conditions for testing the toxicity of chemicals to earthworms, although it is difficult to obtain identical natural soils from different countries.

Table 1.2. Methods for testing the toxicity of chemicals to earthworms, and sampling for earthworms, in the field.

Type of test	Species	Experimental method	Comments and Conclusions	Reference
Field experiment	Unspecified	A minimum plot size of 10m x 10m was used and the test chemicals were applied at two rates to cropped vegetation. A chemical toxic to earthworms such as benomyl or chlordane was used as a standard. The rate, time and method of application of the test chemicals were performed as recommended by the manufacturer. Earthworms were sampled between August and November by formalin extraction.	This method was developed from that of Edwards and Lofty (1971), designed for industrial use and adopted by the UK Pesticides Safety Precautions Scheme. Sampling was timed to coincide with maximum earthworm activity in the soil.	Anonymous 1983
Field experiment	Unspecified	Plots measuring 3m x 3m were ploughed out of old grassland and the test chemical was broadcast or applied to the soil as a spray and rotavated in immediately. Earthworms were extracted using formalin after 150 days.	This plot size may be too small to prevent a substantial reinvasion of earthworms during the course of the experiment (Edwards and Brown, 1982; Martin, 1976). The use of permanent grassland sites usually ensures that the initial population of earthworms is large. Species data are often lacking from the reports of early field experiments (Lofs-Holmin and Bostrom, 1985).	Edwards and Lofty 1971
Field experiment	L. terrestris, A. caliginosa and L. festivus (Savigny)	Plots measuring 6m x 6m were arranged in randomised blocks. The test chemicals were used at the manufacturers' recommended rate and at 10 times this rate. Benomyl was used as a toxic standard. The test chemicals were applied to a cut grass sward but were not incorporated. Earthworms were sampled in the spring and autumn by formalin extraction (i.e. after 30, 180 and 395 days).	This method was recommended by the authors as a standard technique for measuring the toxicity of chemicals to earthworms in the field. Earthworms appeared to become more numerous in plots treated with herbicides that caused leaf litter to become more abundant. This antagonised the toxic effects of some of the test chemicals.	Edwards and Brown 1982
Field experiment	Unspecified	Plots measuring 3.04m x 3.04m were arranged in a Latin square and the test chemicals were applied to a cut grass sward but not incorporated. Earthworms were sampled after 21 days by formalin extraction.	This method (adapted from Edwards and Lofty [1971]) simulated farm conditions, where the chemicals often reach the soil by penetrating an existing cover of vegetation.	Thompson 1971

Table 1.2 continued

Type of test	Species	Experimental method	Comments and Conclusions	Reference
Baiting	A. caliginosa, A. rosea (Savigny) and L. castaneus (Savigny)	Perforated plastic one litre pots, containing various mixtures of organic materials and mineral soil, were buried at field sites and recovered after a given period. The earthworms that were attracted to these media were counted.	Equal quantities of clay, and farmyard manure or sewage, was the most desirable mixture for earthworms. A moist proteinaceous mixture attracted L. castaneus, whilst that with a lower organic matter content favoured A. rosea. A. caliginosa was less discriminating and was attracted to a wider range of baits. The method appeared to be a reliable, non-destructive, qualitative method of sampling populations of earthworms in the field. The disappearance of leaf litter from nylon gauze bags has also been used as an indirect measure of earthworm numbers (Broadbent and Tomlin, 1982). The disadvantage of such methods is that they rely upon the feeding behaviour of the earthworms. This behaviour may vary and influence the estimate of the size of the population of earthworms.	Lofs-Holmin 1979
Formalin extraction	Unspecified	Formaldehyde was obtained as a 40% industrial reagent, and 4.5 litres of a 0.2% a.i. solution was watered onto 0.37m^2 quadrates (0.6m x 0.6m). A second application, 20 minutes later, recovered most of the remaining earthworms. Potassium permanganate was originally used as the expellant in this method and is still used occasionally (Stuurman and Kamp, 1971).	Formalin expels earthworms from the soil efficiently, but such samples are biased against the deep dwelling species except L. terrestris. These samples are also affected by the developmental stage of the earthworms, the soil conditions and the season (Bouche 1969). Samples obtained by expellants or handsorting were less accurate than those from soil washes floated in $MgSO_4$ solution (Raw, 1960b), or from a formalin extraction of the deeper soil layers combined with a wash of the top 20 cm of soil (Bouche and Gardner, 1984). Handsorting is still used in some studies (Lofs-Holmin, 1982a; Ma, 1982) but can be laborious and inaccurate for deep burrowing or small species. A method in which soil cores were immersed in a solution of formalin in the field, was found to be almost as efficient as handsorting and much less laborious (Springett, 1981).	Raw 1959

Table 1.2 continued

Type of test	Species	Experimental method	Comments and Conclusions	Reference
Electrical extraction	Unspecified	A 35.2cm diameter ring of 15 steel electrodes, each electrode measuring 0.79 cm in diameter, held 7.2 cm apart with a similar electrode in the centre, was used to collect earthworms from a well defined volume of soil. A current of 0.4A (240V) was applied for 20 minutes.	This method was based upon earlier techniques (Edwards and Lofty, 1975; Satchell, 1955c) and it was found that shallow dwelling species and juveniles were extracted more efficiently than deep dwelling species and adults. The moisture content of the soil affected the efficiency with which the earthworms were extracted and the technique was slow to operate.	Rushton and Luff 1984

Table 1.3. Methods for testing the toxicity of chemicals to earthworms in the laboratory

Type of test and measurement of toxicity obtained	Species	Experimental method	Comments and Conclusions	Reference
*Electrophysiological measurement (EC_{50} conduction velocity of a nerve fibre)(1)	L. terrestris, E. fetida and 10 other species	The conduction velocity of impulses in giant nerve fibres was recorded in unanaesthetised earthworms at 22-26°C, using an external electrode grid. The earthworms had previously been exposed to test chemicals in the filter paper contact test that was developed in the present study (Goats and Edwards, 1982, 1985).	This technique gave a rapid indication of sublethal neurotoxicity but the results were difficult to relate to toxicity in the field and an elaborate apparatus was required. The electrophysiological method was a simplification of that described by Drewes, Callahan and Fender (1983).	Drewes and Callahan (1985) Drewes, Vining and Callahan (1984)
Topical application (LD_{50})	L. terrestris	A suspension of the test chemical was applied to the epidermis of an earthworm using a paint brush or a microapplicator.	Mucous exudates impair the contact between the test chemical and the earthworm (Stringer and Wright, 1976), although such tests have been used successfully with insects (e.g. Taylor 1982).	Aspock and An Der Lan (1963). (From Ebing and Haque 1979) Fisher (1984)
Injection into the coelemic cavity (LD_{50})	L. terrestris	The test chemical was injected in 5 µl of ethanolic solution into the coelomic cavity of a cooled earthworm, which was observed subsequently in soil for 15 days at 15°C.	This technique injured the body, was slow to use and did not deposit the test chemicals in a reproducible manner (Stenerson, 1981). The results from this method were difficult to relate to the toxicity of chemicals to earthworms in the field and the effect of the test chemical could not be differentiated from that of the solvent.	Nakatsugawa and Nelson (1972) Stenersen, Gilman and Vardanis (1973) Fisher (1984)
Injection into the coelomic cavity (LD_{50})	E. fetida	The technique of Stenersen et al. (1973) was modified slightly and 1 µl of the test chemical was injected into the coelomic cavity. The effects were observed in soil for 5 days.	The disadvantages of this method are the same as those mentioned above.	Gilman and Vardanis (1974)

Table 1.3 continued

Type of test and measurement of toxicity obtained	Species	Experimental method	Comments and Conclusions	Reference
Voluntary feeding upon treated food material (LD_{50})	L. terrestris	One cm^2 discs of apple leaf were pre-soaked in water for 2 days to soften them and treated with the test chemical in a settling tower. The earthworms were observed for any toxic or antifeedant effect of the test chemical for 2 days.	This method did not distinguish between antifeedant or toxic effects, or between the uptake of the chemical over the cuticle or the gut of the earthworm. There are few suitable species for this type of test. Fayolle (1979) successfully used a method in which the test chemical was applied to a food that consisted of leaf litter and bran.	Stringer and Wright (1973)
Forced feeding (LD_{50})	L. terrestris	The test chemical was suspended in a 1.5% agar-agar gel and injected into the oesophagus of an earthworm. The earthworm had been anaesthetised previously in a 10% aqueous ethanol solution. A motor driven Hamilton syringe, fitted with a blunt glass needle, was used to make the injection and the effects were observed subsequently for an unspecified period in a moistened plastic box.	The technique was slow, awkward to use and only suitable for species of earthworms with large bodies, although no regurgitation of the gel was seen. This method assessed uptake of the chemical over the gut alone. The use of an anaesthetic (also employed in other methods) was undesirable, as it can interact with the toxic effects of the test chemical.	Stringer and Wright (1976)
*Filter paper contact (Accumulation)	A. caliginosa and L. rubellus (Hoffmeister)	Filter paper discs, held in 9cm diameter Petri dishes, were treated with the test chemical which was dissolved in 1 ml n-hexane. The paper was wetted with 2 ml H_2O after the solvent had evaporated and the earthworms were exposed to chemicals on the paper for 8 days at 20°C.	This type of method is often used for testing toxicity to insects (Morrison, 1950; Parkin, 1951; Taylor, 1982). L. rubellus and A. caliginosa survived under these conditions for 3 and 20 days respectively. The authors assumed that the test chemical was only taken up over the cuticle and that the earthworms did not eat the filter paper. I have seen earthworms consume filter paper under similar conditions.	Reinecke and Nash (1984)

Table 1.3 continued

Type of test and measurement of toxicity obtained	Species	Experimental method	Comments and Conclusions	Reference
*Filter paper contact (LC_{50})	L. rubellus and E. fetida	The method used was very similar to the 48 hour filter paper contact test developed in the present work (Goats and Edwards 1982, 1985). Organic solvents were removed from the filter paper by applying a jet of hot air for three minutes.	The test chemicals were ranked according to their toxicity to earthworms in a manner similar to that of Goats and Edwards (1985), although the removal of the solvent using hot air could decompose some test chemicals. E. fetida was less sensitive to chemicals than L. rubellus although regarded by these workers as very suitable for this type of test. The results from this method appeared to correlate poorly with previous studies of toxicity made using fish and rats.	Roberts and Dorough (1984)
Immersion (LC_{50})	L. terrestris	The earthworms were observed for 24 hours following immersion for 5 hours in an aqueous solution of the test chemical.	Stable suspensions of non-water soluble chemicals in water are difficult to produce. The duration and route of exposure make it difficult to extrapolate from the results of this type of test, to predict the toxicity of chemicals to earthworms in the field. Dean-Ross (1983) criticised immersion tests for giving an underestimate of the toxicity of chemicals to earthworms in the field.	Lebrun, DeMedts and Wauthy (1981)
Immersion (LC_{50})	L. terrestris	The earthworms were exposed to the test chemicals by immersion for 2 hours in an aqueous solution of the chemical at 22°C, followed by a further 24 hours in peat moss that was wetted with an unspecified amount of the immersion solution.	The conditions of exposure were defined poorly and made the data difficult to interpret. The test species may be L. rubellus and not L. terrestris as reported by the authors (Martin, 1982). Ghabbour and Imam (1967) also used this method with A. calignosa, Pheretima californica and an Alma sp.	Martin and Wiggans (1959)

Table 1.3 continued

Type of test and measurement of toxicity obtained	Species	Experimental method	Comments and Conclusions	Reference
Immersion (LC_{50})	L. terrestris, A. caliginosa, L. rubellus and E. fetida.	The earthworms were exposed to the test chemicals for 30 minutes in 2 ml of an ethanolic solution of the chemical. The effects were observed by placing the earthworms in soil for 80 days.	The mortality of the earthworms and their ability to work the soil were used to measure the toxicity of the chemicals to earthworms. Solvents that remain in the test system may also exert a toxic effect. This method suffers from similar disadvantages to those of Martin and Wiggins (1959) and Lebrun et al (1981), in that it is difficult to predict the effects of chemicals upon earthworms in the field from such results.	Stenersen, 1979a
*Laboratory soil (LC_{50})	E. fetida	An artificial soil was used that consisted of sand, kaolinitic clay, peat and $CaCO_3$ in the ratio 69:20:10:1, with a moisture content of 35% and a pH of 6±0.5. Ten adult earthworms were contained within 650 g (wet weight) soil at 20±2°C. The test chemical was applied as a spray and the deposit incorporated mechanically. Mortality was assessed after 7 or 14 days.	This was an early version of the artificial soil test that was developed during the present study (Goats and Edwards 1982, 1985). The test was submitted to, and adopted by, the U.K. Pesticides Safety Precautions Scheme.	Anonymous, 1983.
Laboratory soil (LC_{50})	L. terrestris and E. eugeniae (Grinsburg)	Two types of soil, an Everglades muck and an Arredondo loamy sand were sprayed with test chemical and the deposit incorporated uniformly. Ten mature earthworms were contained within 750g (wet weight) of soil for 32 days at 27-30°C. Corn meal and enough water to maintain the soil at 75% of field capacity was added every 2 days. Any dead earthworms were removed during these inspections.	The results of this study were not analysed statistically. Both the species of earthworm and type of soil influenced the results. Mortality was higher in the sandy soil and E. eugeniae was the more susceptible species. The authors reported that dead earthworms released toxins that affected the assessments of toxicity and that an unsuitable particle size distribution in the soil or a build-up of excretory products may increase mortality in the controls. Milne and DuToit (1976) also reported that soils with a high organic matter content may reduce the toxicity of chemicals to earthworms in the laboratory.	Caseley and Eno, 1966

Table 1.3 continued

Type of test and measurement of toxicity obtained	Species	Experimental method	Comments and Conclusions	Reference
Laboratory soil (Accumulation)	L. terrestris	A sieved, pesticide-free natural soil was sprayed with test chemical and the deposit incorporated mechanically. Approximately 8.75 kg of soil (wet weight) was contained within a wooden box and held 10 mature earthworms for 180 days.	The earthworms survived well in these conditions and this method was adapted for use in further studies (Edwards and Lofty, 1973). The test chemical was applied as a spray, although I have found that this method of application is a compromise between achieving a deposit that is distributed uniformly in the soil and the loss of test chemical through spray drift.	Edwards and Jeffs 1974
Laboratory soil (Sublethal effects)		Slices of soil 9 cm thick were sandwiched between plates of glass. The behaviour of the earthworms could be observed for extended periods in soils that had been treated with test chemical or were untreated.	The assessment of the toxicity of chemicals to earthworms was subjective, but this method allowed sublethal effects upon the behaviour of the earthworms to be observed directly.	Edwards and Jeffs 1974
Laboratory soil (LC_{50})	A. chlorotica (Savigny) and E. fetida	A natural clay soil and a sand were sieved together (ratio 2:1) to give a medium with a pH of 7 at a moisture content of 25%. 20-25 adult A. chlorotica or 20 E. fetida were contained within 3.5 kg soil (wet weight) at 15° or 22.5°C respectively, for 7 days.	This test attempted to reproduce a population density of earthworms that was equivalent to 2 tonnes.ha^{-1} in the field. The relationship between the rate of application of a chemical in the field and the resultant concentration of chemical in the soil (and hence the relationship between the results of this test and those from field experiments) was based upon the unusual assumption that a chemical will only penetrate the top 1 cm of the soil in the field. E. fetida would not enter the soil and the type of soil was defined poorly. The author observed that dead earthworms affected the assessment of toxicity and that a 7 day period of exposure was too short to predict accurately the toxicity of chemicals to earthworms in the field.	Fayolle, 1979

Table 1.3 continued

Type of test and measurement of toxicity obtained	Species	Experimental method	Comments and Conclusions	Reference
Laboratory soil (LC_{50})	L. terrestris	The media consisted of 900g sandy loam soil, 100g air dried and milled peat moss with 2g maize flour and 35% H_2O. 1.5kg (wet weight) of the soil contained 6 earthworms at 10-15°C in darkness for 14 days. Chemicals were incorporated into the soil without using solvents.	The organic matter content of this soil was very high when compared with that found in most arable soils. The use of this type of test in darkness would encourage the earthworms to escape from the media.	Haque and Ebing 1983a
*Laboratory soil (LC_{50})	E. fetida	An artificial soil was prepared from 835g quartz sand, 100g air dried and milled peat moss, 50g bentonitic clay, 10g $CaCO_3$, 5g air dried cow manure, 50 ml of soil leachate and 450 ml H_2O. 0.5 kg of this mixture contained 6 earthworms at 22°C in the light for 14 days. Test chemicals were incorporated into the soil without using solvents.	This method was adapted from that developed in the present study (Goats and Edwards 1982, 1985). The media were less easy to standardise because animal faeces and soil infusions were incorporated into the soil.	Haque and Ebing 1983a
Laboratory soil (LC_{50})	L. terrestris	Several variants of this medium were described which consisted generally of 850g soil, 50g (dry weight) rabbit faeces and 100 ml H_2O. 0.5 kg (wet weight) of soil contained 5 earthworms at 13°C in the dark for 14 days. Test chemicals were incorporated into the soil without using solvents.	The animal faeces were difficult to standardise as discussed previously and were toxic above a certain concentration. The determination of the end-point relied upon a subjective estimate of the condition of the body of the earthworm using the categories flaccid, moribund and dead. Subjective assessments of toxicity of this type can be unreliable and reduce the reproducibility of the results that are obtained.	Karnak and Hamelink 1982

Table 1.3 continued

Type of test and measurement of toxicity obtained	Species	Experimental method	Comments and Conclusions	Reference
Laboratory soil (Sublethal effects)	L. terrestris, A. caliginosa, A. chlorotica and A. rosea.	Several variants of the soil were described which consisted of mixtures of a natural mineral soil, farmyard manure and clay with a moisture content of 35%. 250 ml of the media contained one juvenile earthworm for 90 days at 10°C. Weight gain and sexual maturity were recorded at 7 or 15 day intervals. Test chemicals were incorporated into the soil as an aqueous drench, or without the use of any solvents.	The growth rate and the period required for an earthworm to reach sexual maturity were considered to be good indicators of sub-lethal toxicity to earthworms. The author also suggested that earthworms should be cultured under standard conditions before use in a toxicity test. Other workers go further and recommend that earthworms are acclimatised to the conditions within the test before the test chemical is applied (Tomlin 1977).	Lofs-Holmin 1980
Laboratory soil (Sublethal effects)	A. caliginosa	A mixture of clay and farmyard manure containing 30% H_2O was drenched with an aqueous solution of test chemical. Five adult earthworms were held in one litre of the mixture for 26 days at 15°C and the number of cocoons that were produced was recorded.	The rate of reproduction was found to be a reliable indicator of the sublethal toxicity of chemicals to earthworms. The method was slow to use but allowed the toxicity of chemicals to earthworms in the field to be predicted accurately. E. fetida was considered to be unsuitable as a test species as it was less susceptible to test chemicals, and lived in a different habitat to the species found in arable soils. However, recent studies have shown that the responses of E. fetida and L. terrestris to chemicals are correlated highly (Heimbach 1985a, 1985b).	Lofs-Holmin 1982b
Laboratory soil (LC_{50})	A. caliginosa	One kg of air-dried soil from Auckland, New Zealand, was mixed with 8.8g of powdered grass and moistened to 25% H_2O content (wet weight). The test chemical was incorporated into the soil without using solvents and 65g of soil contained one earthworm for 7 days at 20°C.	The volume of soil was very low and in a short duration test such as this it was probably not necessary to add food. The author reported that a 7 day test was too short to predict accurately the toxicity of chemicals to earthworms in the field.	Martin 1982

Table 1.3 continued

Type of test and measurement of toxicity obtained	Species	Experimental method	Comments and Conclusions	Reference
Laboratory soil (LC_{50})	L. terrestris	An air-dried potting soil was wetted with an unspecified amount of water, and the test chemical was incorporated without the use of solvents. Ten earthworms were contained within the soil at 40°C and 70% relative humidity in darkness Mortality was assessed after 3 days.	This study supported field experiments using the same chemicals, but the experimental conditions were reported poorly. In the field, the method of application altered the toxicity of the test chemical to earthworms, and by using band instead of broadcast applications, the toxicity of chemicals to earthworms was reduced. Thus the method by which a test chemical is formulated and applied in the field should be considered when attempting to extrapolate from the assessments of the toxicity of chemicals to earthworms made in the laboratory.	Ruppel and Laughlin 1977
Laboratory soil substitute (LC_{50})	L. terrestris	The medium was paper based and 0.45kg of this material was drenched with one litre of an aqueous solution of the test chemical. An unspecified amount of the medium contained 10 earthworms for 42 days at 15°C.	No information was given concerning the adsorptive capacity of the media or whether it was ingested by the earthworms. The authors recognise that the conditions of the test do not reflect those found in the field. In a similar type of experiment, dampened vermiculite was used successfully to test the toxicity of chemicals to L. mauritii in the laboratory (Bharathi and Subba Rao 1984).	Cathey 1982

Table 1.3 continued

Type of test and measurement of toxicity obtained	Species	Experimental method	Comments and Conclusions	Reference
Laboratory soil substitute (LC_{50})	L. terrestris, A. chlorotica and E. fetida	The medium was a paste of amorphous silica and water. 180 g of silica was added to 414g of an aqueous solution of the test chemical and the resultant paste was mixed with 2850g of 2cm diameter glass balls, the latter provided support. An unspecified number of earthworms were contained within this mixture which was ventilated continuously with humid air.	All the species that were tested survived in, and ingested the medium. The technique was awkward to use and required sampling for mortality destructively at the end of the test. I found that the medium had a high adsorptive capacity, contrary to the reports of other workers (Dean-Ross 1983). The method was simplified subsequently and renamed "Artisol" (Bouche 1982). Without adequate ventilation, the medium became acidic and killed E. fetida, but such ventilation can cause the loss of volatile test chemicals. The authors also report supplementary experiments in which E. fetida survived for 11 days in gravel	Ferriere, Fayolle and Bouche 1981

(1) EC_{50} = The effective concentration, at which 50% of the earthworms tested showed a response to the chemical

Few critical reviews of the methods for testing toxicity to earthworms exist; thus I have attempted to consider this subject thoroughly. Ebing and Haque (1979) considered the methods that were then available for testing the toxicity of chemicals to earthworms and attempted to select a standard testing method and reference compound that would be suitable for the OECD. Although offering suggestions for the improvement of the existing techniques, which included the standardisation of the conditions of exposure (the test media, period of exposure and environmental conditions) and of the earthworms (species, physiological age and population density), these workers could not recommend that any particular method for testing the toxicity of chemical to earthworms be adopted. My work attempted to correct these deficiencies, reviewed the available methods for testing toxicity to earthworms, defined the advantages and disadvantages of each and then evaluated the promising methods experimentally.

The standard of the experimental procedures and reports that exist in the literature were reviewed by Lofs-Holmin and Bostrom (1985). These workers found that the common methodological failings included poor replication, absence of experimental controls or statistical analysis of the results, whilst the reports often failed to mention the species of earthworm used, the formulation or concentration of the test chemical or to describe adequately the test conditions. These workers and others (Bouche, 1985; Dean-Ross, 1983; Ebing and Haque, 1979; Goats and Edwards, 1985) have called for a greater standardisation and quality of the techniques that are used to test toxicity to earthworms.

1.8. The objectives of the study

1. The initial part of this study was concerned with the development of methods suitable for general use in testing the toxicity of chemicals to

earthworms. The existing testing methods were evaluated and those that appeared potentially useful were investigated experimentally. Each of these methods proved to be inadequate and it was necessary to develop new methods of testing the toxicity of chemicals to earthworms. Certain guidelines that assisted the development of these new methods were formulated initially in consultation with expert scientists from several European countries and included:

a) The test species of earthworms should be E. fetida.

b) The test should be standardised (to allow the results from different countries to be compared), sensitive (a small change in the variable of the test, e.g. the concentration of the test chemical, leading to a large change in response) and reproducible (a small change in the conditions of exposure, e.g. temperature, producing a minimal change in response).

c) The period of exposure should be short (to reduce operating costs, and to avoid decomposition of the test chemical and mortality in the controls).

d) The method should be capable of testing any chemical without elaborate formulation techniques.

e) The test should be economical and not require specialised apparatus or operator skill.

2. The reproducibility and performance of the new methods were criticised and evaluated by collaborating scientists in a large number of laboratories worldwide.

3. Field experiments were done using five chemicals applied at two rates at two sites in soils of differing organic matter content. The effect of these chemicals upon populations of earthworms was observed after one and six months post-treatment.

4. The relationship between the assessments of the toxicity of chemicals to earthworms made in the laboratory and the field was clarified. This was done by testing several chemicals in seven laboratory tests and in two field experiments, and comparing the results.

5. The new methods for testing the toxicity of chemicals to earthworms allowed differences in the susceptibility to chemicals between L. terrestris, A. longa, A. caliginosa, E. fetida andrei and E. fetida fetida to be quantified.

6. The differences in susceptibility to chlordane and iodoacetamide seen between E. fetida andrei and E. fetida fetida were investigated further. The uptake and metabolism of these chemicals by earthworms of both sub-species were studied using radiochemical and chromatographic techniques.

CHAPTER 2

THE EVALUATION OF THE PERFORMANCE OF EXISTING METHODS FOR TESTING TOXICITY TO EARTHWORMS

2.1 Introduction

The methods for testing the toxicity of chemicals to earthworms can be divided into those tests conducted in the laboratory and those done in the field. Field experiments were used in early studies and gave a reliable estimate of the toxicity of chemicals to natural populations of earthworms. Such tests were expensive to use and gave results that were poorly reproducible without extensive replication. Field tests for the toxicity of chemicals cannot be standardised completely, because the experimental conditions depend largely upon the available sites and seasons. These disadvantages made field testing inappropriate for ecotoxicological purposes.

Laboratory methods of testing the toxicity of chemicals to earthworms seemed capable of being sensitive, reproducible, standardised and inexpensive, although the methods that existed before my studies had serious disadvantages (Ebing and Haque, 1979). The methods that were already available (Table 1.3) were evaluated critically (Table 2.1) and those techniques which appeared to be most suitable for further development were used experimentally to test the toxicity of chemicals to earthworms.

Several test methods were rejected without an experimental investigation. Chemicals injected into the coelom of the earthworm using a hypodermic needle caused mechanical damage, and it was difficult to place the test chemical accurately, which reduced the reproducibility of the method (Stenersen, 1981). Furthermore, the test chemical was injected in

a solvent, which for non-water soluble chemicals may influence the assessment of toxicity. The method was slow and exposed the earthworm to the test chemical in a manner that was unrepresentative of that found in the field.

Methods that rely upon the earthworms voluntarily ingesting a treated food material did not differentiate between repellent (antifeedant) or toxic effects and were thus neither sensitive nor reproducible. This type of exposure to the test chemical was unrepresentative of that experienced by the majority of species of earthworm found in arable soil.

Chemicals may be applied topically to the body surface of the earthworm, although the mucous sheath that surrounds the body can impair contact with the test chemical. Furthermore, it is difficult to estimate the dose of the chemical that is applied to the earthworm, the method is slow to operate, and the results may be variable and difficult to relate to the toxicity of chemicals to earthworms seen in the field.

Electrophysiological methods of testing toxicity to earthworms were sensitive and reproducible, but required elaborate apparatus and also gave estimates of toxicity that were difficult to relate to the effects of chemicals upon earthworms in the field.

Testing methods that had no more than two main disadvantages (Table 2.1) were assessed experimentally. Contact tests in which the earthworms crawl over a damp surface treated with the test chemical, or methods in which the earthworms are immersed in solutions of chemicals had few serious disadvantages. However, the toxicity of non-water soluble chemicals can be difficult to measure in an immersion test, particularly when the additional toxic effect of formulants is to be avoided. The results from these methods can be difficult to relate to the assesments of the toxicity of chemicals to earthworms in the field.

Table 2.1. The advantages and disadvantages of methods for testing the toxicity of chemicals to earthworms.

Type of method	Quality					
Laboratory methods using a lethal end-point	Sensitive	Reproducible	Standardised	Adaptable for use with any chemical	Economical	Indicates toxicity in the field
Topical application	-	-	+	+	-	-
Injection into coelom	+	-	+	-	-	-
Voluntary feeding	-	-	+	+	-	-
Forced feeding	+	+	+	+	-	-
Contact methods	+	+	+	+	+	-
Immersion	+	+	+	-	+	-
Soil	+	+	-	+	+	+
Soil substitutes	+	+	+	+	+	+
Laboratory methods using a sublethal end-point						
Growth or reproduction in soil	+	+	-	+	-	+
Electrophysiological measurement of nervous activity	+	+	+	+	-	-
Field methods	+	-	-	+	-	+

+ Quality present; - Quality absent.

The method in which a suspension of the test chemical in an agar-agar gel was forced into the oesophagus of the earthworm using a syringe and needle was able to give an LD_{50}, whereas the other tests measured toxicity only as an LC_{50}. The apparatus was designed originally for use with L.terrestris, but was modified to accommodate E. fetida. This method seemed likely to predict the toxicity of chemicals to the litter feeding earthworms in the field, but the method by which the earthworms were exposed to the chemicals was unrepresentative of that experienced by many species found in arable land.

Tests of the toxicity of chemicals to earthworms using soil in the laboratory can provide data on the influence of soil type upon the toxicity of a chemical and give results that are related closely to those obtained in the field, however natural soils are difficult to standardise and this type of method can be laborious to use.

Soil substitutes such as fine silica, sand, anti-bumping granules (small glass beads) and vermiculite can also be used. Vermiculite is very absorbant and as this property would probably affect the assessment of toxicity, a test using this material was not included in the experimental investigations. The methods for testing the toxicity of chemcials to earthworms using the remaining materials appeared to have few disadvantages and were studied experimentally.

2.2 Materials, methods and results

2.2.1 Materials

The compounds tested were of technical grade unless otherwise stated and included chlordane, carbaryl, benomyl, thiophanate-methyl and triazophos. The physico-chemical data, chemical name and suppliers of these compounds, the commercially formulated pesticides and other chemicals of analytical grade that were tested including pentachlorophenol,

chloroacetamide and potassium bromide are presented in a tabulated form (Table A5.4). All the solvents were of analytical grade.

The earthworms used in the tests were sexually mature E. fetida andrei, weighing 0.4-0.6 g with an empty gut. These were grown in the laboratory from cocoons at 20°C in the dark, in culture boxes that contained pressed cow manure that had matured for three weeks beforehand. The earthworms were extracted from the manure by hand after approximately nine weeks, washed and allowed to empty their guts by overnight storage on damp filter paper before use in the experiments.

2.2.2 General methods

Several aspects of the experimental method were common to more than one testing technique and are described below. The method for each test is included with the results in a following section.

Where possible the test chemicals were used in an aqueous solution, although non-water soluble chemicals were also studied. The non-water soluble chemicals were suspended or dispersed in water using a method adapted from that of McIntosh, Bateman, Chamberlain, Dawson and Burrel (1981). The chemical was dissolved in a small volume of a suitable organic solvent and 200 µl of this solution was squirted quickly into a rapidly swirling mixture of 199.6 ml H_2O and 0.2 ml 10% Tween 80 solution in ethanol. The dispersions that were produced were stable for at least 48 hours.

The cation exchange capacity (CEC) of the test media was considered likely to influence the assessment of the toxicity of chemicals to earthworms. The CEC of the filter paper, sand, silica powder and natural soil were measured using a standard method (Bascomb, 1964) at a reference pH (8.1) and at the natural pH of the media in distilled water. By taking measurements at two pH's, this technique indicated the extent of pH dependent adsorption.

Death was assessed mechanically and the earthworms were considered to have died after failing to twitch when the anterior two or three segments were prodded firmly with a spatula. The effect of compounds that produced paralysis before death could not be assessed in this way and with these chemicals, death was recorded when the earthworm was unable to restore turgor pressure to sections of the body that had been squashed gently using a spatula.

Every experiment was accompanied by an untreated control. Where organic solvents had been used to apply the test chemicals, the solvents were included in the control treatment in a manner similar to that used with the test chemical. Where mortality in the controls exceeded 1%, or the nearest estimate to this that was possible, the results were discarded and the experiment re-run. Other workers have accepted 10% mortality in the controls (Haque and Ebing, 1983a; Lofs-Holmin, 1982b), but I considered that this was too high for the present series of experiments.

All the tests were done at 20 ± 2°C in a cooled incubator (Gallenkamp and Company Ltd).

The mortality data was processed by a computed probit analysis using the maximum likelihood programme (MLP). The data were converted into logarithms before analysis and thus the results appear on a log scale unless specified to the contrary. The differences between replicate sets of data were analysed using a chi-squared test. Where the differences between individual probit lines were not significant ($P>0.05$) for parallelism and position of the Y-intercept, these data were combined and a single probit line fitted. If the chi-squared test for parallelism alone was not significant, a combined gradient was calculated.

Under certain circumstances, the standard errors obtained by assuming a binomial distribution required adjustment. When a chi-squared test indicated that the heterogeneity of the data of an individual probit line was significant ($P<0.05$), i.e. the data did not conform to a binomial

distribution, the standard error of the LC_{50} or LT_{50} was adjusted using the following calculation

$$\text{Adjusted S.E.} = \text{S.E. } LC_{50} \text{ or } LT_{50} \sqrt{\frac{\chi^2 \text{ heterogeneity}}{\text{D.F. heterogeneity}}}$$

When the chi-squared value for the total heterogeneity of replicated probit data was significant ($P<0.05$), subsequent chi-squared tests for parallelism and the position of the Y-intercept would be inaccurate, so the differences between these probit lines were examined using an F test.

$$F = \frac{\dfrac{\chi^2 \text{ parallelism or Y-intercept position}}{\text{D.F. parallelism or Y-intercept position}}}{\dfrac{\chi^2 \text{ total heterogeneity}}{\text{D.F. total heterogeneity}}}$$

2.2.3 Immersion test

Method

The chemicals that were tested included chlordane, carbaryl, pentachlorophenol, chloroacetamide and thiophanate-methyl as the technical compounds, and chlordane (Chlordane 25), thiophanate-methyl (Cercobin) and triazophos (Hostathion) as commercially formulated products. These chemicals were dissolved or suspended in distilled water, in which one earthworm was immersed in 50 ml contained in a 100 ml glass crystallising dish. Four replicates were used at each of six concentrations of the test chemical in a logarithmic dilution series. The dishes were covered with a loosely fitting lid and mortality was determined after a 24 hour period of exposure under artificial light (Figure 2.1 and Plate 2.1).

Results

The LC_{50}'s of the test chemicals, in a decreasing order of toxicity to earthworms, were pentachlorophenol > chlordane > carbaryl > chloroacetamide > thiophanate-methyl (Table A7.1 and Figure 2.3). The gradients of the probit lines can also be ranked in decreasing order as follows; thiophanate-methyl > chlordane > chloroacetamide > pentachlorophenol > carbaryl.

The probit lines of the individual replicates of carbaryl and of triazophos were not significantly different and could be combined to give a single probit line for each chemical. Only the gradients of the probit lines for the individual replicates of the other test chemicals could be combined to give a common gradient for each of those compounds. The chemicals formulated commercially were more toxic to earthworms than the same chemical as the technical compound. No mortality occurred in the controls during a 72 hour period of immersion.

The method was easy to use and the assessment of mortality was simple, although it was difficult to produce a long-lived dispersion of some of the technical compounds in distilled water. These suspensions and those of the commercial formulations began to settle out after 48 hours. This did not influence the assessment of toxicity with the present method, but indicated that this type of test has only a limited application for assessing longer term effects.

Figure 2.1

Immersion Test

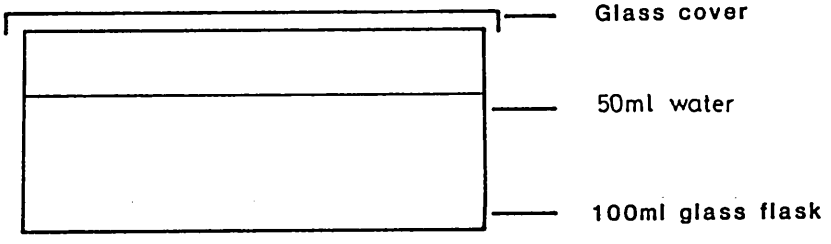
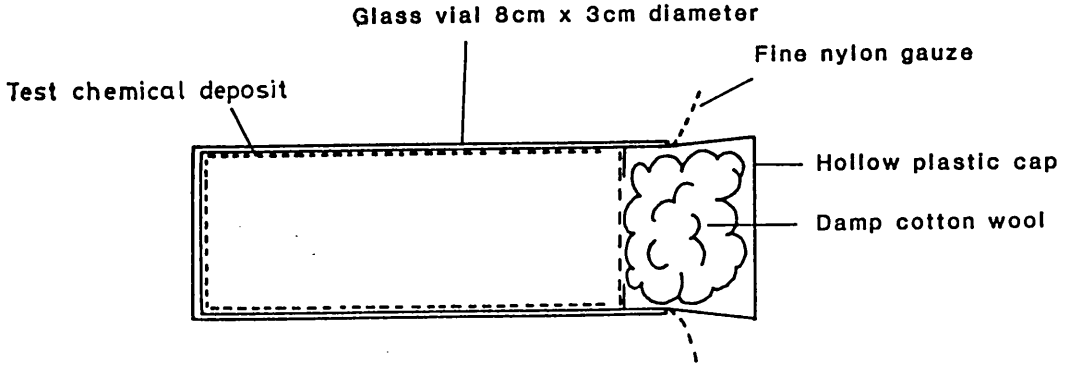


Figure 2.2

Glass Contact Test



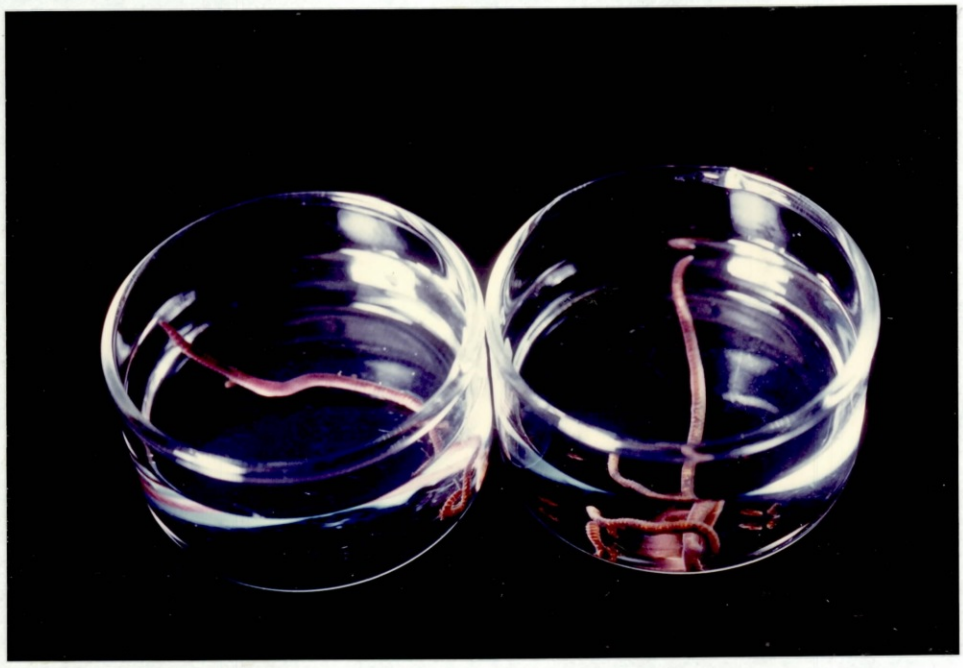


Plate 2.1. Immersion Test

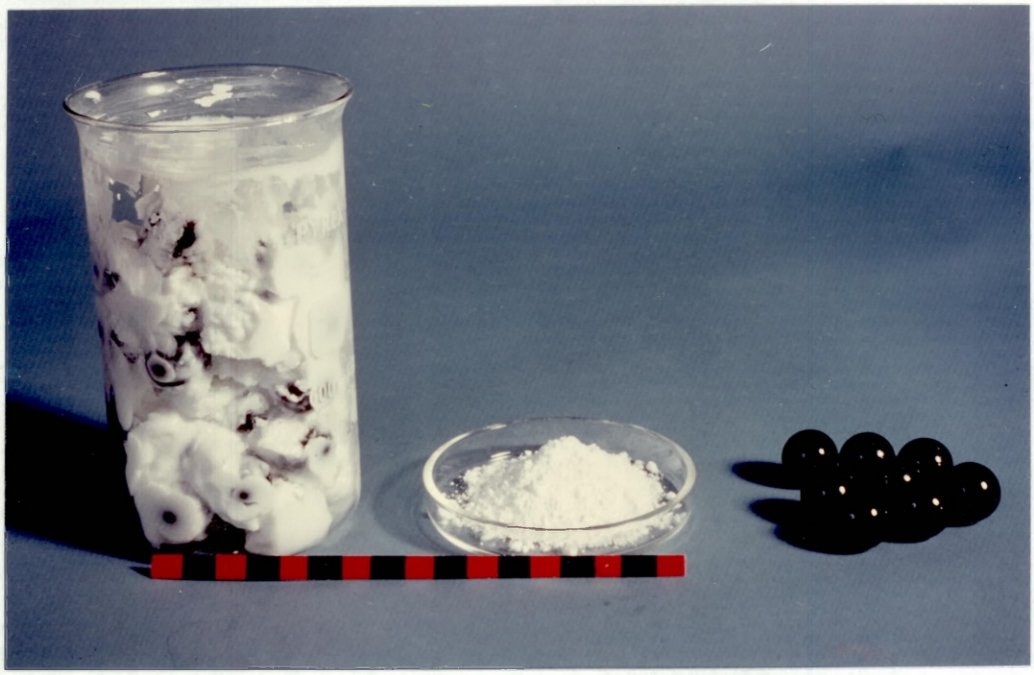
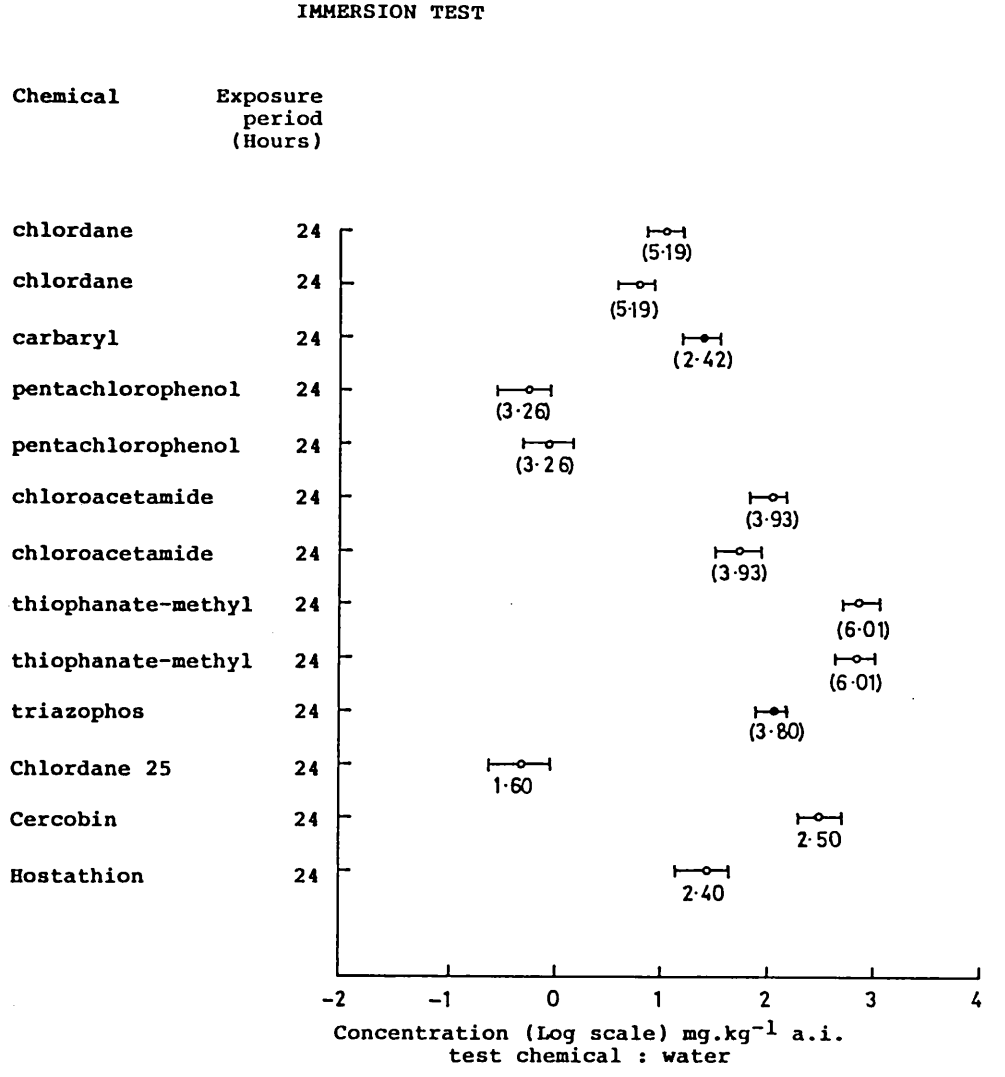


Plate 2.2. Silica paste and glass ball test.

Scale: 1 cm

Figure 2.3



KEY

⊢o⊣ x	Individual LC_{50} estimate [o], gradient [x] and fiducial limits
⊢o⊣ (x)	Individual LC_{50} estimate [o], common gradient for more than one replicate [(x)] and fiducial limits
⊢●⊣ (x)	Common LC_{50} estimate [●], gradient [(x)] and fiducial limits for more than one replicate
⊢----⊣	Range within which a poorly defined LC_{50} falls
No LC_{50} →	LC_{50} at an undefined concentration that was greater than those studied
← No LC_{50}	LC_{50} at an undefined concentration that was less than those studied
(No LC_{50})	More than one LC_{50} at an undefined concentration

2.2.4 Glass contact test

Method

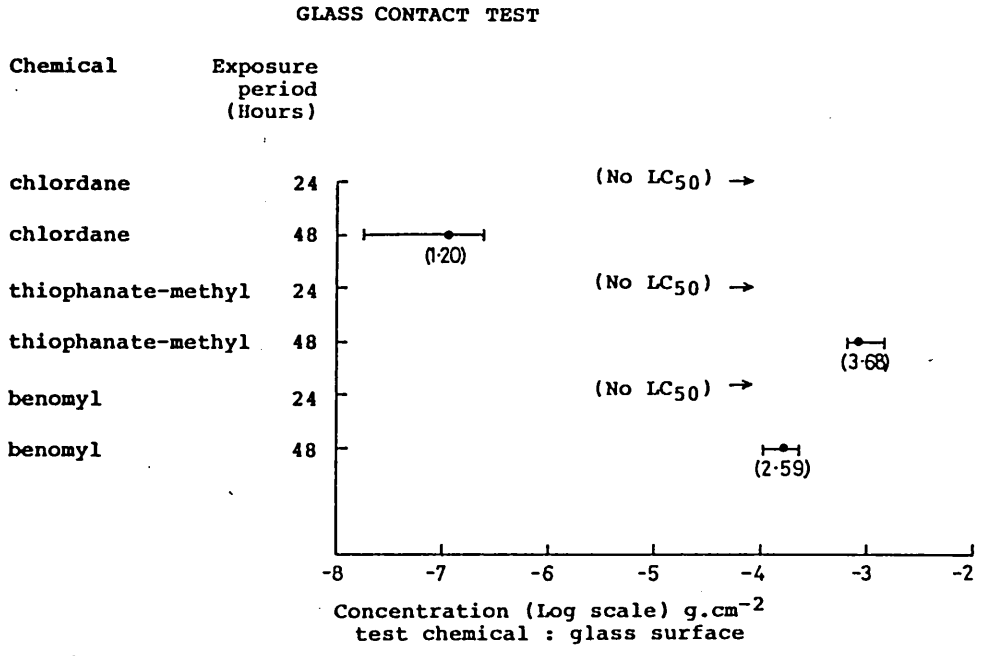
Chlordane, thiophanate-methyl and benomyl were dissolved in suitable volatile organic solvents and one ml of each of these solutions was pipetted into separate 8 cm x 3 cm diameter glass vials (internal surface area 82.5 cm^2). Each chemical was represented by 10 replicates at five concentrations in a logarithmic dilution series. The solutions were swirled in the vial to give a uniform deposit and dried gently with compressed air. One earthworm was put into each vial, which was then sealed with a plastic cap containing a wad of damp cotton wool to humidify the air. Mortality was determined after a 24 or 48 hour period of exposure conducted under artificial light (Figure 2.2).

Results

After 48 hours, chlordane was the most toxic chemical to earthworms and was followed in a decreasing order of toxicity by benomyl and thiophanate-methyl, however the gradients of the probit lines appeared inversely related to toxicity and the steepest gradient seen with thiophanate-methyl, followed by benomyl and chlordane (Table A7.2 and Figure 2.4).

The probit lines of the individual replicates of the test compounds did not differ significantly and were combined to give a single probit line for each compound. Mortality was absent from the controls during the 48 hour period of exposure, although the method for humidifying the air appeared to be inadequate. Uniform deposits of test chemical were applied with difficulty to the glass surface, particularly at high concentrations, as the deposit tended to flake off. In other respects, the method was easy to use and the assessment of mortality was uncomplicated.

Figure 2.4



KEY

⊢o⊣ x	Individual LC_{50} estimate [o], gradient [x] and fiducial limits
⊢o⊣ (x)	Individual LC_{50} estimate [o], common gradient for more than one replicate [(x)] and fiducial limits
⊢●⊣ (x)	Common LC_{50} estimate [●], gradient [(x)] and fiducial limits for more than one replicate
⊢----⊣	Range within which a poorly defined LC_{50} falls
No LC_{50} →	LC_{50} at an undefined concentration that was greater than those studied
← No LC_{50}	LC_{50} at an undefined concentration that was less than those studied
(No LC_{50})	More than one LC_{50} at an undefined concentration

2.2.5 Filter paper contact test using glass vials

Method

Chlordane, pentachlorophenol, thiophanate-methyl, benomyl and triazophos were dissolved in distilled water or a suitable volatile organic solvent. One ml of each of these solutions was pipetted into a separate 8 cm x 3 cm diameter glass vial, the side and end walls of which had been lined with Whatman No.1 cellulose filter paper (lined internal surface area 82.5 cm^2). The solution was swirled around in the vial to give a uniform deposit and the organic solvent that remained was evaporated gently using compressed air. The filter papers that were dried in this manner were moistened with 1 ml of distilled water. Ten replicates were used at each of five concentrations of the test chemical in a logarithmic dilution series and one earthworm was placed in each vial, which was then sealed with a plastic cap. Mortality was determined after a 24, 48 or 72 hour period of exposure conducted under artificial light (Figure 2.5). The cation exchange capacity of the filter paper was measured in order to estimate the amount of test chemical likely to bind to this surface.

Results

Chlordane was the most toxic compound to earthworms after 48 hours and was followed, in a decreasing order of toxicity by triazophos, pentachlorophenol, benomyl and thiophanate-methyl. The steepest probit line gradient was seen with triazophos, whilst the other chemicals had less steep gradients that could be ranked in the following decreasing order: pentachlorophenol, chlordane, benomyl and thiophanate-methyl (Table A7.3 and Figure 2.7). Pentachlorophenol, thiophanate-methyl, benomyl and triazophos failed to give an LC_{50} after a 24 hour period of exposure. The assessments of mortality made after 48 hours were used to compare the results from this test with those of the other methods, as the only chemicals for which mortality was assessed after 72 hours were the

Figure 2.5

Filter Paper in Vials Test

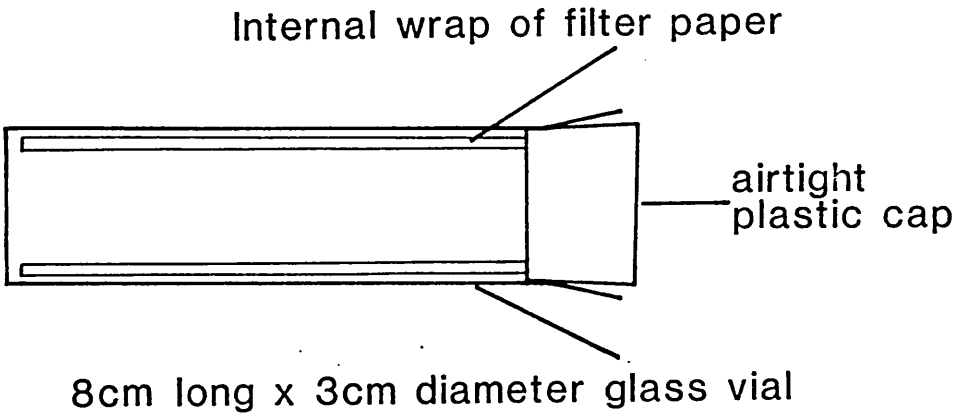


Figure 2.6

Filter Paper In Petri Dish Test

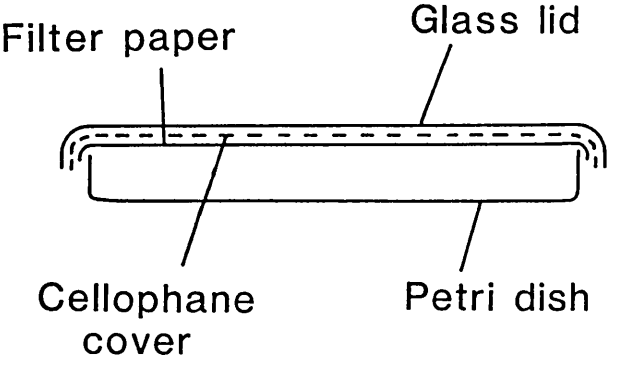
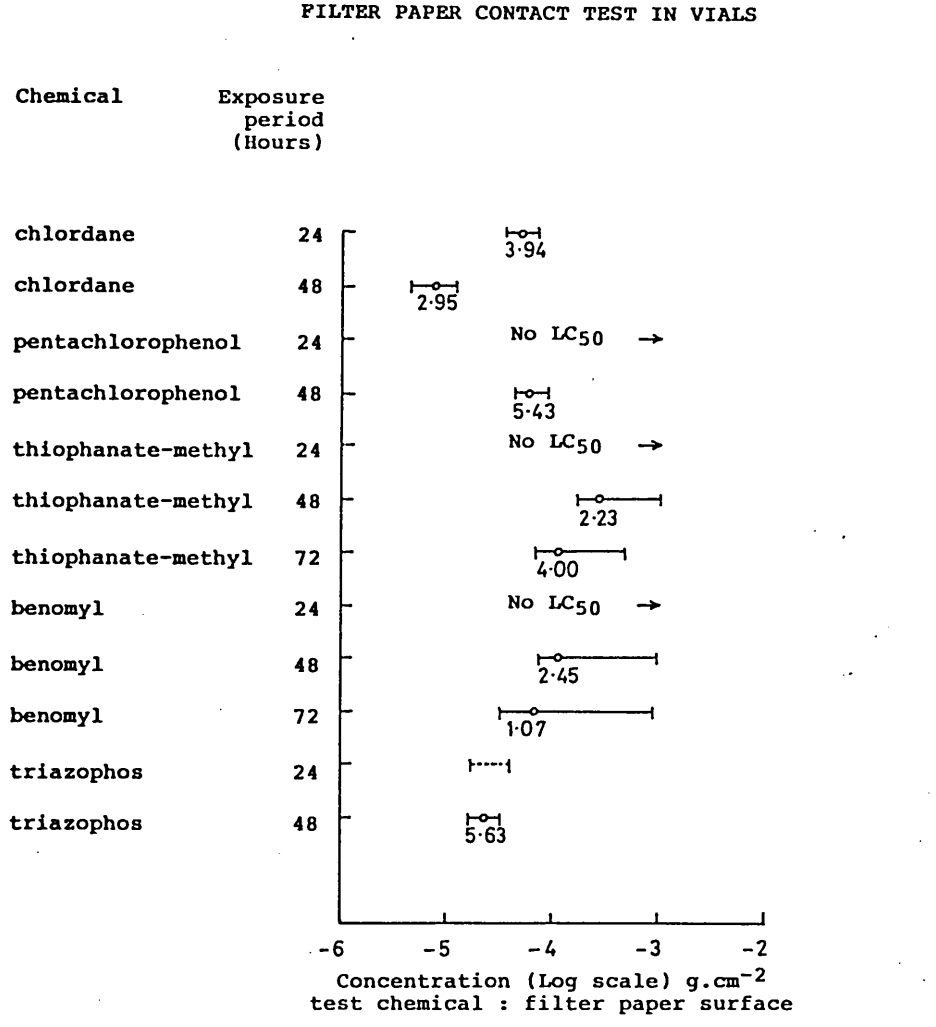


Figure 2.7



KEY

⊢o⊣ x	Individual LC_{50} estimate [o], gradient [x] and fiducial limits
⊢o⊣ (x)	Individual LC_{50} estimate [o], common gradient for more than one replicate [(x)] and fiducial limits
⊢●⊣ (x)	Common LC_{50} estimate [●], gradient [(x)] and fiducial limits for more than one replicate
⊢----⊣	Range within which a poorly defined LC_{50} falls
No LC_{50} →	LC_{50} at an undefined concentration that was greater than those studied
← No LC_{50}	LC_{50} at an undefined concentration that was less than those studied
(No LC_{50})	More than one LC_{50} at an undefined concentration

fungicides. Mortality was absent in the controls after 72 hours and the CEC of the filter paper was zero at the standard pH (8.1) and at the natural pH (7.0). The method was easy to use, the deposit of test chemical remained distributed uniformly on the filter paper and mortality was assessed conveniently. The earthworms grazed the filter paper occasionally and therefore these estimates of toxicity are not due necessarily to uptake over the cuticle alone.

2.2.6 Filter paper contact test using Petri dishes

Method

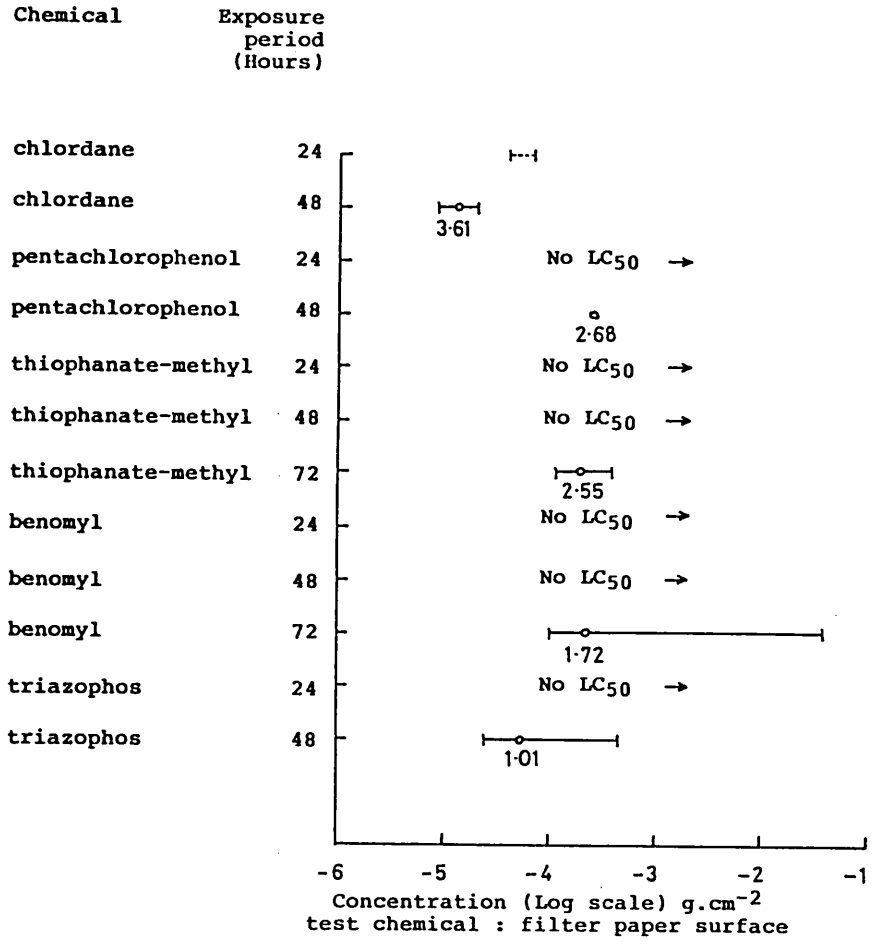
The chemicals tested were chlordane, pentachlorophenol, thiophanate-methyl, benomyl and triazophos. These compounds were dissolved in distilled water or a suitable volatile organic solvent. One ml of these solutions were pipetted separately onto 9 cm diameter discs of Whatman No.1 cellulose filter paper which were supported on 9 cm diameter discs of cellophane held individually in 8.5 cm diameter glass Petri dish lids (64 cm^2 of filter paper was treated with test chemical, but only 57 cm^2 was exposed to the earthworms when the apparatus was assembled and the bottom of the dish was fitted into the lid). Any volatile organic solvent that had been used was evaporated in a gentle airflow and the filter paper dried in this way was moistened with 1 ml distilled water. One earthworm was placed between the cellophane and filter paper layers, which were pulled taut by inserting the Petri dish base into the lid (Figure 2.6). The other experimental conditions were the same as those described for the method using filter paper in glass vials.

Results

The estimates of the toxicity of chemicals to earthworms that were obtained with this method (Table A7.4 and Figure 2.8) indicated that chlordane was more toxic to earthworms than triazophos after a 48 hour

Figure 2.8

FILTER PAPER CONTACT TEST IN PETRI DISHES



KEY

⊢o⊣ x	Individual LC_{50} estimate [o], gradient [x] and fiducial limits
⊢o⊣ (x)	Individual LC_{50} estimate [o], common gradient for more than one replicate [(x)] and fiducial limits
⊢●⊣ (x)	Common LC_{50} estimate [●], gradient [(x)] and fiducial limits for more than one replicate
⊢---⊣	Range within which a poorly defined LC_{50} falls
No LC_{50} →	LC_{50} at an undefined concentration that was greater than those studied
← No LC_{50}	LC_{50} at an undefined concentration that was less than those studied
(No LC_{50})	More than one LC_{50} at an undefined concentration

period of exposure, and that both of these chemicals were more toxic than pentachlorophenol. Thiophanate-methyl and benomyl did not give data that could be processed by probit analysis. The gradients of the probit lines for chlordane were the most steep, followed in a decreasing order of steepness by pentachlorophenol and triazophos. None of the test chemicals gave an LC_{50} after a 24 hour period of exposure. Mortality was absent in the controls after 72 hours. The method was difficult to use because the filter paper tended to split when wet. Furthermore, the test chemicals were distributed less evenly on the filter paper in the Petri dishes than on a similar material in a glass vial, because the solution of the chemical could not be swirled around the dish to give a uniform deposit. The earthworms tended to escape from the apparatus, the filter paper dried out rapidly unless the dishes were stored in humid conditions and it was necessary to dismantle the apparatus to assess the mortality of the earthworms at the end of the test.

2.2.7 Sand test

Method

The chemicals tested for toxicity to earthworms using this method were chlordane, pentachlorophenol and thiophanate-methyl, which were dissolved in 5 ml of distilled water or a suitable volatile organic solvent and mixed with 50 g of fine quartz sand (British Industrial Sand 110 Grade. [Table A5.3]). Any volatile organic solvents that had been used were removed by a process of spreading and mixing the sand in a gentle flow of air. The sand that was dried in this manner was moistened with 15 ml distilled water, but where the test chemical had been applied in an aqueous solution, only 10 ml of water were added. Each replicate consisted of 50 g (dry weight) of sand that had been treated with the test chemical, held in a 100 ml glass crystallising dish with a loosely fitting lid and containing three earthworms. Four replicates were used at each of six

Figure 2.9

Sand Test

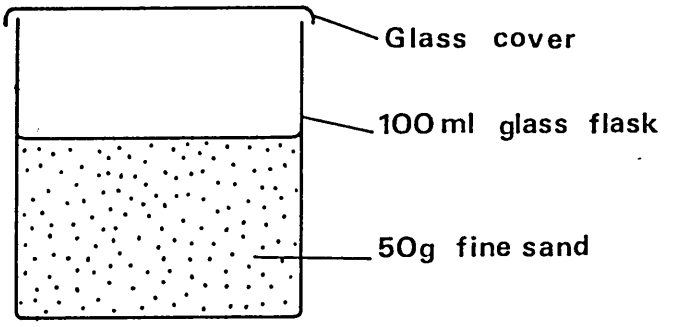


Figure 2.10

Antibumping Granule Test

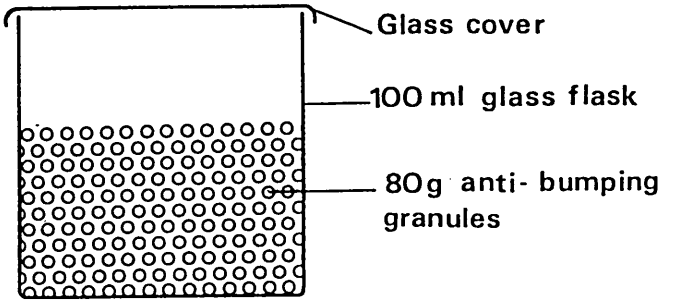
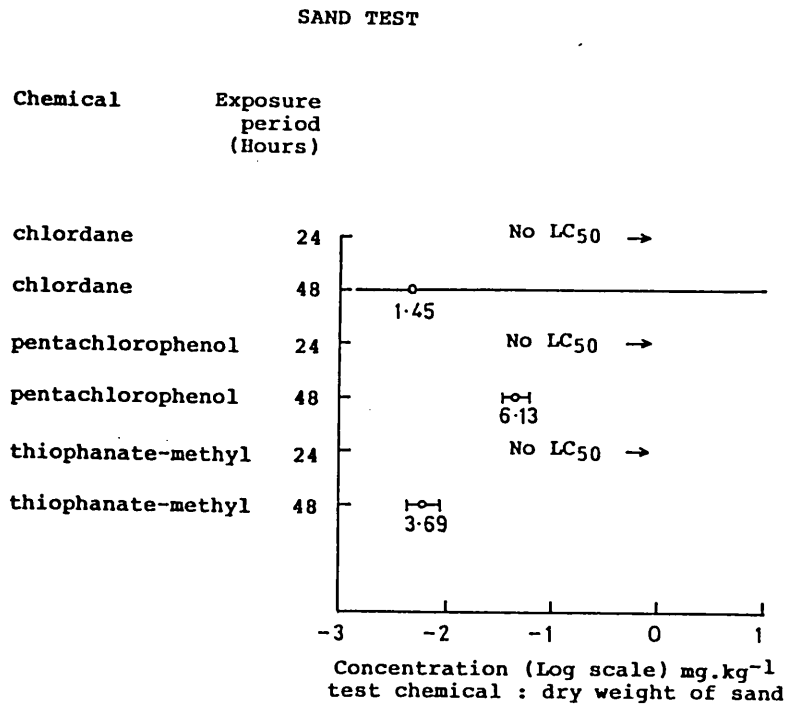


Figure 2.11



KEY

⊢o⊣ x	Individual LC_{50} estimate [o], gradient [x] and fiducial limits
⊢o⊣ (x)	Individual LC_{50} estimate [o], common gradient for more than one replicate [(x)] and fiducial limits
⊢●⊣ (x)	Common LC_{50} estimate [●], gradient [(x)] and fiducial limits for more than one replicate
⊢----⊣	Range within which a poorly defined LC_{50} falls
No LC_{50} →	LC_{50} at an undefined concentration that was greater than those studied
← No LC_{50}	LC_{50} at an undefined concentration that was less than those studied
(No LC_{50})	More than one LC_{50} at an undefined concentration

concentrations of the chemical in a logarithmic dilution series. The earthworms were extracted from the sand by hand and mortality was assessed after a 24 or 48 hour period of exposure under artificial light (Figure 2.9). The cation exchange capacity of the sand was measured in order to estimate the amount of test chemical that would be adsorbed by it.

Results

None of the test chemicals gave an LC_{50} after a 24 hour period of exposure, although after 48 hours the results could be analysed statistically and the gradients of the probit lines appeared to be related inversely to the toxicity to earthworms of the test chemicals (Table A7.5 and Figure 2.11). Chlordane, thiophanate-methyl and pentachlorophenol were ranked in a decreasing order of toxicity to earthworms by this test, although the LC_{50} for chlordane was ill defined and had widely separated fiducial limits. Although there was no mortality in the controls, the earthworms began to look unhealthy during the 48 hour period of exposure and attempted to escape from the sand. The death of one earthworm had an adverse effect upon the survival of others in the same container. The sand was unlikely to adsorb a significant amount of the test chemicals, because it had a CEC of zero at the standard pH (8.1) and natural pH (6.7). The procedure for removing volatile organic solvents from the sand was slow and it was difficult to determine the point at which the solvent had evaporated completely.

2.2.8 Antibumping granule test

Method

Antibumping granules are small glass pellets (2 mm diameter) with a rough, sand-blasted surface. The rough surface of the granules seemed likely to support a uniform deposit of test chemical, whilst allowing

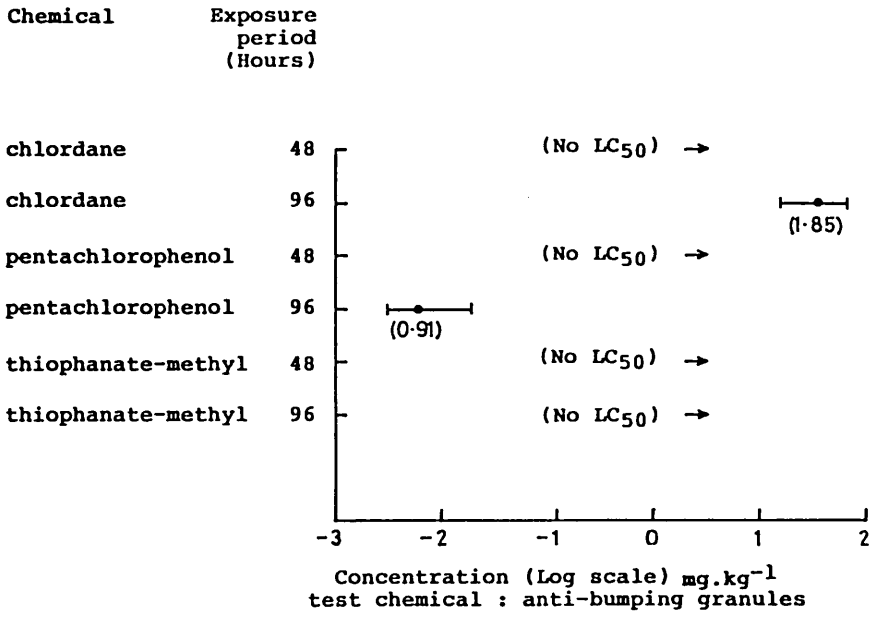
the earthworms to move freely in the spaces between the granules. The earthworms were reluctant to enter this medium and were therefore buried under 1 cm of granules at the beginning of each experiment. The test chemicals used were chlordane, pentachlorophenol and thiophanate-methyl. These were dissolved in 5 ml of a volatile organic solvent and mixed with 80 g of the granules. The solvents were removed by spreading and mixing the granules in a gentle airflow. This process was similar to that used in the sand test and presented difficulties that were common to both tests. Each replicate consisted of 80 g (dry weight) granules that had been treated with the test chemical, held in a 100 ml glass crystallising dish and containing five earthworms. Four replicates were used at a minimum of six concentrations of the test chemical in a logarithmic dilution series. The dishes were covered by a loosely fitting lid that held a wad of damp cotton wool to humidify the air. Each test ran for 48 or 96 hours under artificial light, after which the earthworms were extracted from the granules by hand and mortality was assessed (Figure 2.10).

Results

None of the test chemicals gave data that were suitable for calculating an LC_{50} after a 48 hour period of exposure. The LC_{50}'s after a 96 hour period of exposure indicated that pentachlorophenol was more toxic to earthworms than chlordane, and that pentachlorophenol had the steepest probit line gradient (Table A7.6 and Figure 2.12). The probit lines for the replicates of each of these chemicals did not differ significantly after a 96 hour period of exposure and the replicates were therefore combined to give a single probit line for each compound. Thiophanate-methyl failed to produce mortality data that could be processed by probit analysis. Mortality did not occur in the controls during the 96 hour period of exposure, although the earthworms failed to penetrate far into the granules, looked very unhealthy and attempted to escape from the

Figure 2.12

ANTI-BUMPING GRANULE TEST



KEY

⊢o⊣ x	Individual LC_{50} estimate [o], gradient [x] and fiducial limits
⊢o⊣ (x)	Individual LC_{50} estimate [o], common gradient for more than one replicate [(x)] and fiducial limits
⊢●⊣ (x)	Common LC_{50} estimate [●], gradient [(x)] and fiducial limits for more than one replicate
⊢----⊣	Range within which a poorly defined LC_{50} falls
No LC_{50} →	LC_{50} at an undefined concentration that was greater than those studied
← No LC_{50}	LC_{50} at an undefined concentration that was less than those studied
(No LC_{50})	More than one LC_{50} at an undefined concentration

apparatus. The death of one earthworm affected adversely the survival of others in the same container and the test chemical was applied uniformly to the granules with difficulty. Furthermore the deposit of the test chemical tended to flake off the surface of the granules. The method for maintaining a humid atmosphere within the tests vessels failed to prevent some desiccation of the earthworms.

2.2.9 Silica paste and glass ball test

Method

This method was based upon a simplified version (Bouche, 1982) of the method designed by Ferriere, Fayolle and Bouche (1981). The test chemicals included chlordane, carbaryl, pentachlorophenol, chloroacetamide, thiophanate-methyl and potassium bromide. These compounds were dissolved or suspended in 215 ml distilled water and mixed with 90 g of air-dried (4.5-5.0% H_2O) finely milled (surface area 450 $m^2.g^{-1}$) silica ('Levilite': Rhone-Poulenc Chimie Fine, Strasbourg, France) to produce a paste. This paste was combined with 1425 g of 2 cm diameter glass balls (Societe Preciver, Maisons-Alfort, France) which provided support and allowed aeration of the media. Each replicate of this mixture was held in a 2 litre glass flask, loosely covered by a plastic film and containing ten earthworms. Four such replicates were used at six concentrations of each test chemical in a logarithmic dilution series. The test ran for 14 days under artificial light (Plate 2.2 and Figure 2.13). The earthworms were separated from the media at the end of the experiment to allow an assessment of mortality by washing the contents of the flasks through a 1 mm mesh sieve. The amount of test chemical that was likely to adsorb onto the particles of silica was estimated by measuring the cation exchange capacity of the silica paste.

Figure 2.13

Silica Paste: Glass Ball Test

(Artisol)

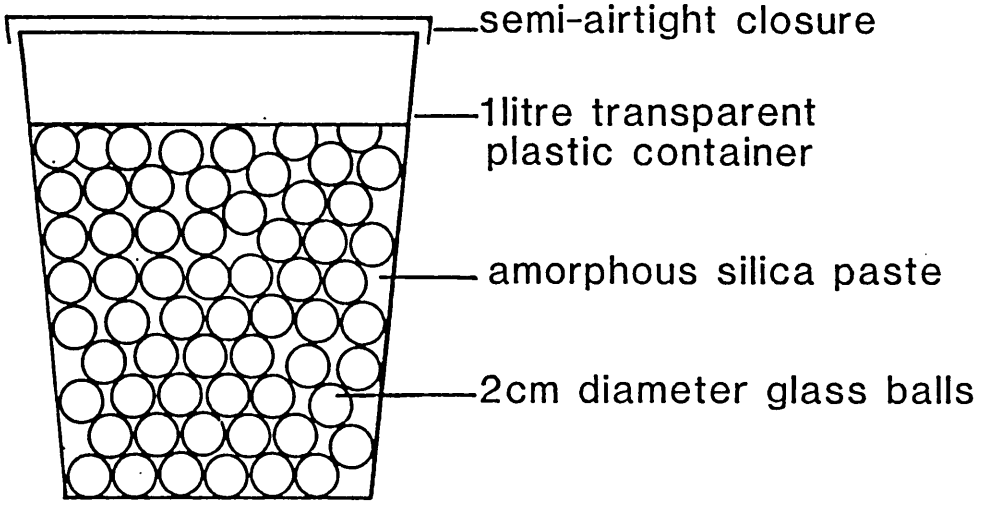


Figure 2.14

Natural Soil Test

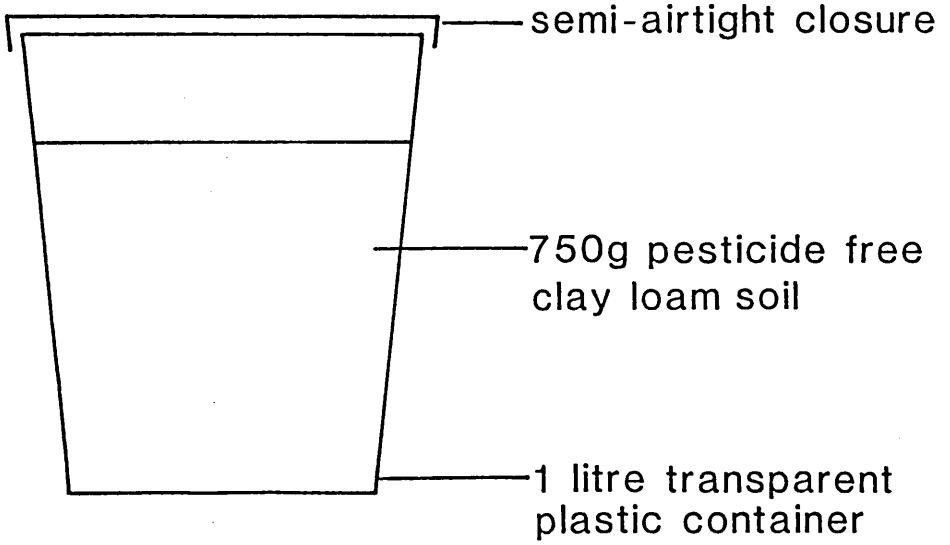
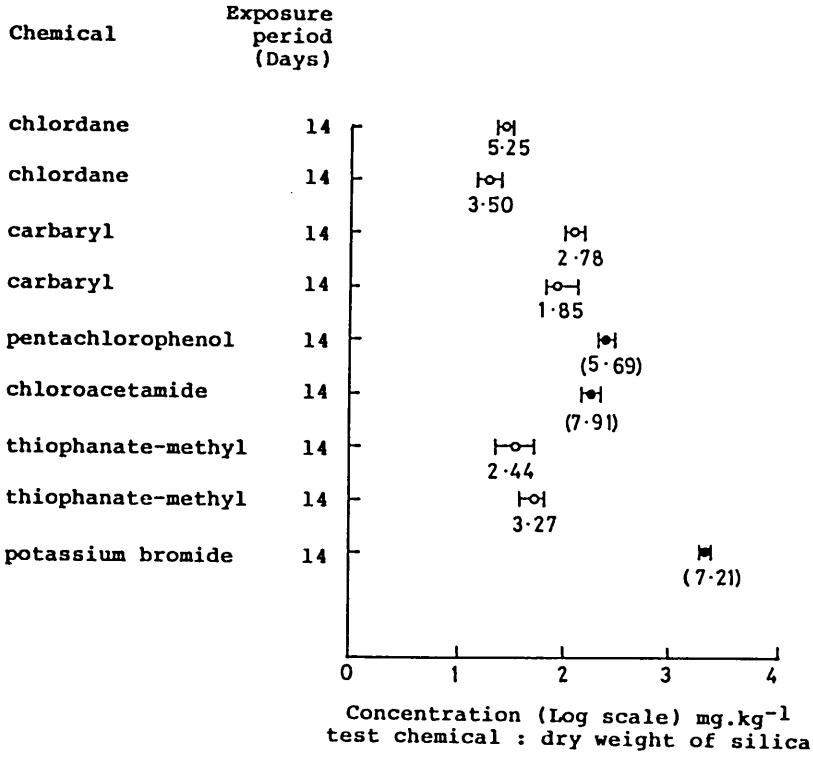


Figure 2.15

SILICA PASTE AND GLASS BALL TEST



KEY

⊢o(x)⊣	Individual LC_{50} estimate [o], gradient [x] and fiducial limits
⊢o(x)⊣	Individual LC_{50} estimate [o], common gradient for more than one replicate [(x)] and fiducial limits
⊢●(x)⊣	Common LC_{50} estimate [●], gradient [(x)] and fiducial limits for more than one replicate
⊢----⊣	Range within which a poorly defined LC_{50} falls
No LC_{50} →	LC_{50} at an undefined concentration that was greater than those studied
← No LC_{50}	LC_{50} at an undefined concentration that was less than those studied
(No LC_{50})	More than one LC_{50} at an undefined concentration

Results

After a 14 day period of exposure the compound that was most toxic to earthworms was chlordane, followed in a decreasing order of toxicity by thiophanate-methyl, carbaryl, chloroacetamide, pentachlorophenol and potassium bromide (Table A7.7 and Figure 2.15). The gradient of the probit lines was steepest for chloroacetamide, and less steep in a decreasing order for potassium bromide, pentachlorophenol, chlordane, thiophanate-methyl and carbaryl. The probit lines for individual replicates of pentachlorophenol, chloroacetamide and potassium bromide did not differ significantly and the replicates were combined to give a single probit line for each chemical. Mortality did not occur in the controls after 14 days and the earthworms appeared to remain healthy in, and to ingest, the media. The CEC of the amorphous silica was 79 and 302 meq.kg^{-1} at the natural pH (5.0) and standard pH (8.1) respectively. This indicated that the silica had an adsorptive capacity similar to that of a natural arable soil and that this capacity was pH dependent. The method was awkward to use and the media became particularly unwieldy when mixed with the glass balls. The method for extracting the earthworms from the media after the 14 day period of exposure was destructive and allowed only a single assessment of mortality, thus preventing the continuation of an unresolved test.

2.2.10 Natural soil test

Method

The soil was collected from Geescroft Field, Rothamsted Experimental Station, and was a pesticide-free sandy clay loam (Anon., 1981b). Using standard methods the soil was estimated to contain 2.53% organic matter (Kalembasa and Jenkinson, 1973) and to have a pH of 7.3 (Anon., 1981d). These values agree closely with estimates made previously by Johnston, Poulton and McEwen (1981). The soil was air-dried to about 4% water

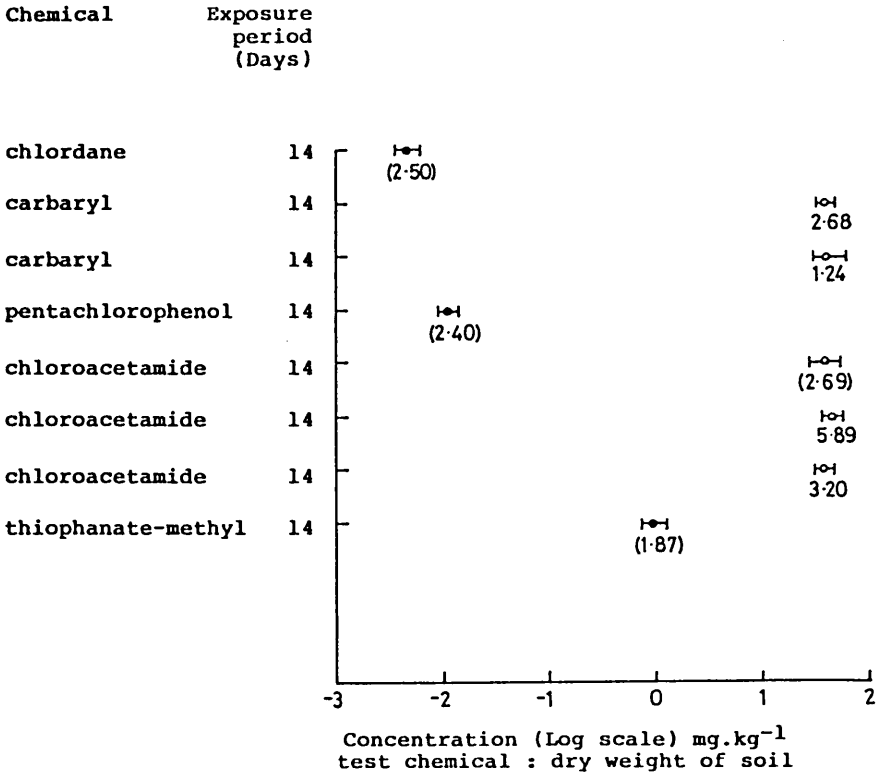
content, passed through a 5 mm sieve and then moistened with distilled water to 20% water content. The test chemicals were chlordane, carbaryl, pentachlorophenol, chloroacetamide and thiophanate-methyl, which were dissolved in distilled water or a suitable volatile organic solvent and were applied to the soil, which was spread out evenly on a tray, using a chromatography spray unit at the rate of 10 ml solution to 750 g soil (wet weight). The soil was left undisturbed in a gentle air-flow for a few minutes to allow the volatile organic solvents to evaporate. The deposit of the test chemical was then mixed thoroughly into the soil by hand using a spatula. This mixture was placed in a one litre plastic flask and 10 earthworms were held in each of four replicates at six concentrations of each chemical in a logarithmic dilution series. The containers were covered with a perforated plastic film (Figure 2.14). The earthworms were extracted by hand after a 14 day period of exposure under artificial light and mortality was assessed. An estimate of the amount of test chemical likely to be adsorbed onto the soil particles was made by measuring the cation exchange capacity of the soil at a standard pH (8.1) and at the natural pH (7.3).

Results

This test indicated that chlordane was the chemical most toxic to earthworms, followed in a decreasing order of toxicity by pentachlorophenol and thiophanate-methyl. Carbaryl and chloroacetamide were less toxic than the other chemicals, but it was not possible to distinguish the ranking of toxicity between these latter two chemicals. The steepness of the gradients of the probit lines was correlated positively with the toxicity of the chemicals to earthworms (Table A7.8 and Figure 2.16). The probit lines that were calculated for the individual replicates of chlordane, pentachlorophenol and thiophanate-methyl did not differ significantly and the replicates were combined to give single lines for each

Figure 2.16

NATURAL SOIL TEST



KEY

⊢o⊣ x	Individual LC_{50} estimate [o], gradient [x] and fiducial limits
⊢o⊣ (x)	Individual LC_{50} estimate [o], common gradient for more than one replicate [(x)] and fiducial limits
⊢●⊣ (x)	Common LC_{50} estimate [●], gradient [(x)] and fiducial limits for more than one replicate
⊢----⊣	Range within which a poorly defined LC_{50} falls
No LC_{50} →	LC_{50} at an undefined concentration that was greater than those studied
← No LC_{50}	LC_{50} at an undefined concentration that was less than those studied
(No LC_{50})	More than one LC_{50} at an undefined concentration

chemical. The earthworms looked unhealthy in the untreated soil after a 14 day period of exposure, although mortality was absent in the controls. The CEC of the soil was 167 and 187 meq.kg^{-1} at the natural pH and the standard pH respectively, which was similar to that of many arable soils. Thus the adsorptive capacity of the soil was not influenced greatly by pH. The collection and preparation of the soil was laborious and it proved difficult to extract the earthworms at the end of the test to assess mortality. This was because the soil settled into a compact mass through the action of the earthworms.

2.2.11 Forced feeding test

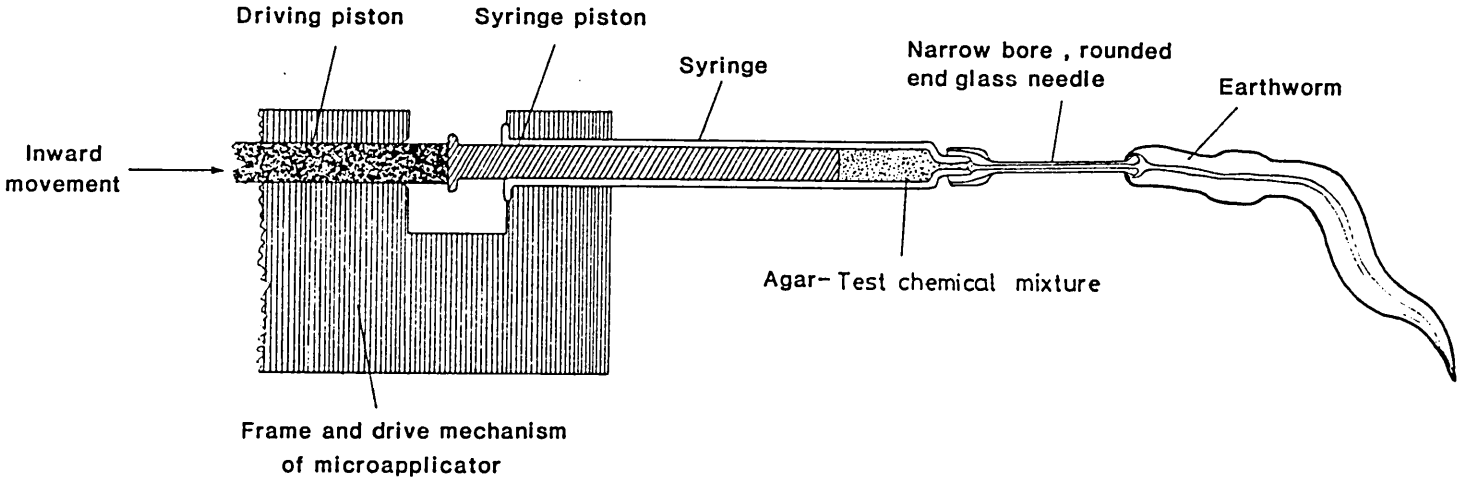
Method

This method was based upon that of Stringer and Wright (1976) which was modified for use with E. fetida. Chlordane, thiophanate-methyl and benomyl were dissolved or suspended in distilled water and these solutions or suspensions were then incorporated into a 1.5% agar-agar gel. The gel was produced by using as little heat as possible, to avoid thermal decomposition of the test chemical. When the gel was cool it was loaded into a 1 ml Hamilton syringe, fitted with narrow bore glass needle that had a rounded end (Plate 2.3). The syringe was mounted in an electrically driven Burkard Arnold micro-applicator Type LV65 (Burkard Manufacturing Company Ltd.) operated by a foot pedal (Figure 2.17).

The earthworms were anaesthetised by a 2 minute immersion in a 10% aqueous ethanol solution. This solution was produced by adding ethanol to water in which the earthworms were immersed gradually, which avoided a defensive reaction by the earthworm. This reaction of E.fetida was characterised by a loss of coelomic fluid. The needle was inserted into the oesophagus of an anaesthetised earthworm and by operating the drive mechanism of the syringe, 5 µl of gel containing the test chemical was forced into the gut of the earthworm (Plate 2.4). The earthworms were

Figure 2.17

Forced Feeding Test



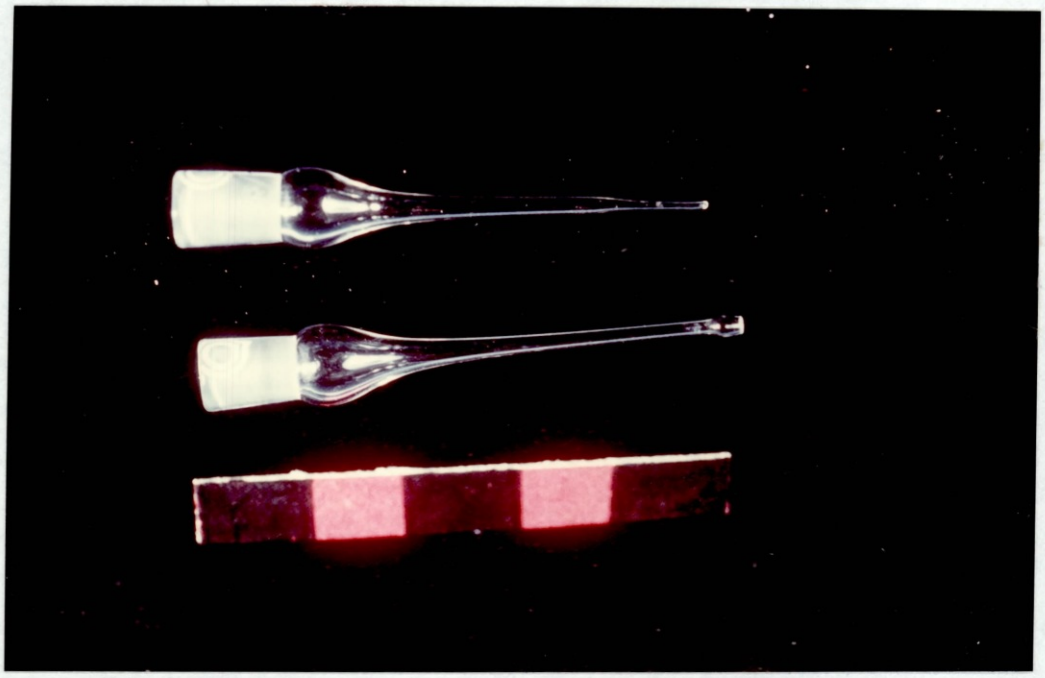


Plate 2.3. Forced feeding test: Glass syringe needles.

Scale: 1 cm

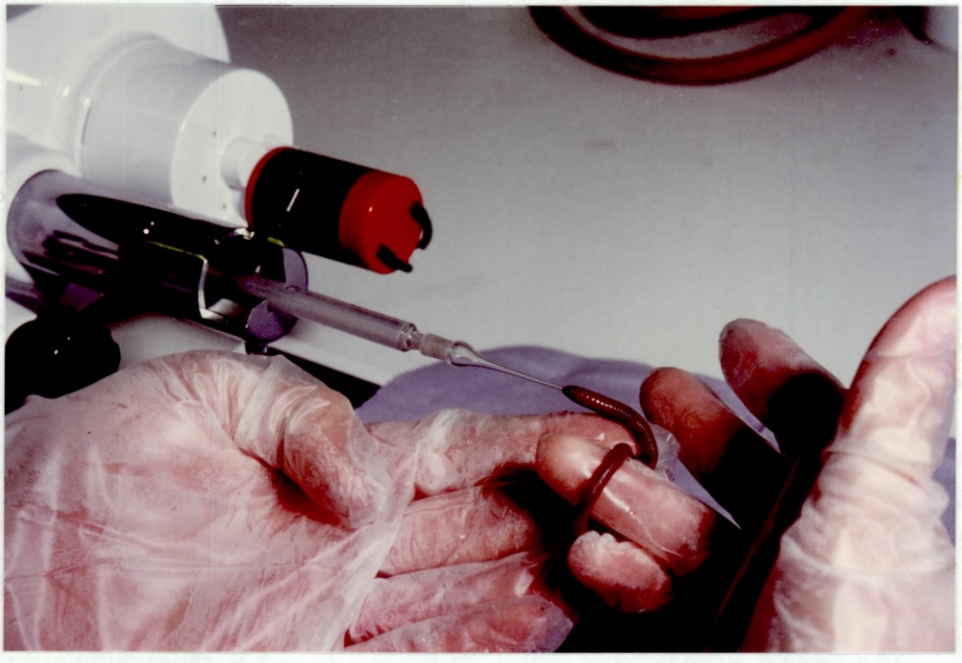
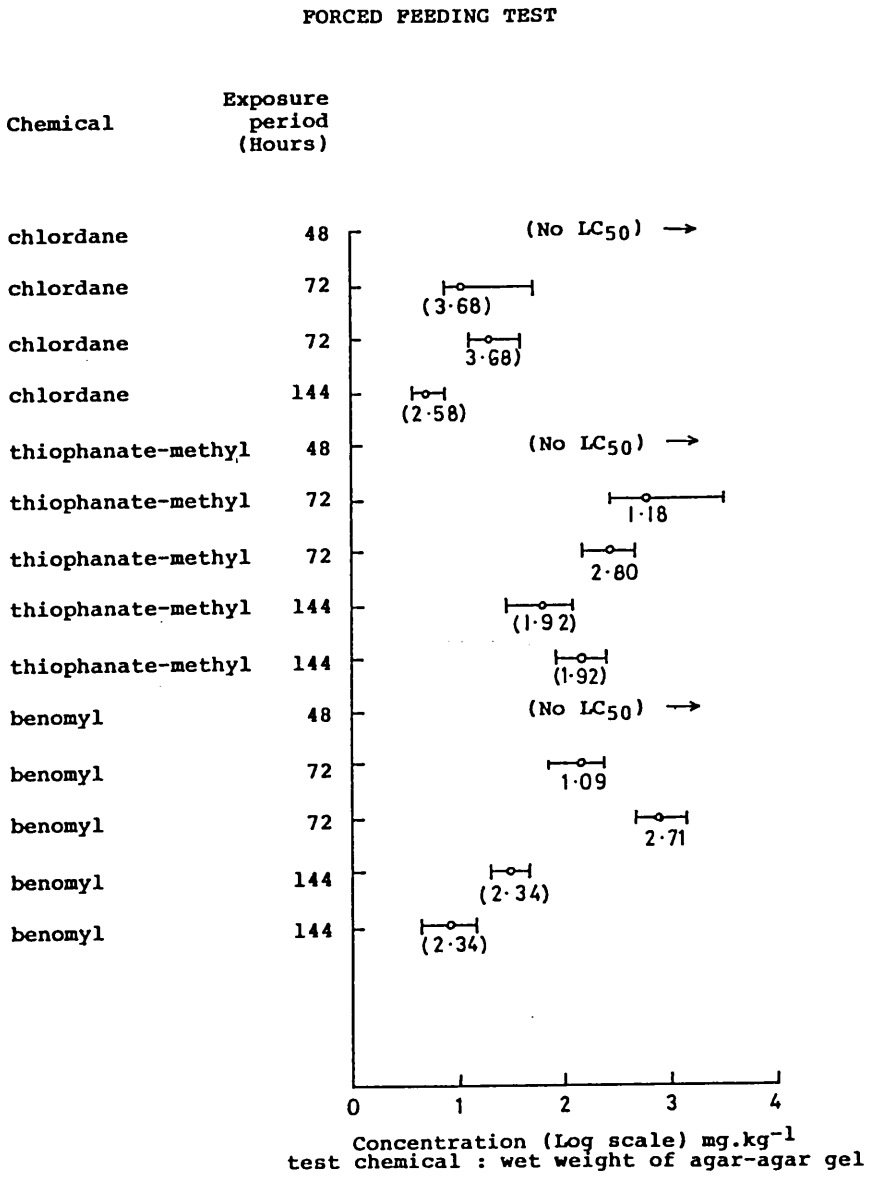


Plate 2.4. Forced feeding test: The syringe was held in a microdrive unit which forced agar-agar gel through a glass needle inserted into the oesophagus of the worm.

Figure 2.18



KEY

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⊢o⊣ (x)	Individual LC_{50} estimate [o], common gradient for more than one replicate [(x)] and fiducial limits
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← No LC_{50}	LC_{50} at an undefined concentration that was less than those studied
(No LC_{50})	More than one LC_{50} at an undefined concentration

immediately placed in distilled water for 10 minutes to recover from the effects of the alcohol, and then transferred to boxes lined with damp filter paper. Ten earthworms were used at each of six concentrations of the test chemical in a logarithmic dilution series, and the effects of the chemicals upon the earthworms were determined after a 48, 72 and 144 hour period of exposure in darkness.

Results

After a 48 hour period of exposure none of the test chemicals gave mortality data that could be processed by probit analysis. The replicated results obtained with each chemical after 72 hours were more dissimilar than those collected after 144 hours, and therefore the latter data were the most suitable for the purposes of this study. The chemical that was most toxic to earthworms after a 144 hour period of exposure was chlordane, followed in decreasing order of toxicity by benomyl and thiophanate-methyl. Chlordane also showed the steepest gradient of the probit lines whilst the steepness of the gradient of the probit lines, for the other chemicals appeared to be related directly to their toxicity to earthworms (Table A7.9 and Figure 2.18). The probit lines for the replicates of chlordane assessed after a 144 hour period of exposure did not differ significantly and were combined to give a single probit line. The data for the other chemicals allowed only a common value to be calculated for the gradients of the probit lines. Mortality did not occur in the controls after 144 hours and it was simple to assess mortality. The earthworms did not regurgitate the gel or show any obvious discomfort during the treatment. The technique was slow to use, particularly with a small species of earthworm such as E. fetida, and demanded certain specialised apparatus and manual dexterity.

2.3. Discussion

The performance of those methods for testing the toxicity of chemicals to earthworms in the laboratory that existed prior to my studies was evaluated, to select a sensitive and reproducible method that would fulfil the requirements of the regulatory authorities.

The methods were assessed initially for sensitivity and reproducibility of the results, economy of materials and labour, ease with which the conditions of the test could be standardised and the ability of the test to assess the toxicity of any chemical to earthworms without elaborate formulation techniques. The tests that were rejected on these criteria without an experimental investigation included the injection of chemicals into the coelomic cavity, topical application, voluntary feeding upon a treated substrate, electrophysiological measurement of nervous activity and any method in which sublethal toxicity was assessed or toxicity to earthworms in the field was measured.

The remaining methods included an immersion test, contact tests in which the chemicals were applied to a glass or a filter paper surface over which the earthworms moved, a natural soil test, a method using silica paste as a soil substitute and a forced feeding test. These methods were used experimentally to assess the toxicity of chemicals to earthworms. Several of these test chemicals were known to be toxic to earthworms and included chlordane (Edwards and Lofty, 1973; Hopkins and Kirk, 1957), carbaryl (Edwards and Lofty, 1973; Stenersen, 1979a), thiophanate-methyl (Roark and Dale, 1979; Stringer and Wright, 1973) and benomyl (Lofs-Holmin, 1980; Stringer and Wright, 1973).

The period of exposure that was required to produce a reliable LC_{50} differed between tests and may be a function of the intimacy of contact that was achieved between the earthworm and the deposit of the test chemical. A 24 hour LC_{50} was obtained consistently with the immersion test, whilst the filter paper contact test in vials and in Petri dishes gave

a 48 hour LC_{50} with all the chemicals except the benzimidazole fungicides. The sand test gave a 48 hour LC_{50} with all the chemicals. A longer period of exposure was required for the forced feeding test which gave a reliable estimate of the LC_{50} after 72 hours, and the antibumping granule test which gave an LC_{50} after 96 hours (although the latter test failed to define the LC_{50} for thiophanate-methyl). The remaining tests, which included the silica paste-glass ball and natural soil methods, gave an estimate of the LC_{50} for each of the test chemicals after a 14 day period of exposure.

A testing method that was capable of giving a reliable estimate of an LC_{50} rapidly was desirable as this would reduce the operating costs, although the precision with which an LC_{50} was determined decreased as the duration of the test was reduced. The benzimidazole fungicides are slow acting poisons (Stringer and Wright, 1976) and the tests that used a short period of exposure rarely gave mortality data for these compounds which were suitable for probit analysis. This indicates a major disadvantage to the use of methods of short duration.

The LC_{50}'s of the chemicals obtained using the various tests could not be compared easily, because of the differences that exist between the conditions in which the earthworms are exposed to the chemicals in each. These differences make it necessary to adopt a variety of units of concentration in which to express the results. One way to compare these varied data is to use the ranking of the toxicity of the chemicals to earthworms indicated by the various methods (Table 2.2). With the exception of the sand and silica paste-glass ball tests, all the tests indicated that thiophanate-methyl was considerably less toxic than chlordane. Chlordane was more toxic than pentachlorophenol in all the tests except the immersion and antibumping granule methods. Thiophanate-methyl was less toxic than pentachlorophenol in all tests except the glass contact, sand, silica paste-glass ball and forced feeding

Table 2.2. The toxicity of chemicals to earthworms estimated using tests in the laboratory and ranked according to the LC_{50}'s.

Method	Exposure Period	Ranked toxicity of chemicals. 1 = most toxic.						
		Chlordane	Carbaryl	Pentachloro-phenol	Chloro-acetamide	Thiophanate-methyl	Benomyl	Triazophos
Immersion	24 hours	2	3	1	4	6	-	5
Glass contact	48 hours	1	-	-	-	3	2	-
Filter paper contact in vials	48 hours	1	-	3	-	5	4	2
Filter paper contact in Petri dishes	48 hours	1	-	3	-	-	-	2
Sand	48 hours	1	-	3	-	2	-	-
Antibumping granule	96 hours	2	-	1	-	-	-	-
Silica paste-glass ball	14 days	1	3	5	4	2	-	-
Natural soil	14 days	1	4	2	5	3	-	-
Forced feeding	144 hours	1	-	-	-	3	2	-

tests. Thus, some similarity between the ranking of the toxicity of chemicals to earthworms was seen using these tests, although it is difficult to draw conclusions concerning the efficiency of the tests from this data. Tests which use chemically inert media and expose the earthworms to chemicals for a short period of time seem to give similar estimates of toxicity.

The relationship that exists between the LC_{50}'s of a single chemical, estimated using several test methods, is poor even when the comparison is limited to test methods in which the routes of exposure are similar. For instance, the 48 hour LC_{50} for chlordane (these results and those that follow are expressed as the antilog of the LC_{50}'s, or the antilogged mean of the replicated LC_{50}'s, calculated previously on a log scale using probit analysis) obtained using the glass contact, filter paper in a vial and filter paper in a Petri dish tests were 0.08, 7.49 and 12.86 $\mu g.cm^{-2}$ respectively, although the chemical would appear to make contact with the earthworm in a similar way in each test.

Supplementary experiments (Appendix 2) have shown that hexachlorobenzene accumulated in earthworm tissue from a glass surface at a more rapid and uniform rate than from a deposit on moist filter paper. These observations may explain the differences in the toxicity of chlordane to earthworms that were seen between the contact tests, although benomyl and thiophanate-methyl were more toxic as a deposit on filter paper. The low toxicity of these fungicides on a glass surface may arise from the tendency of these materials to flake off the glass and accumulate in a narrow band at the base of the vial. The earthworms are able to avoid chemicals that are redistributed in this manner and thus appear to be less sensitive to them.

The filter paper contact test using vials gave a 48 hour LC_{50} for benomyl of 123 $\mu g.cm^{-2}$, whilst that calculated by Roberts and Dorough (1984) using a similar test system was 9.1 $\mu g.cm^{-2}$, and by Heimbach

(1984) was 86 $\mu g.cm^{-2}$. The filter paper in vials test gave a 48 hour LC_{50} for pentachlorophenol of 59.89 $\mu g.cm^{-2}$ and for chlordane of 7.49 $\mu g.cm^{-2}$, whilst those determined by Heimbach (1984) were 4.2 $\mu g.cm^{-2}$ and 1.3 $\mu g.cm^{-2}$ respectively.

The immersion, sand and antibumping granule tests presented the chemical to the earthworm in a similar way to the other contact tests, although the intimacy of the contact between the earthworm and the chemical in the latter tests was less. The 24 hour LC_{50} for chlordane was 8.63 $\mu g.g^{-1}$ using the immersion test, the 48 hour LC_{50} was 4.85 $ng.g^{-1}$ using the sand test and the 96 hour LC_{50} was 36.30 $\mu g.g^{-1}$ using the antibumping granule test. Although the data from these tests are given in similar units of concentration, that from the immersion test represents a different measure of the toxicity of the chemicals to earthworms from the results obtained with the mineral media. This is because the test chemical adhered to the granules of the mineral media as a superficial deposit. Furthermore, the earthworms tried to avoid burrowing in the granular substrates and thus reduced the contact between the earthworm and the test chemical.

A stable dispersion of the test chemical in water was difficult to achieve for the immersion test, although previous reports concerning this type of test do not address this problem (Ghabbour and Imam, 1967; Lebrun et al., 1981; Martin and Wiggans, 1959). The toxicity to earthworms of the commercially formulated chemicals estimated using the immersion test was considerably greater than that of the same chemicals tested as the technical compound. Whilst formulants may speed up the action of chemicals that affect earthworms slowly, the use of formulating agents in testing for toxicity is undesirable, because the influence of these agents may be unpredictable. In another study, earthworms were immersed for 30 minutes in a dilute ethanolic solution of carbaryl, followed by a period of observation for 80 days in soil (Stenersen, 1979a). An LC_{50} of

200-800 $\mu g.g^{-1}$ was obtained which was dissimilar to the 24 hour LC_{50} for carbaryl estimated using the immersion test in my experiments of 25.19 $\mu g.g^{-1}$. Such differences between these LC_{50}'s clearly arise from the dissimilar testing techniques that were used.

The natural soil and silica paste-glass ball tests gave estimates of the toxicity of chemicals to earthworms using a longer period of exposure than the other methods. The media used in these tests had a high adsorptive capacity and were probably ingested by the earthworms. These tests gave 14 day LC_{50}'s for chlordane that were dissimilar, and were estimated using the natural soil method as 4.62 $ng.g^{-1}$ and with the silica paste-glass ball method as 23.89 $\mu g.g^{-1}$. Chlordane, pentachlorophenol and thiophanate-methyl seemed to be more toxic to earthworms in the natural soil than in the silica paste, although these differences were unlikely to have arisen from a disparity between the adsorptive capacities of the media, which were similar at a pH near neutrality. The differences between these estimates of toxicity may be due to the inability of E. fetida to tolerate a mineral soil and, indeed, the estimate of the toxicity of chlordane in the natural soil was similar to that seen previously in sand. E. fetida showed obvious signs of discomfort in sand. The conditions within certain mineral soils differ considerably from those known to favour the growth of this species (Kaplan et al., 1980) and these findings were supported by Fayolle (1979), who also reported that E. fetida would not enter a mineral soil used as a medium for testing toxicity in the laboratory.

The 14 day LC_{50}'s for carbaryl and thiophanate-methyl which I estimated using the silica paste-glass ball test were 112.33 and 44.57 $\mu g.g^{-1}$ respectively, which compared poorly with the 14 day LC_{50} for carbaryl of 25 $\mu g.g^{-1}$ and the 7 day LC_{50} for thiophanate-methyl of 1056 $\mu g.g^{-1}$ reported in similar studies (Bouche, 1984a). Heimbach (1984) used the silica paste-glass ball test in a collaborative exercise that compared the

efficiency of this test with that of my own methods for testing the toxicity of chemicals to earthworms (Chapter 5). My assessments of toxicity using the silica paste-glass ball test agree closely with those of Heimbach (1984) and for which the 14 day LC_{50}'s, given in this order, were for chlordane 23.89 and 15 $\mu g.g^{-1}$, carbaryl 112.33 and 151 $\mu g.g^{-1}$, pentachlorophenol 276.98 and 316 $\mu g.g^{-1}$, chloroacetamide 183.46 and 75 $\mu g.g^{-1}$ and potassium bromide 2089.63 and 1000 $\mu g.g^{-1}$.

The results from the forced feeding method were additionally calculated to give a 144 hour LD_{50} for chlordane of 0.025 $\mu g.worm^{-1}$, although this assessment of toxicity cannot be related easily to the data obtained using the other tests. The 144 hour LD_{50} for benomyl using E. fetida was 0.08 $\mu g.worm^{-1}$, which compares poorly to that obtained with L. terrestris of 13.9 $\mu g.worm^{-1}$ by Stringer and Wright (1976) after an unspecified period of exposure. Thus, on this evidence and after taking into account the differences in body weight between these species, E. fetida would appear to be more susceptible to benomyl than L. terrestris.

The order in which the gradients of the probit lines for the various chemicals were ranked by the test methods shows little similarity. The inverse relationship that occurred occasionally between the toxicity of a chemical to earthworms and the gradient of the probit line was probably an artifact that arose from expressing the mortality data on a logarithmic concentration scale. Chemicals of low toxicity to earthworms often show an effect over a wide range of concentrations (indicated by a shallow gradient of the probit line), because such chemicals frequently act slowly and allow differences to appear between the susceptibility of individuals.

The reproducibility of an assessment of toxicity may be estimated by using a chi-squared test to analyse the differences between the probit line gradient and the position of the Y-intercept of data from repeated experiments. Where this analysis was possible, the various tests showed a similar reproducibility, which indicated that the laboratory procedures,

environmental conditions and populations of earthworms used during the experiments were of consistent and uniform quality.

The ease with which the tests could be carried out was a criterion which assisted in the selection of suitable methods. Some of the tests had practical disadvantages which may be summarised as follows. The earthworms were unhealthy in the sand, antibumping granule and natural soil media, but did well in a medium consisting of silica paste and glass balls. A suitable humidity was difficult to maintain within the glass contact and antibumping granule test containers, furthermore the deposit of the test chemical could not be applied uniformly to these surfaces, particularly at the higher concentrations. Methods in which the test chemical was deposited on a moistened filter paper surface overcame these problems, although the paper tended to split and was more difficult to treat evenly with chemicals when used in a Petri dish.

The physical characteristics of the media used in the silica paste-glass ball method made this test awkward to use. Earthworms were extracted from the media at the end of the test by a process which necessarily destroyed the media and thus prevented the continuation of an experiment in which too few partial mortalities (where the percentage of the mortality of earthworms seen in the replicates of any one experimental condition was greater than 0%, but less than 100%) had occurred to allow probit analysis of the results. The media had the advantage that it was standardised easily and was ingested readily by the earthworms (although E. fetida was reported to ingest less silica than L. terrestris or A. chlorotica [Ferriere et al., 1981]). The media also allowed individual earthworms to die without affecting the survival of others in the same container. This contrasts with the results obtained with the glass contact, sand, antibumping granule and filter paper contact tests. In these tests, the death of one earthworm in a test container caused others in the same vessel to die as the corpse released toxic substances.

The soil collected from the field and used in the natural soil test also allowed the earthworms to die independently and was handled more easily than the mixture of silica paste and glass balls. By recreating the conditions that occur in the field more accurately than many artificial media, a test for the toxicity of chemicals to earthworms using a natural soil was expected to provide a better estimate of the toxicity of chemicals to earthworms in the field (Dean-Ross, 1983). The disadvantage of the natural soil test lay in the inability of E. fetida to tolerate the mineral soil that was collected from Geescroft Field.

The forced feeding method was slow and difficult to operate, particularly when modified for use with a small earthworm such as E. fetida, although it gave an estimate of the toxicity of a known dose of the test chemical. Thermolabile chemicals could not be tested using this technique.

In conclusion, the methods that appeared to be most suitable for further development included the filter paper contact test in vials, the silica paste-glass ball test and the natural soil test. The disadvantages present in the silica paste-glass ball and the natural soil methods were overcome by the development of a test using an artificial soil. The filter paper contact test was developed by refining the filter paper contact test using glass vials described above, although some modifications were necessary.

CHAPTER 3

DEVELOPMENT OF THE FILTER PAPER CONTACT TEST

3.1. Introduction

A comparison of the efficiency of several methods for testing the toxicity of chemicals to earthworms (Chapter 2), indicated that a filter paper contact test gave reproducible results and might be suitable for ecotoxicological testing. A filter paper contact test exposes the earthworms to a deposit of the test chemical on a moist filter paper surface that lines the inner side wall of a small glass vial.

The species of earthworm used in this toxicity test was E. fetida, a species adapted to live in organic materials like animal dung. Although the filter paper contact test could not reproduce an environment similar to that in which E. fetida occurs naturally, the conditions within the test had to be such that the earthworms remained healthy in the absence of the test chemicals.

The environmental conditions can also affect the toxicity to earthworms of the test chemical. This topic is extensive and I shall consider only those conditions likely to influence the assessment of toxicity to earthworms when using the filter paper contact test. The effect of such environmental conditions upon the toxicity of chemicals to organisms has been reviewed comprehensively by Ebeling (1963), Goring, Laskowski, Hamaker and Meikle (1975) and Harris (1972a).

Temperature affects the toxicity of chemicals to organisms. Occasionally pesticides are more toxic at low temperatures (e.g. copper [Ma, 1984], DDT, DNOC, methoxychlor, nicotine, pyrethrum and rotenone [Guthrie, 1950]), although it is more common to find that the toxicity of a chemical increases as the temperature rises (e.g. aldrin, dieldrin, HCH,

heptachlor, parathion, toxaphene [Harris, 1972a; Hoffman and Lindquist, 1949; Hoffman, Roth and Lindquist, 1949], chlordane [Harris, 1972b], chlorpyriphos, diazinon and methomyl [Harris, 1971]).

The toxicity of carbon disulphide doubled with a 10°C rise in temperature due to an increase in vapour pressure (Jones, 1933), but the effect of temperature upon the toxicity of other soil fumigants such as 1,3-dichloropropene was less predictable (McKenry and Naylor, 1975).

The toxicity to earthworms of chemicals like endosulfan or benomyl, did not appear to depend upon temperature between 10-15°C (Haque and Ebing, 1983a), although other reports suggest that the toxicity of benomyl is correlated positively with the temperature of the soil between 5-15°C (Blackshaw, 1980).

An increase in vapour pressure is not the only cause of a temperature dependent change in the toxicity of a chemical to invertebrates. The toxicity to insects of a deposit of DDT on glass was correlated positively with temperature up to 21°C, above which this correlation became negative, although the toxicity of aldrin and heptachlor was correlated positively with temperature throughout the range tested. These observations indicated that DDT was adsorbed more strongly onto glass, and was less volatile than the other chemicals, at higher temperatures (Harris, 1971).

The rate of evaporation of chemicals from water (such as that which could occur from the moisture film surrounding a filter paper) was influenced by both the vapour pressure and the solubility of the compound in water (Kenaga, 1972), together with the relative humidity of the atmosphere (Harris, 1972a; Kenaga, 1972). These properties were affected by an increase in temperature.

Earthworms are poikilothermic and temperature can affect the rate at which metabolic processes occur. This can influence the behaviour of the earthworm and its response to a toxic chemical (Hassall, 1982).

Other variables that can influence an assessment of toxicity to earthworms include the duration of the test. The period of exposure of LC_{50} contact tests with insects often falls within the range between 24 hours (Morrison, 1950) and 140 hours (Parkin, 1951), but is rarely longer.

The illumination of the test and the formulation of the test chemical may also affect assessments of toxicity to invertebrates (Busvine, 1957).

The accuracy of an LC_{50} estimate of toxicity to an organism improves as the number of replicates used at each concentration of the test chemical is increased, and in insect bioassays it is considered unwise to use less than 15-20 individuals per replicate (Busvine, 1957). However, tests using earthworms have to avoid overcrowding because the death of one earthworm in a small container can affect others in the same vessel. Mortality data from tests in which the death of one earthworm affects the survival of another cannot be processed using probit analysis, as this technique assumes that each death is independent. Therefore, only one earthworm could be used in each vial for the filter paper contact test and a high level of replication was necessary to reduce the variability of the resultant estimates of toxicity.

The filter paper contact test had to be sensitive, reproducible, inexpensive and capable of testing the toxicity of any chemical to E. fetida. The optimum conditions for the test were assessed in a study of the effect upon assessments of toxicity of several experimental variables which included a) the body weight and sexual maturity of the earthworm, b) temperature, c) area of filter paper, d) type and grade of filter paper, e) period of exposure, f) number of replicates, g) illumination, h) ventilation of the vial and i) the use of formulating agents. Once the conditions of the test had been defined, the method was used to assess the toxicity of numerous chemicals to earthworms.

3.2. Materials, methods and results

3.2.1. Materials

The pesticides used were of technical grade and included chlordane, dieldrin, triazophos, carbaryl, benomyl and thiophanate-methyl. The solvents were of analytical grade, as were the other test chemicals which included cadmium acetate, chloroacetamide, copper sulphate, lead acetate, pentachlorophenol and potassium bromide. The details of the solvents that were used for the test chemicals and the suppliers of these chemicals, together with the specifications of the apparatus for the filter paper contact test are given in tabular form (Tables A5.1 and A5.4).

The earthworms were sexually mature E. fetida andrei weighing between 0.4-0.6 g and cultured from cocoons in boxes containing pressed cow manure (matured for 3 weeks beforehand), at 20°C in the dark. The earthworms were extracted by hand after approximately 9 weeks, washed and allowed to empty their guts by storing them on damp filter paper overnight before being used in the experiments.

3.2.2. General methods

Where possible, the test chemicals were applied to the filter paper as a solution or suspension in water. These suspensions were produced by an adaptation of the method of McIntosh et al. (1981) in which 200 µl of a solution of the test chemical, dissolved in a suitable organic solvent, was added to 199.6 ml of rapidly swirling distilled water that contained 200 µl of 10% Tween 80 (a detergent) in ethanolic solution.

Mortality was assessed mechanically by prodding the anterior segments of the earthworm with a spatula or by gently squashing the body. The absence of a twitch reaction or the inability to restore turgor pressure to the flattened segments was taken to indicate death. The latter method of assessment was used for test chemicals that paralysed the earthworms.

Every experiment was accompanied by an untreated control and, where a solvent had been used to apply the test chemical, by a solvent blank. The vials containing the solvent blank were treated with solvent alone in a manner similar to that used when applying the test chemical. Mortality in the controls was not tolerated in any of the experiments and those in which it occurred were re-run. The experiments were conducted in a cooled incubator (Gallenkamp and Company Ltd.) that had a thermal stability at 20°C of ± 1°C. Lighting within the incubator was provided by two 50 W fluorescent lamps ('Daylight', Thorn EMI Ltd.). Experiments in which the vials were open to the atmosphere were done in a transparent plastic box that contained a filter paper wick (0.5 m^2 filter paper per m^3 air) standing in a jar of water to humidify the air.

The data were processsed, where possible, by a computed probit analysis using the maximum likelihood programme. The details of this analysis and the appropriate data corrections were the same as those described for previous experiments (Section 2.2.2).

3.2.3. Method for the filter paper contact test

The filter paper contact test was developed from a prototype method described previously (Section 2.2.5). The development programme consisted of observing the effect upon the toxicity of chemicals to earthworms that was caused by altering a single experimental parameter at a time. The descriptions of the experimental methods used in the investigations contain only details of deviations from this basic method, the other conditions may be assumed to have remained unchanged.

The test chemical was dissolved or suspended in distilled water, or dissolved in a suitable volatile organic solvent. One ml of this was pipetted onto a Whatman No.1 cellulose filter paper that lined internally the side walls of a glass vial. The vial was made of colourless, transparent soda glass and measured approximately 8 cm x 3 cm diameter. The area of

the side wall was 75 cm^2 (Plate 3.1). The solution of test chemical was swirled in the vial to give a uniform deposit and any volatile organic solvent that remained was evaporated gently using a jet of compressed air. The evaporation of the organic solvent was achieved most efficiently by passing the air through a Pasteur pipette placed near the internal base of the vial (Plate 3.2). Care was taken not to overdry the deposit which could cause a loss of volatile test chemical. Filter paper that had been dried in this manner was moistened with 1 ml distilled water. Each vial contained one earthworm, and ten replicates were used at each of five or more concentrations of the test chemical in a logarithmic dilution series that extended between 1.0 - 5000 $\mu g.cm^{-2}$ (Plate 3.3). The vials were sealed with a polypropylene cap, laid horizontally in the dark at 20° ± 1°C, and mortality was determined after 48 hours.

The LT_{50} assessments of toxicity were made at a concentration of test chemical similar to the 48 hour LC_{50}, and mortality was assessed hourly for 8 hours and thereafter at 12 hour intervals. Most experiments were repeated twice or more.

3.2.4 The effect of the body weight and sexual maturity of earthworms upon the assessments of toxicity using the filter paper contact method

Method

The earthworms were graded into weight categories when they had an empty gut. These categories of body weight were separated by 0.1 ± 0.05 g increments between 0.1 - 0.7 g. In all other respects the earthworms were prepared as described previously. Estimates of the LT_{50} were made for chlordane, chloroacetamide and copper sulphate at concentrations of 1, 8 and 50 $\mu g.cm^{-2}$.

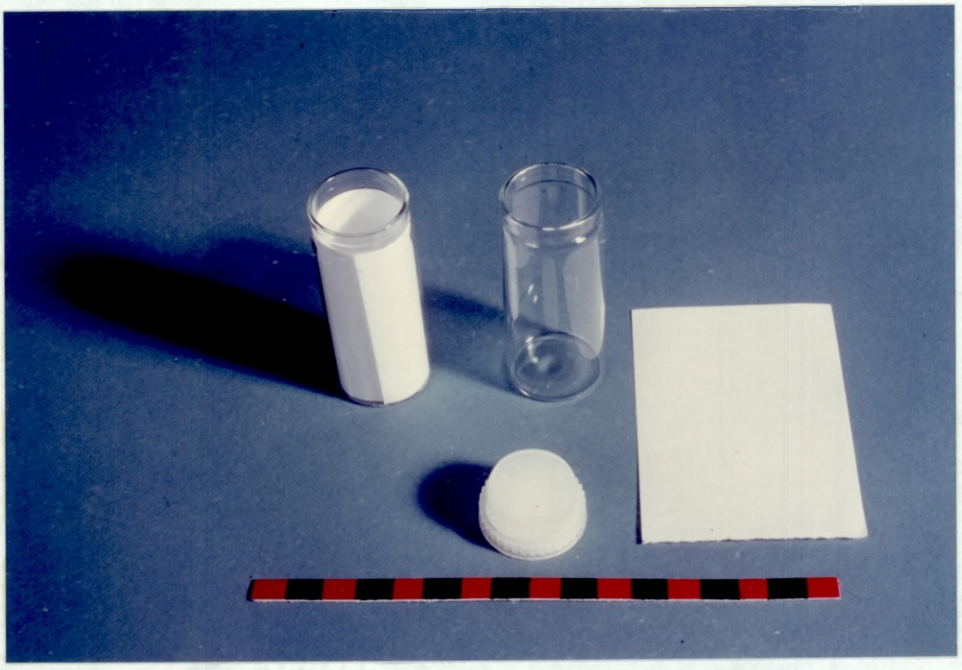


Plate 3.1. Filter paper contact test materials: glass vial, cap and filter paper. (Scale: 1 cm).

Plate 3.2. Filter paper contact test: evaporation of volatile organic solvent using compressed air.

Plate 3.3. Filter paper contact test: the apparatus in use showing two replicates of each concentration of the test chemical and a control.

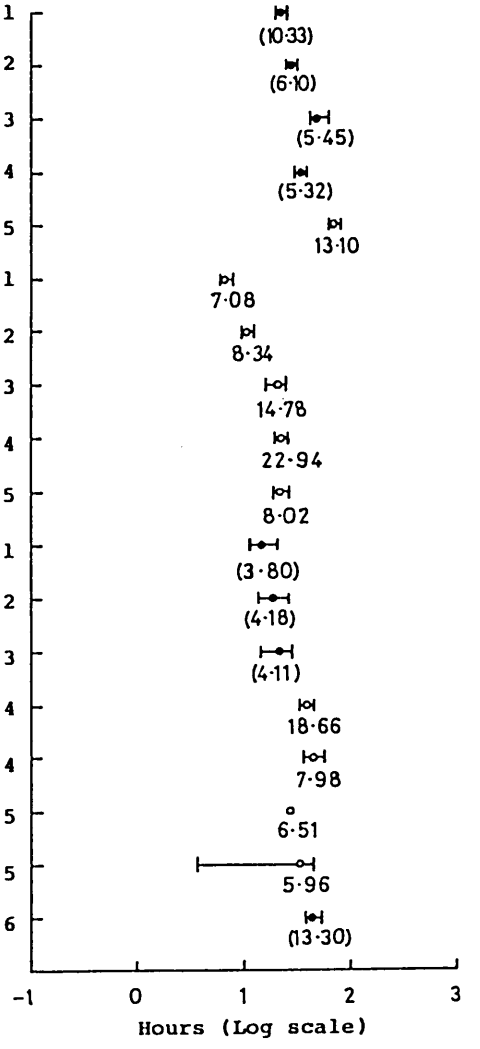
Results

Earthworm body weight correlated negatively with the susceptibility of the earthworms to the chemicals. The change in susceptibility due to an increase in body weight was less marked for chloroacetamide and copper sulphate with earthworms that weighed more than 0.3 or 0.4 g respectively (Tables A7.10.1-A7.10.3 and Figure 3.1). However, the susceptibility of earthworms weighing between 0.3 - 0.4 g did not appear to conform to this inverse relationship. This was particularly clear for chlordane and copper sulphate and corresponded to the period of puberty as the earthworms began to mature sexually at a body weight of 0.4 - 0.5 g. Although the gradients of the probit lines (and hence the range of periods of exposure within which the earthworms succumbed to the effects of the chemical) were not related to the body weight of the earthworms, a chi-squared test indicated that the replicated LT_{50} estimates for copper sulphate and

Figure 3.1

THE EFFECT OF WORM BODY WEIGHT AND SEXUAL MATURITY UPON FILTER PAPER CONTACT TOXICITY ASSESSMENTS WITH E. FETIDA

Chemical	Concentration ($\mu g.cm^{-2}$)	Body weight (g)
chlordane	1	0.1
chlordane	1	0.2
chlordane	1	0.3
chlordane	1	0.4
chlordane	1	0.5
chloroacetamide	8	0.1
chloroacetamide	8	0.2
chloroacetamide	8	0.3
chloroacetamide	8	0.4
chloroacetamide	8	0.5
copper sulphate	50	0.1
copper sulphate	50	0.2
copper sulphate	50	0.3
copper sulphate	50	0.4
copper sulphate	50	0.4
copper sulphate	50	0.5
copper sulphate	50	0.5
copper sulphate	50	0.6



Concentration expressed in $\mu g.cm^{-2}$
(Test chemical : filter paper surface)

KEY

⊢○⊣ x	Individual LT_{50} estimate [o], gradient [x] and fiducial limits
⊢○⊣ (x)	Individual LT_{50} estimate [o], common gradient for more than one replicate [(x)] and fiducial limits
⊢●⊣ (x)	Common LT_{50} estimate [●], gradient [(x)] and fiducial limits for more than one replicate
⊢····⊣	Range within which a poorly defined LT_{50} falls
No LT_{50} →	LT_{50} at an undefined period of exposure that was greater than those studied
← No LT_{50}	LT_{50} at an undefined period of exposure that was less than those studied
(No LT_{50})	More than one LT_{50} at an undefined period of exposure

chlordane differed significantly with earthworms that weighed more than 0.3 and 0.4 g respectively. Small earthworms were most susceptible to these chemicals, but proved to be difficult to extract from the culture medium and to handle subsequently.

3.2.5 The effect of test temperature upon the assessment of toxicity to earthworms

Method

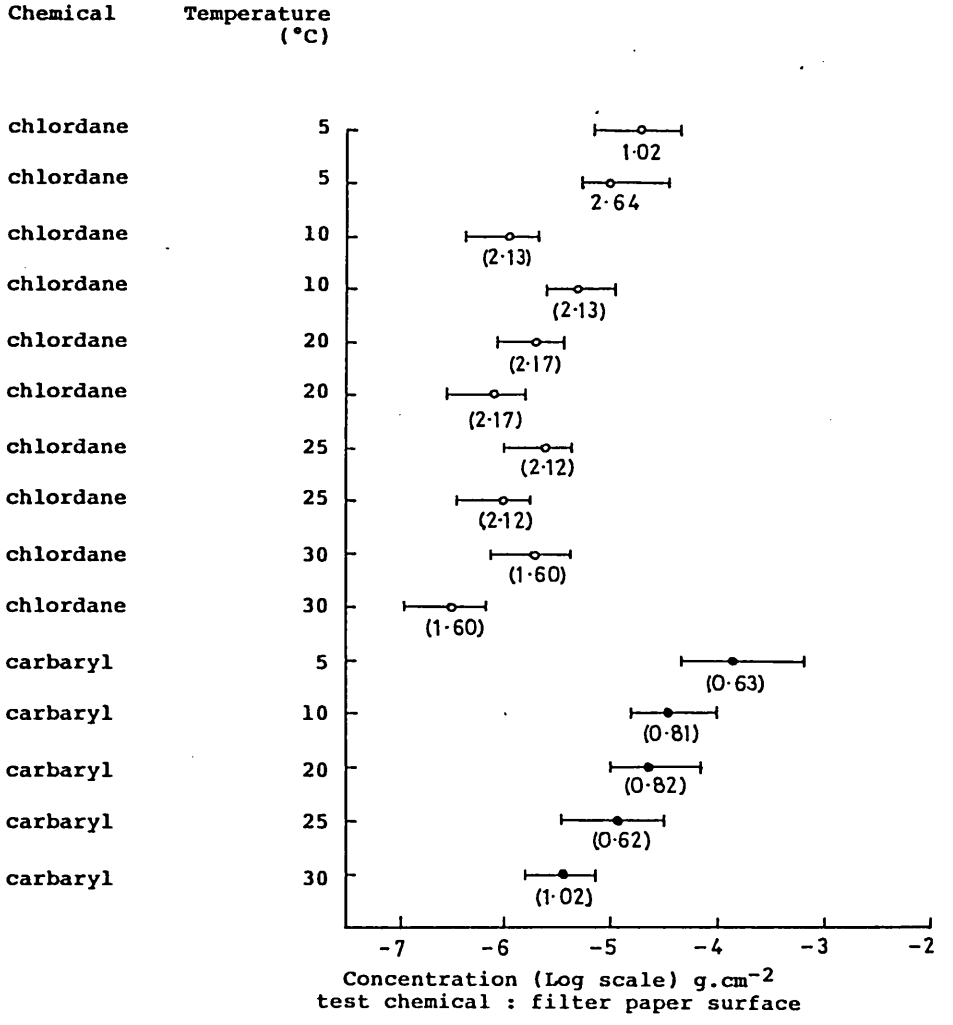
The toxicity of chlordane and carbaryl was estimated as an LC_{50} at 5°, 10°, 20°, 25° and 30°C. Chlordane was used in this experiment because it was known to have a toxicity to insects (and thus, probably to earthworms) that was dependent upon temperature (Harris, 1972b), but it was not known whether the toxicity of carbaryl to earthworms was affected by temperature.

Results

Both chlordane and carbaryl became more toxic as the temperature increased (Table A7.11 and Figure 3.2), although this effect was seen most clearly with carbaryl. The toxicity of chlordane changed little above 20°C, but the replicates differed significantly when tested by the chi-squared test and this observation must be interpreted with care. The gradients of the probit lines (and hence the range of concentrations within which the earthworms succumbed to the effects of the test chemical) did not appear to be related to the temperature at which the experiment was conducted.

Figure 3.2

THE EFFECT OF TEST TEMPERATURE UPON CONTACT TOXICITY ASSESSMENTS WITH *E. FETIDA*



KEY

⊢o⊣ x	Individual LC_{50} estimate [o], gradient [x] and fiducial limits
⊢o⊣ (x)	Individual LC_{50} estimate [o], common gradient for more than one replicate [(x)] and fiducial limits
⊢●⊣ (x)	Common LC_{50} estimate [●], gradient [(x)] and fiducial limits for more than one replicate
⊢----⊣	Range within which a poorly defined LC_{50} falls
No LC_{50} →	LC_{50} at an undefined concentration that was greater than those studied
← No LC_{50}	LC_{50} at an undefined concentration that was less than those studied
(No LC_{50})	More than one LC_{50} at an undefined concentration

3.2.6 The effect of filter paper area upon the assessments of toxicity to earthworms

Method

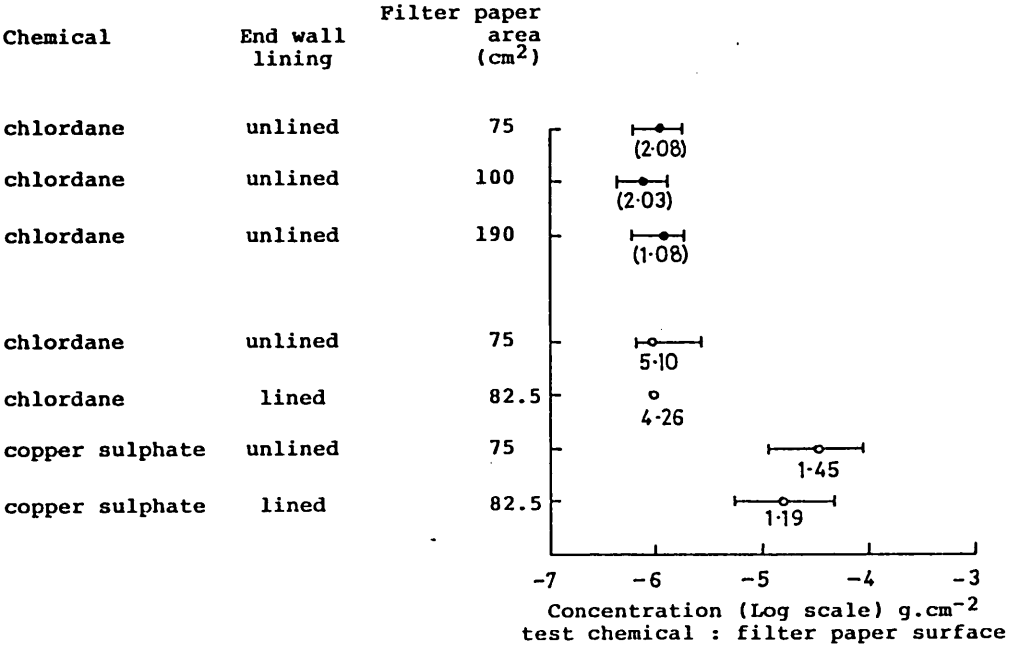
The toxicity of chlordane was estimated as an LC_{50} in vials of three sizes (8 cm x 3 cm diameter, 8 cm x 4 cm diameter and 11 cm x 5.5 cm diameter) which had internal side wall areas of 75, 100 and 190 cm^2 respectively that were lined with filter paper. The test chemical was applied to these areas of filter paper in 1.0, 1.3 and 2.5 ml solvent respectively. An experiment was also conducted with chlordane and copper sulphate to compare the effect upon toxicity to earthworms of an additional filter paper to cover the end wall of an 8 cm x 3 cm diameter vial. This gave a total filter paper area of 82.5 cm^2.

Results

The area of filter paper that lined the side walls of the vial appeared to have little effect upon the toxicity of chlordane to earthworms (Table A7.12.1 and Figure 3.3). The toxicity of chlordane was also unaffected by covering the end wall of the vial (Table A7.12.2 and Figure 3.3), although copper sulphate appeared to be slightly more toxic to earthworms in the vials with an end wall which was lined. In several experiments, the earthworms were observed to curl up in the angle between the side wall and base of the vial to avoid the filter paper that was impregnated with the test chemical.

Figure 3.3

THE EFFECT OF FILTER PAPER AREA UPON CONTACT TOXICITY ASSESSMENTS WITH *E. FETIDA*



KEY

⊢o⊣ x	Individual LC_{50} estimate [o], gradient [x] and fiducial limits
⊢o⊣ (x)	Individual LC_{50} estimate [o], common gradient for more than one replicate [(x)] and fiducial limits
⊢●⊣ (x)	Common LC_{50} estimate [●], gradient [(x)] and fiducial limits for more than one replicate
⊢----⊣	Range within which a poorly defined LC_{50} falls
No LC_{50} →	LC_{50} at an undefined concentration that was greater than those studied
← No LC_{50}	LC_{50} at an undefined concentration that was less than those studied
(No LC_{50})	More than one LC_{50} at an undefined concentration

3.2.7 The effect of the physical characteristics and the material from which the filter paper was made upon the assessment of toxicity to earthworms

Method

The thickness, density and capacity to retain water (Table 3.1) of cellulose and glass fibre filter paper can affect the availability of a test chemical applied to them. The toxicity of chlordane was measured as an LC_{50} using three cellulose papers, and at a concentration of 78.5 $\mu g.cm^{-2}$ was assessed as an LT_{50} using Whatman No.1 Grade cellulose and Whatman GF/A Grade glass fibre filter papers.

Results

The retention of water within the cellulose filter paper was a function of the density and the thickness. The glass fibre paper had the lowest density and was very absorbent. The toxicity of chlordane to earthworms appeared to be correlated negatively with the capacity of the cellulose filter paper to retain water (Table A7.13 and Figure 3.4) and the replicated estimates of the LC_{50} did not differ significantly for any of these cellulose papers. The LT_{50}'s for chlordane that were estimated using cellulose and glass fibre filter papers were very similar (Table A7.14 and Figure 3.5). The glass fibre filter papers were stiff and proved to be difficult to use in this type of test.

Table 3.1. The physical characteristics of Whatman cellulose and glass-fibre filter papers.

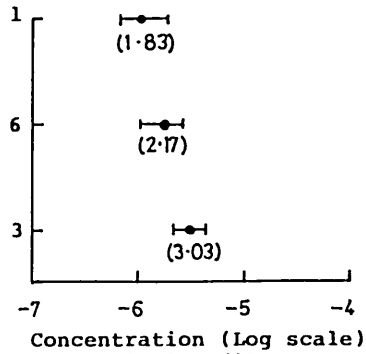
Filter paper material	Whatman Grade	Density $g.m^{-2}$	Thickness mm	Water retention $g.m^{-2}$ (1)
Cellulose	1	87	0.18	120
Cellulose	3	185	0.39	350
Cellulose	6	100	0.18	140
Glass fibre	GF/A	53	0.26	275

(1) Determined by immersing a pre-weighed 15 cm diameter circle of each filter paper in water for 30 seconds, removing the paper and allowing the surface water to drip away before re-weighing.

Figure 3.4

THE EFFECT OF WHATMAN FILTER PAPER GRADE UPON CONTACT TOXICITY ASSESSMENTS WITH E. FETIDA

Chemical	Filter paper material	Whatman filter paper grade
chlordane	cellulose	1
chlordane	cellulose	6
chlordane	cellulose	3



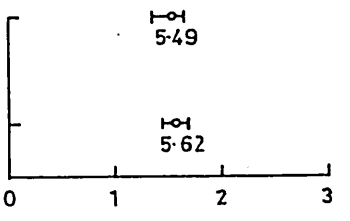
KEY

⊢o⊣ x	Individual LC_{50} estimate [o], gradient [x] and fiducial limits
⊢o⊣ (x)	Individual LC_{50} estimate [o], common gradient for more than one replicate [(x)] and fiducial limits
⊢●⊣ (x)	Common LC_{50} estimate [●], gradient [(x)] and fiducial limits for more than one replicate
⊢----⊣	Range within which a poorly defined LC_{50} falls
No LC_{50} →	LC_{50} at an undefined concentration that was greater than those studied
← No LC_{50}	LC_{50} at an undefined concentration that was less than those studied
(No LC_{50})	More than one LC_{50} at an undefined concentration

Figure 3.5

THE EFFECT OF WHATMAN FILTER PAPER MATERIAL UPON CONTACT TOXICITY ASSESSMENTS WITH E. FETIDA

Chemical	Concentration ($\mu g.cm^{-2}$)	Filter paper material and Whatman grade
chlordane	78.5	Cellulose Grade 1
chlordane	78.5	Glass fibre Grade GF/A



Concentration expressed in $\mu g.cm^{-2}$
(Test chemical : filter paper surface)

KEY

⊢o⊣ x	Individual LT_{50} estimate [o], gradient [x] and fiducial limits
⊢o⊣ (x)	Individual LT_{50} estimate [o], common gradient for more than one replicate [(x)] and fiducial limits
⊢●⊣ (x)	Common LT_{50} estimate [●], gradient [(x)] and fiducial limits for more than one replicate
⊢----⊣	Range within which a poorly defined LT_{50} falls
No LT_{50} →	LT_{50} at an undefined period of exposure that was greater than those studied
← No LT_{50}	LT_{50} at an undefined period of exposure that was less than those studied
(No LT_{50})	More than one LT_{50} at an undefined period of exposure

3.2.8 The effect of the period of exposure upon the assessments of toxicity to earthworms

Method

The LC_{50} of carbaryl was estimated after periods of exposure of 24, 48, 72 and 96 hours.

Results

The toxicity of carbaryl appeared to be correlated negatively with the length of the period of exposure (Table A7.15 and Figure 3.6), although this conclusion must remain tentative, because the data for 24 and 96 hours were not suitable for probit analysis as they contained too few partial mortalities.

3.2.9 The effect of the number of replicates upon the assessments of toxicity to earthworms

Method

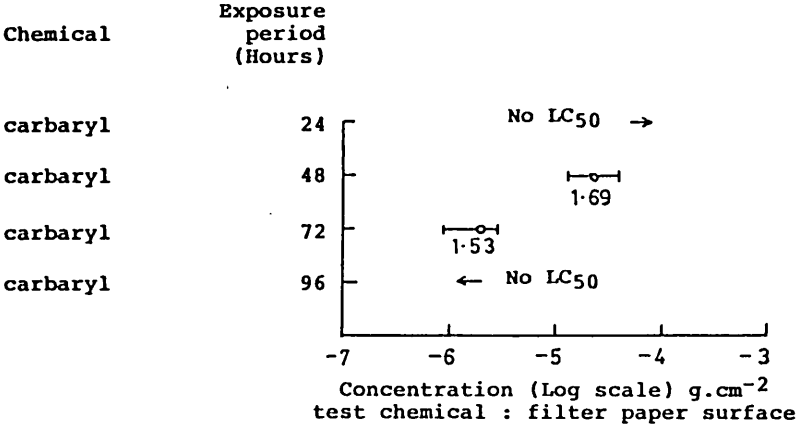
The toxicity of carbaryl was assessed as an LC_{50} using 5, 10, 15, 20 and 25 replicates at each concentration.

Results

The LC_{50} of carbaryl was largely unaffected by the number of replicates (Table A7.16 and Figure 3.7), although it was not possible to estimate whether the replicates differed significantly at some levels of replication but not at others by using the chi-squared test. The fiducial limits of the LC_{50}'s did not vary when experiments were done with more than 10 replicates. This number of replicates was taken, in the absence of other data, to indicate the minimum amount of replication that was necessary to give a reproducible estimate of the LC_{50}.

Figure 3.6

THE EFFECT OF EXPOSURE PERIOD UPON CONTACT TOXICITY ASSESSMENTS WITH E. FETIDA

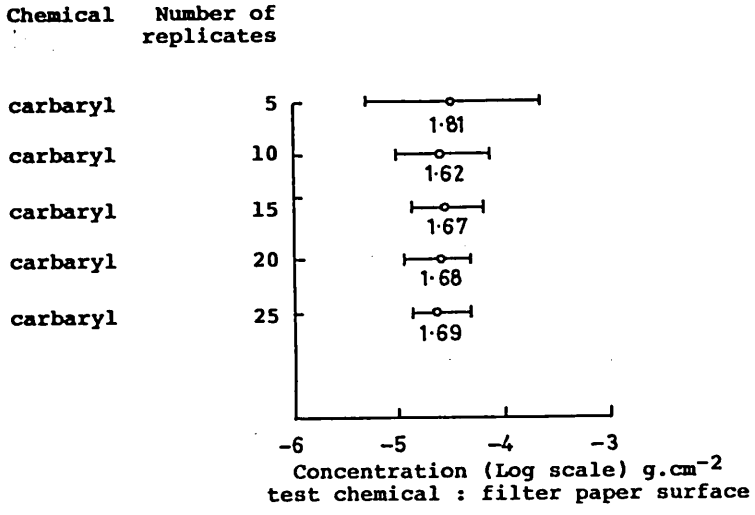


KEY

⊢o⊣ x	Individual LC_{50} estimate [o], gradient [x] and fiducial limits
⊢o⊣ (x)	Individual LC_{50} estimate [o], common gradient for more than one replicate [(x)] and fiducial limits
⊢●⊣ (x)	Common LC_{50} estimate [●], gradient [(x)] and fiducial limits for more than one replicate
⊢----⊣	Range within which a poorly defined LC_{50} falls
No LC_{50} →	LC_{50} at an undefined concentration that was greater than those studied
← No LC_{50}	LC_{50} at an undefined concentration that was less than those studied
(No LC_{50})	More than one LC_{50} at an undefined concentration

Figure 3.7

THE EFFECT OF THE NUMBER OF REPLICATES UPON CONTACT TOXICITY ASSESSMENTS WITH E. FETIDA



KEY

⊢o⊣ x	Individual LC_{50} estimate [o], gradient [x] and fiducial limits
⊢o⊣ (x)	Individual LC_{50} estimate [o], common gradient for more than one replicate [(x)] and fiducial limits
⊢●⊣ (x)	Common LC_{50} estimate [●], gradient [(x)] and fiducial limits for more than one replicate
⊢---⊣	Range within which a poorly defined LC_{50} falls
No LC_{50} →	LC_{50} at an undefined concentration that was greater than those studied
← No LC_{50}	LC_{50} at an undefined concentration that was less than those studied
(No LC_{50})	More than one LC_{50} at an undefined concentration

3.2.10 The effect of the illumination and ventilation of the vials upon assessments of toxicity to earthworms

Method

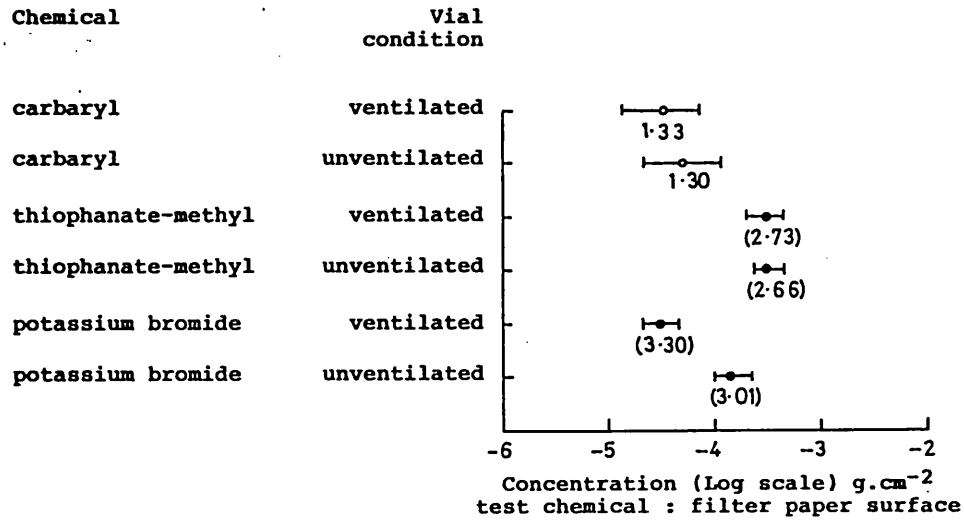
Carbaryl, thiophanate-methyl and potassium bromide were non-volatile chemicals known to be toxic to earthworms. The LC_{50}'s of these compounds were estimated in darkness using vials with a) an airtight cap, or b) a cap that allowed ventilation through a 1 cm diameter circular hole covered with a fine nylon gauze. The ventilated vials were used in a humid environment that prevented desiccation of the earthworms. An additional experiment was done to estimate the LT_{50} of chlordane with *E. fetida* at a concentration of 5 $\mu g.cm^{-2}$ in vials that were either ventilated or sealed, and illuminated or in darkness.

Results

Carbaryl and potassium bromide appeared to be more toxic to earthworms in the ventilated vials (Table A7.17 and Figure 3.8), although thiophanate-methyl had a similar LC_{50} whether the vials were ventilated or not. The estimates of the LT_{50} for chlordane also appeared to be unaffected by the ventilation or illumination of the vial (Table A7.18 and Figure 3.9), although the replicates of the assessments that were done in the ventilated vials gave LT_{50}'s that differed significantly when analysed using a chi-squared test.

Figure 3.8

THE EFFECT OF VIAL VENTILATION UPON CONTACT TOXICITY ASSESSMENTS



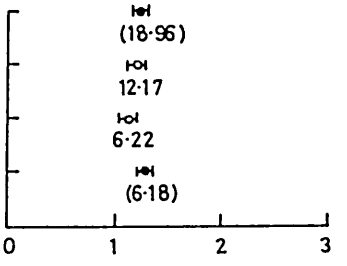
KEY

⊢o⊣ x	Individual LC_{50} estimate [o], gradient [x] and fiducial limits
⊢o⊣ (x)	Individual LC_{50} estimate [o], common gradient for more than one replicate [(x)] and fiducial limits
⊢●⊣ (x)	Common LC_{50} estimate [●], gradient [(x)] and fiducial limits for more than one replicate
⊢----⊣	Range within which a poorly defined LC_{50} falls
No LC_{50} →	LC_{50} at an undefined concentration that was greater than those studied
← No LC_{50}	LC_{50} at an undefined concentration that was less than those studied
(No LC_{50})	More than one LC_{50} at an undefined concentration

Figure 3.9

THE EFFECT OF ILLUMINATION AND VIAL VENTILATION UPON CONTACT TOXICITY ASSESSMENTS WITH *E. FETIDA*

Chemical	Concentration ($\mu g.cm^{-2}$)	Vial condition
chlordane	5	Unventilated : Dark
chlordane	5	Ventilated : Dark
chlordane	5	Ventilated : Dark
chlordane	5	Unventilated : Light



Concentration expressed in $\mu g.cm^{-2}$
(Test chemical : filter paper surface)

KEY

⊢○⊣ x	Individual LT_{50} estimate [o], gradient [x] and fiducial limits
⊢○⊣ (x)	Individual LT_{50} estimate [o], common gradient for more than one replicate [(x)] and fiducial limits
⊢●⊣ (x)	Common LT_{50} estimate [●], gradient [(x)] and fiducial limits for more than one replicate
⊢----⊣	Range within which a poorly defined LT_{50} falls
No LT_{50} →	LT_{50} at an undefined period of exposure that was greater than those studied
← No LT_{50}	LT_{50} at an undefined period of exposure that was less than those studied
(No LT_{50})	More than one LT_{50} at an undefined period of exposure

3.2.11 The effect of wetters, emulsifiers and other formulating agents upon the assessments of toxicity to earthworms

Method

The formulants present in pesticides that are prepared commercially can affect the toxicity of the active ingredient. The LT_{50}'s of technical benomyl, Benlate wettable powder (E.I. DuPont de Nemours and Company), technical benomyl with Teepol, Teepol and xylene were estimated at concentrations of the pesticide of 0.5 $\mu g.cm^{-2}$ a.i., and at concentrations of the wetting and emulsifying agents of 500 $\mu g.cm^{-2}$. The manufacturers of Benlate would not disclose which formulants were used in the production of this fungicide. I decided, therefore, to test Teepol and xylene with this fungicide, because these chemicals are used frequently as wetting and emulsifying agents respectively in the manufacture of pesticides.

Results

Technical benomyl, Benlate w.p. and technical benomyl with Teepol had a similar toxicity to earthworms (Table A7.19 and Figure 3.10). These treatments were slightly more toxic than Teepol alone, although xylene alone was extremely toxic at the concentration used and for which the LT_{50} was too short to be measured accurately. The toxicity of technical benomyl and Teepol appeared to be slightly additive.

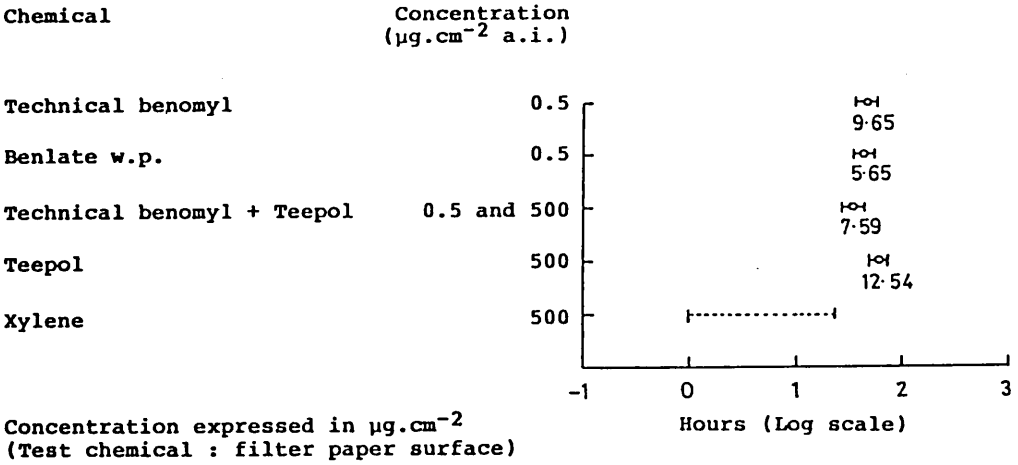
3.2.12 Assessment of the toxicity of chemicals to earthworms using the filter paper contact test

Method

The filter paper contact test was used to estimate the LC_{50}'s of chlordane, dieldrin, triazophos, carbaryl, benomyl, thiophanate-methyl, pentachlorophenol, trichloroacetic acid, chloroacetamide, potassium bromide, copper sulphate, lead acetate and cadmium acetate.

Figure 3.10

THE EFFECT OF FORMULANTS UPON THE TOXICITY OF BENOMYL TO E. FETIDA IN THE FILTER PAPER CONTACT TEST



Concentration expressed in µg.cm^{-2}
(Test chemical : filter paper surface)

KEY

⊢o⊣ x	Individual LT_{50} estimate [o], gradient [x] and fiducial limits
⊢o⊣ (x)	Individual LT_{50} estimate [o], common gradient for more than one replicate [(x)] and fiducial limits
⊢●⊣ (x)	Common LT_{50} estimate [●], gradient [(x)] and fiducial limits for more than one replicate
⊢····⊣	Range within which a poorly defined LT_{50} falls
No LT_{50} →	LT_{50} at an undefined period of exposure that was greater than those studied
← No LT_{50}	LT_{50} at an undefined period of exposure that was less than those studied
(No LT_{50})	More than one LT_{50} at an undefined period of exposure

Results

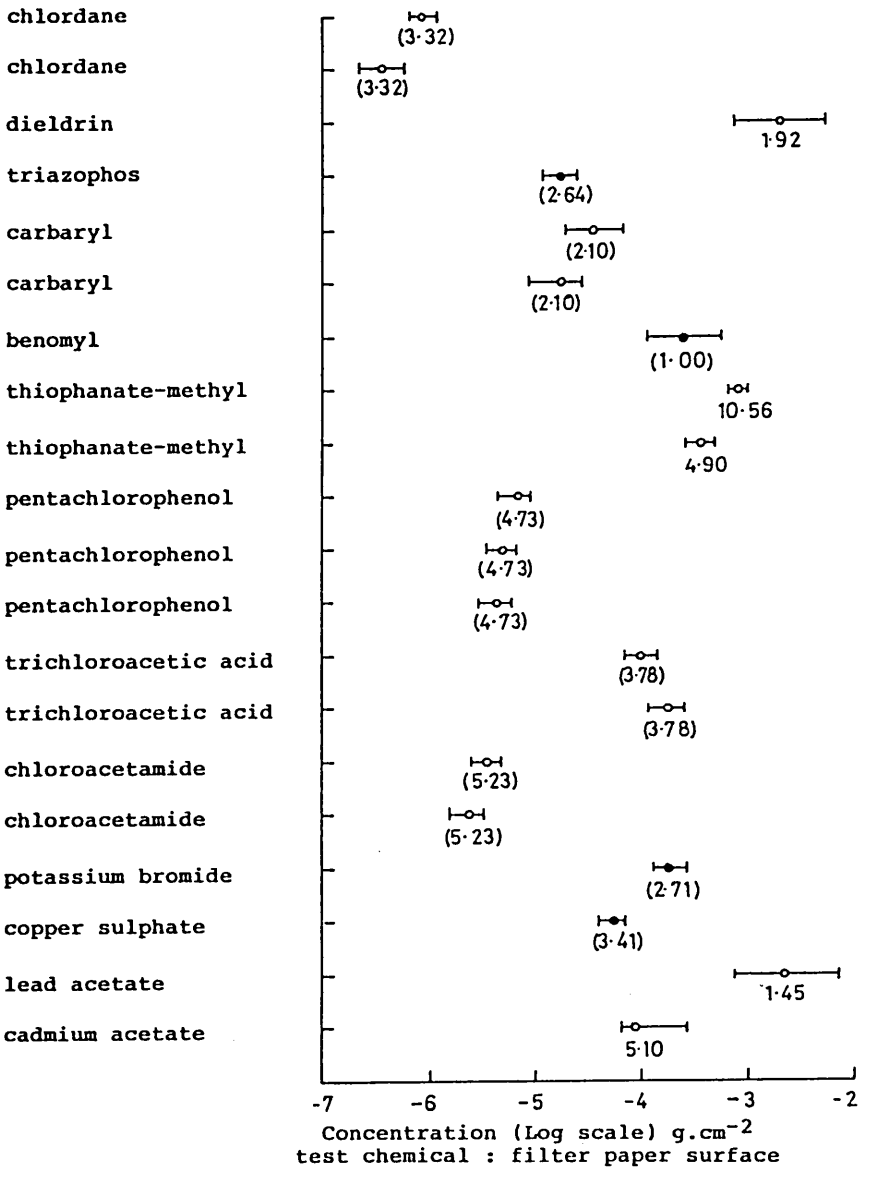
The assessments of toxicity (Table A7.20 and Figure 3.11) allowed the chemicals to be ranked according to their LC_{50}'s, within categories of toxicity to earthworms that had been assigned arbitrarily (Table 3.2).

Table 3.2. Categories of the toxicity of chemicals to earthworms assigned to the results obtained using the filter paper contact test.

Category of toxicity	Range of the LC_{50}'s within this category $\mu g.cm^{-2}$	Chemical
Very toxic	<0.99	Chlordane
Toxic	1.0 - 9.9	Chloroacetamide Pentachlorophenol Triazophos Carbaryl Copper sulphate Cadmium acetate
Moderately toxic	10.0 - 99.9	Trichloroacetic acid Potassium bromide Benomyl Thiophanate-methyl
Slightly toxic	>100.0	Dieldrin Lead acetate

Figure 3.11

FILTER PAPER CONTACT TEST TOXICITY ASSESSMENTS WITH E. FETIDA



KEY

⊢o⊣ x	Individual LC_{50} estimate [o], gradient [x] and fiducial limits
⊢o⊣ (x)	Individual LC_{50} estimate [o], common gradient for more than one replicate [(x)] and fiducial limits
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⊢----⊣	Range within which a poorly defined LC_{50} falls
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← No LC_{50}	LC_{50} at an undefined concentration that was less than those studied
(No LC_{50})	More than one LC_{50} at an undefined concentration

3.3. Discussion

The filter paper contact test provided a simple, rapid and inexpensive method for assessing the toxicity of chemicals to earthworms. These assessments of toxicity correlated poorly (Heimbach, 1984; 1985b) with those from other tests developed subsequently using an artificial soil ($r = 0.55$) or a mixture of silica paste-glass balls ($r = 0.48$), because the filter paper contact test was of short duration and the earthworms were exposed to the test chemicals at the cuticle alone, on a substrate that was non-adsorptive, of neutral pH and quite unlike a soil.

Assessments of the time required to achieve 50% mortality (LT_{50}'s) were used extensively in this study and proved to be a valuable tool, permitting multiple sampling for mortality and providing a more refined estimate of toxicity to earthworms than that obtained from the single sample taken during a lethal concentration (LC_{50}) study. However, LT_{50} assessments indicate the toxicity of a chemical to earthworms in the field less accurately than an assessment based upon concentration (Besch, 1976b). Hence the latter type of measurement was adopted in the final filter paper contact method.

The developmental stage and size of the test organisms can affect the assessment of the toxicity of chemicals (Busvine, 1957). In this study, young earthworms were found to be more susceptible to the chemicals than older earthworms. This may be because young earthworms have a thinner cuticle, greater surface area to volume ratio, more limited metabolic activity and excretory capacity, and are less able to avoid toxic chemicals than adult earthworms. These results are supported by similar observations on L. terrestris (Lebrun et al., 1981), although other workers using E. fetida failed to find any effect of body weight upon susceptibility to benomyl or chloroacetamide (Heimbach and Edwards,

1983). This latter paper contained insufficient detail to permit an explanation of such contradictory results.

The relationship between body weight and susceptibility to chemicals was non-linear for all the chemicals tested, furthermore, earthworms weighing 0.3 and 0.4 g were particularly susceptible to chlordane and copper sulphate respectively. Well fed earthworms of this body weight were beginning to mature sexually and it is possible that the metabolic changes occurring at this time limited the ability of the earthworms to tolerate toxic chemicals.

The size of earthworm that appeared best suited to the tests developed in my study weighed between 0.4 - 0.6 g. These earthworms could be handled easily and were large enough to avoid the unpredictable changes in susceptibility that occured with younger earthworms. E. fetida was able to achieve this body weight in laboratory cultures within 9 weeks of hatching from the cocoon (Neale, 1984).

Temperature can exert an unpredictable effect upon the results of a bioassay (Harris, 1972a; Parkin, 1951) and must therefore be standardised. In my study, the test temperature was correlated positively with the toxicity of chemicals to earthworms. Tests at low temperatures required a long period of exposure for sufficient earthworms to die to give an estimate of the LC_{50}, whilst at higher temperatures the rate of decomposition and evaporation of the test chemical was accelerated. The toxicity of chlordane to earthworms changed little above 20°C and as extremes of temperature were undesirable, a test temperature of 20° ± 2°C was recommended. This temperature was within the range that allowed the optimum growth and survival of E. fetida (Kaplan et al., 1980) and was readily available in existing controlled environment facilities. By recommending a temperature that approximated to room temperature, the filter paper contact test could be used in the absence of such facilities. This temperature is also similar to that used in other studies of toxicity to earthworms (Anon, 1983; Ebing and Haque, 1983a).

The area of the filter paper had little effect upon the toxicity of the chemicals that were studied here. A large area of filter paper exposes the earthworms to more of the test chemical than a smaller area of filter paper. This can have the effect of potentiating the toxicity of compounds that are accumulated strongly. Small areas of filter paper are also easier to treat uniformly and are less expensive to use than large areas, since these require larger test containers.

The density and thickness of the filter paper affected the amount of the solution of the test chemical that was absorbed. The similar amounts of chemical were more toxic on the least absorbent paper, presumably because more of the solution of test chemical was in contact with the earthworm. A low density cellulose filter paper (Whatman Grade No.1) was recommended, which was easy to handle and retained a small volume of water.

The length of the period of exposure of the earthworm to the chemicals affected the assessments of toxicity. The filter paper contact test gave an LC_{50} after 48 and 72 hours only. Data that was collected after longer or shorter periods of exposure often included results showing 100% or 0% mortality which were unsuitable for probit analysis. A review of the principles of testing the toxicity of chemicals to organisms (Besch, 1976b) concluded that the gradient of the probit lines from most acute toxicity tests became stable only after 96 hours. This contrasts with my observation that the variability in the assessments of the toxicity of chemicals to earthworms seems to be increased by the use of extremely short or long periods of exposure, however this conclusion must remain tentative. In short duration tests there was less mortality in the controls and a uniform distribution of the test chemical was maintained. Short tests are also less expensive to use than a long duration tests. A good compromise between these various considerations was a test duration of 48 hours.

An assessment of the toxicity of chemicals can be made more precise by increasing the number of replicates that are used (Busvine, 1957). The LC_{50} for carbaryl changed little as the number of replicates was raised from 5 to 25, although the range of the fiducial limits about the LC_{50} decreased considerably as the number of replicates was increased from 5 to 10. The fiducial limits do not provide the best measure of the repeatability of a result (Stephan, 1977), but in the absence of further data the optimum number of replicates appeared to be 10, above which the range of the fiducial limits decreased minimally.

Ventilation of the vials did not affect the toxicity of chlordane or thiophanate-methyl, although it increased the susceptibility of the earthworms to carbaryl and potassium bromide. This increase in susceptibility may be because the earthworms remained active in a ventilated vial and encountered a greater amount of the test chemical. Other reports have contradicted these observations and indicated that earthworms became more susceptible to toxic chemicals in non-ventilated vials (Stenersen, 1981), probably because the stress caused by the lack of oxygen potentiated the effect of the chemical. A non-ventilated vial was chosen because this prevented the loss of volatile test chemicals and obviated the need for a humid environment which was necessary for a test that used ventilated vials.

The earthworms were more active in darkness. Earthworms that are active make more contact with the treated surface, and become more susceptible to a given concentration of test chemical than less active earthworms. (This effect is analogous to the response of earthworms in airtight and ventilated vials.) Earthworms that were exposed to test chemicals in the dark were found to be more susceptible to the chemicals than those in the light. A dark environment will also avoid photodecomposition of the test chemical and was chosen as the best condition to use.

Formulating agents affected the toxicity of the test chemical, and the wetting and emulsifying agents Teepol and xylene were very toxic to earthworms. These results confirm those of previous studies of the effect of formulants (Parkin, 1951). The toxicity of Teepol seemed to be additive with that of technical benomyl. As pesticides that have been formulated commercially may contain a variety of wetters, stickers and emulsifiers, it is preferable to use technical grade chemicals in toxicity tests. In order to predict the toxicity of chemicals to earthworms in the field, it may be necessary to test the chemical in the formulation that contaminates the environment.

The assessments of the toxicity of the test chemicals indicated that chlordane was the most toxic chemical to earthworms and was followed, in a decreasing order of toxicity, by chloroacetamide, pentachlorophenol, triazophos, carbaryl, copper sulphate, cadmium acetate, trichloroacetic acid, potassium bromide, benomyl, thiophanate-methyl, dieldrin and lead acetate. The probit lines for the repeated estimates of toxicity to earthworms with these chemicals seldom differed significantly for gradient or position of the Y-intercept when analysed using a chi-squared test.

The filter paper contact method has been reported (Goats and Edwards, 1982, 1983, 1985) and used to test a wide range of compounds for toxicity to earthworms (Drewes et al., 1984; Heimbach, 1984, 1985b; Roberts and Dorough, 1984). The work of Roberts and Dorough (1984) confirmed my conclusion that carbaryl was very toxic to earthworms and that thiophanate-methyl was only moderately toxic, although benomyl was found to be more toxic and copper sulphate less toxic to earthworms than in my assessments. These workers also reported that acetone, benzene and ethanol were toxic to earthworms, emphasising the need for an efficient method for removing these solvents from the filter paper after they have been used to apply the test chemicals in the filter paper contact test. These workers modified the method by which such solvents were

evaporated and used a hot air blower. This treatment was probably over-thorough, so the results of their work should be treated with caution especially since they do not present the complete data.

A recent study (Heimbach, 1984) has provided results with the filter paper contact test that may be compared directly to those of my investigation. The 48 hour LC_{50}'s that were determined by my work agree closely with those quoted by Heimbach and, presented in this order, were for chlordane, 0.56, 1.3 $\mu g.cm^{-2}$; carbaryl 24.2, 3.0 $\mu g.cm^{-2}$; benomyl 170.1, 86 $\mu g.cm^{-2}$; pentachlorophenol 6.0, 4.2 $\mu g.cm^{-2}$; chloroacetamide 2.9, 2.4 $\mu g.cm^{-2}$;, potassium bromide 186.5, 530 $\mu g.cm^{-2}$; and copper sulphate 54.3, 30 $\mu g.cm^{-2}$.

In conclusion, the optimum experimental conditions for testing toxicity to earthworms in the filter paper contact test were defined and the method provided a rapid, reproducible, sensitive and inexpensive measure of the toxicity of chemicals to E. fetida.

CHAPTER 4

DEVELOPMENT OF THE ARTIFICIAL SOIL TEST

4.1. Introduction

An artificial soil test was designed to overcome the disadvantages of tests using a natural soil, or silica paste and glass balls for assessing the toxicity of chemicals to earthworms. The natural soil was a medium that seemed unsuitable for E. fetida and the silica paste-glass ball mixture did not reproduce accurately the conditions found in an arable soil and seemed unlikely to predict accurately the toxicity of chemicals to earthworms in the field.

The general characteristics of the artificial soil were defined with assistance from members of the Soil Survey of England and Wales, which is based at Rothamsted Experimental Station. The soil was designed to provide a suitable environment for E. fetida, yet to simulate the conditions that occur in an arable loam soil. Particular emphasis was placed upon achieving a cation exchange capacity and pH similar to those found commonly in field soils. Obviously the organic matter content of the artificial soil needed to be high in order to support this species of earthworm, and such a soil would therefore have a greater capacity for holding water than that of most natural soils.

The toxicity of a chemical to earthworms in soil is influenced by several factors, some of which have been mentioned previously in the description of in the development of the filter paper contact test (Chapter 3). These include the temperature, illumination and ventilation of the test, and the method of formulation of the test chemical. In addition, the toxicity of a chemical depends upon certain characteristics of the soil, such as the content of organic matter, clay and moisture; the pH and the method by which the test chemical is applied to the soil. These

factors can affect the availability and subsequent activity of a chemical (Edwards, 1975a), and also influence the behaviour of the earthworms.

The effect that several components of the soil and the test temperature, the amount of soil in each test container, the period of exposure and the number of replicates had upon the assessment of toxicity to earthworms was estimated experimentally, in order to define the optimum conditions for testing toxicity to earthworms with the artificial soil. These studies used mortality as the end-point. Additional experiments to define these optimum conditions were done using the change in body weight of the earthworms measured under various experimental conditions.

A positive correlation exists between the temperature, and the solubility and rate of volatilisation of chemicals in the soil water (Harris, 1972a; Kenaga, 1972). The other effects of temperature upon the toxicity of a chemical to soil organisms relevant to this study include an inverse relationship which exists between temperature and the adsorption of a chemical onto the soil, and the fact that hydrophobic chemicals desorb more rapidly from wet soils than hydrophilic compounds as the temperature increases (Harris, 1971).

Chemicals can be adsorbed onto soil particles and rendered less toxic. Such interactions are the subject of an extensive literature outside the scope of this present work, but since the type of soil has been shown to affect the toxicity of pesticides to earthworms (Casey le and Eno, 1966; Lofs-Holmin, 1981), this topic will be considered briefly. A more detailed review of the interactions that occur between chemicals and the soil may be found in the works of Bailey and White (1964), Busvine (1957), Ebeling (1963), Goring et al. (1975), Harris (1972a), Hassall (1982) and Kenaga (1975a).

The effect of moisture in the soil upon the toxicity of chemicals can be unpredictable (Harris, 1964, 1966, 1972a) and influence the amount of chemical that is adsorbed onto the particles of soil, and also the biological

activity of chemicals in the aqueous and vapour phases (Bailey and White, 1964). Many compounds, such as methyl bromide (Chisholm and Koblitsky, 1943), are bound more strongly to the soil and become less effective as the amount of moisture in the soil decreases, and some pesticides, for example diazinon, become inactive in soils with a low moisture content (Harris, 1964). There are several explanations for this behaviour. The most likely of these is that water is adsorbed preferentially in a dry soil, causing solutes to crystallise and therefore to become biologically inert. An alternative hypothesis is that weakly polar organic materials can compete more effectively with a limited amount of water for the adsorption sites that are available in a dry soil (Bailey and White, 1964). Some of the volatile herbicides are less effective in wet soils as these chemicals remain dissolved in the soil water, from which they vaporise readily and are lost (Gantz and Slife, 1960).

Soils with a low moisture content can desiccate earthworms and also reduce the amount of a chemical that is accumulated by them. This was shown by the uptake into L. terrestris of the water soluble pesticide aldicarb, which was found to be correlated positively with the moisture content of the soil (Briggs and Lord, 1983). Lumbricid earthworms grow best in soils that contain 20-30% water, although they are reported to be able to tolerate a 50% loss of body weight through dehydration (Grant, 1955). In decaying organic matter, the optimum moisture content of the substrate for E. fetida lies between 70-85% (Kaplan et al., 1980), but it is difficult to compare this requirement with the demand for moisture of other earthworms found in arable soil or of E. fetida in other media.

The organic matter and clay particles in soil can adsorb chemicals. A soil that contained 65% organic matter caused a decrease in the toxicity of DDT, diazinon, heptachlor and parathion to crickets, when compared with the effect that these chemicals had in sand (Harris, 1966). Other chemicals that have been reported to become less toxic in soil containing a

lot of organic matter include aldrin and HCH (Edwards, Beck and Lichtenstein, 1957), chlordane (Harris, 1972b) and various organophosphorus and carbamate pesticides (Harris, 1970, 1972a; Harris and Hitchon, 1970; Harris and Mazurek, 1966). The concentration of a non-polar chemical that accumulates in the tissues of an earthworm is inversely related to the organic matter content of the soil (Lord, Briggs, Neale and Manlove, 1980). Occasionally, the dissolved organic matter that is present in soil, e.g. sodium humate, will help chemicals to enter aqueous solution (Wershaw, Burcar and Goldberg, 1969) and become more available to earthworms.

Particles of clay have a large surface area and an adsorptive capacity dependent upon pH. Kaolinitic clays are non-expanding and have a smaller active surface area (reflected in a lower cation exchange capacity) than montmorillonitic clays (Brown, 1974). The low cation exchange capacity of a kaolinitic clay makes it less likely to adsorb chemicals, and for this reason kaolinites were chosen as a component of the artificial soil. Many pesticides are deactivated by clays (White, 1976) including aldrin, chlordane, dieldrin (Weise, 1964), heptachlor and DDT (Harris, 1966). The latter two chemicals were deactivated more completely by the clay in a dry soil.

The main component of the artificial soil was a fine quartz sand. Sand has a very low adsorptive capacity and has been recommended as a substrate suitable for model ecosystems, because it allows reproducible assessments of toxicity to organisms to be obtained (Moriarty, 1977) which are more sensitive than those done with soil. However, both sand and silt can reduce the toxicity to insects of chemicals such as diazinon and parathion (Harris, 1966) and should not be considered as inert.

The pH of a soil can alter the adsorptive properties of some of the soil components, and in particular that of the clay fraction (Brown, 1974) which may in turn affect the availability and toxicity to earthworms of

chemicals that are incorporated into the soil. The activity of microorganisms that can detoxify chemicals is also influenced by the pH of the soil (Torstensson, 1975), which can affect the persistence of chemicals in the soil.

The tests reported previously for the toxicity of chemicals to earthworms using soil in the laboratory have used many different periods of exposure, numbers of replicates and amounts of soil in each of the test vessels. The details of these aspects of the tests have been summarised (Table 1.3) and clearly do not show a consensus of opinion. Thus the selection of the optimum conditions for testing toxicity to earthworms in the artificial soil was often made without the benefit of an established precedent.

The most suitable composition of the artificial soil, and the optimum values of the other conditions of the test, were assessed in a series of experiments done in a manner similar to that described for the development of the filter paper contact test. These investigations altered a single experimental condition of a prototype method in turn, to observe the effect that these changes had upon the assessment of the toxicity of a particular chemical to earthworms.

4.2. Materials, methods and results

4.2.1 Materials

The pesticides used as test chemicals were of technical grade and included chlordane, triazophos, carbaryl, benomyl and thiophanate methyl. The solvents and the other test chemicals, which included pentachlorophenol, trichloroacetic acid, chloroacetamide, potassium bromide and copper sulphate were of analytical grade. The suppliers of these chemicals and the details of the solvents that were used for them, together with the specifications for the components of the artificial soil and the other

apparatus of the test appear in a tabular form (Tables A5.2-A5.4).

The test earthworms were sexually mature E. fetida andrei weighing between 0.4-0.6 g with an empty gut. These were grown from cocoons and cultured for approximately 9 weeks in matured, pressed cow manure at 20°C in the dark. To avoid errors in the assessment of the toxicity of chemicals to earthworms that can occur due to a sudden change in the conditions in which the earthworms are cultured (Tomlin, 1977), and also to allow them to evacuate their gut contents, the earthworms were placed in an untreated artificial soil for 24 hours prior to use.

4.2.2 General methods

The test chemicals were applied to the soil as a spray in aqueous solution, dissolved in a suitable volatile organic solvent, or suspended in water using a method adapted from that of McIntosh et al. (1981) in which the test chemical was dissolved in 0.2 ml of a suitable organic solvent and pipetted quickly into a rapidly stirred mixture of 199.6 ml water and 0.2 ml of 10% Tween 80 in ethanol. The cation exchange capacity of the soil was measured using a standard method (Bascomb, 1964) at a reference pH (8.1) and at the natural pH of the soil in distilled water. The mortality of the earthworms was assessed mechanically, by prodding the anterior segments with a spatula and assuming the absence of a twitch to indicate death. Each experiment was accompanied by an untreated control and also, where an organic solvent had been used to apply the test chemical, by a solvent blank. Any experiments in which mortality occurred in the controls were discarded and re-run. The experiments were done at 20°C ± 1°C in a constant temperature room with illumination provided by four 100 W fluorescent lamps ('Daylight', Thorn EMI Ltd.) suspended 30 cm above the shelves that supported the test vessels. The data was analysed by a computed probit analysis using the maximum likelihood programme. The full details of this analysis and of the techniques that were used to correct the data were described previously (Section 2.2.2).

4.2.3 The method for the artificial soil test

The first artificial soil used consisted of 68% quartz sand, 20% kaolinitic clay, 10% air dried and milled sphagnum peat and 2% calcium carbonate (dry weight), contained 35% water and had a pH of 7. Individuals of E. fetida became unhealthy in this mixture and the experiments that were done in it were re-run using an improved artificial soil that is described below.

This improved artificial soil consisted of 71% fine quartz sand, 20% kaolinitic clay, 8% air dried and milled sphagnum peat and 1% $CaCO_3$ when expressed as a percentage of the dry weight of the soil (Plate 4.1). Distilled water was added to the soil to give a moisture content of 48% (wet weight) and 50 kg lots of the medium were mixed thoroughly in a clean cement mixer. When ready for use, the artificial soil had a pH of 6.8 and a cation exchange capacity at the natural pH (6.8) and the standard pH (8.1) of 162 and 152 $meq.kg^{-1}$ respectively (Plate 4.2). The hydrated material remained usable for extended periods and for at least three months, when stored within airtight plastic bags at 4°C in darkness.

The test chemical was dissolved or suspended in water or a suitable volatile organic solvent and applied as a spray (using a chromatographic atomiser) to 3 kg of soil (wet weight) that was spread thinly on a stainless steel tray in a fume cupboard. Organic solvents were evaporated under a gentle airflow and the deposit of the chemical that remained was incorporated manually into the soil.

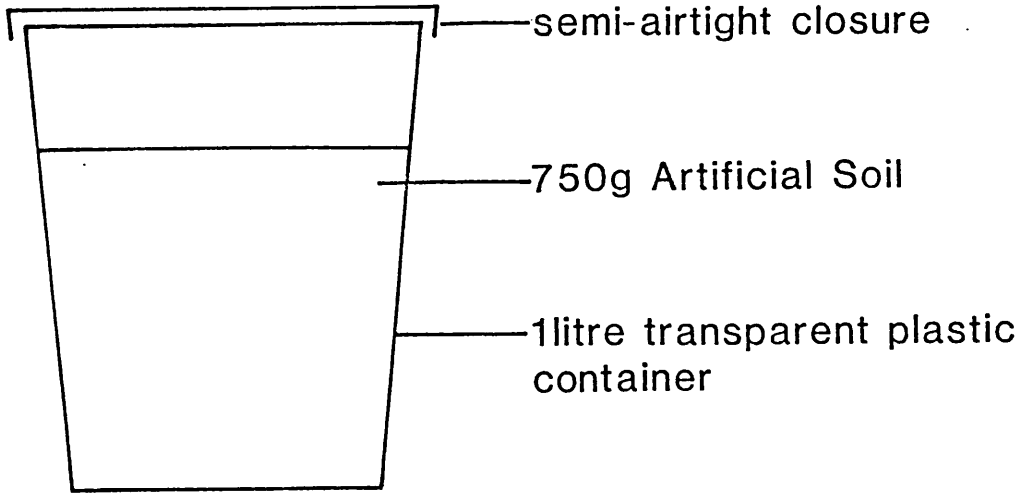
Chemicals that would not dissolve or disperse in any suitable solvent were incorporated directly into the soil in the following manner. The test chemical was ground with 10 g of dry sand in a pestle and mortar, and this mixture was incorporated into 2.99 kg of hydrated artificial soil from which 1% of the sand had been omitted (this was equivalent to 10 g sand [dry weight] omitted from 2.99 kg artificial soil [wet weight]).

Plate 4.1. Artificial soil test: the components of the soil were (Left-Right) quartz sand, kaolinitic clay, airdried and milled peat and calcium carbonate.

Plate 4.2. Artificial soil test: a one litre plastic flask containing 750 g hydrated artificial soil is shown together with a sample of the soil.

Figure 4.1

Artificial Soil Test



Four replicates were used for each concentration of the test chemical and each replicate consisted of 0.75 kg of artificial soil (wet weight) held in a one litre transparent, colourless plastic flask with a loose fitting lid (Figure 4.1). The assessments of the LC_{50} were generally made using a minimum of five concentrations of the test chemical within the range 0.1-1000 $mg.kg^{-1}$ (test chemical:dry weight of artificial soil) in a logarithmic dilution series. The upper concentration limit was extended to 10,000 $mg.kg^{-1}$ for experiments in which the toxicity of trichloroacetic acid and copper sulphate was assessed. Each test vessel held ten earthworms, buried initially under 2 cm of soil to minimise the possibility of escape from chemicals that had a repellent effect. After 14 days, the soil was turned out onto a tray, the live earthworms were extracted by hand sorting and the numbers of dead earthworms counted. The assessments of an LT_{50} were made at a concentration similar to the 7 or 14 day LC_{50} and mortality was estimated daily. Most experiments using both types of assessment were repeated at least twice.

The following descriptions of the experimental methods will only contain details of the deviations from the prototype technique outlined above.

4.2.4. The effect of exposure to the artificial soil and to a natural soil upon the body weight of earthworms.

Method

L. terrestris, A. longa and A. caliginosa were collected during the autumn from the edge of Park Grass field, Rothamsted Experimental Station, using the formalin extraction method (Raw, 1959; Table 1.2). The majority of the earthworms so collected survived for at least 14 days in the laboratory, provided that they were washed immediately after extraction from the soil in a large volume of water. These earthworms were cultured in 0.05 m^3 wooden boxes filled with pesticide-free loam soil

taken from the field in which they were collected. The E. fetida that were used in this experiment were extracted from the boxes of cow manure that were used to culture this species of earthworm in the laboratory.

Earthworms of each species were placed on damp filter paper overnight, to allow them to void their gut contents and then weighed. Ten individuals of each species with a body weight within ± 0.2 g of the mean body weight for that species, were placed separately into 10 kg of pesticide-free artificial soil, or 10 kg of air-dried and sieved clay-loam soil collected from Geescroft Field, Rothamsted Experimental Station, which had been moistened and contained 20% water. The earthworms remained in these soils for 14 days before being removed, washed and re-weighed with an empty gut.

Results

E. fetida and the soil-inhabiting species of earthworm were able to maintain their body weight for 14 days in the artificial soil and A. caliginosa increased in weight during this period (Table 4.1). The body weight of E. fetida decreased considerably after 14 days in the natural soil, whilst those species that had been collected from an arable soil did not lose weight.

4.2.5 The effect of the moisture content of the artificial soil upon the body weight of earthworms

Method

Pesticide-free artificial soils containing 0, 5, 10, 15, 20, 25, 30, 40 and 50% water (wet weight) were prepared. Ten E. fetida (with empty guts) were weighed and placed on top of 1 kg batches of these soils held in plastic flasks. After 7 days, the earthworms were extracted and re-weighed (with empty guts).

Table 4.1. The effect of the type of soil used to culture earthworms upon body weight. A comparison of the artificial soil and a natural soil.

Type of Soil	Species	Initial body weight (g)	Body weight after 14 days (g)	Change in body weight during 14 days (g)
Artificial soil	L. terrestris	0.95	0.98	+0.03
	A. longa	0.77	0.70	-0.07
	A. caliginosa	0.62	0.79	+0.17
	E. fetida andrei	0.58	0.59	+0.01
Natural clay loam soil (Geescroft Field)	L. terrestris	1.43	1.45	+0.02
	A. longa	0.97	0.96	-0.01
	A. caliginosa	0.63	0.61	-0.02
	E. fetida andrei	0.40	0.24	-0.16

Nb. The weight of the earthworms is expressed as the mean of the body weight of ten earthworms.

Results

E. fetida burrowed into all the soils except that which was completely dry. In dry soil the earthworms died and the soil which contained only 5% water caused a severe loss of body weight (Figure 4.2). In soils that contained 10% moisture or more (up to the maximum water holding capacity of this soil, namely 48%), the earthworms showed a similar and minimal decline in body weight.

4.2.6. The effect of the amount of organic matter in the artificial soil upon the body weight of earthworms

Method

Artificial soils containing 0, 5, 10 and 20% of air-dried and milled peat (dry weight) were prepared, with the amount of sand in the soil increased or decreased to compensate for the changes in the amount of peat that was present. The organic matter in the soil determined largely the amount of moisture that was retained, and as changes in the moisture content of the soil could also affect the change in body weight of the earthworms, soils of differing organic matter content were wetted to the maximum holding capacity (which was equivalent to a soil that contained 8% [dry weight] organic matter moistened to 48% [wet weight]). Ten E. fetida (with empty guts) were weighed and placed on top of 1 kg batches of these pesticide-free soils held in plastic flasks. After 7 days the earthworms were extracted and re-weighed (with empty guts).

Results

E. fetida burrowed into each of the soils, but lost weight only in the soil from which organic matter was absent. The loss of weight was negligible in those soils that contained more than 5% organic matter (Figure 4.3).

Figure 4.2

THE EFFECT OF MOISTURE CONTENT OF ARTIFICIAL SOIL UPON THE BODY WEIGHT OF E. FETIDA

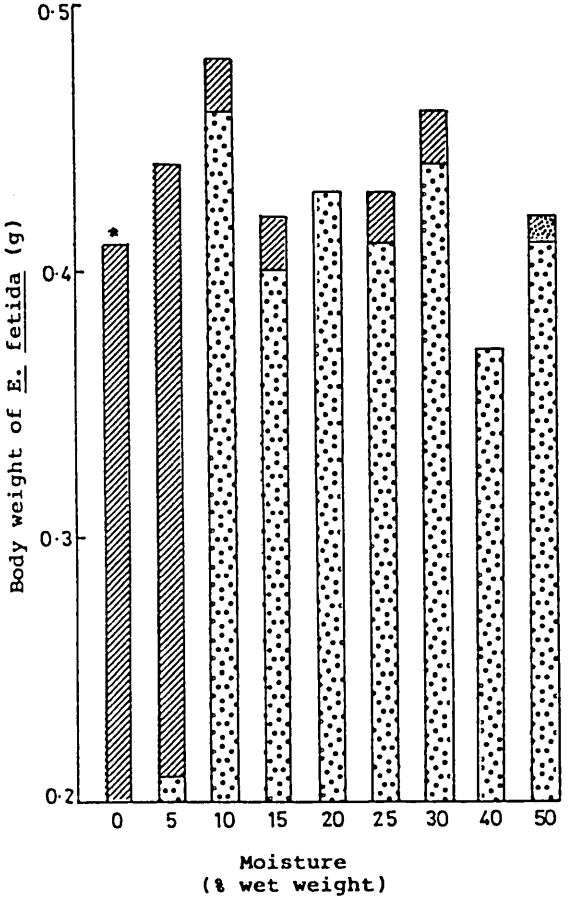
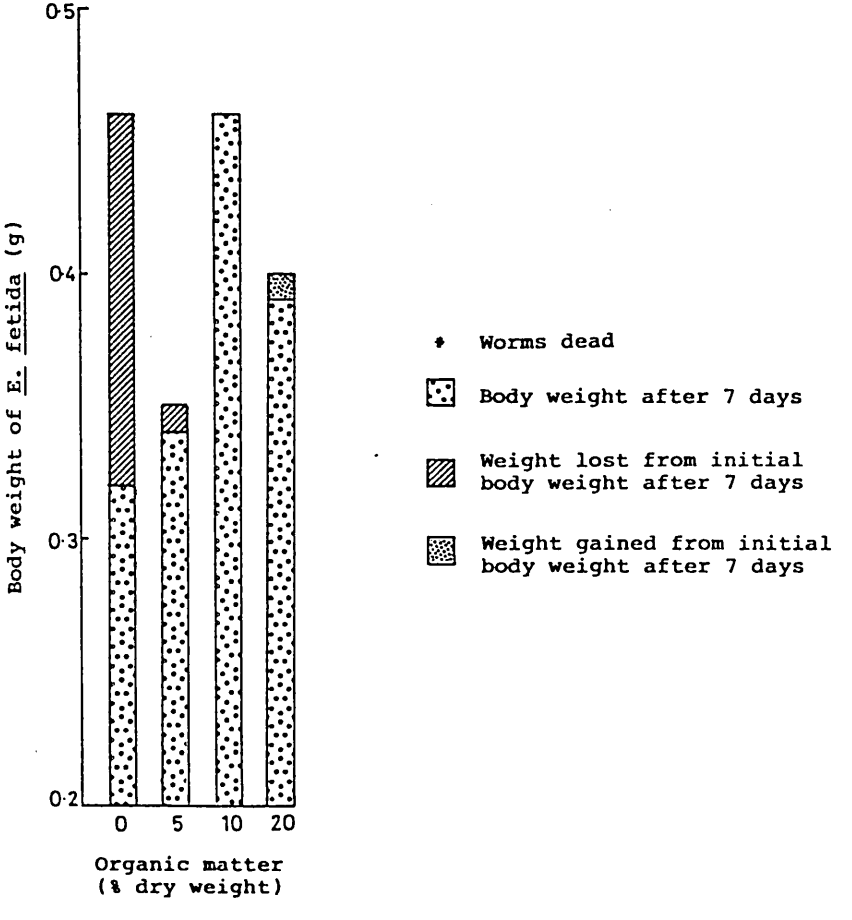


Figure 4.3

THE EFFECT OF ORGANIC MATTER CONTENT OF ARTIFICIAL SOIL UPON THE BODY WEIGHT OF E. FETIDA



4.2.7 The effect of the kaolinitic clay content of the artificial soil upon the body weight of earthworms

Method

Ten E. fetida were weighed with empty guts and placed on top of one kg batches of pesticide-free artificial soils that contained 0, 10, 20 and 30% (dry weight) kaolinitic clay. The amount of sand in the mixture was altered to compensate for the changes in the amount of clay present. The earthworms were extracted after 7 days and re-weighed with empty guts.

Results

E. fetida burrowed into all the soils and suffered a reduction in body weight in each (Figure 4.4), however these losses of body weight were small and similar in each of the soils.

4.2.8 The effect of the pH of the artificial soil upon the body weight of earthworms

Method

Pesticide-free artificial soils were prepared with pH's of 3.7, 6.2, 7.7 and 8.0, as determined by a standard method (Anon., 1981d). The most acidic soil was prepared by adding an appropriate amount of 0.01 M hydrochloric acid to an artificial soil that did not contain any calcium carbonate. The more alkaline soils which had pH's of 6.2, 7.7 and 8.0, were prepared by adding 0.5, 2.0 and 10% calcium carbonate (dry weight) respectively to soils from which this compound had been omitted. The amount of sand in the mixture was varied to compensate for the changes in the amount of calcium carbonate present. Ten E. fetida were weighed with empty guts and placed on top of one kg batches of these artificial soils and left for 7 days. After this period had elapsed the earthworms were extracted and re-weighed with empty guts.

Figure 4.4

THE EFFECT OF KAOLINITIC CLAY CONTENT OF ARTIFICIAL SOIL UPON THE BODY WEIGHT OF E. FETIDA

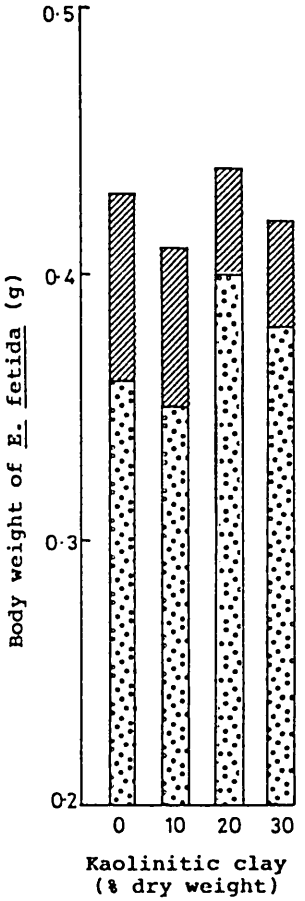
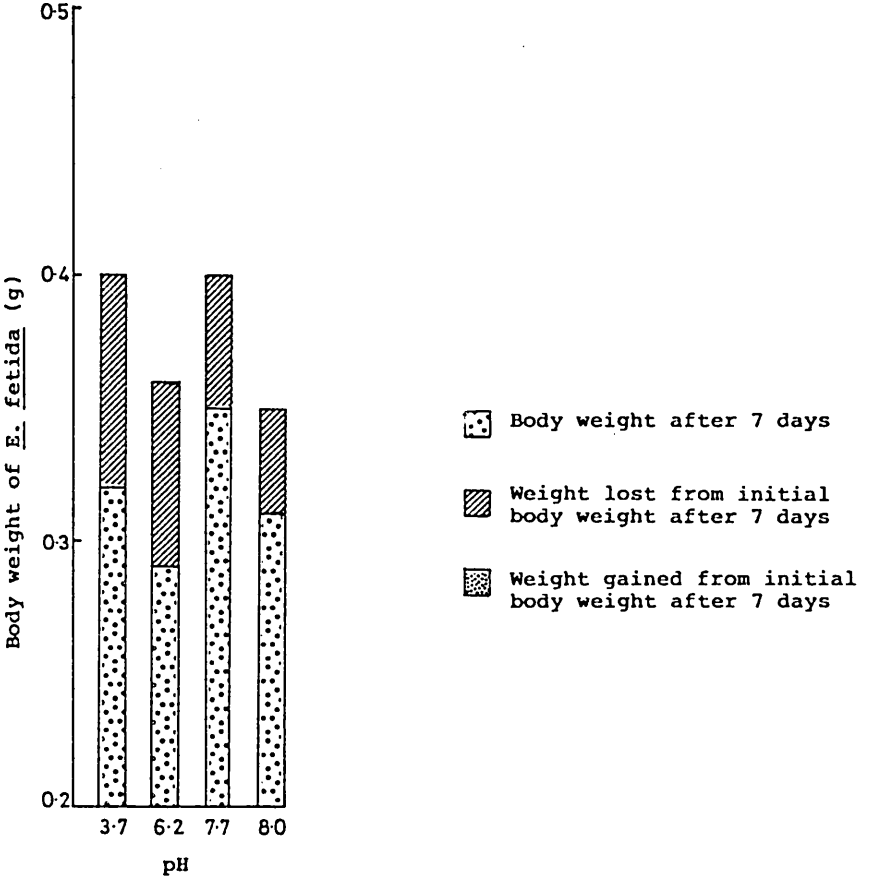


Figure 4.5

THE EFFECT OF pH OF ARTIFICIAL SOIL UPON THE BODY WEIGHT OF E. FETIDA



Results

A preliminary study had indicated that mature earthworms lost more weight ($g.g^{-1}$ tissue [wet weight]) than juveniles in acid soils, although both mature and immature earthworms burrowed into each of the test soils. The present experiments showed that the loss of body weight was similar in each of the test soils (Figure 4.5), although the amount lost in soils of pH 3.7 and 6.2 was slightly greater than that lost in soils with a pH of 7.7 and 8.0.

4.2.9 The effect of the moisture content of the artificial soil upon assessments of toxicity to earthworms

Method

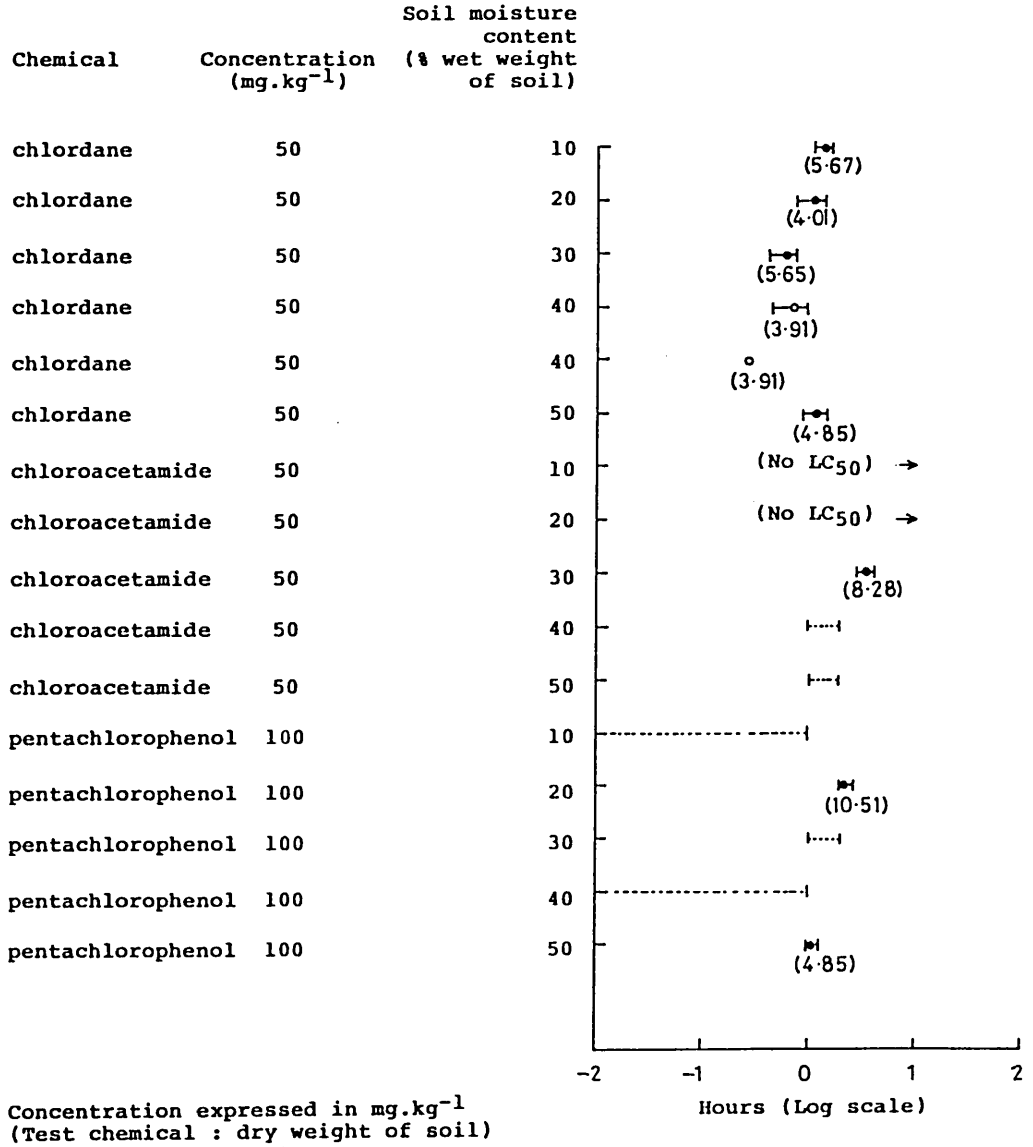
The LT_{50}'s of chlordane and chloroacetamide at a concentration of 50 $mg.kg^{-1}$, and pentachlorophenol at a concentration of 100 $mg.kg^{-1}$ (test chemical:dry weight of soil), were assessed in artificial soils that contained 10, 20, 30, 40 and 50% water (wet weight).

Results

The toxicity of chlordane and pentachlorophenol to earthworms was not influenced much by these changes in the moisture content of the soil. Chloroacetamide was more toxic in the wetter soils, but had little effect upon the earthworms in media that contained less than 30% water (Tables A7.21.1-A7.21.3 and Figure 4.6). The reproducibility of the test (inferred from the significance of the differences for parallelism and Y-intercept position that appeared between the probit lines for replicates when tested by chi-squared) did not appear to be related to the moisture content of the soil.

Figure 4.6

THE EFFECT OF ARTIFICIAL SOIL MOISTURE CONTENT UPON TOXICITY ASSESSMENTS WITH E. FETIDA



Concentration expressed in $mg.kg^{-1}$
(Test chemical : dry weight of soil)

KEY

⊢○⊣ x	Individual LT_{50} estimate [o], gradient [x] and fiducial limits
⊢○⊣ (x)	Individual LT_{50} estimate [o], common gradient for more than one replicate [(x)] and fiducial limits
⊢●⊣ (x)	Common LT_{50} estimate [●], gradient [(x)] and fiducial limits for more than one replicate
⊢·····⊣	Range within which a poorly defined LT_{50} falls
No LT_{50} →	LT_{50} at an undefined period of exposure that was greater than those studied
← No LT_{50}	LT_{50} at an undefined period of exposure that was less than those studied
(No LT_{50})	More than one LT_{50} at an undefined period of exposure

4.2.10 The effect of the organic matter content of the artificial soil upon assessments of toxicity to earthworms

Method

Artificial soils were prepared that contained 0, 5, 10 and 20% organic matter (dry weight), and larger or smaller amounts of sand were used to compensate for these changes in the composition of the medium. The LT_{50}'s of chlordane, chloroacetamide and pentachlorophenol were assessed at concentrations of 50, 50 and 100 $mg.kg^{-1}$ respectively (test chemical:dry weight of artificial soil).

Results

Only the results that were obtained with chlordane gave a sufficient number of observations of partial mortality to be suitable for probit analysis, but all three compounds became less toxic to earthworms as the amount of organic matter in the soil increased (Tables A7.22.1-A7.22.3 and Figure 4.7). The difference between the toxicity of chlordane to earthworms in soils that contained 5% and 10% organic matter was greater than the difference in the toxicity of this compound seen between soils that contained 10% and 20% organic matter. A chi-squared test of the differences that occurred between the probit lines that were calculated for repeated experiments with chlordane, failed to indicate any relationship between the reproducibility of the test and the amount of organic matter in the soil.

4.2.11 The effect of the kaolinitic clay content of the artificial soil upon assessments of toxicity to earthworms

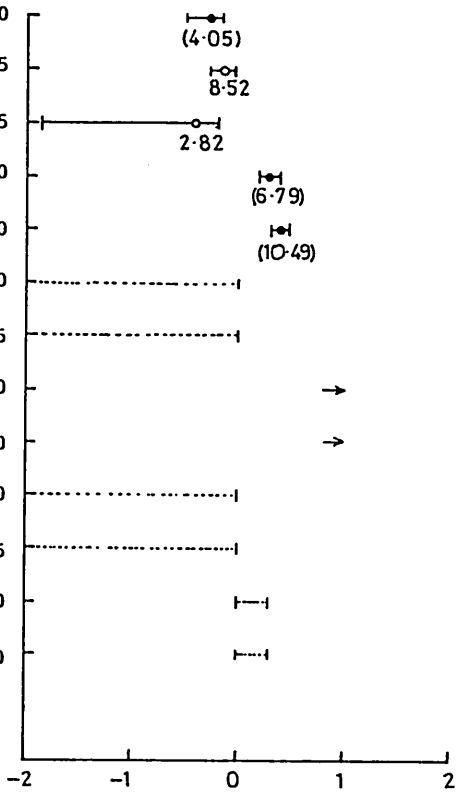
Method

Artificial soils were prepared that contained 0, 10, 20 and 30% kaolinitic clay (dry weight), and in which the amount of sand was increased or decreased to compensate for changes in the amount of clay.

Figure 4.7

THE EFFECT OF ARTIFICIAL SOIL ORGANIC MATTER CONTENT UPON TOXICITY ASSESSMENTS WITH *E. FETIDA*

Chemical	Concentration ($mg.kg^{-1}$)	Soil organic matter content (% dry weight of soil)
chlordane	50	0
chlordane	50	5
chlordane	50	5
chlordane	50	10
chlordane	50	20
chloroacetamide	50	0
chloroacetamide	50	5
chloroacetamide	50	10
chloroacetamide	50	20
pentachlorophenol	100	0
pentachlorophenol	100	5
pentachlorophenol	100	10
pentachlorophenol	100	20



Concentration expressed in $mg.kg^{-1}$
(Test chemical : dry weight of soil)

KEY

⊢o⊣ x	Individual LT_{50} estimate [o], gradient [x] and fiducial limits
⊢o⊣ (x)	Individual LT_{50} estimate [o], common gradient for more than one replicate [(x)] and fiducial limits
⊢●⊣ (x)	Common LT_{50} estimate [●], gradient [(x)] and fiducial limits for more than one replicate
⊢····⊣	Range within which a poorly defined LT_{50} falls
No LT_{50} →	LT_{50} at an undefined period of exposure that was greater than those studied
← No LT_{50}	LT_{50} at an undefined period of exposure that was less than those studied
(No LT_{50})	More than one LT_{50} at an undefined period of exposure

The toxicity of chlordane, chloroacetamide and pentachlorophenol to earthworms in these soils was estimated as an LT_{50} at concentrations of 50, 50 and 100 $mg.kg^{-1}$ respectively (test chemical: dry weight of artificial soil).

Results

The clay content of the soil appeared to have little influence upon the toxicity to earthworms of any of the test chemicals (Tables A7.23.1-A7.23.3 and Figure 4.8). Only with chlordane did the variation in the reproducibility of the test seem to be related to the clay content of the soil, as indicated by the significance of the differences between the probit lines that were calculated for repeated experiments and analysed by a chi-squared test. An artificial soil with a clay content of more than 20% gave assessments of toxicity with chlordane for which the reproducibility was decreased.

4.2.12 The effect of the pH of the artificial soil upon assessments of toxicity to earthworms

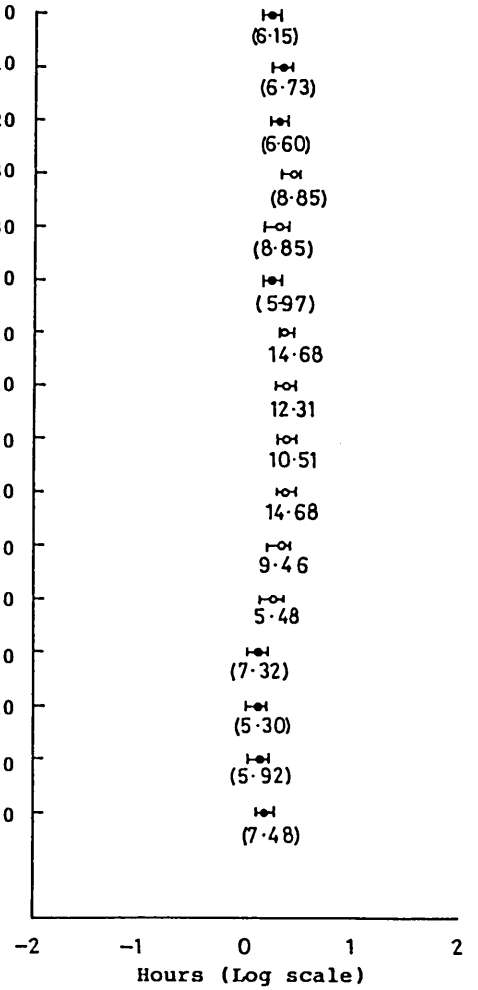
Method

The LT_{50}'s for chlordane, chloroacetamide and pentachlorophenol were assessed at concentrations of 50, 50 and 100 $mg.kg^{-1}$ respectively (test chemical:dry weight of artificial soil) in artificial soils with a pH of 3.7, 6.2, 7.7 and 8.0. These soils were prepared by adding dilute HCl or $CaCO_3$ to a medium that did not contain any $CaCO_3$, by the method which was described previously for the experiments in which the change in the body weight of earthworms was measured under similar conditions (Section 4.2.8).

Figure 4.8

THE EFFECT OF ARTIFICIAL SOIL KAOLINITIC CLAY CONTENT UPON TOXICITY ASSESSMENTS WITH E. PETIDA

Chemical	Concentration ($mg.kg^{-1}$)	Soil kaolinitic clay content (% dry weight of soil)	
chlordane	50	0	(6·15)
chlordane	50	10	(6·73)
chlordane	50	20	(6·60)
chlordane	50	30	(8·85)
chlordane	50	30	(8·85)
chloroacetamide	50	0	(5·97)
chloroacetamide	50	10	14·68
chloroacetamide	50	10	12·31
chloroacetamide	50	20	10·51
chloroacetamide	50	20	14·68
chloroacetamide	50	30	9·46
chloroacetamide	50	30	5·48
pentachlorophenol	100	0	(7·32)
pentachlorophenol	100	10	(5·30)
pentachlorophenol	100	20	(5·92)
pentachlorophenol	100	30	(7·48)



Concentration expressed in $mg.kg^{-1}$
(Test chemical : dry weight of soil)

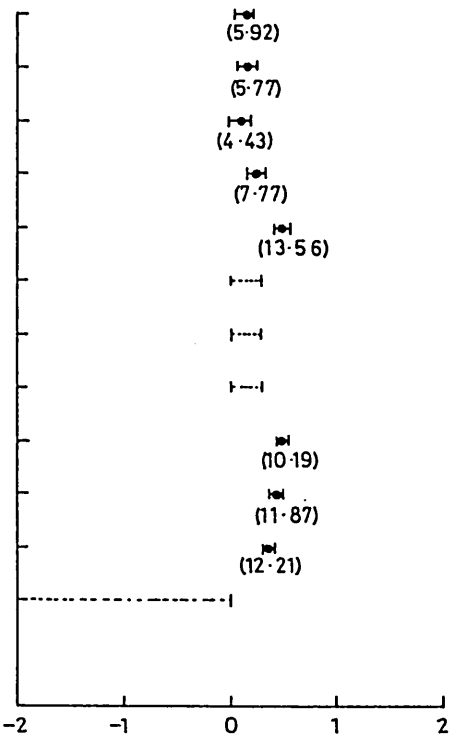
KEY

⊢o⊣ x	Individual LT_{50} estimate [o], gradient [x] and fiducial limits
⊢o⊣ (x)	Individual LT_{50} estimate [o], common gradient for more than one replicate [(x)] and fiducial limits
⊢●⊣ (x)	Common LT_{50} estimate [●], gradient [(x)] and fiducial limits for more than one replicate
⊢····⊣	Range within which a poorly defined LT_{50} falls
No LT_{50} →	LT_{50} at an undefined period of exposure that was greater than those studied
← No LT_{50}	LT_{50} at an undefined period of exposure that was less than those studied
(No LT_{50})	More than one LT_{50} at an undefined period of exposure

Figure 4.9

THE EFFECT OF ARTIFICIAL SOIL pH UPON TOXICITY ASSESSMENTS WITH E. FETIDA

Chemical	Concentration ($mg.kg^{-1}$)	Soil pH (pH)
chlordane	50	3.7
chlordane	50	6.2
chlordane	50	7.7
chlordane	50	8.0
chloroacetamide	50	3.7
chloroacetamide	50	6.2
chloroacetamide	50	7.7
chloroacetamide	50	8.0
pentachlorophenol	100	3.7
pentachlorophenol	100	6.2
pentachlorophenol	100	7.7
pentachlorophenol	100	8.0



Concentration expressed in $mg.kg^{-1}$
(Test chemical : dry weight of soil)

KEY

⊢o⊣ x	Individual LT_{50} estimate [o], gradient [x] and fiducial limits
⊢o⊣ (x)	Individual LT_{50} estimate [o], common gradient for more than one replicate [(x)] and fiducial limits
⊢●⊣ (x)	Common LT_{50} estimate [●], gradient [(x)] and fiducial limits for more than one replicate
⊢····⊣	Range within which a poorly defined LT_{50} falls
No LT_{50} →	LT_{50} at an undefined period of exposure that was greater than those studied
← No LT_{50}	LT_{50} at an undefined period of exposure that was less than those studied
(No LT_{50})	More than one LT_{50} at an undefined period of exposure

Results

Although the toxicity of chlordane to earthworms appeared to be unaffected by the pH of the soil, chloroacetamide and pentachlorophenol were less toxic in the most acidic soil (Tables A7.24.1-A7.24.3 and Figure 4.9). Thus, the toxicity of chloroacetamide and pentachlorophenol seem to be correlated positively with the pH of the soil, although the reproducibility of the test (inferred from the significance of the differences between the gradient and the position of the Y-intercept of probit lines calculated for the data from repeated experiments and tested by chi-squared) did not appear to be dependent upon the pH of the soil.

4.2.13 The effect of the temperature of the artificial soil upon assessments of toxicity to earthworms

Method

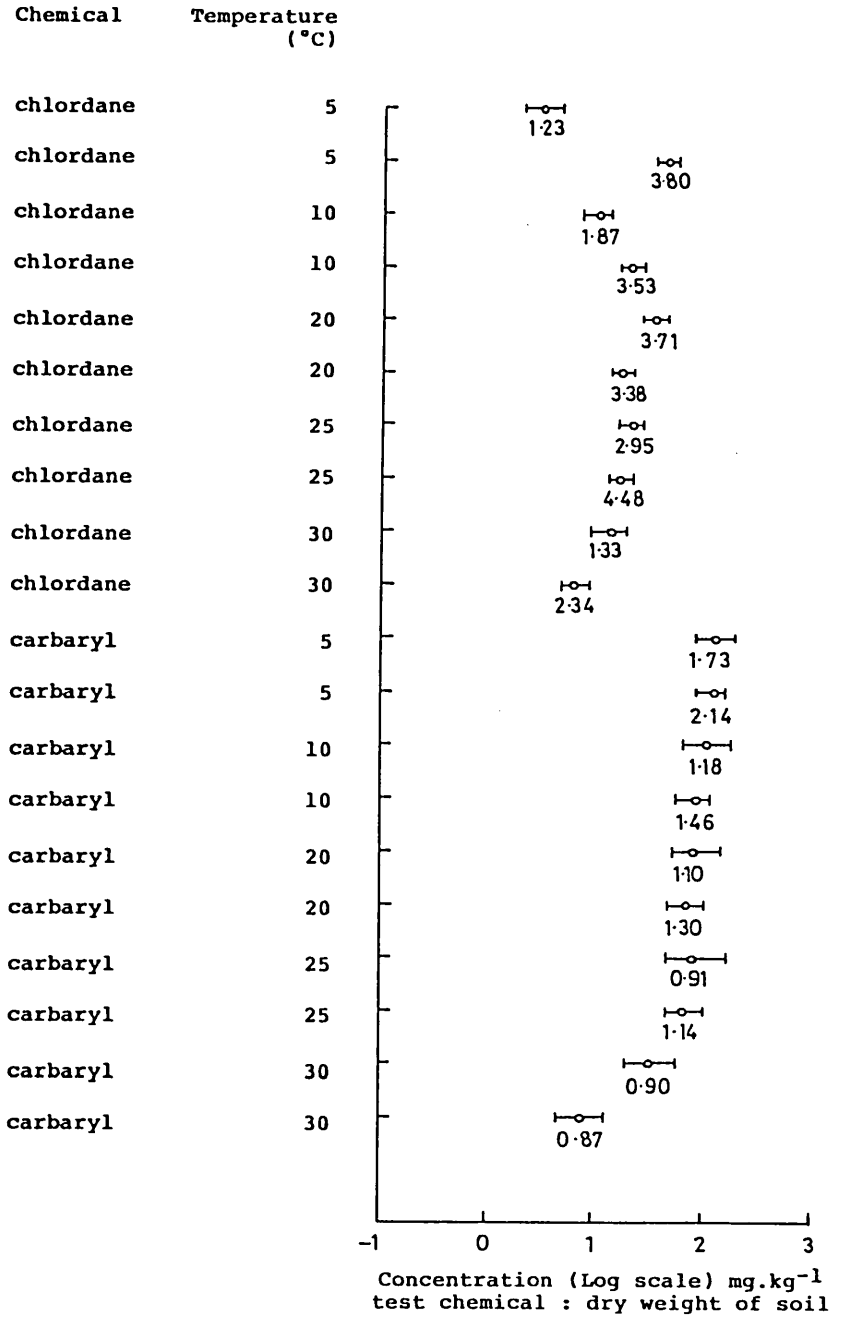
The LC_{50} of chlordane and of carbaryl was estimated at a test temperature of 5, 10, 20, 25 and 30°C.

Results

The toxicity of chlordane and carbaryl to earthworms was correlated positively with the test temperature (Table A7.25 and Figure 4.10) between 20° and 30°C, although the toxicity of chlordane was correlated negatively with the temperature of the test between 5° and 20°C. The repeated estimates of the toxicity of both chemicals were calculated to give probit lines that differed significantly for parallelism and Y-intercept position when tested using chi-squared, and thus the reproducibility of the test did not appear to be related to the temperature at which it was done.

Figure 4.10

THE EFFECT OF ARTIFICIAL SOIL TEMPERATURE UPON TOXICITY ASSESSMENTS WITH E. FETIDA



KEY

⊢o⊣ x	Individual LC_{50} estimate [o], gradient [x] and fiducial limits
⊢o⊣ (x)	Individual LC_{50} estimate [o], common gradient for more than one replicate [(x)] and fiducial limits
⊢●⊣ (x)	Common LC_{50} estimate [●], gradient [(x)] and fiducial limits for more than one replicate
⊢----⊣	Range within which a poorly defined LC_{50} falls
No LC_{50} →	LC_{50} at an undefined concentration that was greater than those studied
← No LC_{50}	LC_{50} at an undefined concentration that was less than those studied
(No LC_{50})	More than one LC_{50} at an undefined concentration

4.2.14 The effect of the quantity of artificial soil upon the assessment of toxicity to earthworms

Method

The toxicity of chlordane was estimated as an LC_{50} using 0.1, 0.2, 0.4, 0.8 and 1.0 kg of artificial soil in each test vessel.

Results

The amount of artificial soil held in each test vessel did not appear to affect significantly either the assessment of the toxicity of chlordane to earthworms (Table A7.26 and Figure 4.11) or the reproducibility of the method as inferred from the results of a chi-squared test for the significance of the differences between the gradient and the position of the Y-intercept of the probit lines calculated for repeated experiments.

4.2.15 The effect of the period of exposure upon assessments of toxicity to earthworms in the artificial soil

Method

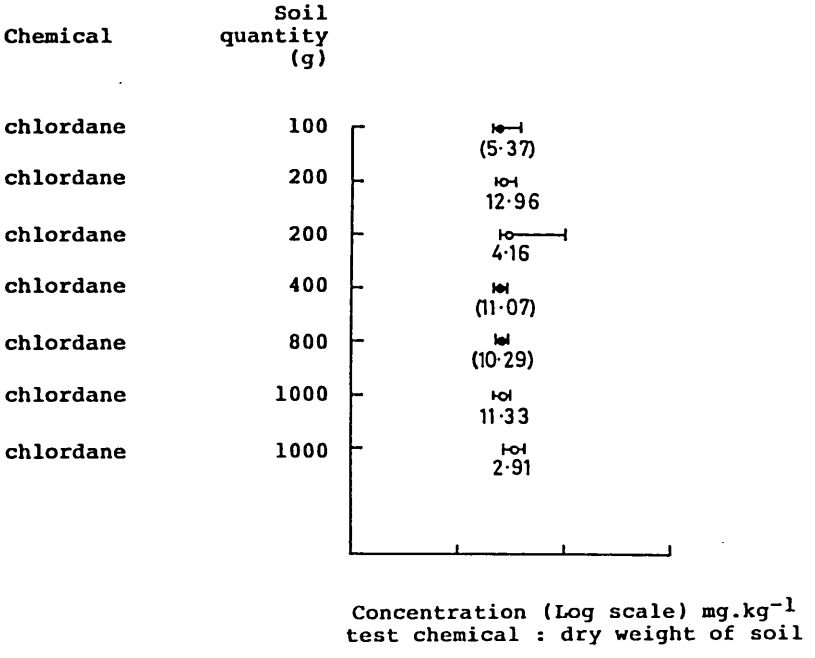
The LC_{50} for carbaryl was estimated after the earthworms had been exposed to an artificial soil that contained this chemical for 7, 14, 20, 40 and 50 days.

Results

The toxicity of carbaryl to earthworms was correlated positively with the period of exposure (Table A7.27 and Figure 4.12) and the greatest change in the LC_{50} occurred between 14-40 days. The fiducial limits were wider for the higher LC_{50}'s which were estimated using a short period of exposure, although this effect is less clear in the diagram due to the logarithmic concentration scale that is used.

Figure 4.11

THE EFFECT OF ARTIFICIAL SOIL QUANTITY IN EACH TEST VESSEL UPON TOXICITY ASSESSMENTS WITH E. FETIDA

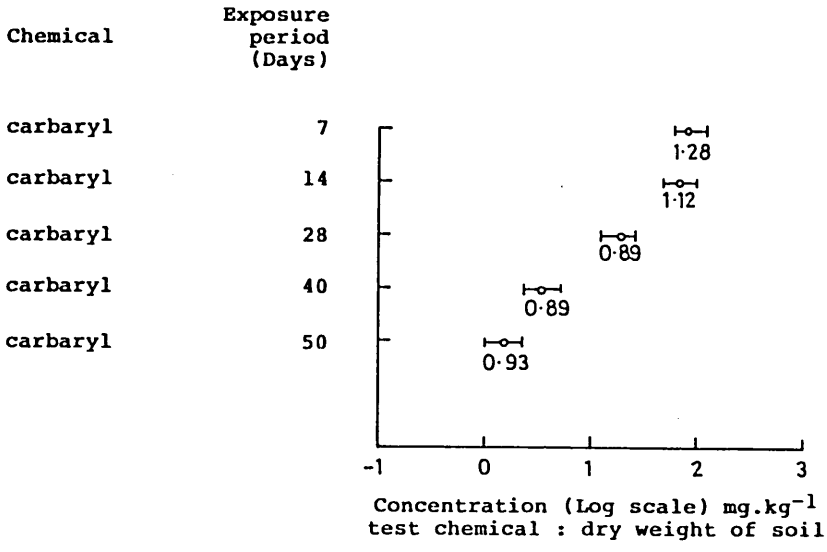


KEY

⊢o⊣ x	Individual LC_{50} estimate [o], gradient [x] and fiducial limits
⊢o⊣ (x)	Individual LC_{50} estimate [o], common gradient for more than one replicate [(x)] and fiducial limits
⊢●⊣ (x)	Common LC_{50} estimate [●], gradient [(x)] and fiducial limits for more than one replicate
⊢----⊣	Range within which a poorly defined LC_{50} falls
No LC_{50} →	LC_{50} at an undefined concentration that was greater than those studied
← No LC_{50}	LC_{50} at an undefined concentration that was less than those studied
(No LC_{50})	More than one LC_{50} at an undefined concentration

Figure 4.12

THE EFFECT OF EXPOSURE PERIOD IN ARTIFICIAL SOIL UPON TOXICITY ASSESSMENTS WITH E. FETIDA



KEY

⊢o⊣ x	Individual LC_{50} estimate [o], gradient [x] and fiducial limits
⊢o⊣ (x)	Individual LC_{50} estimate [o], common gradient for more than one replicate [(x)] and fiducial limits
⊢●⊣ (x)	Common LC_{50} estimate [●], gradient [(x)] and fiducial limits for more than one replicate
⊢----⊣	Range within which a poorly defined LC_{50} falls
No LC_{50} →	LC_{50} at an undefined concentration that was greater than those studied
← No LC_{50}	LC_{50} at an undefined concentration that was less than those studied
(No LC_{50})	More than one LC_{50} at an undefined concentration

4.2.16 The effect of the number of replicates upon the assessment of toxicity to earthworms in the artificial soil

Method

Estimates of the LC_{50} for carbaryl to earthworms were made using 2, 4, 6, 8 and 10 replicates at each concentration of the test chemical.

Results

The differences that occurred between the LC_{50}'s for carbaryl determined at various levels of replication were small (Table A7.28 and Figure 4.13), although it is difficult to draw a conclusion about the effects of replication upon the reproducibility of the test in the absence of repeated experiments. The fiducial limits were of similar width for the LC_{50}'s that were estimated in the experiments made using four or more replicates, and were somewhat wider for the LC_{50} that was estimated using only two replicates.

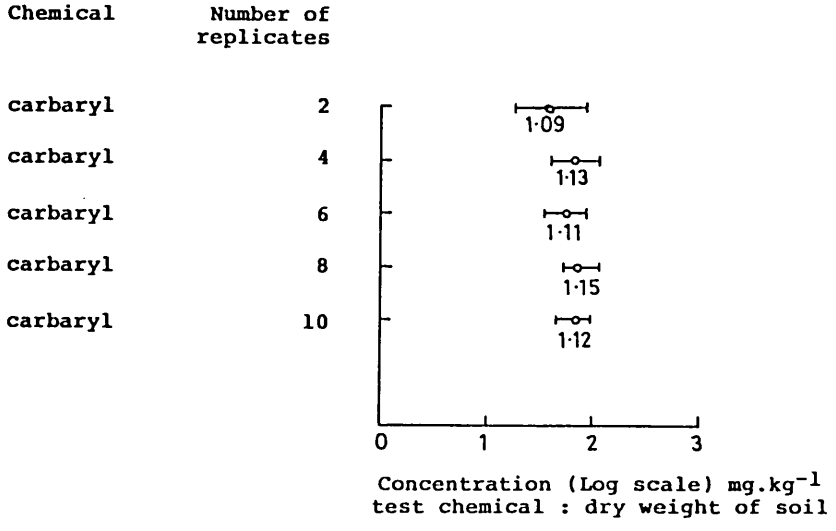
4.2.17 The effect of the method of application of the test chemical to the artificial soil upon the assessment of toxicity to earthworms

Method

Three methods of applying the test chemical to the artificial soil were studied, and each of these was a modification of an existing method. The methods included a) spraying 10 ml of a solution or suspension of the test chemical onto the soil in water or a suitable volatile organic solvent. The spray was generated by a chromatographic atomiser, and the deposit of the test chemical that was left on the soil was incorporating manually (after Edwards and Jeffs, 1974), b) mixing the test chemical directly with 2.99 kg artificial soil, by manually incorporating a premix consisting of 10g sand and an appropriate amount of the test chemical ground together previously using a pestle and mortar (after Davis, 1971), and c) drenching the test chemical into the soil as a solution or suspension in

Figure 4.13

THE EFFECT OF THE NUMBER OF REPLICATES UPON TOXICITY ASSESSMENTS IN THE ARTIFICIAL SOIL TEST WITH E. FETIDA



KEY

⊢o⊣ x	Individual LC_{50} estimate [o], gradient [x] and fiducial limits
⊢o⊣ (x)	Individual LC_{50} estimate [o], common gradient for more than one replicate [(x)] and fiducial limits
⊢●⊣ (x)	Common LC_{50} estimate [●], gradient [(x)] and fiducial limits for more than one replicate
⊢----⊣	Range within which a poorly defined LC_{50} falls
No LC_{50} →	LC_{50} at an undefined concentration that was greater than those studied
← No LC_{50}	LC_{50} at an undefined concentration that was less than those studied
(No LC_{50})	More than one LC_{50} at an undefined concentration

10 ml distilled water or a suitable volatile organic solvent. The test chemical was then distributed uniformly throughout the medium by mixing and spreading the soil by hand continually using a spatula in a gentle airflow, and any volatile organic solvent was allowed to evaporate. The amount of water lost from the soil during this process was estimated by weighing the soil before and after the application of the chemical and correcting any decrease in weight by adding distilled water (Davis, 1971).

The LC_{50}'s for chlordane and chloroacetamide that had been applied by the three different methods were estimated after 14 days.

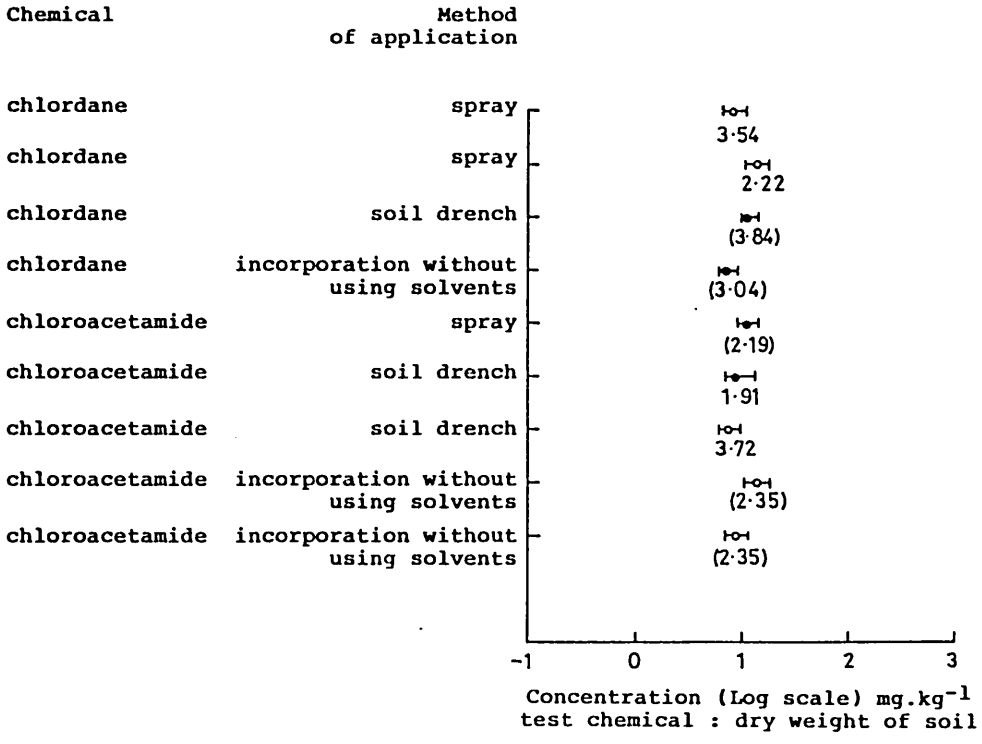
Results

The LC_{50}'s for chlordane and chloroacetamide, applied as a spray, a drench or incorporated into the soil without using any solvents were similar (Table A7.29 and Figure 4.14). A chi-squared test for the significance of the differences between the probit lines calculated for the data from repeated experiments, indicated that the most reproducible results were obtained using a) the method of incorporation without solvents with both chlordane and chloroacetamide, b) the application of chlordane using a drench and c) the application of chloroacetamide using a spray.

These methods of application had some practical disadvantages. Sprays tended to drift, and the volatile organic solvents that were used to drench the test chemicals were only removed from the soil with difficulty. Viscous liquids like technical chlordane could not be applied easily to the artificial soil without using solvents, and the method of application in which the test chemicals were ground with sand before being mixed into the soil was slow to operate.

Figure 4.14

THE EFFECT OF THE METHOD BY WHICH THE TEST CHEMICAL WAS APPLIED TO THE ARTIFICIAL SOIL UPON TOXICITY ASSESSMENTS WITH E. FETIDA



KEY

⊢o⊣ x	Individual LC_{50} estimate [o], gradient [x] and fiducial limits
⊢o⊣ (x)	Individual LC_{50} estimate [o], common gradient for more than one replicate [(x)] and fiducial limits
⊢●⊣ (x)	Common LC_{50} estimate [●], gradient [(x)] and fiducial limits for more than one replicate
⊢----⊣	Range within which a poorly defined LC_{50} falls
No LC_{50} →	LC_{50} at an undefined concentration that was greater than those studied
← No LC_{50}	LC_{50} at an undefined concentration that was less than those studied
(No LC_{50})	More than one LC_{50} at an undefined concentration

4.2.18 Assessments of the toxicity of chemicals to earthworms using the artificial soil test

Method

The final version of the artificial soil test using optimal conditions was used to estimate the LC_{50}'s of chlordane, triazophos, carbaryl, benomyl, thiophanate-methyl, pentachlorophenol, trichloroacetic acid, chloroacetamide, potassium bromide and copper sulphate.

Results

The LC_{50}'s of the test chemicals (Table A7.30 and Figure 4.15) were ranked within arbitrarily assigned categories of toxicity to earthworms (Table 4.2).

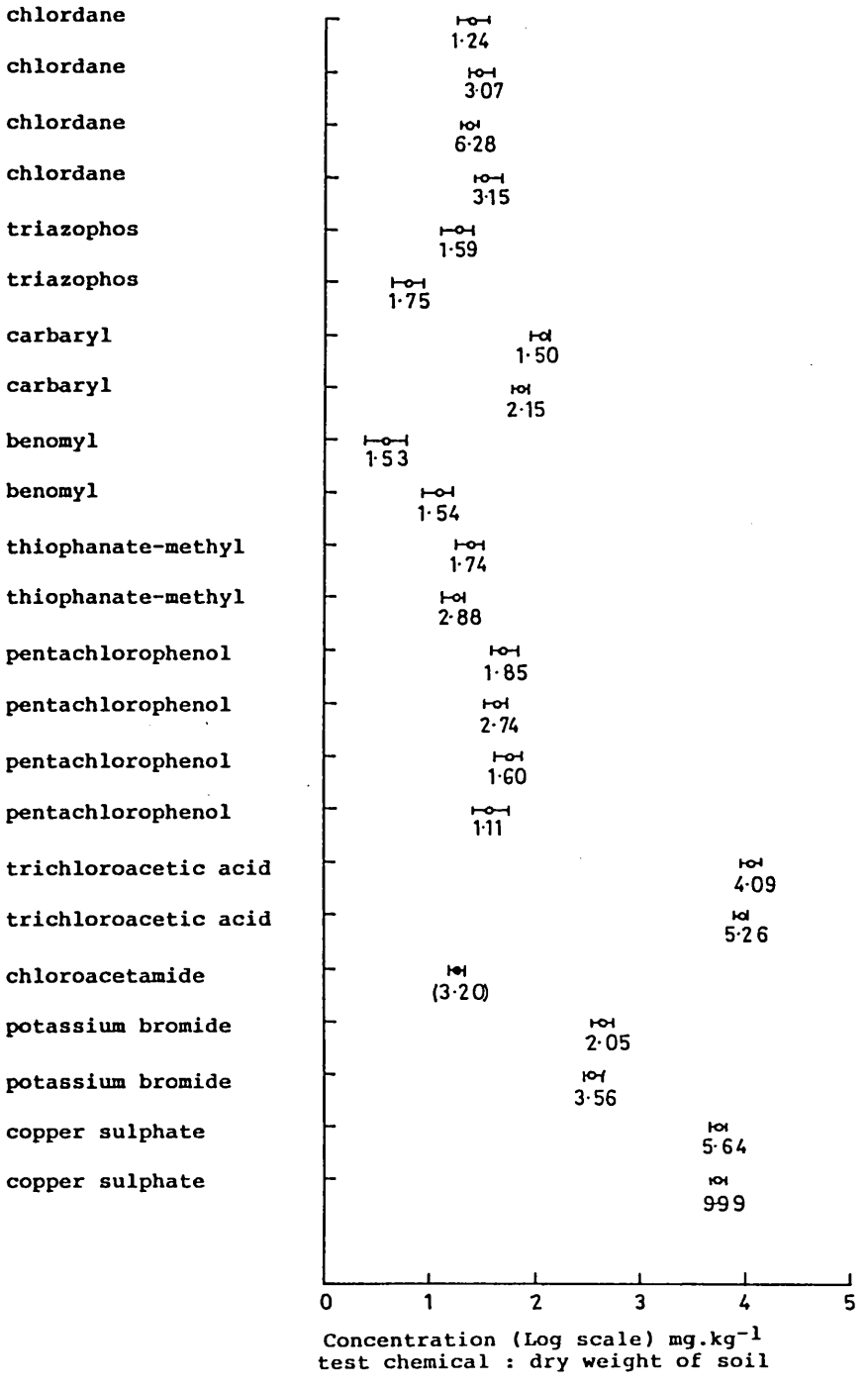
4.3. Discussion

The artificial soil had properties that were similar to those of a natural sandy loam (Avery, 1980), with a pH of 6.8 and a cation exchange capacity of 152 and 187 $meq.kg^{-1}$ at the natural pH (6.8) and a standard pH (8.1) respectively. The soil became saturated at a moisture content of approximately 50% (wet weight).

I decided to develop a standardised mineral artificial soil, because this type of medium showed promise for predicting accurately the toxicity of chemicals to earthworms in the field. *E. fetida* would not tolerate the very sandy soils that I used in my preliminary experiments, and, unlike *L. terrestris*, *A. longa* and *A. caliginosa*, lost weight rapidly in artificial soils that had a low organic matter content. Therefore a soil that contained a large amount of peat was essential for the survival of *E. fetida*, although this gave the artificial soil a water holding capacity higher than that found in many natural soils. Organic matter has a high cation exchange capacity and to keep the overall adsorptive capacity of the soil similar to that of a natural arable soil, a non-expanding kaolinitic clay

Figure 4.15

TOXICITY ASSESSMENTS WITH E. FETIDA USING THE ARTIFICIAL SOIL TEST



KEY

⊢o⊣ x	Individual LC_{50} estimate [o], gradient [x] and fiducial limits
⊢o⊣ (x)	Individual LC_{50} estimate [o], common gradient for more than one replicate [(x)] and fiducial limits
⊢●⊣ (x)	Common LC_{50} estimate [●], gradient [(x)] and fiducial limits for more than one replicate
⊢----⊣	Range within which a poorly defined LC_{50} falls
No LC_{50} →	LC_{50} at an undefined concentration that was greater than those studied
← No LC_{50}	LC_{50} at an undefined concentration that was less than those studied
(No LC_{50})	More than one LC_{50} at an undefined concentration

Table 4.2. Categories of the toxicity of chemicals to earthworms assigned to the results obtained using the artificial soil test.

Category of toxicity	Range of the LC50's within this category $mg.kg^{-1}$	Chemical
Very toxic	<9.9	Benomyl
Toxic	10.0 - 99.9	Triazophos Chloroacetamide Thiophanate-methyl Chlordane Pentachlorophenol Carbaryl
Moderately toxic	100.0 - 999.9	Potassium bromide
Slightly toxic	>1000	Copper sulphate Trichloroacetic acid

which is rarely found in European soils had to be used, because these clays possess a low cation exchange capacity.

E. fetida was reluctant to burrow into an artificial soil that contained less than 5% water and in these soils lost body weight, although in artificial soils that contained between 10-50% water E. fetida did not lose weight. These studies agree with previous work which concluded that E. fetida was susceptible to desiccation although it could tolerate the loss of 59% of the body water (Grant, 1955), and that in an organic substrate these earthworms required a very moist environment that contained between 70-85% water (Kaplan et al., 1980).

The assessment of the toxicity of chemicals to earthworms can be affected by the moisture content of the artificial soil. Chloroacetamide is a water-soluble chemical that was non-toxic to earthworms in a soil that contained less than 30% water, although chlordane and pentachlorophenol (both of which were almost insoluble in water), were approximately equitoxic in soils of different moisture contents. These observations agree with other studies on chlordane (Harris, 1972b) in which the toxicity of water soluble compounds was correlated positively with the amount of water (between moisture contents of 1-12%) in the soil. Other assessments of the toxicity of benomyl and chloroacetamide to earthworms have been made using this artificial soil containing between 35-51% water (Heimbach and Edwards, 1983), during a study to establish the value of the artificial soil test for estimating the toxicity of pesticides to earthworms. The toxicity of these chemicals appeared to be unaffected by the moisture content of the soil, although these chemicals were insoluble and soluble in water respectively. I recommended that the artificial soil should contain 48% water (wet weight), approximately 2% below the saturation capacity. This moisture content provided very suitable conditions for the survival of E. fetida and potentiated the toxicity of water soluble chemicals, whilst having little effect upon the toxicity of the less water soluble compounds.

E. fetida lost weight rapidly in artificial soils that contained less than 5% organic matter, and also lost weight in the field soil collected from Geescroft Field (Rothamsted Experimental Station) which contained 2.5% organic matter. Thus, for an artificial soil to be suitable for the assessment of the toxicity of chemicals to E. fetida, it should contain a high proportion of organic matter. The type of organic matter that was incorporated into the artificial soil needed to be standardised easily, readily available and to have a low adsorptive capacity. Organic matter can have a very high cation exchange capacity between 2000-4000 $meq.kg^{-1}$(Bailey and White, 1964), although sphagnum moss peats of the type used in this study have a lower adsorptive capacity of approximately 1000 $meq.kg^{-1}$ (Puustjarvi, 1979). Sphagnum moss peats may be defined precisely (Farnham, 1979), although those sold for horticultural use are often blends of amorphous and fibrous peats. The horticultural peat recommended in this study was a blend that was stable with regard to pH, moisture holding capacity and cation exchange capacity.

The toxicity of chlordane, pentachlorophenol and chloroacetamide were correlated negatively with the amount of organic matter in the artificial soil. This confirmed other observations which indicated that chlordane was less toxic in a muck soil than in a loam soil (Harris, 1972b), and that the toxicity of some unspecified pesticides was related inversely to the organic matter content of an artificial soil (Heimbach, 1984). The results of my studies contrast with those of Heimbach and Edwards (1983), who found that the toxicity of benomyl and chloroacetamide was unaffected by the amount of organic matter in the artificial soil within the range 2.5-10% (dry weight). I recommended that 8% organic matter (dry weight) should be included in the soil as a compromise between the development of a medium that was acceptable to E.fetida, and one that had a relatively low adsorptive capacity. I agree with Heimbach (1982) that the amount of organic matter in the artificial soil, the moisture content that is associated

with it and the amount of $CaCO_3$ needed to achieve a suitable pH, could probably be decreased by using a different species of earthworm, although E. fetida is well suited to this type of test. These changes would make the artificial soil more like a natural arable soil by reducing the adsorptive capacity and limiting the neutralisation of acidic test chemicals by alkaline components in the soil, but would raise difficulties in culturing the earthworms.

Clays adsorb chemicals (Bailey and White, 1964; Harris, 1966; Weise, 1964) and show a cation exchange capacity that is additive to that of the organic matter. Kaolinitic clays have a lower adsorptive capacity than montmorillonitic clays (Weber and Weed, 1974) and hence were recommended for use in the artificial soil despite their scarcity in European soils to avoid an overall adsorptive capacity that was undesirably high.

The amount of clay present in the artificial soil, within the range 0-30% (dry weight), did not affect the body weight of E. fetida during the test period. Hence the proportion of clay that was incorporated into the artificial soil was not restricted by the amount that would be tolerated by the earthworms. The assessment of the toxicity of chemicals to earthworms, together with the reproducibility of these assessments with chlordane, chloroacetamide and pentachlorophenol, were also unaffected by the amount of clay in the artificial soil. Similar observations have been made using kaolinite and bentonite (a montmorillonite type) clays, and when these clays were incorporated into the artificial soil in amounts ranging from 5-20% (dry weight), no change was seen in the the toxicity of benomyl or chloroacetamide to earthworms (Heimbach and Edwards, 1983). Thus, a soil was recommended that contained 20% clay, which was similar to the amount found in a natural sandy loam soil (Avery, 1980).

E. fetida was sensitive to the pH of the soil and lost more weight in an acidic soil than in an alkaline soil, although this species of earthworm was able to survive in a soil of pH 3.7. These results agree with

previous investigations but contradict the conclusion that this species will not tolerate a pH of less than 5 (Kaplan et al., 1980). Some workers suggest that E. fetida loses less weight in an acidic peat, because it burrows in this medium less actively than in a more alkaline material and thus conserves energy and biomass (Satchell and Dottie, 1984). My results are consistent with the opinion that the most alkaline conditions tolerated by lumbricid earthworms are within the range from pH 9 for E. fetida (Kaplan et al., 1980) to pH 7.3 for A. caliginosa (Springett and Syers, 1984).

The pH can affect the adsorptive properties of the soil and the state of ionisation of the test chemical (Bailey and White, 1964). The toxicity of chlordane, chloroacetamide and pentachlorophenol to earthworms was unchanged when assessed in artificial soils which had pH's within the range 3.7-8.0. These results agree with similar studies done using benomyl and chloroacetamide, in which the toxicity of these chemicals to earthworms was unaffected in artificial soils that had pH's within the range 4.5-7.5 (Heimbach and Edwards, 1983). The effect of the pH of the soil upon the state of ionisation of the test chemical differs between groups of compounds and can influence the rate at which chemicals are taken up into earthworms (Lofs-Holmin, 1981). Furthermore, the atomic valency state of an element can affect the toxicity that it presents to earthworms and the toxicity of copper chloride to L. rubellus was found to be correlated negatively with the acidity of a sandy soil between pH 4.8 to 7.1 (Ma, 1984). This effect was attributed to the increased availability of cupric ions, although other workers have disputed that toxicity to organisms is related to the valency of the copper atom (Anon., 1972). These differences in the effect of pH upon the test chemicals were minimized by recommending an artificial soil with a pH of 6.8, which was almost neutral and was representative of many soils that occur naturally in arable land. A pH of 6.8 was achieved conveniently by adding 1% calcium carbonate

(dry weight) to the soil and, as this compound can react with acidic test chemicals to affect their toxicity, it was desirable that as little calcium carbonate as possible was used.

The toxicity of the test chemicals was correlated positively with the temperature of the artificial soil, except for that of chlordane which was correlated negatively with the temperature of the soil between 5-20°C. Other studies have shown that the toxicity to earthworms of benomyl and chloroacetamide in the artificial soil was not affected by temperature between 10-26°C (Heimbach and Edwards, 1983). The temperature of the soil also affects the rate at which a chemical evaporates and the vapour pressure that is achieved by it within the soil spaces. Furthermore, the chemicals become adsorbed onto the soil and dissolved in the water within the soil to an extent that is dependent upon the temperature (Bailey and White, 1964). As each of these processes can affect the toxicity of chemicals to earthworms, the choice of the temperature at which the test is conducted was critical. A temperature of 20° ± 1°C was recommended because this optimised the physico-chemical interactions between the soil and test chemical, was within the range of temperatures that were acceptable to E. fetida in cultures in animal manure (Hartenstein, 1982; Kaplan et al., 1980) and in the artificial soil and gave a reliable estimate of the toxicity of chemicals to earthworms within 14 days. The capacity to be able to culture and test E. fetida at the same temperature was advantageous, because a change in temperature between these two conditions could affect the assessment of toxicity (Tomlin, 1977). Artificial soil tests at this temperature use readily available facilities and give results that compare directly with other tests for toxicity to earthworms, most of which have been carried out at similar temperatures.

The quantity of artificial soil used in each test vessel did not affect the assessment of toxicity, although the possibility existed that the effect of chemicals that were bioaccumulated by earthworms would be greater,

and the non-uniform distribution of the test chemical more probable, in a large volume of soil. Furthermore, it was undesirable that the earthworms should be capable of ingesting a large proportion of the soil in the test vessel within the period of the test, because the products of excretion may accumulate to a toxic concentration in the cast material, and the test chemical can become degraded. The estimates of the amount of soil that is ingested by the earthworms during a 14 day period of exposure differ. If one accepts the estimate of Hartenstein, Hartenstein and Hartenstein (1981), the mean gut load of E. fetida is 30-70% of the live body weight (which is 0.4 g, assuming a 50% gut load in a 0.8 g earthworm) and has a transit time of 2.5 hours at 25°C in soil or cellulose substrates. Thus ten earthworms would ingest 537.6 g of soil in 14 days, which is equivalent to 53.8 $g.worm^{-1}$ over this period. Other data (Morgan, 1984) indicated that E. fetida with a live body weight of 0.8 g rarely had a gut contents that weighed more than 0.4 g, and showed that the gut transit time was 45 hours for microcrystalline cellulose. Calculations based upon these data indicate that a single E. fetida ingests 2.99 g of $soil.worm^{-1}$ in 14 days and that 29.9 g of soil would be ingested by the ten earthworms in each test vessel during the course of an experiment that lasts 14 days. The use of 750 g of artificial soil in each test vessel greatly exceeded these minimum amounts, when ten E. fetida were used in each replicate.

Some tests for the toxicity of chemicals to earthworms using soil in the laboratory have used five E. fetida in 1.1 kg soil (Hopkins and Kirk, 1957) or six E. fetida in 0.5 kg soil (Haque and Ebing, 1983a). These quantities of soil, together with those recommended in the present study of 750 g artificial soil, are somewhat lower than the minimum of 20 litres of soil per replicate suggested by Ebing and Haque (1979). The density of the population of earthworms that was achieved in the artificial soil was, however, within the range proposed by these workers and did not exceed that of the natural population by more than ten times, because of the gregarious habits of E. fetida.

The period of exposure was correlated positively with the toxicity of chemicals to earthworms, and the difference between the assessments of toxicity made at several different longer periods of exposure, was less than that between assessments made at several different shorter periods of exposure. Not all long duration tests provide stable estimates of toxicity and at the end of a test, many assessments were still found to be changing after 28 days (Milne and DuToit, 1976), 32 days (Caseley and Eno, 1966) and in my own work after 50 days. Very long tests were undesirable, because the test chemical can volatilise, spontaneously decompose or be detoxified by microorganisms or by the earthworms. Tests of long duration also require a large volume of soil in each test vessel to ensure that the earthworms are able to avoid cast material, and may require the addition of food to avoid mortality in the controls. Foods are difficult to standardise (Ebing and Haque, 1979), may adsorb chemicals and would represent an additional complication of the method. Short tests lasting seven days or less may give an unreliable assessment of the toxicity of chemicals to earthworms (Fayolle, 1979; Martin, 1982), but have the advantage of being economical and of using a period of exposure that fits conveniently into weekly working schedules. These considerations, together with the reports of previous studies of the toxicity of chemicals to earthworms that have given a satisfactory result after periods of exposure of 14 days (Haque and Ebing, 1983a; Karnak and Hamelink, 1982), indicated that the optimum test duration was 14 days.

The reproducibility of an estimate of toxicity can be affected by the number of replicates that are used. The results of my work suggest that the variability of the assessments of the toxicity of chemicals to earthworms in the artificial soil was somewhat lower when four or more replicates were used. Other workers have considered three replicates (Haque and Ebing, 1983a; Hopkins and Kirk, 1957; Karnak and Hamelink, 1982) or four replicates (Hyche, 1956; Ruppel and Laughlin, 1977) to be

adequate for similar types of tests for toxicity to earthworms. Thus, by recommending the use of four replicates in the artificial soil test, I hoped to combine accuracy with a low cost of operation.

The method by which the test chemical was incorporated into the artificial soil did not have much effect upon the assessment of toxicity, although the assessments made with chemicals that were applied using a drench appeared to be the most reproducible. Drenches have been used successfully by other workers (Stringer and Wright, 1973; Davis, 1971) and are simple to use with aqueous solutions of test chemical, but the subsequent removal of volatile organic solvents from the soil is difficult. Chemicals applied by this method tend to concentrate in the upper layers of the soil (Morrod, 1982) and thus still require some form of incorporation. The use of organic solvents can cause chemicals to adsorb more strongly onto the soil colloids (Weise, 1964) or may interact directly with the test chemical, for instance acetone is reported to have an additive or synergistic effect upon the fungicidal properties of benomyl (Burrell and Corke, 1980). The application of the test chemical to the soil in a spray limits the effect of the solvent, but considerable care is required to prevent drift of the spray, which can introduce inaccuracies into the amount of the test chemical that is deposited onto the soil. The method in which the test chemical is incorporated directly into the soil without using any solvents has been used extensively in previous studies (e.g. Haque and Ebing, 1983a; Hockenyos, 1939; Hopkins and Kirk, 1957), but proved laborious to use and not suitable for all chemicals. The technique of application using a spray was recommended because it allowed for chemicals of widely differing properties to be applied to the artificial soil in a rapid and uniform manner. The period that was allowed to elapse between the treatment of the soil with the test chemical and the introduction of the earthworms did not appear to affect the toxicity of compounds such as chloroacetamide or benomyl (Heimbach and Edwards, 1983).

The assessments of the toxicity of chemicals to E. fetida that were made with the artificial soil test agreed generally with those that had been reported previously from other experiments done in the laboratory and the field (Table A1.1). Chemicals such as benomyl and thiophanate-methyl were ranked relatively more toxic than other chemicals when tested in the artificial soil test, although these chemicals were ranked as relatively less toxic than the other compounds from the results that were obtained using the filter paper contact test. This effect was probably due to the slow action of these chemicals, which prevented the manifestation of their true toxicity to earthworms in the filter paper contact test.

Copper sulphate and trichloroacetic acid were more toxic to earthworms in the filter paper contact test than in the artificial soil test. The action of trichloroacetic acid was investigated further (Appendix 3) in an experiment in which the toxicity of trichloroacetic acid and some of the salts of trichloroacetic acid were compared using the filter paper contact test. The results indicated that the acidic properties of trichloroacetic acid were probably responsible for the rapid mortality of the earthworms that were exposed to it on moist filter paper. Thus, it would seem that trichloroacetic acid reacts with certain components of the artificial soil (possibly with calcium carbonate, to give calcium chloroacetate) to form salts that are less toxic to earthworms.

The low toxicity of copper sulphate to earthworms in the artificial soil was probably caused by the adsorption of copper ions onto the soil. Copper ions were found previously to adsorb strongly onto ferro-manganese oxides and organic matter, but were not sequestered by clay minerals (McLaren, Swift and Williams, 1981). Furthermore, the formation of insoluble organic complexes with a variety of materials, reduced the toxicity of copper to fish (Stiff, 1971). Thus copper sulphate was probably adsorbed and deactivated by various components of the artificial soil, amongst which the most important was probably the peat (Verloo, 1980).

Despite the extensive studies of the toxicity of chemicals that have been undertaken with earthworms, very few data exist which can be compared directly with those obtained from the present experiments. This is because previous tests have used different species of earthworm, periods of exposure, types of soil and test chemicals.

The assessments of the toxicity of chemicals to earthworms that were obtained using the artificial soil test were calculated to give a single LC_{50} for the data from repeated experiments, by taking the antilogged mean of the individual LC_{50}'s that had been calculated previously on a logarithmic scale. The mean 14 day LC_{50}'s for benomyl and chloroacetamide using E. fetida in artificial soil tests were 6.72 and 18.41 $mg.kg^{-1}$ respectively, and using the same method the results obtained by Edwards (1985) were 3.5 and 26.0 $mg.kg^{-1}$ respectively. Thus, it would appear that the LC_{50}'s of chemicals calculated from data obtained using the artificial soil test may be reproduced closely in different laboratories, which supports the conclusions drawn from previous work (Heimbach and Edwards, 1983). The results obtained using the artificial soil test and the silica paste-glass ball test appeared to relate poorly. The silica paste-glass ball test gave a mean 14 day LC_{50} for benomyl and carbaryl of 132 and 25 $mg.kg^{-1}$ respectively (Bouche, 1984a), although my results using the artificial soil test with these chemicals showed that the 14 day LC_{50}'s were 6.72 and 85.74 $mg.kg^{-1}$ respectively. These data contrast with the results of Heimbach (1984, 1985b), who concluded that the results obtained using these two methods for testing toxicity to earthworms were correlated strongly.

Some assessments of the toxicity of chemicals to earthworms that were made using an artificial soil similar in composition to my own have now become available. This artificial soil was developed subsequently to my own at a research institute in West Germany (Biologische Bundesanstalt fur Land - und Fortwirtschaft, Braunschweig) that participated in the

evaluation of the artificial soil test (Chapter 5) developed at Rothamsted. This artificial soil consisted of 93.5% fine quartz sand, 5% bentonitic clay, 10% air-dried and finely milled peat, 1% $CaCO_3$, 0.5% air-dried and finely milled cow droppings and had a moisture content of 35%. The toxicity of several chemicals to earthworms was investigated by me using the Rothamsted artificial soil test, and was also studied in two separate experiments with E. fetida using the German method by Heimbach (1984) and Haque and Ebing (1983a). The LC_{50}'s that were generated by these tests are tabulated (Table 4.3) and show that the results from the two artificial soil methods are very similar, although those of Heimbach (1984) relate to a 28 day test and those of Haque and Ebing (1983a) to a 14 day test. (Nb. The correlations between the results obtained from 'an artificial soil' test and from other tests reported by Heimbach [1984, 1985b] were based largely upon the German artificial soil method, although the behaviour of this soil and that of the Rothamsted artificial soil were considered to be identical for the purposes of the comparison.)

The artificial soil developed during my studies was less elaborate than the German soil and does not contain unstandardised components such as cow droppings. Furthermore, the Rothamsted artificial soil probably has a lower adsorptive capacity than the other soil and will thus reproduce more closely the conditions that are present within a natural arable soil. The Rothamsted artificial soil is therefore more likely to predict accurately the toxicity of chemicals to earthworms in the field.

The artificial soil test that I developed combined the advantages that were present in the existing tests for toxicity to earthworms using natural soils or soil substitutes in the laboratory, and was capable of giving a sensitive, reproducible and economical assessment of the toxicity of any chemical to earthworms. This method is now used widely to test for toxicity to earthworms in laboratories that study ecotoxicology.

Table 4.3. The assessment of toxicity to earthworms using artificial soils. A comparison of the results from three independent studies.

Chemical	The 14 day LC_{50}'s reported by three independent sources and expressed in $mg.kg^{-1}$ (test chemical:dry weight of artificial soil)		
	Goats	Heimbach (1984)	Haque and Ebing (1983a)
	Rothamsted artificial soil	German artificial soil	German artificial soil
Chlordane	26.97	42	-
Carbaryl	85.74	174	-
Benomyl	6.72	22	27.2
Pentachlorophenol	45.53	87	-
Chloroacetamide	18.41	24	-
Potassium bromide	419.23	393	-

CHAPTER 5

A COMPARISON OF THE TOXICITY OF CHEMICALS TO EARTHWORMS ESTIMATED IN SEVERAL LABORATORIES

5.1. Introduction

The performance of the filter paper contact, artificial soil and silica paste-glass ball tests was assessed by independent laboratories in order to verify their efficiency. The tests were done by workers in industrial, academic and government research institutes on a voluntary basis, few of whom had any experience of work with earthworms.

Two exercises comparing the efficiency of the test methods were made. The first included laboratories from countries worldwide, but the second was with the help of Western European and North American collaborators only. The methods for the filter paper contact and artificial soil tests that were used in the first exercise were modified as a result of the criticism that arose from it. The performance of the improved Rothamsted test methods, together with a silica paste and glass ball test ('Artisol') that was developed in France, was assessed in a second exercise.

The collaborating laboratories were sent detailed instructions for the test, three unidentified chemicals and a named reference chemical. The reference chemical allowed the differences between the assessments of toxicity to earthworms that were obtained in different laboratories (due to variation in the susceptibility of the earthworms or changes in the test conditions) to be quantified. When the tests were in service, the reference chemical allowed the results that were obtained with the tests to be corrected for such variation in the experimental conditions.

5.2. Materials and methods

5.2.1 General materials and methods

Both of the exercises, or 'Ring' tests, were done in a similar manner. In the first exercise, the three unidentified test chemicals were pentachlorophenol, carbaryl and trichloroacetic acid, with copper sulphate as the reference compound. The unidentified chemicals used in the second exercise were potassium bromide, pentachlorophenol and chlordane, with chloroacetamide as the reference compound. Both chlordane and carbaryl were supplied as technical grade compounds, whilst the other chemicals were of analytical grade. The test chemicals killed earthworms in a variety of ways and represented a wide range of aqueous solubility and toxicity to earthworms (Table 5.1).

Supplies of earthworms were mailed from Rothamsted to the collaborating institutes for the first exercise, but many earthworms died in transit. In the second exercise the participants were asked to obtain their own earthworms locally. No other test materials were supplied from Rothamsted. The earthworms used in the first exercise were adult E. fetida weighing between 0.4-0.6 g, whilst in the second exercise, the earthworms were redefined as adult E. fetida andrei weighing between 0.4-0.6 g. The temperature at which the tests were done was 20° ± 1°C in the first ring test, but this range was extended to 20° ± 2°C in the second ring test.

The mortality data from each ring test test were processed by probit analysis. The protocols for the tests recommended the use of the method of Litchfield and Wilcoxon (1949), which allowed an approximate probit analysis to be made without using a computer, provided two or more partial mortality observations were obtained. The method has no advantage where computers are available (many of the European and North American laboratories reported computed probit results), but offered a simplicity that was advantageous in less developed countries (McIntosh, 1961).

Table 5.1. Characteristics of the chemicals used in the multi-laboratory exercises to assess toxicity to earthworms using three testing methods.

Exercise	Compound	Toxicity category(1)		Aqueous Solubility	Mode of Action
		Filter paper contact test	Artificial soil test		
1	Pentachloro-phenol	Toxic	Toxic	Low	Inhibits respiration (Weinbach and Garbus, 1969)
	Carbaryl	Toxic	Toxic	Low	Inhibits cholin-esterase activity in earthworms (Dikshith and Gupta 1981; Kale and Krishnamoorthy, 1982)
	Trichloro-acetic acid	Moderately toxic	Slightly toxic	High	Precipitates proteins (Cremlyn, 1978)
	Copper Sulphate	Toxic	Slightly toxic	High	Chelates and precipites proteins (Hassall, 1982)
2	Potassium bromide	Moderately toxic	Moderately toxic	High	-
	Pentachloro-phenol	Toxic	Toxic	Low	Inhibits respiration (Weinbach and Garbus, 1969)
	Chlordane	Very toxic	Toxic	Low	Neurotoxic (Hassall, 1982)
	Chloro-acetamide	Toxic	Toxic	High	Probably precipitates proteins

(1) Categories of toxicity to earthworms derived from the use of these tests in previous experiments (Tables 3.2 and 4.2).

The instructions that were sent to the collaborating workers in the first exercise included the option of recalculating the results to account for mortality in the controls by using Abbott's formula (Abbott, 1925). This technique was useful when tests were done in laboratories that could not provide stable experimental conditions or did not have the time to be able to repeat experiments. This correction was not intended to compensate for a severe environmental stress or for an unhealthy population of test earthworms (Stephan, 1977). Mortality in the controls was generally much lower in the second comparison and in which Abbott's correction was not used.

5.2.2 Filter paper contact test

The method was similar to the final version of the filter paper contact test (Section 3.2.3), but included the following additional instructions. A range finding test, using the test chemicals at five concentrations in a logarithmic dilution series between 0.1 - 1000 $\mu g.cm^{-2}$, was followed by a more precise test, using at least five concentrations of the chemicals in a geometric dilution series around the LC_{50} that had been calculated from the range test. The glass vials that were recommended measured 8 ± 0.5 cm x 3 ± 0.25 cm diameter. The report of each test included a description of the type of apparatus used, the state of the earthworms, the conditions under which the earthworms were cultured and the calculated LC_{50} with confidence or fiducial limits, together with the details of any pathological symptoms shown by the earthworms, data corrections used or criticisms of the method.

The criticisms of this method that were received after the first exercise were limited, and the only modification that was made to the method used in the second exercise (in addition to the general changes mentioned previously) was to alter the specification of the glass vials to 8 ± 1 cm x 3 ± 0.5 cm diameter.

5.2.3 Artificial soil test

The method used in the first exercise was very similar to the final version of the artificial soil test (Section 4.2.3), although some of the experimental conditions were, at this time, defined with less precision. The soil contained sand, clay, peat and $CaCO_3$ (Table A5.3) in the proportions 68:20:10:2 and at a moisture content of 35% had a pH of 6.8. Each replicate consisted of 400 g of artificial soil held in a 500 ml glass crystallising dish. The test chemical was applied to the soil as a spray in 8 ml distilled water or a suitable volatile organic solvent. A range finding test was done initially using five concentrations of the test chemical in a logarithmic dilution series between 0.1 - 1000 $mg.kg^{-1}$ (test chemical:dry weight of soil). This was followed by a more precise test, using five concentrations of the test chemical on a geometric dilution series around the LC_{50} that had been calculated from the range finding test. The earthworms were weighed before and after the test and mortality was assessed after 14 days, with optional assessments at 7 and 28 days. The reports of the tests contained similar information to that described for the filter paper contact test.

The method used in the second exercise was modified with the help of the criticism that was received after the first exercise. The ratio of sand, clay, peat and $CaCO_3$ in the soil was changed to 71:20:8:1, and the soil at 48% moisture content had a pH of 6.5 ± 0.5. In addition to the method of applying the test chemical as a spray, provision was made for incorporating the test chemical into the soil without the use of solvents. The chemicals applied in this way were ground with 10 g sand in a mortar and pestle, and this mixture was then incorporated into 3 kg of soil (wet weight, enough for four replicates) by hand using a spatula. Each replicate consisted of 750 g of moist soil and mortality was recorded after 14 days only.

5.2.4 Silica paste and glass ball test ("Artisol")

This method, developed in France by Dr. M.B. Bouche, was evaluated experimentally prior to the development of the filter paper contact and artificial soil tests and was considered worth testing further (Section 2.2.9). This test was used in the second exercise only. A paste, made by blending an aqueous suspension or solution of the test chemical with a fine silica powder, was supported on a matrix of 1.5-2.0 cm diameter glass balls. The mixture used for each replicate was contained in a 2 litre glass jar covered with a perforated plastic film. To each jar ten adult E. fetida andrei which weighed 0.3-0.6 g were added. A range finding test using five concentrations of the test chemical in a logarithmic dilution series between 0.1 - 1000 $mg.kg^{-1}$ (test chemical:dry weight of silica) was followed by a more precise test, using five concentrations of the test chemical in a geometric dilution series around the LC_{50} that had been calculated from the range finding test. Mortality was assessed by extracting the earthworms from the silica paste by washing the media gently through a fine mesh sieve after 14 days.

5.3. Results

The data generated using the tests for toxicity of chemicals to earthworms by the individual collaborating laboratories were expressed as the LC_{50} for each test chemical with confidence or fiducial limits (Tables A6.1-A6.5). The data for each test chemical are summarised to show the mean LC_{50} and the standard deviation of the mean LC_{50} (Tables 5.2-5.6). Some laboratories returned incomplete data, failed to quote confidence or fiducial limits or did not test all the chemicals. Despite a request to the contrary, the collaborators tended to use the filter paper contact test more than the artificial soil or silica paste-glass ball methods.

In the first exercise, pentachlorophenol and carbaryl had similar mean LC_{50}'s estimated using the filter paper contact test, and these chemicals were followed in a decreasing order of toxicity to earthworms by copper sulphate and trichloroacetic acid. Although the standard deviation of the LC_{50}'s increased with decreasing toxicity, this measure of the distribution of the results approximately equalled the LC_{50} of the chemical (Table 5.2). The artificial soil test gave a similar ranking of toxicity but the standard deviation, whilst also increasing as toxicity decreased, was approximately equal to, or considerably greater than the LC_{50} of the chemical (Table 5.3).

The data returned during the second exercise were more complete than in the first for both the filter paper contact and artificial soil tests, although the results obtained for the silica paste-glass ball test were less comprehensive. Pentachlorophenol was included in both 'Ring' tests and the LC_{50} for this chemical given by each of the Rothamsted methods in the first exercise was similar to that determined by these methods in the second exercise. The chemicals were ranked, in a decreasing order of toxicity to earthworms, by the filter paper contact (Table 5.4) and artificial soil tests (Table 5.5) as chlordane, chloroacetamide, pentachlorophenol and potassium bromide. The silica paste-glass ball test ranked the chemicals, in a decreasing order of toxicity to earthworms, as chloroacetamide, pentachlorophenol, chlordane and potassium bromide (Table 5.6). The standard deviations of the LC_{50}'s were approximately half, or equal to the LC_{50}'s of the test chemicals, when assessed by the filter paper contact and artificial soil tests respectively. The standard deviation of the LC_{50}'s obtained with the silica paste-glass ball test were more variable, but in general were similar to those of the artificial soil test. The standard deviations of the LC_{50}'s for pentachlorophenol were reduced considerably by the modifications that were made to the methods after the first exercise.

Table 5.2. Filter paper contact test results from the first multi-laboratory exercise.

Chemical	Number of laboratories responding. (1)	Number of laboratories quoting an LC50	Mean LC50 $\mu g.cm^{-2}$	Standard deviation
Pentachlorophenol	33	33	7.0	11.1
Carbaryl	33	33	7.1	10.4
Trichloroacetic acid	33	33	99.2	62.1
Copper sulphate	34	34	25.4	25.4

Table 5.3. Artificial soil test results from the first multi-laboratory exercise.

Chemical	Number of laboratories responding (1)	Number of laboratories quoting an LC50	Mean LC50 $mg.kg^{-1}$ (2)	Standard deviation
Pentachlorophenol	22	22	75.9	71.5
Carbaryl	22	22	86.9	116.6
Trichloroacetic acid	22	5	2813	4618.7
Copper sulphate	22	10	1684	1821.1

(1) Some laboratories quoted results in a form that could not be used, e.g. as < or > values.

(2) The LC_{50}'s were expressed as $mg.kg^{-1}$ (test chemical:dry weight of artificial soil).

Table 5.4. Filter paper contact test results from the second multi-laboratory exercise.

Chemical	Number of laboratories responding. (1)	Number of laboratories quoting an LC_{50}	Mean $LC50_{-2}$ $\mu g.cm^{-2}$	Standard deviation
Potassium bromide	20	20	460.8	284.3
Pentachlorophenol	20	20	4.0	1.7
Chlordane	20	20	2.3	1.4
Chloroacetamide	20	20	2.7	1.4

Table 5.5. Artificial soil test results from the second multi-laboratory exercise.

Chemical	Number of laboratories responding (1)	Number of laboratories quoting an LC50	Mean $LC50_{-1}$ $mg.kg^{-1}$ (2)	Standard deviation
Potassium bromide	16	16	232.9	235.5
Pentachlorophenol	19	19	96.2	103.7
Chlordane	18	18	58.1	45.9
Chloroacetamide	18	18	33.7	21.8

Table 5.6. Silica paste-glass ball ("Artisol") test results from the second multi-laboratory exercise.

Chemical	Number of laboratories responding (1)	Number of laboratories quoting an LC_{50}	Mean $LC50_{-1}$ $mg.kg^{-1}$ (3)	Standard deviation
Potassium bromide	5	5	1723.0	1104.5
Pentachlorophenol	7	7	98.6	97.3
Chlordane	8	8	144.5	345.8
Chloroacetamide	8	8	74.5	52.4

(1) Some laboratories quoted results in a form that could not be used, e.g. as < or > values.
(2) The LC50's were expressed as $mg.kg^{-1}$ (test chemical:dry weight of artificial soil).
(3) The LC50's were expressed as $mg.kg^{-1}$ (test chemical:dry weight of silica).

Following the first exercise, some of the collaborating scientists criticised a) the choice of test species and absence of a recommended sub-species, b) the recommended body weight of the earthworms, because the range of 0.4-0.6 was considered to be too high, c) the recommended test temperature, because the limits of 20 ± 1°C were considered to be too narrow, d) the comparatively high organic matter content (10%) and the low moisture content (35%) of the artificial soil, e) the amount of $CaCO_3$ (2%) required to achieve a pH of 6.8 in the artificial soil, f) the small amount of artificial soil used for each replicate and g) the use of Abbott's formula to correct for mortality in the controls, which was considered to be unnecessary.

The comments from the second exercise were that a) the earthworms should be acclimatised to the uncontaminated test media before starting the test, b) the organic matter content of artificial soil was still too high, c) both technical grade and formulated chemicals should be tested and d) the silica paste-glass ball test was difficult to use.

5.4 Discussion

The multi-laboratory comparison exercises allowed the three methods to be criticised and permitted the variability between the results obtained in different laboratories to be measured. The criticisms that were made of the test methods were used to modify the filter paper contact and artificial soil tests, and these modifications allowed the reproducibility of the results obtained with both methods to be improved. However, these comments were received during the developmental period of the test methods and therefore these modifications were a synthesis of my own findings and those of the collaborating workers.

The filter paper contact test method remained largely unaltered after the first exercise, except for general changes to the testing conditions

one of the subspecies of E. fetida (E. fetida andrei was found to be more susceptible to toxic chemicals than E. fetida fetida) and b) an increase in the range within which the test temperature was allowed to fluctuate from 20 ± 1°C to 20 ± 2°C. The revised instructions for the filter paper contact test laid greater emphasis on the need for care during the removal of volatile organic solvents, lest volatile test chemicals were also removed. The filter paper contact test was reported by the participants to be the easiest of the three methods to use and gave the least variable assessments of the toxicity of chemicals to earthworms.

The artificial soil test was modified after the first exercise and (in addition to the general changes to the test methods mentioned above), the new medium contained more water, less organic matter and had a lower pH that could be achieved by adding less $CaCO_3$. The artificial soil still contained more organic matter than an average arable soil, which might decrease the toxicity of some chemicals, although this disadvantage was unavoidable since E. fetida was poorly suited to a soil that contained a greater proportion of mineral components. The weight of soil used in each replicate was increased from 400 g to 750 g (wet weight), and an additional method for incorporating the test chemical into the soil, without using solvents, was included in the method. The use of a correction factor to compensate for mortality in the controls was unnecessary and undesirable in the second exercise, because the earthworms survived well in the modified test conditions.

The silica paste-glass ball test was reported to be difficult to use by most of the participants and gave estimates of the toxicity of chemicals to earthworms that were similar to those obtained with the artificial soil test.

The reference compound allowed changes in the assessments of toxicity to earthworms that were due to fluctuations in the conditions of the test to be quantified. Copper sulphate was proposed initially as a

reference chemical, but was adsorbed strongly by the artificial soil and became unavailable to the earthworms. Chloroacetamide was used in the second exercise and showed a consistent and moderate toxicity to earthworms.

CHAPTER 6

THE TOXICITY OF CHEMICALS TO EARTHWORMS IN THE FIELD

6.1 Introduction

Field experiments are a reliable way to assess the toxicity of a chemical to earthworms in the field. Methods for testing the toxicity of chemicals to earthworms in the laboratory are prevalent, because they are inexpensive to use and give a sensitive and reproducible result, although field techniques for testing toxicity to earthworms remain useful.

My field studies at Rothamsted Experimental Station and at the Shell (U.K.) Research Centre, Sittingbourne, were designed to assess the toxicity of several chemicals in different soil types, to all the species of earthworm that were present. These data were compared subsequently with the assessments of toxicity that had been obtained using various tests in the laboratory with the same chemicals (Chapter 7).

The availability, stability and therefore the toxicity of a chemical that is applied in the field can be affected by the moisture, clay and organic matter contents of the soil, together with the pH of the soil, the temperature and the activity of micro-organisms. These interactions are described in detail elsewhere (Section 4.1), although the most important effects of the environment upon the toxicity of a chemical to earthworms are summarised below.

A soil with a low moisture content can reduce the toxicity of water soluble chemicals, whilst compounds that dissolve in water can be leached from a soil that is waterlogged. Chemicals can bind reversibly or irreversibly with the particles of clay and organic matter in the soil, and the proportions in which these two components occur will, to a large extent, dictate the cation exchange capacity of the soil. The pH of the

soil can influence the ionic state of the test chemical as well as the adsorptive capacity of the soil, which will affect the amount of chemical that is available to the earthworms. Soil temperature can affect the vapour pressure of a chemical and its solubility in the soil water, and thereby also influence the rate at which compounds are taken up by earthworms. The loss of chemicals from the soil as a vapour or through the action of soil micro-organisms is also dependent upon soil temperature. Micro-organisms are often instrumental in the detoxification of chemicals, yet the range of organisms that are present in the soil at an experimental site, and their subsequent activity, is difficult to determine and standardise.

Several aspects of the behaviour of earthworms can affect the assessment of the toxicity of chemicals to them in the field. Earthworm ecology has been reviewed extensively (Edwards, 1983a; Edwards and Lofty, 1977; Gerard, 1967; Laird and Kroger, 1981; Wallwork, 1970), and I will describe only those aspects that influence directly the response of earthworms to chemicals.

Light and medium loam soils contain higher numbers of L. terrestris, A. longa, A. caliginosa and L. rubellus than clay or sandy soils, and whilst A. caliginosa appears to be the numerically dominant species in most soil types, it is relatively intolerant of acidic peat soils (Edwards and Lofty, 1977).

The horizontal distribution of earthworms in the soil is limited by certain aspects of the physico-chemical environment such as the temperature, moisture content and pH of the soil, the availability of food and the capacity of the earthworms to reproduce and disperse (Murchie, 1958). Vertical distributions are also determined by changes in the soil conditions such as low winter temperatures, summer drought (Gerard, 1967) and the ability of the earthworms to respond to these changes. Juvenile earthworms are often unable to burrow deeply in the soil

(Edwards and Lofty 1977) and are therefore affected more severely than the adults by unfavourable changes in the soil environment such as the application of toxic chemicals (Martin, 1980, 1983; Saunders and Forgie, 1977). In contrast to this, adult L. terrestris and A. longa can move rapidly into the safety of the deep soil layers using permanent, mucous lined burrows.

The peak period of activity for British earthworm species is from April to May and from August until early December. L. terrestris appears to remain active for most of the year (Gerard, 1967), whilst A. longa enters an obligate diapause from May until October. In other respects, L. terrestris and A. longa have similar seasonal behaviour. A. caliginosa, A. chlorotica and A. rosea can enter a facultative diapause during unfavourable conditions (Evans, 1947), and such resting behaviour should be taken into account before field trials are sampled.

The activity of earthworms in soil is limited mainly by temperature and moisture (Evans and Guild, 1947), although drought induces a more profound quiescence than winter cold (Gerard, 1967).

The temperature of soil preferred by earthworms can vary between species. A. caliginosa and E. fetida prefer soils between 10-23°C and 16-23°C respectively, whilst L. terrestris grows best in soil at 10°C (Edwards and Lofty, 1977). High temperatures will limit the activity of earthworms more than low temperatures and the removal of an insulating layer of vegetation from the field by cultivations can increase the range within which the temperature of the soil fluctuates, and thus affect populations of earthworms adversely (Edwards, 1983a).

Most lumbricid earthworms can withstand the loss of 50% of their body water (Grant, 1955), although earthworms will avoid drying conditions by emigration, for instance L. terrestris and A. longa will burrow more deeply, or by aestivation as seen with A. caliginosa (Gerard, 1960). The optimum moisture content of the soil for the North American species of

earthworms that were found in agricultural land was between 12-30% (Grant, 1955; Olson, 1928).

The acidity of the soil can affect the behaviour of earthworms. L. terrestris tolerated changes in the pH of the soil whilst A. longa, A. caliginosa and A. chlorotica avoided acid conditions (Satchell, 1955b).

L. terrestris is often exposed to a high concentration of pesticides, because this species of earthworm moves on and feeds at the soil surface. This species is more susceptible to benomyl than other lumbricid earthworms (Stringer and Lyons, 1977), and is more vulnerable than A. longa, A. chlorotica and L. rubellus in particular to the sprays of the benzimidazole fungicides that are used in orchards (Stringer and Lyons, 1974). A. caliginosa also lives in the superficial layers of the soil and the adults move on the soil surface in wet weather, becoming particularly vulnerable to pesticides such as methiocarb (Martin, 1982). A. longa seems to be less susceptible to pesticides than many other species of earthworm, because it can burrow deep into the soil (Wheatley and Hardman, 1968) and enters an obligatory diapause during the summer (Gerard, 1967).

Both L. terrestris and A. longa construct permanent burrows (Edwards, 1983a) into which chemicals diffuse slowly, and this probably decreases the exposure of these earthworms to pesticides that are incorporated into the soil (Lord et al., 1980). However, under some conditions such burrows can channel chemicals that are drenched onto the soil, allowing them to percolate rapidly and make contact with the earthworms. Cultivation can disturb such permanent burrows.

Ploughing decreases the numbers of L. terrestris and A. longa in arable soil, although such cultivation does not affect A. caliginosa and often leaves A. chlorotica as the dominant species. The earthen cells in which A. caliginosa aestivates can be destroyed by cultivation and therefore this species is affected more than L. rubellus, which can

oversummer as a cocoon (Martin, 1980). The most important effect of cultivation is to lessen the amount of organic matter that is available in the soil. However, the populations of earthworms can recover to their original size when manure is applied (Edwards, 1983a).

Adult earthworms are mobile and may migrate away from or into field plots. These movements can affect the population of earthworms in the plot and the assessments of the toxicity of chemicals made subsequently (Martin, 1976). L. terrestris and A. caliginosa can spread from an inoculation point in polder soils at a rate of 4.5 m and 9.0 m per annum respectively (Hoogerkamp et al., 1983). This supports previous observations with L. terrestris which suggest that earthworms can move several metres horizontally through the soil in a year (Bouche, 1974). However, Edwards and Brown (1982) report that this species failed to repopulate 6 m square grassland plots within one year of an application of benomyl which eliminated the earthworms. Whilst this fungicide may remain toxic in the soil for several months (Hassall, 1982), the same study showed that the population of L. festivus recovered rapidly to a size equivalent to that which was present before the treatment. These workers suggest that the cocoons of L. festivus survived the effects of the chemical, the concentration of which had fallen to a level tolerable to this species by the time that the cocoons hatched. The young earthworms then grew rapidly in an environment that presented little competition.

A variety of methods have been used to study the toxicity of chemicals to earthworms in the field but, in general, the test compounds were sprayed onto a loam soil, incorporated mechanically and then the plots were reseeded with grass. Occasionally the chemicals were applied to cropped grass without subsequent cultivation. The sizes of the field plots have ranged from 3 m to 10 m squares. These are commonly arranged in randomised blocks or as a Latin square. The earthworms are often sampled using the formalin extraction method (Raw, 1959) between one and

12 months post-treatment, and the data are then processed by an analysis of variance (Barker, 1982; Edwards and Brown, 1982; Thompson, 1971; Tomlin and Gore, 1974). The details of these field experiments and of the techniques for sampling earthworms have been criticised already and described in detail (Table 1.2). This type of investigation was adapted and used in the following study.

The field trials carried out at Rothamsted and Sittingbourne consisted of three randomised block experiments conducted during 1980, 1981 and 1982 on soils of differing type, organic matter content and cation exchange capacity.

The effects of five chemicals upon the populations of earthworms were studied; these chemicals included chlordane, carbaryl, thiophanate-methyl, triazophos and pentachlorophenol. Each of these chemicals had been tested under laboratory conditions and was found to be toxic to earthworms (Section 4.2.18). The toxicity of triazophos and pentachlorophenol to earthworms had not been investigated previously in the field. Chlordane and carbaryl are used commercially as vermicides, and thiophanate-methyl is a fungicide applied in large amounts to cereal crops. Triazophos is used widely as an insecticide and is related structurally to chemicals known to kill earthworms in arable soils. Pentachlorophenol (PCP) is used extensively in industry as a disinfectant and preservative as it has a broad-spectrum biocidal action (Cirelli, 1978). PCP has herbicidal properties (Hassall, 1982) and has been proposed for use as a molluscicide (Gould, 1962), whilst sodium pentachlorophenate was found to be useful as a soil insecticide for the control of termites (Hockenyos, 1939).

The effect of each of these chemicals applied to the soil at two rates, upon the numbers and weights of earthworms collected from the plots was assessed after one and six months post-treatment.

Unfortunately the first in this series of field trials, set up during 1980 on Geescroft Field, Rothamsted Experimental Station (on a sandy clay loam soil containing 2.5% organic matter), was destroyed accidentally by the staff of the experimental farm. Several difficulties prevented me from repeating this experiment and thus the investigation on this type of soil is lacking.

6.2 Materials and Methods

6.2.1. Materials

Chemicals

Commercially formulated chemicals were used wherever possible, as these materials could be suspended in water and applied to the soil as a spray. The solvents that were used in these investigations were of analytical grade.

Rothamsted field site

The trial site was on Appletrees field, a 19 year old mixed sward pasture. The soil was a clay-loam of pH 6.5, containing 5.1% organic matter and with a cation exchange capacity of 193 $meq.kg^{-1}$. The site had not been treated with pesticides for at least 19 years (Plate 6.1).

Sittingbourne field site

A site was obtained at the Shell (U.K.) Research Centre, Sittingbourne, on a 50-60 year old mixed sward pasture that had been woodland previously. The field was used to graze sheep and had never been treated with pesticides. The soil was a flinty clay-loam of pH 6.1, containing 6.9% organic matter with a cation exchange capacity of 211 $meq.kg^{-1}$ (Plate 6.2).

Plate 6.1. Rothamsted Field Site. Appletrees Field, Rothamsted Experimental Station, Harpenden, Hertfordshire.

Plate 6.2. Sittingbourne Field Site. Shell (UK) Research Centre, Sittingbourne, Kent.

6.2.2. The determination of the properties of the soil

The pH of the soil was determined according to a standard method (Anon. 1981d) using a pH meter (Gallenkamp and Company Ltd.) that was readable to 0.05 of a pH unit. Air-dried soil with a volume of 10 ml was ground finely, passed through a 2 mm mesh sieve and then shaken with 25 ml distilled water. The pH electrode was immersed in this suspension for 30 seconds and the pH of the soil read from the meter (the pH meter had been calibrated previously using standard solutions prepared from buffer tablets supplied by the manufacturer).

A modification of the Tinsley I method was used to determine the organic matter content of the soil. The soil was digested at 151°C for 120 minutes using a mixture of sodium dichromate, phosphoric acid and sulphuric acid, the amount of dichromate consumed was then estimated by titration against an acidified solution of ferrous ammonium sulphate (Kalembasa and Jenkinson, 1973).

The cation exchange capacity of the soil was determined at the natural pH of the soil using the method of Bascomb (1964), in which the amount of magnesium that was adsorbed by the soil was measured titrimetrically.

6.2.3. The design and execution of the field experiments

The manufacturers recommended rates of application for the test chemicals (Table 6.1) assisted in the design of the trials. The rate at which the chemicals were applied to the plots and the resultant concentration of the chemical in the soil, together with the 14 day LC_{50}'s of these chemicals for earthworms determined previously using the artificial soil test, are presented in a tabulated form (Table 6.2). Each chemical was applied to the soil at two rates and both of these rates produced a concentration of the chemical in the soil that was lower than that of the 14 day LC_{50} estimated using the artificial soil test. These low rates of

Table 6.1. The commercial pesticides used in the field experiments.

Chemical	Trade name	% a.i.	Formulation (1)	Recommended application rate $kg.ha^{-1}$ and use (2)	Manufacturer or supplier
Chlordane	Chlordane 25	25	e.c.	Earthworm control 11.25	Synchemicals Ltd.
Triazophos	Hostathion	42	e.c.	Cutworm control 1.05	Hoechst A.G.
Carbaryl	Sevin 85	85	w.p.	Earthworm control 3.83	Union Carbide (UK) Ltd.
Thio-phanate-methyl	Cercobin	50	w.p.	Cereal fungicide 0.70	May and Baker Ltd.
Penta-chloro-phenol	Witophen P	100	Flakes	Freshwater snail control ($5\ mg.kg^{-1}$)	Dynamit-Nobel A.G.

(1) e.c. emulsion concentrate, w.p. wettable powder.

(2) The recommended rate of application for the eradication of earthworms, or the rate of application for the use that was most similar to the control of earthworms.

Table 6.2. The rate of application of pesticides used for experiments in the field and laboratory, together with the concentration of these chemicals that resulted in the soil.

	Manufacturers recommended application rate $mg.kg^{-1}$ a.i. (1)	Artificial soil test LC50 $mg.kg^{-1}$ a.i. (2)	Rate of application used at the field site $kg.ha^{-1}$ a.i.	Concentration of chemicals achieved in the soil at the field site $mg.kg^{-1}$ a.i. (1)
Chlordane	5.63	26.97	5 10	2.5 5.0
Triazophos	0.53	10.53	3 6	1.5 3.0
Carbaryl	1.92	85.74	2.5 25	1.25 12.5
Thiophanate-methyl	0.35	20.06	3 6	1.5 3.0
Pentachloro-phenol	5.00	45.53	12.5 75	6.25 37.5

(1) The method for calculating the concentration of the chemical in the soil was based upon that of Thompson and Troeh (1973), cited by Dean-Ross (1983) amongst others. This calculation assumed that for a soil of bulk density 1.3, the weight of an acre-furrow slice (to a depth of 15 cm) was approximately 2×10^6 lbs. When this value was used to calculate the concentration of a chemical in the soil, 1 $lb.acre^{-1}$ was assumed to equal 1 $kg.ha^{-1}$ and therefore 1 $mg.kg^{-1}$ was achieved by an approximate rate of application of 2 $kg.ha^{-1}$. The soils taken from both field sites had a bulk density of approximately 1.25 as determined by the method of Avery and Bascomb (1974).

(2) The 14 day LC_{50}'s were calculated from data that were obtained using the artificial soil test (Table A7.30).

application were expected to demonstrate the lethal and sublethal effects of the chemicals upon the populations of earthworms during the long period of exposure that was used in the field.

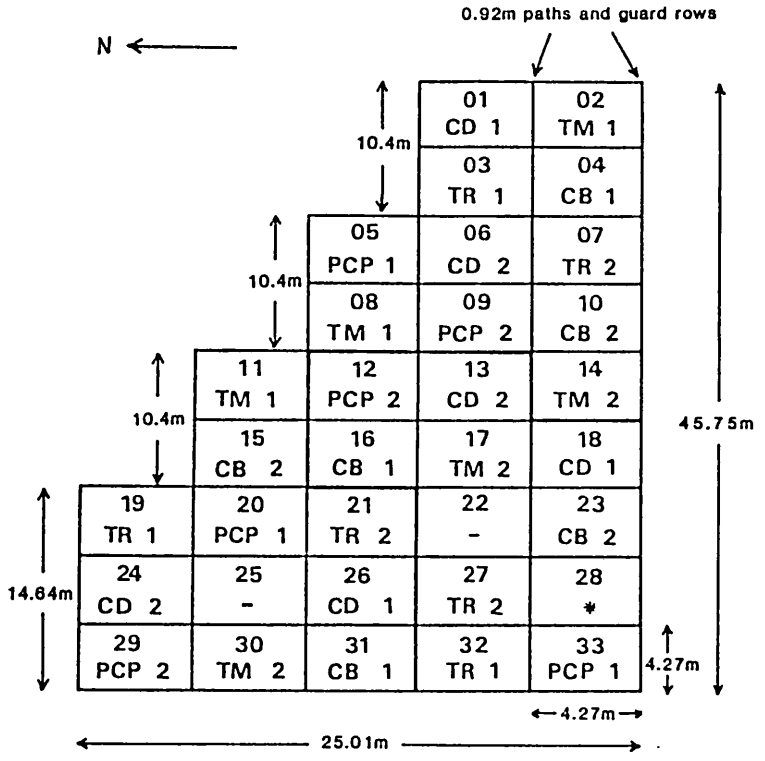
The high rate of application gave a concentration of chemical in the soil that was approximately 15-25% of the 14 day LC_{50} in the artificial soil, although pentachlorophenol was applied at a rate that gave a concentration in the soil similar to the 14 day LC_{50}. By using this approach, I hoped to see some mortality of earthworms due to the effect of each of the chemicals, although no information was available concerning the effect of pentachlorophenol upon earthworms in the field and a manufacturer's recommendation for the rate of application did not exist.

The lower rate of application for the chemicals was half that of the higher rate, except for carbaryl which was known to show a rapid and severe toxicity to earthworms, and for pentachlorophenol which persisted for a long time in the soil. These chemicals were applied at 10% and 17% of the higher rates of application respectively, in an attempt to avoid the complete elimination of the earthworms from these plots.

The trials at Rothamsted and Sittingbourne were designed as randomised block experiments (Figures 6.1 and 6.2 respectively), with each block containing one plot per treatment and an untreated control. However, an error occurred during the application of the chemicals at Rothamsted which, together with inadequate pretreatment sampling of the populations of earthworms at both sites, required that the data were analysed in a more appropriate manner which is described below. The trials had a factorial design because each chemical was applied at two rates, and the overall layout of each trial was tailored to fit the available space. The diagrams indicate that the dimensions of both trials were similar, but that the trial conducted on the experimental farm at Rothamsted was required by the management to be measured in Imperial units.

Figure 6.1

ROTHAMSTED FIELD TRIAL APPLETREES FIELD



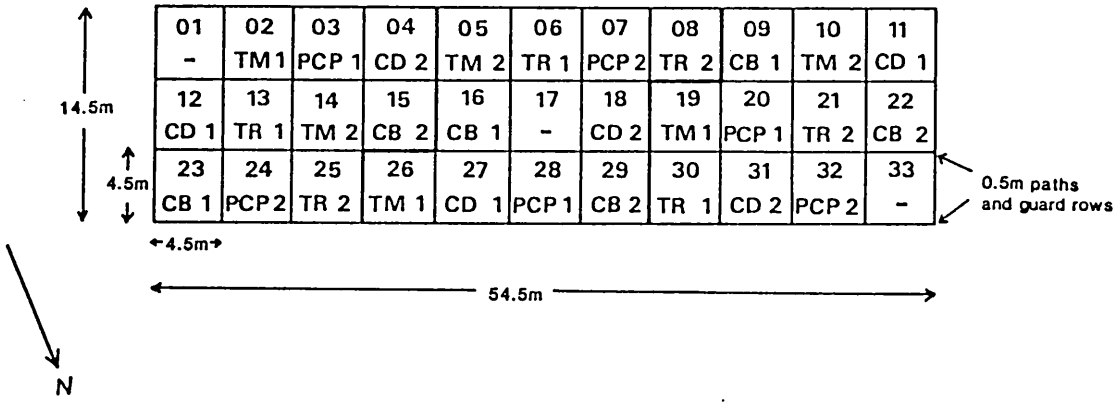
TREATMENTS

-	Control	-
TM 1	Thiophanate-methyl	3 kg.ha^{-1}
TM 2	Thiophanate-methyl	6 kg.ha^{-1}
CD 1	Chlordane	5 kg.ha^{-1}
CD 2	Chlordane	10 kg.ha^{-1}
TR 1	Triazophos	3 kg.ha^{-1}
TR 2	Triazophos	6 kg.ha^{-1}
CB 1	Carbaryl	2.5 kg.ha^{-1}
CB 2	Carbaryl	25 kg.ha^{-1}
PCP 1	Pentachlorophenol	12.5 kg.ha^{-1}
PCP 2	Pentachlorophenol	75 kg.ha^{-1}

* Plot 28 was incorrectly treated, and therefore discounted. The treatment intended for Plot 28 was applied to Plot 2 instead. Plot 2 was originally a control.

Figure 6.2

SITTINGBOURNE FIELD TRIAL SHELL RESEARCH CENTRE



TREATMENTS

-	Control	-
TM 1	Thiophanate-methyl	3 $kg.ha^{-1}$
TM 2	Thiophanate-methyl	6 $kg.ha^{-1}$
CD 1	Chlordane	5 $kg.ha^{-1}$
CD 2	Chlordane	10 $kg.ha^{-1}$
TR 1	Triazophos	3 $kg.ha^{-1}$
TR 2	Triazophos	6 $kg.ha^{-1}$
CB 1	Carbaryl	2.5 $kg.ha^{-1}$
CB 2	Carbaryl	25 $kg.ha^{-1}$
PCP 1	Pentachlorophenol	12.5 $kg.ha^{-1}$
PCP 2	Pentachlorophenol	75 $kg.ha^{-1}$

The trials were done during consecutive years, but the timing of the various operations was similar for each (Table 6.3). The grass at each site was rotovated using a tractor mounted unit, between four and seven days before the chemicals were applied. The plots were marked out with nylon cord to act as a spray guide and the chemicals were applied during the late afternoon in two litres of water per plot, using an I.C.I. hand-pumped backpack sprayer (Cooper-Pegler and Co. Ltd.). The sprayer was fitted with a one metre spray lance, a pressure gauge and a single flat fan nozzle Type F110/100 (Plate 6.3). Pentachlorophenol was insoluble in water and was therefore applied in 0.5 litre methan-1-ol per plot. The alcohol evaporated quickly. The deposits of the chemicals were then incorporated by rotovation during the evening of the day on which they were applied, and the plots seeded with an Italian ryegrass.

At Rothamsted, Plot 28 was sprayed twice by accident and was therefore not sampled. The application of 3 $kg.ka^{-1}$ thiophanate-methyl that should have been sprayed onto this plot was applied instead to Plot 2, which should have been a control plot. This control plot was chosen to receive the replacement treatment, because additional information about the populations of earthworms in the untreated soil could, if necessary, be obtained from the discard areas.

Soil samples (one kg) were taken for the determination of organic matter content and cation exchange capacity before the chemicals were applied. The populations of earthworms were sampled within 0.5 m square (0.25 m^2) quadrats by an extraction method using formalin (Plates 6.4 and 6.5) that was adapted from the method of Raw (1959). The earthworms were extracted from the central area of each plot using two 4.5 litre applications of a 0.2% aqueous solution of formalin, drenched onto 0.25 m^2 of soil at 20 minute intervals. The earthworms expelled by this irritant were collected and preserved in a 5% aqueous solution of formalin prior to identification in the laboratory.

Table 6.3. The timing of the operations at the trial sites.

Operation	Rothamsted Site	Sittingbourne Site
Pasture rotovation	14.4.81	5.4.82
Pre-treatment sampling for earthworms	15.4.81	7.4.82
Treatment application	21.4.81	9.4.82
Treatment incorporation	21.4.81	9.4.82
1 month post-treatment sampling for earthworms	21.5.81	10.5.82
6 months post-treatment sampling for earthworms	15.10.81	11.10.82

Plate 6.3. Application of the test chemical to the soil using a backpack sprayer.

Plate 6.4. Earthworm sampling: applying a dilute solution of formalin to the soil as a drench.

Plate 6.5. Earthworm sampling: collecting the earthworms expelled within the 0.5 m square quadrat.

Pretreatment samples of the populations of earthworms taken from the discard areas bordering the trial, indicated that these populations were high at both sites. Without pretreatment samples of the populations of earthworms within each plot, it was difficult to estimate the initial population density of the earthworms. The subsequent statistical analyses indicated that these populations were distributed non-uniformly across the trial sites before the experiments were set up.

Post-treatment samples were collected after approximately 30 and 180 days (hereafter referred to as the one month and six month samples respectively). At each sampling date, two 0.25 m^2 subsamples were taken from each plot using different areas of soil on each occasion. The sub-samples were collected near the centre of each plot to reduce the effect of any migration of the earthworms.

6.2.4. The analysis of the data obtained from the field experiments

The information recorded for each subplot consisted of the numbers and weights of L. terrestris, A. longa and A. caliginosa, together with the total numbers and weights of the remaining species of earthworm which included A. chlorotica, L. rubellus, L. castaneus, A. rosea and O. cyaneum. These latter species will hereafter be referred to as the 'other' species.

The numbers of A. caliginosa and both the numbers and the weights of L. terrestris, A. longa and the other species, together with the total numbers and weights for all the species, were analysed for both trials. The data for the weights of A. caliginosa were too sparse to be analysed.

The data were transformed into logarithms and differences between treatment means detected using an analysis of variance. A preliminary analysis of variance using the data from the Rothamsted site, without allowing for blocks, produced a pattern of residual variation which indicated that the initial population density differed systematically within

the originally proposed blocks. Therefore, new blocks were defined within which the plots were spatially contiguous and these newly defined blocks used as a covariate in an analysis of variance for the one month sample. The covariate used for the six month sample was the mean value of two subsamples of the population taken from each plot after one month. These analyses of covariance substantially reduced the residual variation seen in preliminary analyses, and allowed the differences between treatments to be estimated with greater precision.

The data collected from the Sittingbourne site after one month was sparse. An initial analysis of variance indicated that the data for L. terrestris alone showed systematic differences in population density, and allowed an analysis of covariance similar to that performed on the Rothamsted site data. The covariance analysis of the data collected after six months from the Sittingbourne site was conducted as described previously for the data from the Rothamsted site.

The data for each trial are presented as the grand mean and the variance ratios of both the treatment and the covariate (including the degrees of freedom for these and for the residual variation), together with the significance of the variance ratio. The arithmetic means of the logarithmically transformed numbers and weights (adjusted for covariates) were calculated, together with the standard error for the difference between the means.

6.3. Results

The description of the results which follows will contain references to an 'increase' or 'decrease' in the numbers or weights of earthworms. This relates to the numbers and weights found in the treated plots, compared to those present in the control plots. When a population is referred to as 'unaffected' by a treatment, this means that the numbers or weights of

earthworms in the treated plots, at the time of sampling, were similar to those in the control plots.

References to the 'high' and 'low' rate of application of a chemical relate to the upper and lower concentrations that were achieved in the soil respectively, at which each of the chemicals were tested. The details of these rates of application appear in a tabular form (Table 6.2).

6.3.1. Rothamsted site. One month post-treatment (21 May)

The numbers and weights of L. terrestris and of the total population of earthworms for the treatments differed significantly ($P<0.01$). The weights of the other species also showed significant ($P<0.05$), but smaller differences due to treatment (Tables 6.4 and 6.8). The effect of the covariate was significant ($P<0.001$) in each of these cases, indicating that the residual sums of squares were reduced by defining new blocks in the analysis.

The mean numbers and weights of the earthworms corresponding with each treatment (Tables 6.5 and 6.9 respectively), indicated that the numbers of L. terrestris were decreased severely by the high rates of chlordane and of carbaryl, whilst being somewhat reduced by both rates of pentachlorophenol. The numbers of A. longa were decreased by all the chemicals, except the low rate of triazophos and both rates of pentachlorophenol. A. caliginosa and the other species were less numerous in plots that had been treated with the high rate of triazophos and both rates of carbaryl. A. caliginosa appeared to be particularly susceptible to carbaryl.

Some treatments apparently increased the number of earthworms. A. longa was more numerous in plots treated with the low rate of triazophos and both rates of pentachlorophenol. The other species were more numerous in plots that had been treated with the low rates of thiophanate-methyl, chlordane, triazophos and pentachlorophenol whilst the

numbers of A. caliginosa were increased by these treatments, and also by the high rate of thiophanate-methyl.

The fluctuations in the numbers and weights of earthworms in response to the treatments appeared to be correlated highly, and almost identical responses were seen for these measurements of the populations of L. terrestris. However, the weights of A. longa found in the plots that were treated with the high rate of triazophos and both rates of pentachlorophenol exceeded those found in controls, although the numbers of A. longa did not. The weights of the other species exceeded those in the controls for the plots that were treated with thiophanate-methyl, pentachlorophenol and the low rates of chlordane and triazophos. These effects only occured in the absence of an increased number of earthworms in the plots that were treated with the high rates of thiophanate-methyl and of pentachlorophenol.

6.3.2. Rothamsted site. Six months post-treatment (15 October)

The differences between the treatments were significant for the numbers and weights of L. terrestris ($P<0.01$), A. longa ($P<0.001$), the other species ($P<0.05$) and for the total population of earthworms ($P<0.001$). The covariate used in this analysis was the mean value of the two subsamples that were collected from each plot after one month, and was significant for the numbers of L. terrestris ($P<0.05$), A. caliginosa ($P<0.001$) and the total population of earthworms ($P<0.01$). The covariate was also significant ($P<0.01$) for the weights of the other species (Tables 6.6 and 6.10). By including this covariate in the analysis of variance, the residual sums of squares was reduced. The numbers and weights of earthworms remaining in the treated plots are presented as mean values (Tables 6.7 and 6.11 respectively).

Rothamsted Field Site. Samples taken one month post-treatment.

Table 6.4. Analysis of variance for the number of earthworms.

Species	Grand Mean	Treatment variance ratio (1)	Covariate variance ratio (1)	Treatment D.F.	Covariate D.F.	Residual D.F.
L.terrestris	0.547	4.993 **	28.154 ***	10	3	18
A.longa	0.120	2.315 NS	2.124 NS	10	3	18
A.caliginosa	0.133	1.177 NS	3.119 NS	10	3	18
Other species	0.343	1.158 NS	2.682 NS	10	3	18
Total population	0.744	4.727 **	21.818 ***	10	3	18

(1) P<0.001 ***

P<0.01 **

P<0.05 *

Rothamsted Field Site. Samples taken one month post-treatment.

Table 6.5. Estimated effect of the treatment upon the mean number of earthworms.(1)

Species	Treatment	Control	Thiophanate-methyl		Chlordane		Triazophos		Carbaryl		Penta-chlorophenol		Standard error for difference between means (2)
	Application rate kg. ha^{-1} a.i.	-	3	6	5	10	3	6	2.5	25	12.5	75	-
L.terrestris		0.72	0.60	0.66	0.69	0.41	0.69	0.64	0.61	0.03	0.50	0.54	0.1525
A.longa		0.20	0.04	0.08	0.07	0.06	0.40	0.12	0.04	-0.04	0.23	0.22	0.1270
A.caliginosa		0.09	0.29	0.27	0.26	0.07	0.21	0.01	-0.03	0.06	0.14	0.10	0.1786
Other species		0.36	0.43	0.35	0.52	0.37	0.49	0.22	0.126	0.08	0.52	0.30	0.2410
Total population		0.86	0.85	0.86	0.93	0.58	0.98	0.72	0.71	0.18	0.81	0.76	0.1776

(1) Means adjusted for covariates. Negative means occur after this adjustment for covariates.

(2) With unequal replication, the S.E.D. of the mean was calculated using the values for the minimum replication.

Rothamsted Field Site. Samples taken six months post-treatment.

Table 6.6. Analysis of variance for the number of earthworms.

Species	Grand Mean	Treatment variance ratio (1)	Covariate variance ratio (1)	Treatment D.F.	Covariate D.F.	Residual D.F.
L.terrestris	0.698	3.496 **	4.288 *	10	1	20
A.longa	0.918	14.119 ***	0.243 NS	10	1	20
A.caliginosa	0.450	2.102 NS	8.860 ***	10	1	20
Other species	0.298	2.493 *	2.300 NS	10	1	20
Total population	1.209	19.108 ***	6.620 **	10	1	20

(1) P<0.001 ***

P<0.01 **

P<0.05 *

Rothamsted Field Site. Samples taken six months post-treatment.

Table 6.7. Estimated effect of the treatment upon the mean number of earthworms.(1)

Species	Treatment	Control	Thiophanate-methyl		Chlordane		Triazophos		Carbaryl		Penta-chlorophenol		Standard error for difference between means (2)
	Application rate $kg.ha^{-1}$ a.i.	-	3	6	5	10	3	6	2.5	25	12.5	75	-
L.terrestris		0.83	0.70	0.57	0.76	0.43	0.76	0.55	0.66	0.87	0.73	0.86	0.1300
A.longa		1.00	1.09	1.07	0.82	0.06	1.03	1.08	0.92	1.03	1.04	0.98	0.1395
A.caliginosa		0.52	0.43	0.37	0.32	-0.01	0.48	0.63	0.48	0.60	0.65	0.52	0.2196
Other species		0.26	0.09	0.34	0.11	-0.02	0.33	0.63	0.41	0.45	0.43	0.23	0.1974
Total population		1.32	1.28	1.26	1.16	0.44	1.30	1.35	1.20	1.40	1.34	1.29	0.1076

(1) Means adjusted for covariates. Negative means occur after this adjustment for covariates.

(2) With unequal replication, the S.E.D. of the mean was calculated using the values for the minimum replication.

Rothamsted Field Site. Samples taken one month post-treatment.

Table 6.8. Analysis of variance for the biomass of earthworms.

Species	Grand Mean	Treatment variance ratio (1)	Covariate variance ratio (1)	Treatment D.F.	Covariate D.F.	Residual D.F.
L.terrestris	0.786	4.693 **	21.331 ***	10	3	18
A.longa	0.103	1.399 NS	1.378 NS	10	3	18
A.caliginosa	-	-	-	-	-	-
Other species	0.162	2.623 *	10.138 **	10	3	18
Total population	0.835	5.359 **	22.993 ***	10	3	18

(1) P<0.001 ***

P<0.01 **

P<0.05 *

Rothamsted Field Site. Samples taken one month post-treatment.

Table 6.9. Estimated effect of the treatment upon the mean biomass of earthworms.(1)

Species	Treatment	Control	Thiophanate-methyl		Chlordane		Triazophos		Carbaryl		Penta-chlorophenol		Standard error for difference between means (2)
	Application rate kg.ha^{-1} a.i.	-	3	6	5	10	3	6	2.5	25	12.5	75	-
L.terrestris		0.92	0.92	0.96	1.09	0.56	0.94	0.88	0.84	0.08	0.69	0.79	0.2244
A.longa		0.11	0.11	0.06	0.03	0.02	0.28	0.15	0.04	-0.03	0.15	0.22	0.1408
A.caliginosa		-	-	-	-	-	-	-	-	-	-	-	-
Other species		0.15	0.27	0.20	0.22	0.10	0.18	0.05	0.11	-0.01	0.29	0.23	0.1008
Total population		0.94	1.00	0.99	1.11	0.59	1.00	0.91	0.88	0.11	0.77	0.91	0.2124

(1) Means adjusted for covariates. Negative means occur after this adjustment for covariates.

(2) With unequal replication, the S.E.D. of the mean was calculated using the values for the minimum replication.

Rothamsted Field Site. Samples taken six months post-treatment.

Table 6.10. Analysis of variance for the biomass of earthworms.

Species	Grand Mean	Treatment variance ratio (1)	Covariate variance ratio (1)	Treatment D.F.	Covariate D.F.	Residual D.F.
L.terrestris	1.077	3.864 **	0.933 NS	10	1	20
A.longa	1.044	13.407 ***	0.024 NS	10	1	20
A.caliginosa	-	-	-	-	-	-
Other species	0.336	2.394 *	6.424 **	10	1	20
Total population	1.402	11.256 ***	2.149 NS	10	1	20

(1) P<0.001 ***

P<0.01 **

P<0.05 *

Rothamsted Field Site. Samples taken six months post-treatment.

Table 6.11. Estimated effect of the treatment upon the mean biomass of earthworms.(1)

Species	Treatment	Control	Thiophanate-methyl		Chlordane		Triazophos		Carbaryl		Penta-chlorophenol		Standard error for difference between means (2)
	Application rate $kg.ha^{-1}$ a.i.	-	3	6	5	10	3	6	2.5	25	12.5	75	-
L.terrestris		1.28	1.16	0.85	1.10	0.67	1.22	0.80	1.14	1.26	1.17	1.26	0.1861
A.longa		1.15	1.19	1.18	0.91	0.06	1.21	1.25	1.05	1.15	1.17	1.20	0.1618
A.caliginosa		-	-	-	-	-	-	-	-	-	-	-	-
Other species		0.64	0.27	0.32	0.18	0.00	0.32	0.53	0.35	0.45	0.40	0.34	0.1762
Total population		1.64	1.48	1.38	1.33	0.68	1.52	1.45	1.41	1.57	1.50	1.53	0.1339

(1) Means adjusted for covariates. Negative means occur after this adjustment for covariates.

(2) With unequal replication, the S.E.D. of the mean was calculated using the values for the minimum replication.

The numbers of L. terrestris were decreased by the high rates of thiophanate-methyl, chlordane and triazophos. The high rate chlordane virtually eliminated A. longa, A. caliginosa and the other species, whilst the high rate of carbaryl appeared to have little effect upon any of the species. A. caliginosa was somewhat less numerous in the plots that had been treated with both rates of thiophanate-methyl and the low rate of chlordane. The other species responded to the treatments in a manner similar to A. caliginosa, but were affected less by the high rate of thiophanate-methyl. The responses of the numbers and the weights of the earthworms in the treated plots, compared with those in the controls, appeared to be correlated highly and were almost identical for L. terrestris and A. longa. However, the increase in the numbers of the earthworms seen for the other species in plots that were treated with the high rate of thiophanate-methyl, both rates of triazophos and carbaryl, and the low rate of pentachlorophenol, was not reflected in an increase in the weights of these earthworms.

6.3.3. Sittingbourne site. One month post-treatment (10 May)

Significant differences between the treatments were seen for the numbers and weights of L. terrestris ($P<0.001$) and for the total population of earthworms ($P<0.01$). The newly defined blocks were used as a covariate, although this was significant for the numbers ($P<0.001$) and weights ($P<0.01$) of L. terrestris alone. Analyses of variance (allowing for covariates) permitted the residual sums of squares to be reduced (Tables 6.12 and 6.16), and the numbers and weights of the earthworms corresponding with each treatment are presented as the mean values (Tables 6.13 and 6.17 respectively).

The high rates of chlordane and carbaryl virtually eliminated L. terrestris and A. longa. Thiophanate-methyl and the low rates of triazophos and pentachlorophenol also decreased the numbers of A. longa,

although this species became more numerous in those plots that were treated with the low rates of chlordane and carbaryl.

The numbers and weights of the populations of earthworms responded to the treatments in a similar manner, and these values appeared to be correlated highly for both L. terrestris and A. longa. Very low numbers of A. caliginosa and of the other species were collected from the plots at this sampling date.

6.3.4. Sittingbourne site. Six months post-treatment (11 October)

The differences between the treatments were significant in terms of the numbers of A. caliginosa and the weights of L. terrestris ($P<0.05$). The covariate used in these analyses was the mean value of the two subsamples taken from each plot after one month, although this was significant ($P<0.05$) only for the numbers of L. terrestris (Tables 6.14 and 6.18). Thus, the analyses of variance (allowing for covariates) allowed the residual sums of squares of the data for L. terrestris to be decreased. The numbers and weights of the earthworms in the treated plots are presented as the mean values (Tables 6.15 and 6.19 respectively).

The numbers and weights of both L. terrestris and A. longa were apparently almost unaffected by the treatments, although the weights of L. terrestris were slightly decreased by the high rate of thiophanate-methyl. A. caliginosa was less numerous in the plots that were treated with chlordane and triazophos. However, the numbers of the other species of earthworm appeared to increase in those plots that were treated with chlordane, pentachlorophenol and the low rates of thiophanate-methyl and triazophos. The high rate of triazophos reduced the numbers and the weights of the population of the other species of earthworm.

The numbers and the weights of the populations of the earthworms appeared to respond to the treatments in a similar manner, for those populations for which data was available.

Sittingbourne Field Site. Samples taken one month post-treatment.

Table 6.12. Analysis of variance for the number of earthworms.

Species	Grand Mean	Treatment variance ratio (1)	Covariate variance ratio (1)	Treatment D.F.	Covariate D.F.	Residual D.F.
L.terrestris	0.632	9.019 ***	16.945 ***	10	3	19
A.longa	0.061	0.782 NS	-	10	-	22
A.caliginosa	-	-	-	-	-	-
Other species	0.018	1.667 NS	-	10	-	22
Total population	0.654	3.442 **	-	10	-	22

(1) P<0.001 ***

P<0.01 **

P<0.05 *

Sittingbourne Field Site. Samples taken one month post-treatment.

Table 6.13. Estimated effect of the treatment upon the mean number of earthworms.(1)

Species	Treatment	Control	Thiophanate-methyl		Chlordane		Triazophos		Carbaryl		Penta-chlorophenol		Standard error for difference between means (2)
	Application rate $kg.ha^{-1}$ a.i.	-	3	6	5	10	3	6	2.5	25	12.5	75	-
L.terrestris		0.67	0.73	0.60	0.76	0.40	0.63	0.76	0.81	-0.00	0.74	0.86	0.1126
A.longa		0.08	0.00	0.05	0.16	0.00	0.05	0.08	0.15	0.00	0.00	0.10	0.0946
A.caliginosa		-	-	-	-	-	-	-	-	-	-	-	-
Other species		0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.05	0.0371
Total population		0.76	0.68	0.65	0.74	0.43	0.67	0.87	0.78	0.00	0.77	0.85	0.1889

(1) Only the mean numbers of L. terrestris were adjusted for covariates. Negative means occur after the adjustment for covariates.

(2) With unequal replication, the S.E.D. of the mean was calculated using the values for the minimum replication.

Sittingbourne Field Site. Samples taken six months post-treatment.

Table 6.14. Analysis of variance for the number of earthworms.

Species	Grand Mean	Treatment variance ratio (1)	Covariate variance ratio (1)	Treatment D.F.	Covariate D.F.	Residual D.F.
L.terrestris	1.022	0.976 NS	5.240 *	10	1	21
A.longa	1.312	0.707 NS	0.095 NS	10	1	21
A.caliginosa	1.054	2.687 *	-	10	-	22
Other species	0.839	2.046 NS	0.084 NS	10	1	21
Total population	1.692	1.332 NS	0.082 NS	10	1	21

(1) P<0.001 ***

P<0.01 **

P<0.05 *

Sittingbourne Field Site. Samples taken six months post-treatment.

Table 6.15. Estimated effect of the treatment upon the mean number of earthworms.(1)

Species	Treatment	Control	Thiophanate-methyl		Chlordane		Triazophos		Carbaryl		Penta-chlorophenol		Standard error for difference between means (2)
	Application rate $kg.ha^{-1}$ a.i.	-	3	6	5	10	3	6	2.5	25	12.5	75	-
L.terrestris		0.95	1.09	0.93	1.02	1.13	1.08	1.01	1.01	1.01	1.00	1.01	0.0889
A.longa		1.30	1.31	1.34	1.16	1.35	1.28	1.24	1.40	1.34	1.37	1.32	0.1101
A.caliginosa		1.20	1.09	1.07	0.92	0.96	1.00	0.79	1.05	1.09	1.24	1.19	0.1137
Other species		0.77	0.84	0.73	1.05	1.07	0.89	0.48	0.75	0.79	0.96	0.90	0.1702
Total population		1.69	1.72	1.68	1.65	1.74	1.69	1.54	1.71	1.69	1.77	1.73	0.0779

(1) Means adjusted for covariates. Negative means occur after this adjustment for covariates.

(2) With unequal replication, the S.E.D. of the mean was calculated using the values for the minimum replication.

Sittingbourne Field Site. Samples taken one month post-treatment.

Table 6.16. Analysis of variance for the biomass of earthworms.

Species	Grand Mean	Treatment variance ratio (1)	Covariate variance ratio (1)	Treatment D.F.	Covariate D.F.	Residual D.F.
L.terrestris	0.809	5.419 ***	7.855 **	10	3	19
A.longa	0.084	0.778 NS	-	10	-	22
A.caliginosa	-	-	-	-	-	-
Other species	0.005	1.743 NS	-	10	-	22
Total population	0.838	3.889 **	-	10	-	22

(1) P<0.001 ***

P<0.01 **

P<0.05 *

Sittingbourne Field Site. Samples taken one month post-treatment.

Table 6.17. Estimated effect of the treatment upon the mean biomass of earthworms.(1)

Species	Treatment	Control	Thiophanate-methyl		Chlordane		Triazophos		Carbaryl		Penta-chlorophenol		Standard error for difference between means (2)
	Application rate kg.ha^{-1} a.i.	-	3	6	5	10	3	6	2.5	25	12.5	75	-
L.terrestris		0.97	0.97	0.80	0.89	0.56	0.87	0.94	1.07	0.04	0.83	0.97	0.1720
A.longa		0.12	0.00	0.05	0.22	0.00	0.08	0.14	0.17	0.00	0.00	0.15	0.1275
A.caliginosa		-	-	-	-	-	-	-	-	-	-	-	-
Other species		0.31	0.00	0.00	0.00	0.00	0.00	0.00	0.16	0.00	0.00	0.01	0.0110
Total population		1.02	0.92	0.87	0.88	0.58	0.91	1.10	1.03	0.00	0.86	1.03	0.2225

(1) Only the mean weights of L. terrestris were adjusted for covariates. Negative means occur after the adjustment for covariates.

(2) With unequal replication, the S.E.D. of the mean was calculated using the values for the minimum replication.

Sittingbourne Field Site. Samples taken six months post-treatment.

Table 6.18. Analysis of variance for the biomass of earthworms.

Species	Grand Mean	Treatment variance ratio (1)	Covariate variance ratio (1)	Treatment D.F.	Covariate D.F.	Residual D.F.
L.terrestris	1.258	2.459 *	2.897 NS	10	1	21
A.longa	1.342	0.704 NS	0.001 NS	10	1	21
A.caliginosa	-	-	-	-	-	-
Other species	0.776	2.279 NS	0.276 NS	10	1	21
Total population	1.676	1.228 NS	0.026 NS	10	1	21

(1) P<0.001 ***

P<0.01 **

P<0.05 *

Sittingbourne Field Site. Samples taken six months post-treatment.

Table 6.19. Estimated effect of the treatment upon the mean biomass of earthworms.(1)

Species	Treatment	Control	Thiophanate-methyl		Chlordane		Triazophos		Carbaryl		Penta-chlorophenol		Standard error for difference between means (2)
	Application rate kg.ha^{-1} a.i.	-	3	6	5	10	3	6	2.5	25	12.5	75	-
L.terrestris		1.24	1.32	0.84	1.25	1.45	1.29	1.23	1.32	1.32	1.30	1.28	0.1442
A.longa		1.31	1.36	1.36	1.12	1.35	1.35	1.29	1.43	1.42	1.40	1.38	0.1416
A.caliginosa		-	-	-	-	-	-	-	-	-	-	-	-
Other species		0.80	0.82	0.75	0.71	0.75	0.76	0.53	0.74	0.86	0.94	0.90	0.1071
Total population		1.69	1.72	1.56	1.56	1.71	1.69	1.64	1.75	1.67	1.74	1.72	0.0895

(1) Means adjusted for covariates. Negative means occur after this adjustment for covariates.

(2) With unequal replication, the S.E.D. of the mean was calculated using the values for the minimum replication.

6.3.5. Phytotoxicity

Although the plots treated with pentachlorophenol at Rothamsted and Sittingbourne were seeded with grass, the soil remained free of vegetation throughout the period of the trial due to the phytotoxicity of this compound. None of the other test chemicals that were tested showed this effect.

6.4. Discussion

L. terrestris was the only species of earthworm present in large numbers at Rothamsted on both sampling dates. The numbers of A. longa, A. caliginosa and of the other species of earthworm varied between the two sampling dates, and were particularly low in the samples that were taken from this site during May, one month after the treatments were applied. At Sittingbourne, L. terrestris was again the only species to be found in large numbers during the spring, although high numbers of all the species were also found at this site during October, six months after treatment.

These species of earthworm are reported to occur in the upper layers of the soil during May (Gerard, 1967), although other observations (Evans and Guild, 1947) suggest that the peak of activity in the spring was past by the time that the first post-treatment samples had been taken from the trials, and my field trials would probably have benefited from beginning one month earlier.

These results support the conclusion that L. terrestris remains active for most of the year (Evans and Guild, 1947), and that several other species become much less active during the summer. A. chlorotica (which constituted a major component of the samples that were analysed as the 'other' species of earthworm) and A. caliginosa are reported to enter a facultative diapause during adverse summer conditions, whilst A. longa shows an obligatory summer diapause that can begin in May (Evans and

Guild, 1947; Satchell, 1967). Furthermore, the low numbers of certain species of earthworms found in samples collected during April in other field experiments have also been attributed to such resting behaviour (Stringer and Lyons, 1977). Thus, the results of the experiments at Rothamsted and Sittingbourne support those obtained previously from studies of the seasonal activity of earthworms.

The number of earthworms that were collected on a particular sampling date determined the type of statistical analysis that could be used to process the data. The numbers and weights of L. terrestris were large enough to allow analyses of variance, adjusted for covariates, to be used to process the data collected on both sampling dates and from both sites. Whilst the other data obtained on both sampling dates from Rothamsted could also be processed in this way, only those collected from Sittingbourne after six months were suitable for this type of analysis.

Significant differences due to treatment occurred in all the data for L. terrestris, except those for the numbers recorded after six months at Sittingbourne. These significant differences between the treatments appeared to be correlated highly with those that occurred in the samples for the total population of earthworms (i.e. the data for all the species of earthworms considered together) and would appear to be largely responsible for them. (An exception to this occured with the weights of the total population of earthworms collected after six months at Sittingbourne, which showed a significant difference due to treatment although the data for L. terrestris did not). The similarity between the response of A. caliginosa and of the other species of earthworm at Rothamsted may have arisen because A. caliginosa is often associated closely with A. chlorotica and A. rosea and has similar habits to these species (Evans and Guild, 1947; Gerard, 1967), both of which formed a major component of the samples collected for the other species of earthworm. Significant differences due to treatment were also shown by

the weights of the other species of earthworms sampled after one month at Rothamsted, and the numbers and weights of A. longa and of the other species of earthworms sampled after six months. The data from the Sittingbourne trial sampled after six months also showed a significant difference between treatments for the numbers of A. caliginosa. The large numbers of earthworms that were collected at both sites during October, six months after treatment, supported the conclusion that this was the optimum period during which to collect earthworms (Evans and Guild, 1947).

The differences between the treatments in the numbers and in the weights of earthworms appear to be correlated highly. Some of the data from the Rothamsted site clearly does not show this relationship, and this includes the numbers of A. longa and of the other species of earthworm found after one month in the plots treated with thiophanate-methyl and pentachlorophenol. The numbers of these earthworms were similar to, or less than, those in the control plots, whilst the weights for these species were similar to, or in excess of, those found in the controls. This suggests that individual earthworms grew more rapidly in the treated plots than in the control plots, a response known to follow from the fungicidal or herbicidal effects of a chemical that increases the amount of plant litter available (Edwards et al., 1971). These results may also indicate that juvenile earthworms were more susceptible to these chemicals than the adults, an effect which has been seen previously with tests for toxicity to earthworms by immersion (Lebrun et al., 1981) or contact (Section 3.2.4) conducted in the laboratory.

Some differences were seen between the numbers and weights of earthworms after six months. The other species of earthworm were more numerous at Rothamsted in the plots that had been treated with the high rate of thiophanate-methyl, the low rate of pentachlorophenol and both rates of triazophos and carbaryl, although the weights of the earthworms

in these plots did not exceed those in the control plots. These observations possibly suggest that some of the chemicals had a sublethal toxic effect upon the adult earthworms (such as the antifeedant properties of the benzimidazole fungicides [Stringer and Wright, 1973]), which combined with the recruitment of young earthworms into the population. This hypothesis is compatible with the observation that the cocoons of A. caliginosa and A. chlorotica hatch between April-August (Gerard, 1967), therefore many young earthworms will avoid chemicals that decompose rapidly in the soil, particularly when such chemicals are applied early in the year.

Occasionally the number of earthworms apparently increased in the treated plots and these increases may be summarised thus. A. caliginosa was more numerous at Rothamsted after one month in the plots that had been treated with thiophanate-methyl, and the low rate of chlordane and triazophos. The data obtained from both trials indicated that A. longa became more numerous after one month in the plots that were treated with the low rates of chlordane, triazophos and carbaryl. At Rothamsted after six months, the numbers of the other species of earthworm exceeded those in the controls in the plots that had been treated with the high rate of thiophanate-methyl, the low rate of pentachlorophenol and both rates of carbaryl and triazophos. At Sittingbourne after six months only A. longa and the other species of earthworm appeared to be more numerous than in the controls, in the plots that were treated with chlordane or pentachlorophenol. Thus, it is possible that A. longa and A. caliginosa were able to avoid the toxic effects of a short-lived chemical and to exploit subsequently the niches vacated by competing species of earthworm. A similar response was seen with the other species of earthworm, but as this occurred only after six months, it would appear that the opportunist response of these earthworms was slower than that of A. longa and A. caliginosa. Earthworms can arrive in such empty ecological niches by

hatching from unharmed cocoons (Edwards and Brown, 1982) or by immigrating from the surrounding untreated soil (Martin, 1976). The horizontal rates of the distribution of earthworms, determined by Bouche (1974) and Hoogerkamp et al. (1983), suggest that the 4 m square plots used in the present study were too small to prevent a substantial immigration of earthworms from the surrounding areas. This conclusion was supported by the observation that L. terrestris was the only species that did not become more numerous in the plots at either site and, in another study, failed to reinvade 6 m square plots from which it had been eradicated one year previously using benomyl (Edwards and Brown, 1982). The apparent increase in the number of earthworms in the treated plots may possibly be an artifact of the sampling technique. A pesticide that potentiates the irritant effect of formalin may result in a more complete expulsion of the earthworms from the soil, and thus bias the estimate of the size of the population.

Most hypotheses that explain an increase in the number of earthworms in a treated plot in terms of the immigration of earthworms or the survival and subsequent hatching of young earthworms from cocoons, require that the chemicals become non-toxic after a short period of time. The residence time of a chemical in the soil is determined by several factors which include the rate at which it is leached, volatilised or degraded in the soil by physical, chemical or microbial processes. In this study, it was not possible to analyse the soil for residues of the test chemicals, and therefore an accurate estimate of the length of time that the chemicals remained in the plots could not be made. Some inferences concerning such residence times may be drawn from the biological effects of the chemicals. Pentachlorophenol remained phytotoxic to the grass at both sites for at least six months, although other studies have shown that this chemical was degraded in the soil within 15-100 days (Kaufman, 1978). Therefore, the phytotoxic and vermitoxic effects of this chemical are likely to have

been caused by persistent metabolites of pentachlorophenol. Chlordane had a toxic effect upon all the populations of earthworms, particularly at the higher rate of application, which persisted for six months in the soil that contained a small amount of organic matter. Similarly, in this soil the numbers of A. caliginosa and of the other species of earthworm were decreased moderately after six months by thiophanate-methyl, and also decreased after six months in the soil that contained more organic matter by triazophos at the higher rate. This would suggest either that these chemicals or their toxic metabolites persisted in the soil for six months, or that the initial toxicity of these chemicals to the earthworms was so severe that the populations could not recover fully within the period of the trial. The latter explanation is less likely because carbaryl eliminated some of the species of earthworm within a month, but these populations had recovered to the pre-treatment levels after six months.

The populations of the earthworms were decreased severely by chlordane after six months in the soil at Rothamsted which contained a small amount of organic matter that was less likely to adsorb this chemical (Edwards, 1974). The other chemicals may also have been adsorbed onto the particles of soil, but did not show a toxicity that was dependent upon the soil type. The effects upon the earthworms of chemicals that were adsorbed onto organic matter can be potentiated by the behaviour of the earthworm. Earthworms may selectively move in and ingest contaminated organic matter, which would increase the exposure of the earthworm to the chemical. This may account for the results obtained in other studies, which showed that the benzimidazole fungicides were more toxic to earthworms in a humus soil rather than in a clay soil (Lofs-Holmin, 1981), despite the antifeedant properties of these chemicals (Stringer and Wright, 1973).

Thiophanate-methyl appeared to have little effect upon the numbers of

L. terrestris in the trials at Rothamsted and Sittingbourne, although the numbers of A. longa were reduced considerably by this treatment after one month at both sites. The size of the population of A. longa in the plots treated with thiophanate-methyl and in the control plots were similar after six months. A. caliginosa was much more numerous after one month in the plots that had been treated with thiophanate-methyl at Rothamsted, but after six months the numbers of this species of earthworm were similar in the treated plots and the control plots at both sites. The other species of earthworm were somewhat less numerous in the plots treated with the low rate of thiophanate-methyl after six months at Rothamsted, but were otherwise unaffected.

A. longa was the most susceptible species of earthworm to thiophanate-methyl, and this species was followed in a decreasing order of susceptibility by L. terrestris, the other species of earthworms and A. caliginosa. The reader should remember that A. caliginosa and the other species of earthworms were almost absent from the samples taken after one month at Sittingbourne, due to the seasonal factors that were described earlier. Thus, the ranking of these species as being relatively tolerant of a chemical, may be related to the small number of earthworms that were collected and not reflect the true susceptibility of these earthworms to the chemicals. Previous investigations of the toxicity of the benzimidazole fungicides to earthworms have often been done in orchards. In these studies, L. terrestris was found to be more susceptible to the fallout of fungicides applied to the trees as a spray than A. caliginosa, whilst A. longa was more susceptible than L. terrestris (Stringer and Lyons, 1974; 1977). This disparity between my results and those of the orchard studies may arise from the behaviour of these earthworms. L. terrestris fed upon the plant litter on the floor of the orchard and was exposed to a higher concentration of the fungicides which ran off the trees than A. longa, and thus appeared to be more susceptible to this chemical. Other workers

have shown that the litter feeding habits of L. rubellus rendered this species of earthworm more susceptible to methiocarb baits than A. caliginosa, (Barker, 1982) which feeds upon fungal mycelia and well decomposed, buried litter (Edwards and Lofty, 1977). However, the study sites at Rothamsted and Sittingbourne did not have an obvious layer of litter on top of the soil, and so the variation in feeding strategies of these species is unlikely to explain the differences in susceptibility to thiophanate-methyl that were seen between them. This difference in the susceptibility between A. longa and L. terrestris may be explained by the avoidance behaviour of these species. The earthworms of both these species are deep dwelling, but L. terrestris may be more sensitive to the presence of contamination in the soil, and thus able to retreat from polluted layers more quickly, than A. longa. This would give the appearance of L. terrestris being less susceptibility to thiophanate-methyl than A. longa.

The results of my studies indicate that thiophanate-methyl has a moderate and non-persistent toxicity to earthworms in the field, which supports the results of previous field studies (King and Dale, 1977; Stringer and Lyons, 1974; Stringer and Wright, 1973).

The populations of most species of earthworm in the plots treated with the high rate of chlordane decreased after one month at both sites. The low rate of chlordane only reduced the numbers of A. longa at Rothamsted after one month, but after six months had elapsed this treatment had a wider effect and caused a slight reduction in the numbers of A. caliginosa and of the other species of earthworm. The high rate of chlordane virtually eliminated the populations of earthworms at Rothamsted, although L. terrestris was the least affected species. Thus, A. longa was the species that was most susceptible to the effects of chlordane after one month, but at the later sampling date A. caliginosa and the other species of earthworm seemed to be the most affected. The effects of chlordane

appeared quickly and persisted for six months, supporting the results of other investigations (Doane, 1962; Legg, 1968; Lidgate, 1966), and the toxicity to earthworms of this chemical was less severe in the soil that contained the most organic matter.

The effects of triazophos upon earthworms in the field have not been reported previously, although this chemical appeared to be moderately toxic to E. fetida in the artificial soil test (Section 4.2.18; Haque and Ebing, 1983a). Triazophos had little effect upon the numbers of L. terrestris after one month at Rothamsted, although at the high rate it reduced severely the size of the populations of A. caliginosa and, to a lesser extent, those of A. longa and of the other species of earthworm.

After six months at Rothamsted the numbers of L. terrestris only were reduced slightly by the high rate of triazophos. The numbers of A. longa were reduced slightly at Sittingbourne by the low rate of triazophos after one month, whilst those of A. caliginosa and the other species of earthworm were reduced by the high rate after six months. A. caliginosa appeared to be more susceptible to triazophos than any of the other species of earthworm at this sampling date, whilst L. terrestris was apparently almost unaffected. Thus triazophos had very little effect upon the populations of earthworms after six months at either site and at these rates of application.

Carbaryl decreased the numbers of all the species of earthworm at both sites severely when applied at the high rate. This effect occurred only after one month, and after six months the populations had returned to a size similar to that in the control plots. The low rate of carbaryl was very toxic to A. caliginosa at the Rothamsted site and, in a decreasing order of susceptibility affected A. longa, the other species of earthworm and L. terrestris. These results confirm other reports in which A. caliginosa was found to be particularly susceptible to the effects of carbaryl in tests done in the laboratory (Stenersen, 1979a). My results

agree with those of previous studies which have shown that carbaryl was very toxic to earthworms (Edwards and Thompson, 1969; Legg, 1968; Thompson, 1971) and had a rapid but non-persistent effect (Legg, 1968; Stuurman and Kamp, 1971).

The toxicity of pentachlorophenol to earthworms had not been investigated previously, despite the widespread industrial and agricultural use of this chemical and its sodium salt (Cirelli, 1978). The results from both the Rothamsted and Sittingbourne trials show that pentachlorophenol had very little effect upon populations of earthworms. The size of some of these populations of earthworms appeared to be increased slightly by this treatment, although such effects may be due to the increased amount of plant litter that was available, following the herbicidal action of this chemical. Similar effects were reported previously from experiments with the herbicide cyanazine (Edwards, 1970; Edwards, Lofty and Stafford, 1971, 1972). The introduction of more stringent EEC and OECD ecoprotection legislation has prompted other field studies with pentachlorophenol, which have been done using similar methods and rates of application to those of my work. The preliminary results of these studies support my findings and indicate that pentachlorophenol is not very toxic to earthworms (Rombke, 1984).

In conclusion, these field experiments have shown that chlordane and carbaryl are very toxic to earthworms in the field, and that the effects of chlordane were persistent whilst those for carbaryl were apparently non-persistent. Thiophanate-methyl and triazophos were moderately toxic to earthworms, but had an effect which was non-persistent. Pentachlorophenol did not appear to be toxic to earthworms. The effects of these chemicals upon earthworms were sometimes more severe in the soil that contained less organic matter. A. caliginosa was the species of earthworm apparently most susceptible to the effects of these chemicals. A. longa and the other species of earthworm were less affected, whilst L. terrestris was the species that seemed to be most tolerant of the chemicals.

CHAPTER 7

A COMPARISON OF ASSESSMENTS OF THE TOXICITY OF CHEMICALS TO EARTHWORMS MADE IN THE FIELD AND IN THE LABORATORY

7.1. Introduction

From the results obtained using tests for the toxicity of chemicals to earthworms in the laboratory, it is often difficult to predict the toxicity of chemicals to earthworms in the field (Edwards and Lofty, 1973). Few studies have attempted to draw a comparison between these two types of assessment, and the majority of such comparisons have been done using aquatic organisms.

The relationship between the toxicity data obtained using earthworms in the laboratory and in the field, and which appears in the literature (Table A1.1) is unclear. This uncertainty arises from the variety of species of earthworm, periods of exposure and routes of exposure that have been used previously to test the toxicity of chemicals to earthworms, and because few chemicals have been studied for toxicity to earthworms using both types of test.

A recent review attempted to draw a comparison between the assessments of the toxicity of benomyl, carbaryl, carbofuran and some organophosphorus chemicals to earthworms made in the laboratory and field from reports in the literature (Dean-Ross, 1983). This concluded that immersion tests (in which earthworms were submerged in a solution of the test chemical for a relatively short period of time, e.g. 24 hours) underestimated the toxicity that benomyl and the carbamate insecticides showed to earthworms in the field. Other workers have supported the conclusion that immersion tests indicate poorly the toxicity of chemicals to earthworms in the field (Edwards and Lofty, 1973). Dean-Ross (1983) also

reported that tests using soil in the laboratory underestimated the toxicity of the organophosphorus insecticides to earthworms in the field, although such soil tests predicted accurately the toxicity of the carbamate compounds including benomyl.

The present study compared the toxicity of several chemicals assessed using tests for toxicity in the laboratory, with the effects of these chemicals upon earthworms in two field experiments that were conducted upon different soil types. Five chemicals of differing toxicity to earthworms were used in both these types of test.

7.2. Materials and Methods

The chemicals used in these experiments were chlordane, carbaryl, thiophanate-methyl, triazophos and pentachlorophenol. Chloroacetamide was used additionally in several of the laboratory tests, because this chemical has been recommended for use as a reference compound (Edwards, 1982; Inglesfield, 1984). These chemicals were of technical grade, except for pentachlorophenol and chloroacetamide, which were of analytical grade. The earthworms used in the laboratory were adult E. fetida andrei weighing 0.4-0.6 g with an empty gut.

The experimental data for this comparison of the results obtained using tests for the toxicity of chemicals to earthworms in the laboratory and the field were drawn from investigations that are described fully in previous sections (Chapters 2,3,4 and 6), and to which the reader is referred for a detailed consideration of the experimental procedures. A synopsis of the methods is given below.

Methods for testing toxicity to earthworms in the laboratory

Immersion test: The earthworms were immersed in an aqueous solution or suspension of the test chemical for 24 hours (Section 2.2.3).

Filter paper contact test: The earthworms were exposed to the test chemical on a damp cellulose filter paper that lined internally the side wall of a small glass vial. The period of exposure was 48 hours (Section 3.2.3).

Silica paste-glass ball test: The test chemicals were incorporated uniformly into a paste of amorphous silica and water, supported on a matrix of glass balls. The earthworms were exposed to this mixture for 14 days (Section 2.2.9).

Sand test: The test chemicals were distributed evenly in a fine quartz sand that was moistened with water. The earthworms were exposed to this medium for 48 hours (Section 2.2.7).

Artificial soil test: An artificial soil consisting of sand, kaolinitic clay, sphagnum peat and calcium carbonate was moistened and treated uniformly with the test chemicals. The mortality of the earthworms was assessed after 14 days (Section 4.2.3).

Natural soil test: The earthworms were exposed to the test chemicals in a sieved sandy clay-loam soil obtained from the field. The period of exposure was 14 days (Section 2.2.10).

Forced feeding test: The earthworms were force-fed with the test chemicals, which were suspended in an agar-agar gel held in a motor-driven syringe that was fitted with a rounded-end glass needle. Gel was impelled into the oesophagus of an anaesthetised earthworm. The effects of the chemicals upon the earthworms were recorded after six days (Section 2.2.11).

Methods for testing toxicity to earthworms in the field

Two field experiments were done during 1981 and 1982 at Rothamsted Experimental Station and at the Shell (UK) Research Centre at Sittingbourne. The trials were on sites having a clay-loam soil containing 5.1% organic matter, and a flinty clay-loam soil containing 6.9% organic matter respectively. Each trial was a randomised block, the chemicals were applied at two rates in early spring and the populations of all the earthworms present were sampled using the formalin extraction method in late spring (one month post-treatment) and autumn (six months post-treatment). The data were processed using analyses of variance and adjusted for covariates (Section 6.2).

7.3. Results

7.3.1 Laboratory tests

The assessments of the toxicity of chemicals to earthworms done in the laboratory are presented (Table 7.1) as the antilog of the mean LC_{50}'s calculated from the individual LC_{50}'s (expressed as a logarithmic scale) from repeated experiments. (These data are summarised from Tables A7.1, A7.5, A7.7, A7.8, A7.9, A7.20 and A7.30). The toxicity to earthworms of the test chemicals was ranked according to these mean LC_{50}'s. This ranking varied considerably between the different methods, as did the ratio of each particular LC_{50} to the LC_{50} of chlordane. Chlordane was chosen as the reference chemical because it showed a well defined and severe toxicity to earthworms in tests done in the laboratory and in the field.

Table 7.1. The ranking of the toxicity of chemicals to earthworms using tests in the laboratory.

Testing method and the units in which the LC50 is expressed	Duration days	The ranked toxicity to earthworms of the test chemicals shown in a decreasing order of toxicity, together with the mean LC50 and the ratio of this mean LC50 to that of chlordane (shown in brackets).
Immersion $mg.kg^{-1}$ (Test chemical:water)	1	pentachlorophenol > chlordane > carbaryl > chloroacetamide > triazophos > thiophanate-methyl 0.69(0.1) 8.63(-) 25.19(2.9) 81.78(9.5) 109.02(12.6) 754.67(87.5)
Filter paper contact in vials. $\mu g.cm^{-2}$ (Test chemical: filter paper surface)	2	chlordane > chloroacetamide > pentachlorophenol > triazophos > carbaryl > thiophanate-methyl 0.56(-) 2.85(5.1) 5.28(9.4) 16.91(30.2) 24.17(43.2) 539.50(963.4)
Silica paste:glass ball $mg.kg^{-1}$ (Test chemical: dry weight of silica paste)	14	chlordane > thiophanate-methyl > carbaryl > chloroacetamide > pentachlorophenol 23.89(-) 44.57(1.9) 112.33(4.7) 183.46(7.7) 276.98(11.6)
Sand $mg.kg^{-1}$ (Test chemical: dry weight of sand)	2	chlordane > thiophanate-methyl > pentachlorophenol 0.005(-) 0.006(1.2) 0.044(9.1)
Artificial soil $mg.kg^{-1}$ (Test chemical: dry weight of soil)	14	triazophos > chloroacetamide > thiophanate-methyl > chlordane > pentachlorophenol > carbaryl 10.53(0.4) 18.41(0.7) 20.06(0.7) 26.96(-) 45.53(1.7) 85.74(3.2)
Natural soil $mg.kg^{-1}$ (Test chemical: dry weight soil)	14	chlordane > pentachlorophenol > thiophanate-methyl > carbaryl > chloroacetamide 0.005(-) 0.011(2.2) 0.954(190.8) 43.114(8622.8) 43.299(8659.8)
Forced feeding $mg.kg^{-1}$ (Test chemical: wet weight of agar-agar gel)	6	chlordane > thiophanate-methyl 4.93(-) 92.97(18.9)

Laboratory toxicity test assessments

Test chemical LC50 and ratio of test chemical LC50 : chlordane LC50

(Bar = LC_{50}; figure above bar = ratio of test chemical LC_{50} : chlordane LC_{50})

Figure 7.1

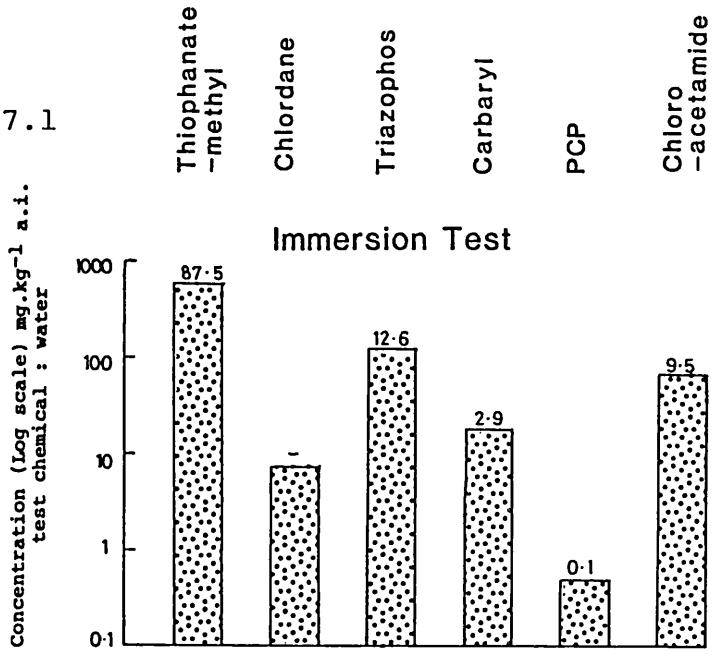


Figure 7.2

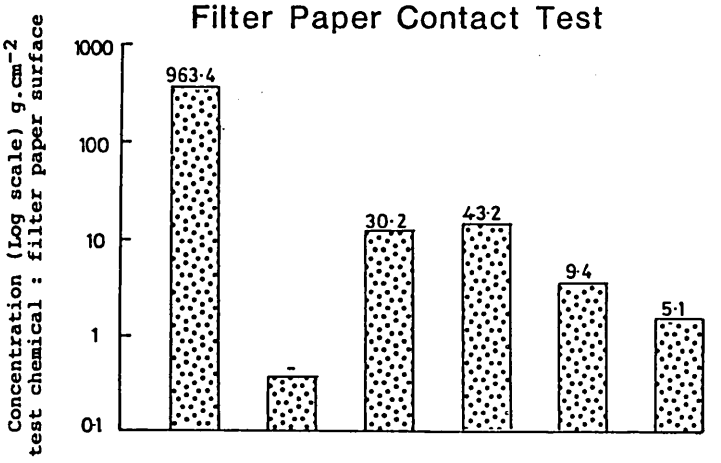
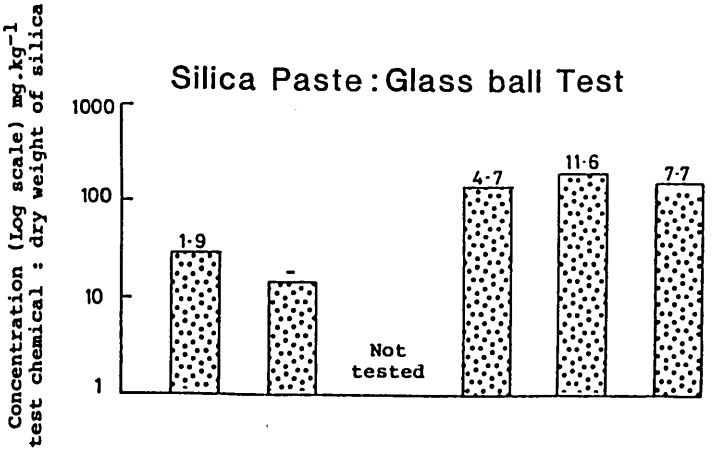


Figure 7.3



Laboratory toxicity test assessments

Test chemical LC50 and ratio of test chemical LC50 : chlordane LC50

(Bar = LC_{50}; figure above bar = ratio of test chemical LC_{50} : chlordane LC_{50})

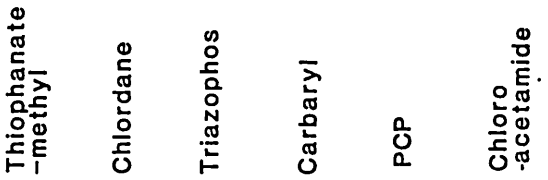


Figure 7.4

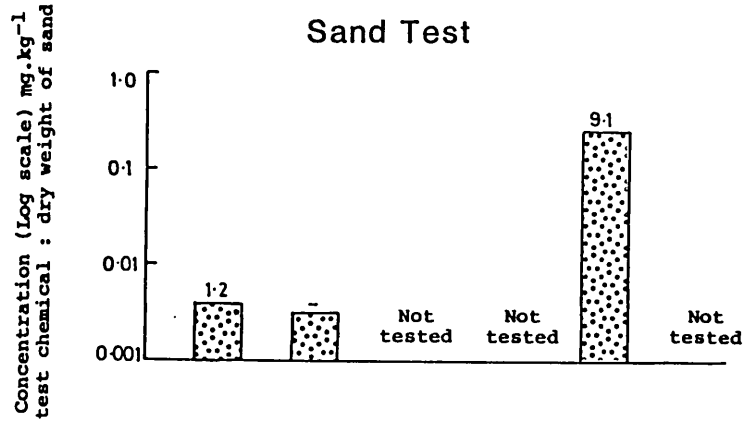


Figure 7.5

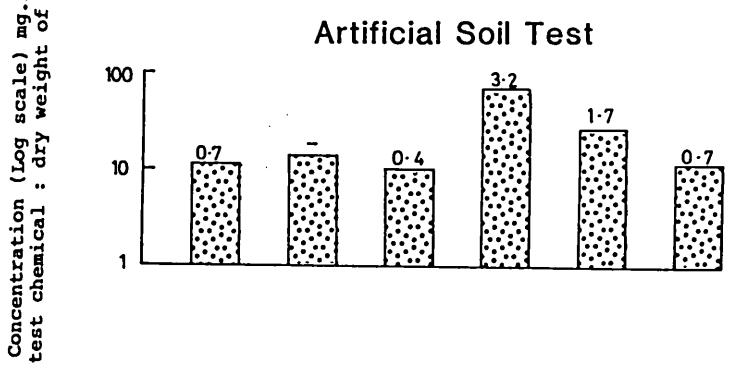


Figure 7.6

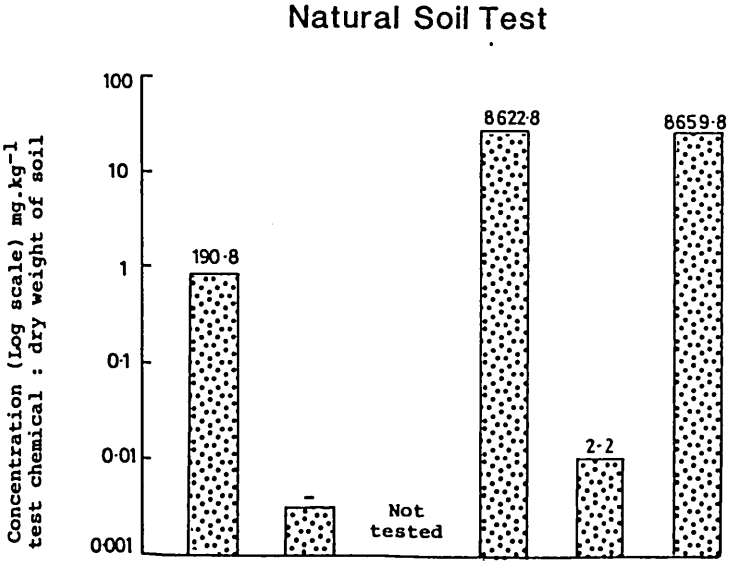
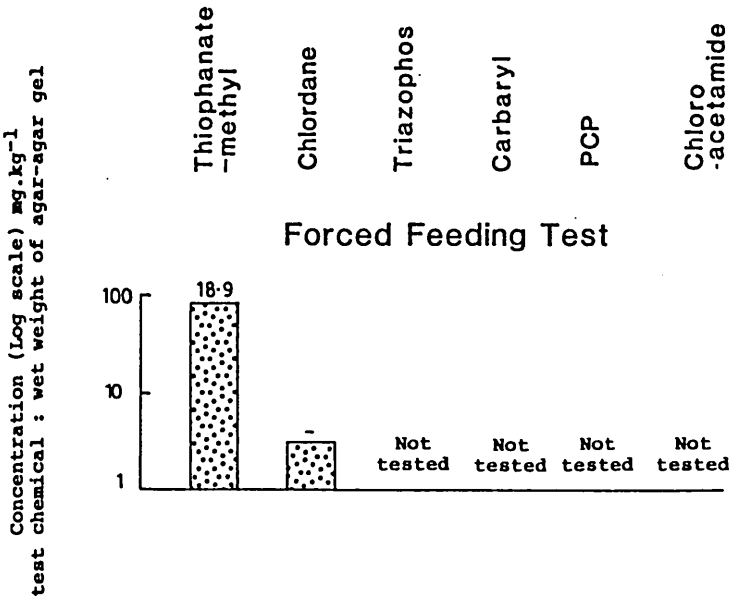


Figure 7.7

Laboratory toxicity test assessments

Test chemical LC50 and ratio of test chemical LC50 : chlordane LC50

(Bar = LC_{50}; figure above bar = ratio of test chemical LC_{50} : chlordane LC_{50})



The differences between the assessments of the toxicity of chemicals to earthworms obtained using tests in the laboratory probably arose from the different conditions of exposure presented by the methods. The factors which influenced these assessments of toxicity included the amount and duration of contact between the earthworm and the test chemical, the uptake route of the test chemical (over the cuticle or gut of the earthworm), the influence of the environment (e.g. the adsorptive capacity of the media) within the test upon the availability of the chemical and the ability of the earthworm to survive the conditions within the test.

Certain similarities between the results that were obtained using the various test methods can be identified clearly when these data are presented diagrammatically (Figures 7.1-7.7). The assessments of the toxicity of the chemicals to earthworms made using the immersion, filter paper contact, natural soil and forced feeding tests indicated that the LC_{50}'s for thiophanate-methyl and carbaryl were much greater than the LC_{50} for chlordane. Furthermore, the LC_{50} for pentachlorophenol was considerably less than that seen for carbaryl. Other similarities occurred between the results obtained using the silica paste-glass ball, sand and artificial soil tests. These tests indicated that the LC_{50}'s for thiophanate-methyl and chlordane were approximately equal to or slightly less than that of pentachlorophenol, and that pentachlorophenol had a toxicity to earthworms that was approximately equal to that of carbaryl.

7.3.2 Field tests

The effects of the test chemicals upon the populations of all the earthworms in the field are shown as the mean numbers and weights (allowing for covariates) of earthworms in the plots at two dates of sampling (Table 7.2 and Figure 7.8). These data are summarised from Tables 6.5, 6.7, 6.9, 6.11, 6.13, 6.15, 6.17 and 6.19.

The variation in the numbers and the weights of earthworms due to the effect of the treatments appeared to be correlated highly at both field sites. The samples from the plots treated with thiophanate-methyl showed only a slight reduction in the numbers of earthworms after one month at Sittingbourne. Earthworms were less numerous in the plots that had been treated with the high rate of chlordane after one month at Rothamsted and at Sittingbourne. Six months after the chemicals had been applied, both rates of chlordane depressed the numbers of earthworms at Rothamsted, whilst those at Sittingbourne had almost returned to the level of the controls. Triazophos applied at the low rate caused a slight increase in the numbers of earthworms, and at the high rate caused a slight decrease at Rothamsted after one month, whilst at Sittingbourne the opposite effect was seen. The numbers of earthworms in the plots treated with triazophos had returned to levels similar to those in the controls after six months at both sites. Carbaryl had no effect on the numbers of earthworms at either site after six months, although the earthworms were less numerous after one month in the plots treated with both rates of carbaryl at Rothamsted, and in the plots treated with the high rate of carbaryl at Sittingbourne. Pentachlorophenol appeared to have little effect upon the numbers of earthworms at either site.

Some of the differences between the assessments of the toxicity of chemicals to earthworms made at the two field sites are probably related to the organic matter content of the soils. The soil at the Sittingbourne site contained more organic matter than that at Rothamsted.

Table 7.2. Estimated mean numbers and weights of the total population of earthworms, sampled on two dates in the field plots of the experiments at Rothamsted and Sittingbourne.

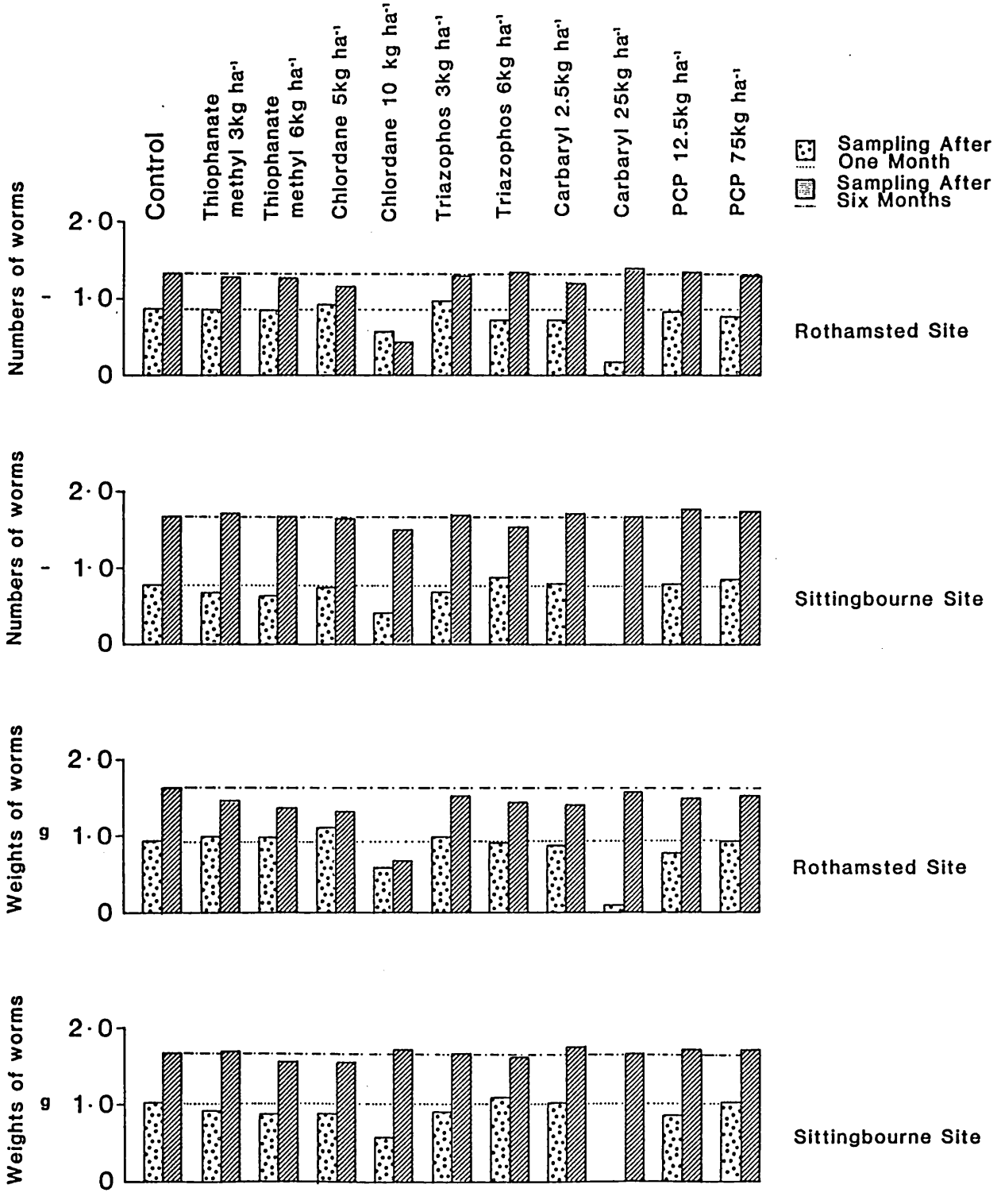
Site	Date of sampling and data recorded for all species of earthworm	Treatment and rate of application. kg.ha^{-1}a.i.											
		Control	Thiophanate-methyl		Chlordane		Triazophos		Carbaryl		Penta-chloro-phenol		Standard error for the difference between means (1)
		-	3	6	5	10	3	6	2.5	25	12.5	75	
Rothamsted	Numbers:One month	0.86	0.85	0.86	0.93	0.58	0.98	0.72	0.71	0.18	0.81	0.76	0.1776
	Numbers:Six months	1.32	1.28	1.26	1.16	0.44	1.30	1.35	1.20	1.40	1.34	1.29	0.1076
	Weights:One month	0.94	1.00	0.99	1.11	0.59	1.00	0.91	0.88	0.11	0.77	0.91	0.2124
	Weights:Six months	1.64	1.48	1.38	1.33	0.68	1.52	1.45	1.41	1.57	1.50	1.53	0.1339
Sittingbourne	Numbers:One month	0.76	0.68	0.65	0.74	0.43	0.67	0.87	0.78	0.00	0.77	0.85	0.1889
	Numbers:Six months	1.69	1.72	1.68	1.65	1.74	1.69	1.54	1.71	1.69	1.77	1.73	0.0779
	Weights:One month	1.02	0.92	0.87	0.88	0.58	0.91	1.10	1.03	0.00	0.86	1.03	0.2225
	Weights:Six months	1.69	1.72	1.56	1.56	1.71	1.69	1.64	1.75	1.67	1.74	1.72	0.0895

1) With unequal replication, the S.E.D. of the mean was calculated using the values for the minimum replication.

NB. All the samples showed significant differences due to treatment ($P<0.01$), with the exception of those taken after six months at Sittingbourne. The full details of these analyses are presented together with a complete description of the field experiments in Chapter 6.

Figure 7.8

Rothamsted and Sittingbourne Field Sites: Total Numbers and Weights of all Earthworms in Treated and Control Plots at Two Sampling Dates



7.4. Discussion

The methods for testing the toxicity of chemicals to earthworms in the laboratory that were developed during this study needed to be able to predict the toxicity of chemicals to earthworms in the field. This type of extrapolation is difficult (Haque and Ebing, 1983a) and is necessarily based upon several approximations (Dean-Ross, 1983).

A quantitative relationship between the results from tests done in the laboratory and the field has been defined using data collected with several leaf-feeding insect pests (Sun, 1966). This technique could not be applied to the results obtained with earthworms because over 200 chemicals had to be tested by Sun to clarify this relationship. Sun found that the toxicity indices (the ratio of the toxicity of a test chemical, calculated as an LD_{50} or as the application rate recommended for the control of the insect in the field, to the toxicity of a standard chemical measured using a similar method [Sun, 1950]) of chemicals determined using tests done in the laboratory and in the field with these insects were related linearly.

The total numbers and weights of the earthworms in the plots of the field experiments provided a synoptic assessment of the effects of the test chemicals upon earthworms. These effects can be summarised thus. Chlordane and carbaryl were very toxic to earthworms in the field and had effects that were persistent and non-persistent respectively. Thiophanate-methyl and triazophos were moderately toxic, but the effect of these chemicals appeared to be non-persistent. Pentachlorophenol did not seem to be toxic to earthworms at the rates of application used in this study.

A laboratory test that is capable of predicting the toxicity of chemicals to earthworms in the field should indicate that chlordane and carbaryl are very toxic to earthworms, and that in comparison, thiophanate-methyl and triazophos are considerably less toxic.

Pentachlorophenol should be assessed as slightly toxic to earthworms.

The order in which the toxicity of the test chemicals to earthworms was ranked by the laboratory and field tests gave some insight into the relationship between these two types of assessment.

The immersion, silica paste-glass ball and artificial soil tests indicated that chlordane and carbaryl had a similar and severe toxicity to earthworms. The filter paper contact, silica paste-glass ball, sand and artificial soil tests assessed pentachlorophenol as considerably less toxic to earthworms than chlordane, although pentachlorophenol was shown to be less toxic than thiophanate-methyl only in the silica paste-glass ball, sand and artificial soil tests. Only the artificial soil method indicated that pentachlorophenol was less toxic to earthworms than triazophos, and that thiophanate-methyl and triazophos had a similar toxicity to earthworms. Both thiophanate-methyl and triazophos were shown to be considerably less toxic than chlordane in the immersion and filter paper contact tests, whilst the natural soil and forced feeding tests indicated that of these two chemicals, only thiophanate-methyl was less toxic to earthworms than chlordane.

Thus, the silica paste-glass ball, artificial soil and sand tests gave a similar and good indication of the toxicity of chemicals to earthworms in the field, but tended to minimise the differences that were seen between the toxicity of these chemicals to natural populations of earthworms. The artificial soil test gave the most accurate prediction of the toxicity of chemicals to earthworms in the field. The predictions of toxicity to earthworms in the field given by these laboratory methods are most accurate for those chemicals that have a very high or very low toxicity to earthworms, and are less reliable with chemicals of moderate toxicity.

The assessments of toxicity given by the silica paste-glass ball and artificial soil tests have been shown to correlate highly ($r = 0.91$

[Heimbach, 1984, 1985b]), and were expressed in units of concentration that could be compared directly with the concentration of the chemicals that was achieved in the field soil. The artificial soil and silica paste-glass ball tests gave LC_{50}'s that were considerably higher than the concentration of the chemicals that was achieved in the soil by the applications made in the field. This difference may arise from the period of exposure used in the laboratory tests, which was much shorter than that used in the field.

Few studies have attempted to compare the toxicity of the same chemicals to earthworms in the laboratory and the field. Edwards (1985) assessed the toxicity of several compounds to earthworms in the artificial soil and in a natural soil in the laboratory, and compared these results with those obtained using a standard method for testing toxicity to earthworms in the field (Edwards and Brown, 1982). The differences that existed between the concentration of benomyl and paraquat that were necessary to kill earthworms in the laboratory and in the field were similar to the difference between these assessments of toxicty seen in my study, for which the LC_{50} determined in the laboratory was approximately 5-10% of that necessary to kill earthworms in the field.

The filter paper contact, silica paste-glass ball and artificial soil tests each indicated that chloroacetamide should be of moderate toxicity to earthworms in the field. Furthermore, my results have shown that the LC_{50} of chloroacetamide using E. fetida in the artificial soil was 18.4 $mg.kg^{-1}$, which agrees closely with the work of Edwards (1985) who reported an LC_{50} for chloroacetamide estimated in the artificial soil of 26 $mg.kg^{-1}$. At this concentration of chloroacetamide in the field, Edwards showed that the populations of L. terrestris and A. caliginosa were unaffected. The low toxicity of chloroacetamide to earthworms in the field is probably due to the adsorption of this chemical onto soil particles, followed by rapid decomposition through the action of micro-organisms (Heimbach, 1982).

Whilst the artificial soil test and the other methods that used substitutes for soil in the laboratory tended to underestimate the toxicity of chemicals to earthworms in the field, this study and others (Dean-Ross, 1983) show that the natural soil test overestimated the toxicity to earthworms observed in the field. This effect was reported previously with tests using natural soil to assess the toxicity of chemicals to L. terrestris (Edwards and Lofty, 1973), although no explanation for this observation was offered. In my studies, this overestimation of the toxicity of chemicals to earthworms in the field by a natural soil test can be attributed to the inability of E. fetida to tolerate the mineral soil that was used in the laboratory, although other studies have used this species of earthworm in a natural mineral soil successfully (Edwards, 1985).

The immersion and filter paper contact tests identified some aspects of the toxicity of the test chemicals to natural populations of earthworms, although immersion tests have been criticised for underestimating such effects in the field (Dean-Ross, 1983). The assessments of the toxicity of chemicals to earthworms made with the filter paper contact test were reported to correlate poorly with the results from the artificial soil test ($r = 0.55$) and the silica paste-glass ball test ($r = 0.48$ [Heimbach, 1984, 1985b]). These conclusions agree with my work, and as the latter tests provided the best indication of the toxicity of chemicals to earthworms in the field it was unlikely that the filter paper contact test would also predict accurately the effect that a chemical would have upon a natural population of earthworms.

The choice of E. fetida as the test species of earthworms has been criticised by some workers (Lofs-Holmin, 1982b; Stenersen, 1979a) on the grounds that this species is unrepresentative of the those living in arable soils, and that it is relatively insensitive to the effects of toxic chemicals. These objections have been answered by recent studies which have indicated that the responses of E. fetida and L. terrestris to chemicals in

the laboratory are correlated highly (r = 0.81 [Heimbach, 1985a, 1985b]), and therefore the choice of E. fetida as the test species is unlikely to impair the ability of tests for the toxicity of chemicals to earthworms done in the laboratory to predict the effects of chemicals upon earthworms in the field.

The accuracy with which laboratory tests for the toxicity of chemicals to earthworms can predict the effects of chemicals upon earthworms in the field might be improved if these methods were extended to measure some aspect of sublethal toxicity as proposed by Goats and Edwards (1985), Lofs-Holmin (1980) and Reinecke and Venter (1985).

CHAPTER 8

INTER- AND INTRA-SPECIFIC VARIATION IN THE RESPONSE OF EARTHWORMS TO TOXIC CHEMICALS

8.1. Introduction

Many workers have shown that species of earthworm differ in their response to toxic chemicals. A. caliginosa was more susceptible to the effects of herbicides in solution than either Pheritima californica (Gates) or an Alma species (Ghabbour and Imam, 1967), and other experiments have shown L. terrestris to be more tolerant of herbicides incorporated into a soil in the laboratory than Eudrilus eugeniae (Caseley and Eno, 1966). The susceptibility of L. rubellus to a wide range of chemicals deposited on damp filter paper was greater than that of E. fetida (Roberts and Dorough, 1984). Investigations such as these have led some workers to suggest that L. terrestris (Dean-Ross, 1983), L. rubellus (Ma, 1984) and A. caliginosa (Edwards, 1982; Lofs-Holmin, 1982b) would be suitable for use in routine tests for toxicity.

The experiments described in this chapter were done with the species L. terrestris, A longa, A. caliginosa and E. fetida, which are also known to differ in their susceptibility to toxic chemicals. L. terrestris was more susceptible to the fungicide captan, mixed with the soil, than was A. caliginosa (Leger and Millette, 1977). The sizes of populations of L. terrestris and A. chlorotica were reduced more severely than those of L. rubellus, L. festivus, A. caliginosa, A. longa, A. rosea, and O. cyaneum (Savigny) by sprays of benomyl and thiophanate-methyl in an orchard (Stringer and Lyons, 1974). Further studies with benomyl alone showed that the size of the populations of L. terrestris were decreased more than those of L. rubellus, L. castaneus, A. rosea, and A. chlorotica, whilst

the numbers of A. longa were virtually unaffected (Stringer and Lyons, 1977). The apparent ability of A. longa to tolerate toxic chemicals in this study was probably due to a summer diapause.

A recent review of the effects of chemicals upon earthworms (Dean-Ross, 1983) concluded that L. terrestris was more susceptible than E. fetida to benomyl and to a variety of insecticides. This supported the results of a study using some cholinesterase inhibiting pesticides (aldicarb, oxamyl, carbaryl, carbofuran, parathion, trichloronate and paraoxon) and the organochlorine chemical HCH. These chemicals appeared to be much less toxic to E. fetida than to either A. chlorotica or L. rubellus in an immersion or a soil test conducted in the laboratory. Futhermore, A. chlorotica and L. rubellus were less susceptible to these chemicals than A. caliginosa (Stenersen, 1979a). Similar results were obtained using chloroacetamide and paraquat mixed with a loamy sand in the laboratory. These chemicals were more toxic to L. terrestris and, in particular, to A. caliginosa than to E. fetida, although these species showed an equal susceptibilty to benomyl (Edwards, 1985). In other studies (Gilman and Vardanis, 1974), carbofuran was found to be less toxic to E. fetida than to L. terrestris when injected directly into the body of the earthworm.

Some workers consider that differences in the susceptibility to chemicals between species of earthworm are specific to each chemical (Haque and Ebing, 1983a), and were this so it would be impossible to extrapolate from the assessments of the toxicity of chemicals made using E. fetida, to the effects that the same chemicals would have upon other species of earthworm. Fortunately the estimates of the LC_{50}'s of chemicals made using L. terrestris in a natural soil test correlated highly with those obtained using E. fetida in an artificial soil test ($r = 0.81$ [Heimbach, 1985a, 1985b]). Thus, the poor relationship found between the estimates of toxicity made using these species of earthworm in previous studies (Haque and Ebing, 1983a) may reflect the inadequacy of some aspect of the

experimental procedures. Furthermore, work has shown that the toxicity of carbofuran in aqueous solution to L. terrestris (Lebrun et al., 1981) and to E. fetida (Stenersen, 1979a) was similar, although these measurements were made using slightly different periods of exposure. In general, A. caliginosa appears to be more susceptible to the toxic effects of chemicals than L. terrestris, and both these species show a greater susceptibility than E. fetida.

Long-lived chemicals and their residues may accumulate in biological systems from a relatively low concentration in the environment and cause toxicity (Carson, 1963). Earthworms can accumulate organochlorine insecticides, persistent compounds such as dioxin (Martinucci et al., 1983) and a variety of other environmental contaminants (Ebing, et al., 1984; Rhett et al., 1985). Some materials, such as sodium and chloride ions, move over the body wall of L. terrestris in response to an electrical gradient (Prusch and Otter, 1977), whilst dissolved organic chemicals such as amino acids appear to accumulate by diffusion as well as by other processes that require energy (Richards and Arme, 1982). Many organic compounds that are electrically neutral accumulate in earthworms from aqueous solution by a passive and reversible process, to achieve a concentration in the tissues similar to that in the external medium (Briggs and Lord, 1983). This passive distribution is determined largely by the partitioning of the chemical between the air, water, soil and tissue of the earthworms, and is the subject of several detailed reviews (Briggs, 1981; Kenaga, 1975a; Moriarty, 1977).

The equilibrium concentration of a non-polar pesticide within an earthworm is determined by the earthworm tissue:water and soil:water partition coefficients of that chemical, which are related to the octan-1-ol:water partition coefficient. (A partition coefficient may be determined by shaking an aqueous solution of the test chemical with an equal volume of an immiscible organic solvent, allowing the phases to

separate, and then dividing the resultant concentration of the chemical that is present in the organic solvent by that in the water). The octan-1-ol:water partition coefficients have been determined for a wide range of chemicals and correlate negatively the aqueous solubility (Chiou, Freed, Schmedding and Kohnert, 1977). Whole earthworms accumulate a concentration of test chemical that is similar to that found in macerated tissue, but as chemicals are taken up much more slowly into whole earthworms, only those chemicals that have a long half-life in soil are likely to achieve the equilibrium concentration within the tissues (Lord et al., 1980). Polar chemicals do not obey this relationship and are accumulated poorly by earthworms.

The effect of organic matter in the soil (the soil fraction that has the greatest influence upon the equilibrium concentration within the tissues of the earthworm) must be considered when attempting to predict the rate of accumulation and subsequent effect of a chemical upon earthworms. These equilibrium concentrations in the tissue of the earthworms, organic matter in the soil and soil water may be calculated using the following formulae:

$$\text{Log } k_{ws} = 0.476 \ (\text{Log } k_{ow}) + 1.04 \qquad (r = 0.88)$$

$$\text{Log } k_{om} = 0.53 \ (\text{Log } k_{ow}) + 0.69 \qquad (r = 0.95)$$

where K_{ws}, K_{ow} and k_{om} are the earthworm:water, octan-1-ol:water and soil organic matter:water partition coefficients respectively (Lord et al., 1980).

Chemicals such as DDT are apparently accumulated by the tissues of L. terrestris at a rate that shows a double maxima at 1.5 and 4.0 months, after which an equilibrium is achieved (Edwards and Jeffs, 1974). Similar results have been obtained with this species of earthworms using tetrachlorobenzene (Lord et al., 1980). The uptake of DDT by Pheretima posthuma also showed a double maxima, but the concentrations that were achieved in the tissues of this species of earthworm were greater than those in L. terrestris (Yadav, Pillai and Agarwal, 1976). Such

observations may indicate that two processes are involved during the accumulation of these pesticides, an initial uptake phase over the gut or body wall, followed by a period of redistribution of the chemical within the tissues of the earthworm (Edwards and Jeffs, 1974).

L. terrestris and A. longa accumulated diazinon and dieldrin from aqueous solution at a rate similar to that shown by the slugs Deroceras reticulatum (Möller) and Arion hortensis (Ferussac). This suggests that one of these groups of invertebrates tested for toxicity in the laboratory may be able to predict toxicity to the other (Lord et al., 1980). Earthworms and slugs also appear to respond in a similar way to pesticides in the field, for instance diazinon and phorate show a minimal and severe toxicity respectively to slugs and to earthworms (Edwards, 1976).

Earthworms limit the toxic effects of chemicals by excreting unchanged material rapidly, or by detoxifying the chemical through metabolic processes. Three classes of enzymes are important in the primary metabolism of chemicals including hydrolases (e.g. esterases, carboxylesterases and phosphatases), glutathione S-transferases and microsomal oxidases. Hydrolases, particularly cholinesterases, have been identified in E. fetida (Stenersen, 1979c, 1980a, 1980b), glutathione S-transferases have been found in L. terrestris, L. rubellus, A. longa, A. caliginosa, A. chlorotica and E. fetida (Stenersen, Guthenberg and Mannervik, 1979) as well as in A. rosea, A. icterica and O. cyaneum (Stenersen and Øien, 1981). Microsomal oxidases occur in L. terrestris (Nakatsugawa and Nelson, 1972; Nelson, Stewart, Morelli and Nakatsugawa, 1976). The detoxification of xenobiotics in earthworms has been discussed fully in a recent review (Stenersen, 1984).

Earthworms can detoxify organochlorine chemicals, and excreted rapidly DDT that had been accumulated previously into an untreated soil. Earthworms were also capable of converting DDT to DDE, and this metabolite was found to be more persistent in the tissues of the earthworm

than DDT (Edwards and Jeffs, 1974). DDT appeared to be metabolised faster in earthworms of the species P. posthuma which had been pretreated with DDT, lindane or dieldrin (Agarwal, Yadav and Pillai, 1978), suggesting that the enzymes capable of detoxifying these chemicals had been induced. Chlordane injected into the coelom of L. terrestris was excreted largely unchanged, although some chlordane was metabolised to oxychlordane and chlordane chlorohydrin (Chio and Sanborn, 1976). These workers could not confirm that this process was due to the action of a microsomal oxidase, although a high concentration of such enzymes (in the form of aldrin epoxidase, which can convert aldrin to dieldrin) was located subsequently in the intestine of L. terrestris (Nelsen et al., 1976). Evidence for the presence of a microsomal oxidase in L. terrestris was strengthened by the recent identification of cytochrome P-450 in the tissues of this earthworm. Cytochrome P-450 is a constituent of the microsomal oxidase system (Liimatainen and Hanninen, 1982).

The organophosphorus insecticide parathion was eliminated at slow but similar rates from L. rubellus and E. fetida, although E. fetida was also able to convert parathion to paraoxon. The paraoxon produced by E. fetida was then oxidised rapidly by additional detoxifying enzymes (Stenersen, 1979b). Other experiments have shown that the carbamate insecticides carbofuran and oxamyl were eliminated rapidly from several species of earthworm, when earthworms that had been pre-treated with these chemicals were placed in an uncontaminated soil (Stenersen and Øien, 1980). E. fetida excreted 95% of the carbofuran accumulated, of which half was unchanged, compared to L. terrestris which only excreted 10% (Gilman and Vardanis, 1974). L. terrestris excreted carbofuran mainly as the unchanged chemical, together with 3-hydroxycarbofuran and small amounts of two unidentified metabolites (Stenersen et al, 1973).

This chapter describes several experiments that were done to study the inter- and intra-specific variation in the response of earthworms to

chemicals. Initially, the differences between the susceptibility of L. terrestris, A. longa, A. caliginosa, E. fetida andrei and E. fetida fetida to a limited range of test chemicals were measured using the filter paper contact and artificial soil tests. The differences that were seen between the susceptibility of E. fetida andrei and E. fetida fetida were then investigated further in a study of the uptake, release and metabolism of chlordane and iodoacetamide. The analytical determinations during the preliminary uptake and metabolism experiments were made using gas-liquid chromatography, however the resolution of this system was inadequate and the subsequent studies were done with radiolabelled chemicals. The concentration of the labelled chemicals was determined using liquid scintillation counting, and the metabolites of these chemicals were separated using thin-layer chromatography.

8.2. Materials, methods and results

8.2.1. Materials and general methods

Chemicals

The non-radiolabelled chemicals used in the experiments were analytical grade alpha-chlordane (Velsicol Chemical Corporation, Chicago, USA), pentachlorophenol, chloroacetamide and iodoacetamide (Sigma Chemical Company). ^{14}C-alpha-chlordane (specific activity 28.1 mCi.mmol^{-1}) was donated by the Midwest Research Institute, Kansas City, U.S.A., and ^{14}C-iodoacetamide (specific activity 54 mCi.mmol^{-1}) was obtained from Amersham International plc. All the other solvents and reagents were of analytical or, where appropriate, scintillation grade.

Earthworms

Earthworms of the species L. terrestris, A. longa and A. caliginosa were collected from the edge of Park Grass Field, Rothamsted Experimental

Station, using the formalin extraction method (Raw, 1959). Earthworms expelled from the soil by this technique were immersed immediately in a large volume of cold water to reduce the toxic effects of the formalin. After 15 minutes the earthworms were transferred to 25 litre plastic boxes containing 15 kg of moist, pesticide-free loam soil collected from Geescroft Field, Rothamsted Experimental Station. Each box was covered with a wooden lid and contained either 30 L. terrestris, 30 A. longa or 50 A. caliginosa. The boxes were stored at 10°C in darkness. These methods of culture were adapted from standard techniques (Tomlin, 1977). Dead earthworms were removed during a weekly inspection of the cultures and at the same time, 100 g of weathered elm leaves were incorporated into the top 5 cm of soil in each box (provided that the foodstuff from the previous week had been consumed). The earthworms were extracted from the soil by hand and before use in the experiments, were placed on a damp filter paper overnight to expel their gut contents.

E. fetida andrei and E. fetida fetida were grown from cocoons in a mixed culture. The cocoons were scattered onto pressed cow manure that had matured for 3 weeks beforehand, and were then stored in boxes at 20°C in the dark for up to nine weeks. After this period the earthworms were extracted and prepared for the experiments in a similar manner to that described above for the other species.

Only sexually mature earthworms within a well defined range of body weights (with an empty gut) were used in the experiments. These were for L. terrestris 1.5 ± 0.5 g, A. longa 1.25 ± 0.5 g, A. caliginosa 0.75 ± 0.25 g and E. fetida 0.5 ± 0.1 g.

Media and apparatus for the toxicity tests

The components of the artificial soil, and the other apparatus that was used for the artificial soil and filter paper contact tests have been described previously (Tables A5.1 - A5.3).

Gas-liquid chromatography

The gas-liquid chromatograph used was a Pye (Series 104) machine coupled to an electron capture detector. The column contained 5% SE30 Chromosorb W, the carrier gas was nitrogen flowing at a rate of 4.5 litres.min^{-1} and the column and detector oven temperatures were 190° and 300°C respectively.

Liquid scintillation counter and scintillant

A Hewlett Packard liquid scintillation counter was employed together with a standard scintillant mixture consisting of 8 g 2-phenyl-5-(4-biphenylyl)-1,3,4-oxadiazole, 0.5 g 2-(4-biphenylyl)-6-phenyl benzoxazole, 500 ml Triton X-100 and one litre toluene.

Filter paper contact and artificial soil tests

The methods for the filter paper contact and artificial soil tests were the final versions that have been described in detail previously (Sections 3.2.3 and 4.2.3 respectively). Any deviations from these methods are described in the appropriate section, otherwise the tests may be assumed to have been used in an unmodified form.

8.2.2. The body weight, dimensions and volume of earthworms

Method

The individual body weight of the four species of earthworm used in these experiments was determined after the earthworms had been extracted from the field or from culture in animal manure, and allowed to empty their guts. Callipers were used to measure the resting diameter and length of the body of individual earthworms that fell within the range of body weights that were used for each species in the experiments. These measurements allowed the body volume to be calculated, by assuming that the shape of the body of the earthworm approximated a regular cylinder. All the measurements were made using ten individuals of each species.

Results

The earthworms collected at random from the field or from culture in animal manure in the laboratory gave mean body weights (Table 8.1) which indicated that L. terrestris was the heaviest earthworm, followed in a decreasing order of mean body weight by A. longa, A. caliginosa and E. fetida. These species had body weights in a ratio to that of L. terrestris of 1 : 1.13, 1 : 1.64 and 1 : 2.64 respectively. The standard deviation of the mean of the body weights was greatest for L. terrestris, indicating that in a natural population, this species of earthworm showed a more variable individual body weight than that of the other species that were studied.

The body diameter, length and calculated body volume were similar for L. terrestris and A. longa, and for A. caliginosa and E. fetida (Table 8.2 and Plate 8.1). E. fetida andrei and E. fetida fetida (Plate 8.2) appeared to have almost identical physical dimensions. L. terrestris was the largest earthworm and had a calculated body volume that was in the ratio of 1 : 1.47, 1 : 8.24 and 1 : 9.89 to that of A. longa, A. caliginosa and E. fetida respectively.

8.2.3. Inter-specific variation in the susceptibility of earthworms to chemicals, estimated using the artificial soil test

Method

The LC_{50}'s of chlordane, pentachlorophenol and chloroacetamide were estimated using the artificial soil test for L. terrestris, A. longa, A. caliginosa and E. fetida andrei. The replicates at each concentration of the test chemical consisted of 750 g of artificial soil in each test vessel, containing three individuals of any one of the species collected from an arable soil, or five E. fetida andrei. The data were processed by probit analysis.

Table 8.1. The body weight of earthworms collected from the field (a) and from culture in the laboratory (b).

Species	Mean body weight (g)	Standard deviation of mean body weight (g)
L. terrestris	1.578	1.434
A. longa	1.399	0.424
A. caliginosa	0.965	0.091
E. fetida andrei	0.598	0.057

Table 8.2. The dimensions of the body (c) and the calculated volume (d) of earthworms collected from the field and from cultures in the laboratory.

Species	Mean diameter (cm)	Mean length (cm)	Mean volume (cm^3)
L. terrestris	0.7	9.0	3.46
A. longa	0.5	12.0	2.36
A. caliginosa	0.3	6.0	0.42
E. fetida andrei	0.3	5.0	0.35

(a) Earthworms were collected from the field in the spring, as the juveniles of the previous year approached maturity. The mean body weight is therefore higher than that seen in a previous study (Section 4.2.4).

(b) E. fetida andrei was cultured in animal manure under laboratory conditions.

(c) The dimensions of the earthworms were measured with the body at rest.

(d) The volume of the body was calculated on the assumption that it was a regular cylinder

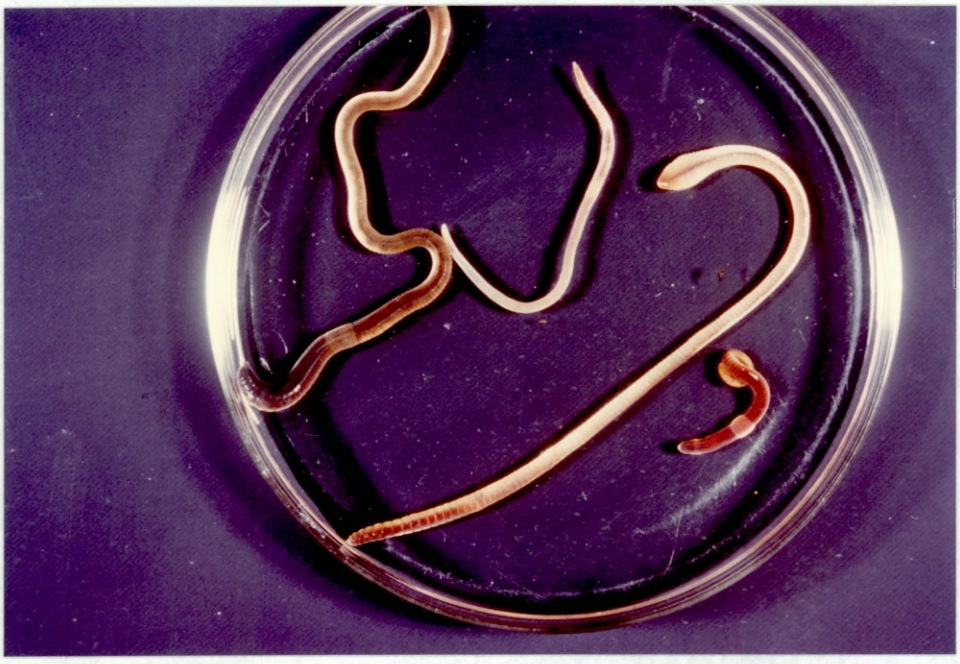


Plate 8.1. Relative sizes of lumbricid earthworms. Left to Right. A. longa, A. caliginosa, L. terrestris and E. fetida andrei (15 cm diameter dish).

Plate 8.2. Eisenia fetida. Left to right: Adult E. fetida andrei, juvenile E. fetida andrei, juvenile E. fetida fetida (15 cm diameter dish).

Results

The antilogged mean of the LC_{50}'s from repeated experiments (calculated on a log scale) for L. terrestris, A. longa, A. caliginosa and E. fetida andrei (Table A7.31) were, for chlordane 3.00, 3.40, 1.29, 11.11 $mg.kg^{-1}$ (Figure 8.1), pentachlorophenol 101.82, 35.39, 3.92, 13.80 $mg.kg^{-1}$ (Figure 8.2) and chloroacetamide 38.00, 38.05, 30.12, 8.88 $mg.kg^{-1}$ respectively (Figure 8.3).

The LC_{50}'s and gradients of the probit lines for chlordane were similar with L. terrestris and A. longa. Both these species gave probit lines with chlordane for which the gradients were significantly different ($p>0.05$) when tested by chi-squared. A. caliginosa was more susceptible than these larger earthworms to chlordane and also gave gradients of the probit lines that were generally steeper than those seen for the other species, although the individual replicates with A. caliginosa differed significantly. E. fetida andrei was less susceptible than the other earthworms to this chemical and gave a probit line with a shallow gradient, for which the replicates were not significantly different.

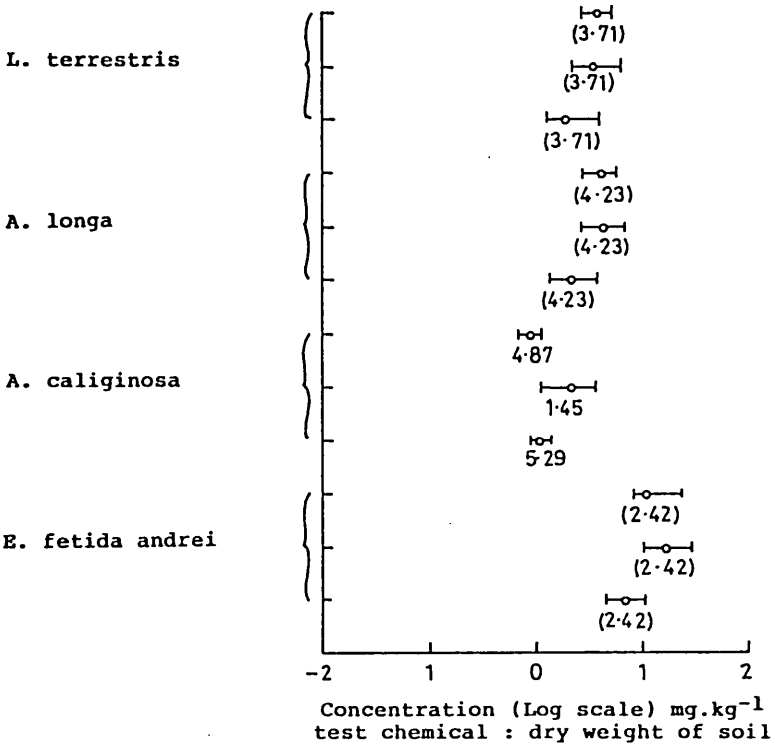
Pentachlorophenol was most toxic to A. caliginosa, and the other species in a decreasing order of susceptibility, were E. fetida andrei, A. longa and L. terrestris. None of the mean LC_{50}'s or gradients of the probit lines showed any particular similarities between species, and only the gradients of the probit lines for replicates with A. longa did not differ significantly when tested using chi-squared.

L. terrestris, A. longa and A. caliginosa had a similar and lower susceptibility to chloroacetamide than E. fetida andrei. The replicates for each of these species did not differ significantly for either the gradient of the probit lines or for the position of the Y-intercepts. These data were therefore combined into a single probit line for each species.

The gradients of the probit lines for E. fetida andrei with chlordane and chloroacetamide were less steep than those for the other species,

Figure 8.1

INTER-SPECIFIC VARIATION IN THE SUSCEPTIBILITY OF EARTHWORMS TO CHLORDANE IN THE ARTIFICIAL SOIL TEST

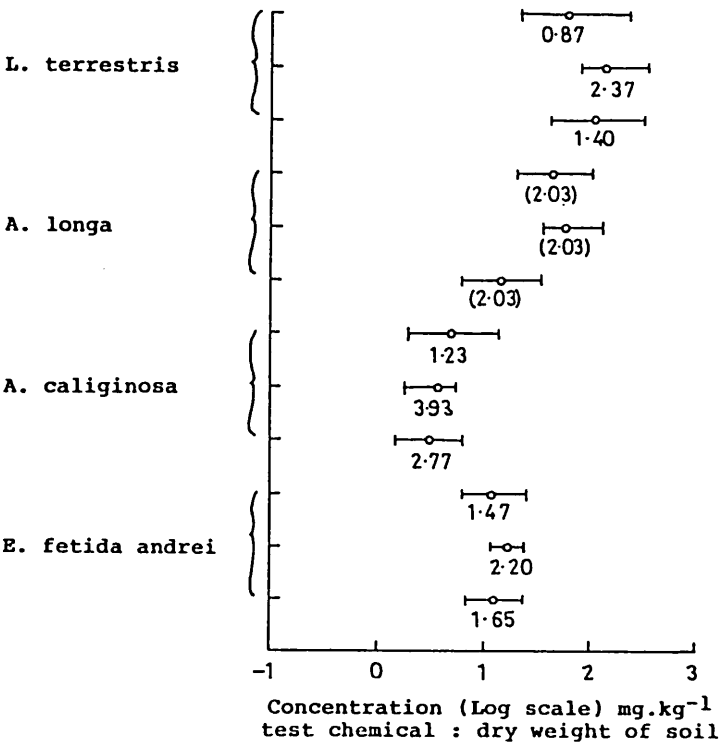


KEY

⊢o⊣ x	Individual LC_{50} estimate [o], gradient [x] and fiducial limits
⊢o⊣ (x)	Individual LC_{50} estimate [o], common gradient for more than one replicate [(x)] and fiducial limits
⊢●⊣ (x)	Common LC_{50} estimate [●], gradient [(x)] and fiducial limits for more than one replicate
⊢----⊣	Range within which a poorly defined LC_{50} falls
No LC_{50} →	LC_{50} at an undefined concentration that was greater than those studied
← No LC_{50}	LC_{50} at an undefined concentration that was less than those studied
(No LC_{50})	More than one LC_{50} at an undefined concentration

Figure 8.2

INTER-SPECIFIC VARIATION IN THE SUSCEPTIBILITY OF EARTHWORMS TO PENTACHLOROPHENOL IN THE ARTIFICIAL SOIL TEST

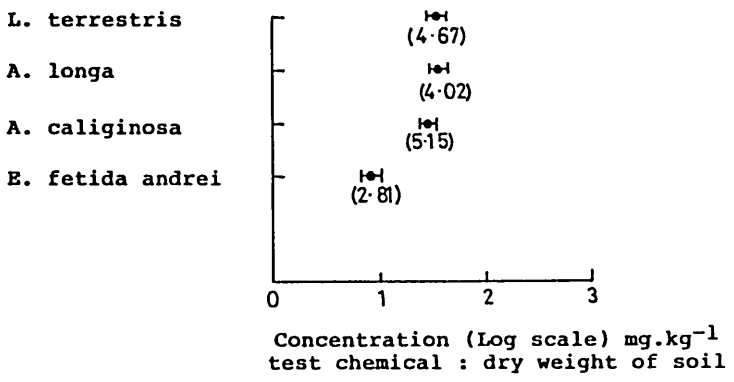


KEY

⊢o⊣ x	Individual LC_{50} estimate [o], gradient [x] and fiducial limits
⊢o⊣ (x)	Individual LC_{50} estimate [o], common gradient for more than one replicate [(x)] and fiducial limits
⊢●⊣ (x)	Common LC_{50} estimate [●], gradient [(x)] and fiducial limits for more than one replicate
⊢----⊣	Range within which a poorly defined LC_{50} falls
No LC_{50} →	LC_{50} at an undefined concentration that was greater than those studied
← No LC_{50}	LC_{50} at an undefined concentration that was less than those studied
(No LC_{50})	More than one LC_{50} at an undefined concentration

Figure 8.3

INTER-SPECIFIC VARIATION IN THE SUSCEPTIBILITY OF EARTHWORMS TO CHLOROACETAMIDE IN THE ARTIFICIAL SOIL TEST



KEY

⊢o⊣ x	Individual LC_{50} estimate [o], gradient [x] and fiducial limits
⊢o⊣ (x)	Individual LC_{50} estimate [o], common gradient for more than one replicate [(x)] and fiducial limits
⊢●⊣ (x)	Common LC_{50} estimate [●], gradient [(x)] and fiducial limits for more than one replicate
⊢----⊣	Range within which a poorly defined LC_{50} falls
No LC_{50} →	LC_{50} at an undefined concentration that was greater than those studied
← No LC_{50}	LC_{50} at an undefined concentration that was less than those studied
(No LC_{50})	More than one LC_{50} at an undefined concentration

which indicated that in this experiment, E. fetida andrei was susceptible to these chemicals over the widest range of concentrations.

8.2.4. Inter- and intra-specific variation in the susceptibility of earthworms to chemicals, estimated using the filter paper contact test

Method

The LT_{50}'s (i.e. the period of exposure required to kill 50% of the population of earthworms that were tested at a single concentration of a chemical) for chlordane at a concentration of 1.0 $\mu g.cm^{-2}$, pentachlorophenol at 150 $\mu g.cm^{-2}$ and both chloroacetamide and iodoacetamide at 10 $\mu g.cm^{-2}$, were estimated for A. caliginosa, E. fetida andrei and E. fetida fetida by using the filter paper contact test. The results were processed by probit analysis.

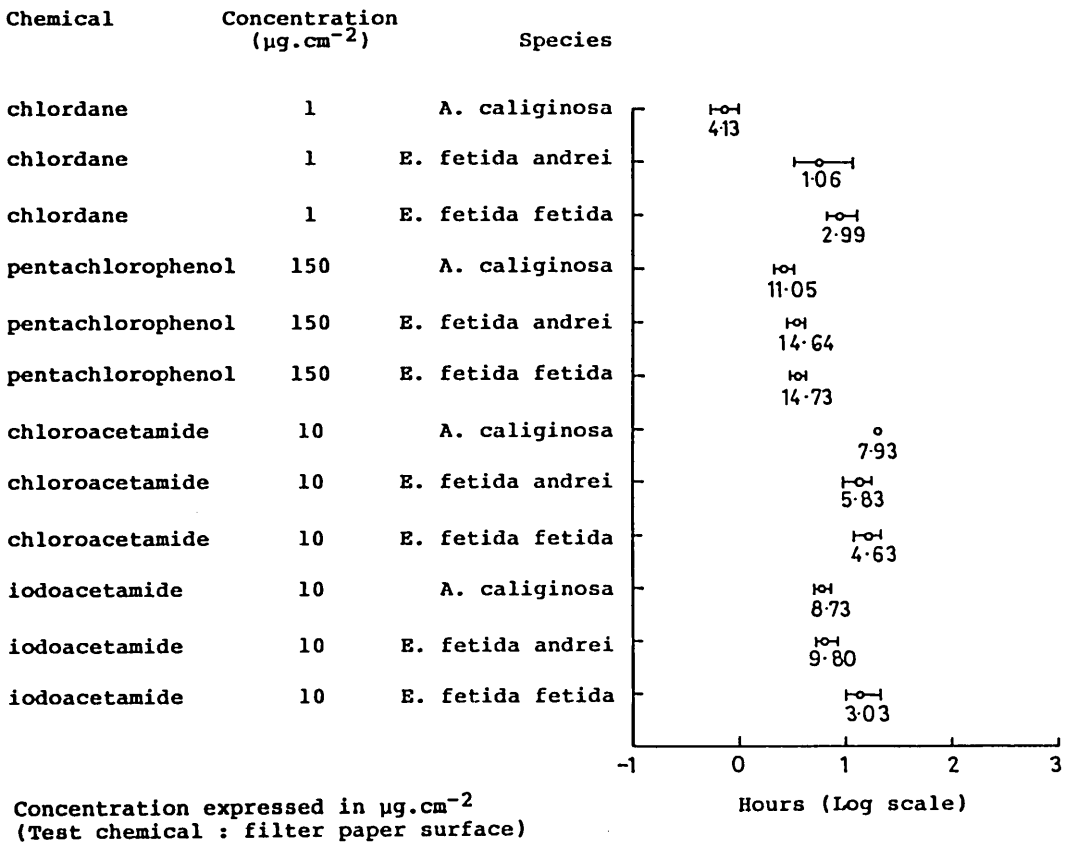
Results

The antilogged LC_{50}'s, calculated on a log scale (Tables A7.32.1 - A7.32.4 and Figure 8.4), were for chlordane 0.78, 5.73, 8.99 hours; pentachlorophenol 2.72, 3.45, 3.82 hours; chloroacetamide 20.64, 13.25, 16.72 hours and iodoacetamide 6.20, 6.58, 13.94 hours with A. caliginosa, E. fetida andrei and E. fetida fetida respectively.

The susceptibility of these species and sub-species of earthworm to pentachlorophenol and chloroacetamide was similar, although A. caliginosa was marginally more susceptible to pentachlorophenol, and marginally less susceptible to chloroacetamide than E. fetida. A. caliginosa was however, considerably more susceptible to chlordane than E. fetida andrei, which was slightly more susceptible than E. fetida fetida. A. caliginosa and E. fetida andrei gave similar LT_{50}'s for iodoacetamide and were somewhat more susceptible to this chemical than E. fetida fetida. The gradients of the probit lines obtained with A. caliginosa were generally similar to, or steeper than those for E. fetida, whilst those for E. fetida andrei were

Figure 8.4

INTER- AND INTRA-SPECIFIC VARIATION IN THE SUSCEPTIBILITY OF EARTHWORMS TO TEST CHEMICALS ASSESSED USING THE FILTER PAPER CONTACT TEST



Concentration expressed in $\mu g.cm^{-2}$
(Test chemical : filter paper surface)

KEY

⊢o⊣ x	Individual LT_{50} estimate [o], gradient [x] and fiducial limits
⊢o⊣ (x)	Individual LT_{50} estimate [o], common gradient for more than one replicate [(x)] and fiducial limits
⊢●⊣ (x)	Common LT_{50} estimate [●], gradient [(x)] and fiducial limits for more than one replicate
⊢····⊣	Range within which a poorly defined LT_{50} falls
No LT_{50} →	LT_{50} at an undefined period of exposure that was greater than those studied
← No LT_{50}	LT_{50} at an undefined period of exposure that was less than those studied
(No LT_{50})	More than one LT_{50} at an undefined period of exposure

similar to, or steeper than those for E. fetida fetida. E. fetida fetida would seem therefore, to succumb to the test chemicals within a less well defined period of exposure than that seen for the other species and subspecies.

8.2.5. Intra-specific variation in the susceptibility of earthworms to chemicals, estimated using the filter paper contact test

Method

The LT_{50}'s for chlordane and iodoacetamide were estimated for E. fetida andrei and E. fetida fetida at concentrations of 5 $\mu g.cm^{-2}$ and 10 $\mu g.cm^{-2}$ respectively, together with the LC_{50}'s for these chemicals, using the filter paper contact test. The data was analysed by probit analysis.

Results

The antilogged LT_{50}'s calculated on a log scale (Tables A7.33.1 and A7.33.2 and Figure 8.5) for E. fetida andrei and E. fetida fetida were for chlordane 25.27, 26.23 hours and for iodoacetamide 8.87, 4.59 hours respectively.

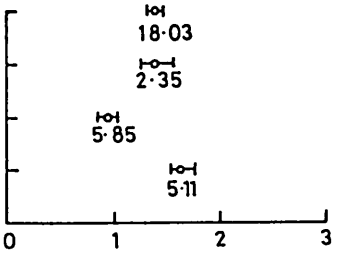
The antilogged mean of the LC_{50}'s for repeated experiments calculated on a log scale (Table A7.34 and Figure 8.6) for chlordane and iodoacetamide with E. fetida andrei were 1.17, 2.52 $\mu g.cm^{-2}$ and with E. fetida fetida 0.95, 2.61 $\mu g.cm^{-2}$ respectively.

Both types of test indicated that E. fetida andrei was considerably more susceptible to iodoacetamide than E. fetida fetida, but that the differences in susceptibility seen between the subspecies for chlordane (whilst showing a similar trend in susceptibility to that seen for iodoacetamide), were less marked particularly with the assessment using an LT_{50}.

Figure 8.5

INTRA-SPECIFIC VARIATION IN THE SUSCEPTIBILITY OF EARTHWORMS TO TEST CHEMICALS IN THE FILTER PAPER CONTACT TEST

Chemical	Concentration ($\mu g.cm^{-2}$)	Species
chlordane	5	E. fetida andrei
chlordane	5	E. fetida fetida
iodoacetamide	10	E. fetida andrei
iodoacetamide	10	E. fetida fetida



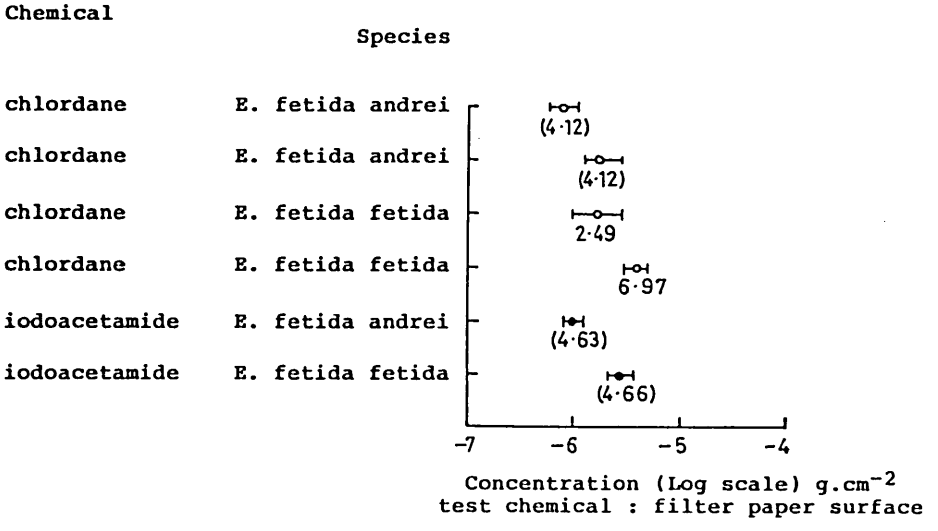
Concentration expressed in $\mu g.cm^{-2}$
(Test chemical : filter paper surface)

KEY

⊢o⊣ x	Individual LT_{50} estimate [o], gradient [x] and fiducial limits
⊢o⊣ (x)	Individual LT_{50} estimate [o], common gradient for more than one replicate [(x)] and fiducial limits
⊢●⊣ (x)	Common LT_{50} estimate [●], gradient [(x)] and fiducial limits for more than one replicate
⊢····⊣	Range within which a poorly defined LT_{50} falls
No LT_{50} →	LT_{50} at an undefined period of exposure that was greater than those studied
← No LT_{50}	LT_{50} at an undefined period of exposure that was less than those studied
(No LT_{50})	More than one LT_{50} at an undefined period of exposure

Figure 8.6

INTRA-SPECIFIC VARIATION IN THE SUSCEPTIBILITY OF EARTHWORMS TO TEST CHEMICALS IN THE FILTER PAPER CONTACT TEST



KEY

⊢o⊣ x	Individual LC_{50} estimate [o], gradient [x] and fiducial limits
⊢o⊣ (x)	Individual LC_{50} estimate [o], common gradient for more than one replicate [(x)] and fiducial limits
⊢●⊣ (x)	Common LC_{50} estimate [●], gradient [(x)] and fiducial limits for more than one replicate
⊢----⊣	Range within which a poorly defined LC_{50} falls
No LC_{50} →	LC_{50} at an undefined concentration that was greater than those studied
← No LC_{50}	LC_{50} at an undefined concentration that was less than those studied
(No LC_{50})	More than one LC_{50} at an undefined concentration

8.2.6. Intra-specific variation in the susceptibility of earthworms to chemicals estimated using the artificial soil test

Method

The LT_{50}'s for chlordane and iodoacetamide were estimated at concentrations of 50 $mg.kg^{-1}$ and 20 $mg.kg^{-1}$ respectively for E. fetida andrei and E. fetida fetida using the artificial soil test. The data were processed using probit analysis.

Results

The antilogged mean of the LT_{50}'s for repeated experiments calculated on a log scale for chlordane and iodoacetamide (Tables A7.35.1 and A7.35.2, and Figure 8.7) with E. fetida andrei were 4.85 and 3.43 hours, and with E. fetida fetida 3.23 and 5.67 hours respectively. E. fetida andrei appeared to be slightly less susceptible to chlordane than E. fetida fetida, although more susceptible to iodoacetamide.

8.2.7 The uptake and release of alpha-chlordane from aqueous solution by E. fetida andrei and E. fetida fetida, measured by gas-liquid chromatography

Methods

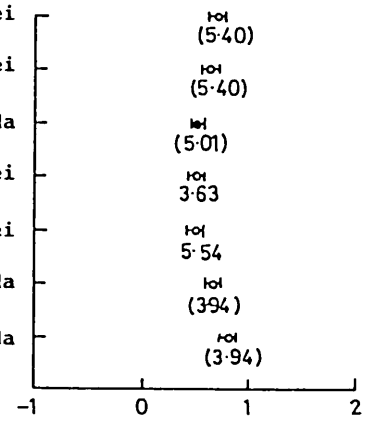
All the glassware, except that used for the extraction procedures that involved organic solvents, was pre-soaked in a 25 $ng.ml^{-1}$ aqueous solution of alpha-chlordane for 24 hours prior to use. This operation was designed to reduce the amount of alpha-chlordane adsorbed onto the glass surfaces of the apparatus.

The adsorptive capacity of the glass had been estimated previously, by measuring the amount of alpha-chlordane remaining in solution after various periods of exposure to a glass surface. This experiment consisted of placing 200 ml of an aqueous solution of 25 $ng.ml^{-1}$ alpha-chlordane into a 250 ml Pyrex conical flask fitted with an airtight stopper. The

Figure 8.7

INTRA-SPECIFIC VARIATION IN THE SUSCEPTIBILITY OF EARTHWORMS TO TEST CHEMICALS IN THE ARTIFICIAL SOIL TEST

Chemical	Concentration ($mg.kg^{-1}$)	Species
chlordane	50	E. fetida andrei
chlordane	50	E. fetida andrei
chlordane	50	E. fetida fetida
iodoacetamide	20	E. fetida andrei
iodoacetamide	20	E. fetida andrei
iodoacetamide	20	E. fetida fetida
iodoacetamide	20	E. fetida fetida



Concentration expressed in $\mu g.cm^{-2}$
(Test chemical : filter paper surface)

KEY

⊢o⊣ x	Individual LT_{50} estimate [o], gradient [x] and fiducial limits
⊢o⊣ (x)	Individual LT_{50} estimate [o], common gradient for more than one replicate [(x)] and fiducial limits
⊢●⊣ (x)	Common LT_{50} estimate [●], gradient [(x)] and fiducial limits for more than one replicate
⊢····⊣	Range within which a poorly defined LT_{50} falls
No LT_{50} →	LT_{50} at an undefined period of exposure that was greater than those studied
← No LT_{50}	LT_{50} at an undefined period of exposure that was less than those studied
(No LT_{50})	More than one LT_{50} at an undefined period of exposure

concentration of alpha-chlordane was measured initially and after 4, 8 and 24 hours by the method described below.

An aqueous solution containing 25 $ng.ml^{-1}$ alpha-chlordane was prepared by dissolving 0.025 mg crystalline alpha-chlordane in 0.5 ml acetone, and then pipetting this solution quickly into one litre of rapidly stirred distilled water. To extract alpha-chlordane from aqueous solution, a 10 ml aliquot of the solution was shaken with 5 ml n-hexane for 30 seconds in a boiling tube. Two ml of the resultant alpha-chlordane in n-hexane solution was stored in small, tightly sealed glass tubes that contained 0.1 g anhydrous Na_2SO_4 at 4°C until analysed.

The extraction of alpha-chlordane from the tissues of the earthworms was done using a procedure similar to that of an existing method (Edwards and Jeffs, 1974), in which the earthworms were removed from the solution of alpha chlordane, gently blotted dry, weighed and dropped individually into liquid nitrogen. The tissues of the earthworms became brittle and were ground in a mortar and pestle that had been pre-cooled to -10°C, with an amount of anhydrous Na_2SO_4 that was four times the body weight of the earthworm. This mixture was added to 100 ml acetone:n-hexane (1:1 v/v) and shaken mechanically for 2 hours in a tightly stoppered flask. The product was filtered through a Whatman No.1 cellulose filter paper held in a Gooch funnel, and the filtrate shaken subsequently with 100 ml 2% aqueous Na_2SO_4 solution for one minute in a separating funnel. The aqueous and organic phases were allowed to separate and the 50 ml n-hexane fraction was collected and stored over 1 g anhydrous Na_2SO_4 in a sealed sample bottle at 4°C.

One untreated earthworm of each subspecies was extracted as described above, to provide an analysis of the extractable components present within the tissues. This showed whether the lipid components of the body of the earthworms differed between these subspecies.

The gas-liquid chromatograph described earlier was calibrated using extracts of a 25 $ng.ml^{-1}$ alpha-chlordane solution, and of five other solutions diluted in a logarithmic series from this. The samples were injected into the chromatograph in 2 µl aliquots and the column was washed with 2 µl of n-hexane between each sample.

The experiment to measure the uptake and release of alpha-chlordane from aqueous solution by earthworms was designed to produce results that could be compared with existing data and was done using a method adapted from that of a previous study (Lord et al., 1980).

A 250 ml Pyrex conical flask with a stopper to prevent the loss of volatile test chemical contained 200 ml of 25 $ng.ml^{-1}$ aqueous alpha-chlordane solution. Two E. fetida andrei and two E. fetida fetida were tied with cotton thread at mouth and anus to ligature the gut, and placed singly in four flasks containing the alpha-chlordane solution at 25°C in the light. The solutions were gently but regularly agitated by hand. A 10 ml sample of the solution in which the earthworms were immersed was taken for an analysis of the concentration of alpha-chlordane before the earthworms were introduced into the flasks, and then hourly for six hours and again after 12 and 24 hours. Following this period of uptake, one earthworm of each subspecies was killed and the amount of alpha-chlordane present in the tissues of the earthworm was determined by the method of extraction and gas-liquid chromatography described above. The other earthworms were washed briefly in distilled water and then immersed in 200 ml distilled water held in a 250 ml Pyrex conical flask. The conditions of exposure, sampling methods and methods of analysis of the concentration of alpha-chlordane in the aqueous solution or tissue of the earthworms were similar to those used during the uptake phase. The amount of alpha-chlordane and of its metabolites that was excreted by, or diffused from the earthworms, was measured during this release phase.

A control flask with no earthworms, which contained an alpha-chlordane solution during the uptake phase and distilled water during the release phase, was used to estimate the amount of alpha-chlordane adsorbed or desorbed from the pre-treated glass surfaces.

Results

The surface of a 250 ml Pyrex conical flask that was exposed to 200 ml of 25 $ng.ml^{-1}$ aqueous alpha-chlordane solution, adsorbed 99.8% of the alpha-chlordane that was in solution within 24 hours (Table 8.3).

The constituents of the tissues of untreated E. fetida andrei and E. fetida fetida that were soluble in n-hexane:acetone were shown to be very similar by gas-liquid chromatography (Figure 8.8).

During the study of the uptake and release of alpha-chlordane from aqueous solution by the tissues of the earthworms, the alpha-chlordane peak was clearly identified from the background response in those samples extracted from the aqueous solution in which the earthworms were immersed and from the tissues of both sub-species. The concentration of alpha-chlordane in aqueous solution decreased rapidly during the initial 6 hours of the uptake phase, after which the decline became more gradual, until after 24 hours only a very low concentration of alpha-chlordane remained in solution (Table 8.4 and Figure 8.9). The output from the gas-liquid chromatograph was converted into $ng.ml^{-1}$ alpha-chlordane using a calibration curve. E. fetida fetida appeared to reduce the amount of alpha-chlordane in aqueous solution more rapidly than E. fetida andrei, although after 24 hours the concentration of alpha-chlordane remaining in solution was similar for both sub-species. Very little alpha-chlordane was released back into aqueous solution by earthworms of either sub-species and the amount of alpha-chlordane adsorbed by, or desorbed from the pre-treated glassware was minimal.

The amount of alpha-chlordane extracted from the tissues of E. fetida andrei and E. fetida fetida after the uptake phase was approximately 1.3 and 2.0 $mg.g^{-1}$ worm respectively. After the release phase the earthworms contained 0.8 and 1.0 $mg.g^{-1}$ worm respectively (Table 8.5). The limit of resolution for the electron capture detector was 0.8 pg alpha-chlordane in a 2 µl sample, which was equivalent to 0.2 $ng.ml^{-1}$ in the aqueous solution and 20 $ng.worm^{-1}$ in the extracts of the tissues of the earthworms.

Table 8.3. The adsorption of alpha-chlordane from aqueous solution onto a glass surface measured using gas-liquid chromatography.

Exposure period (hours)	alpha-chlordane in aqueous solution ($ng.ml^{-1}$)
0	25
4	0.95
8	0.12
24	0.04

Figure 8.8

GLC profiles of acetone-hexane (1:1v/v)extracts of whole E.fetida .

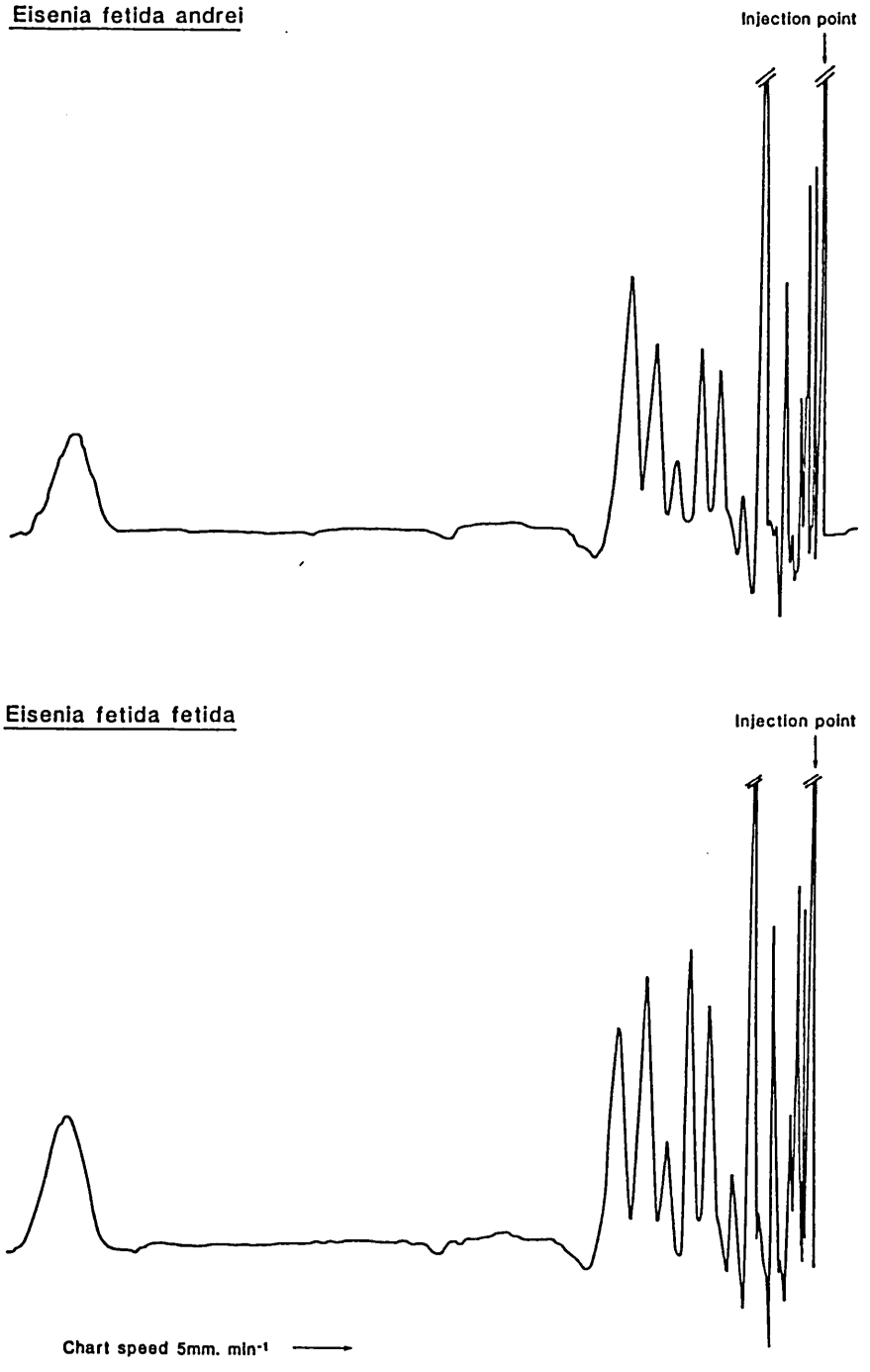


Table 8.4. The uptake and release of alpha-chlordane by earthworms, determined by measuring the concentration of alpha-chlordane in aqueous solution using gas-liquid chromatography.

	UPTAKE (a) $ng.ml^{-1}$									RELEASE (a) $ng.ml^{-1}$								
Exposure period (hours)	0	1	2	3	4	5	6	12	24	0	1	2	3	4	5	6	12	24
E. fetida andrei	25	25	23	16	4.0	3.0	6.0	1.0	0.3	0	0	0.2	0.2	0.2	0.2	0.2	0.2	0.2
E. fetida fetida	25	25	4.0	5.0	2.0	2.0	1.5	0.2	0.2	0	0	0.2	0.2	0.2	0.2	0.2	0.2	0.2
No earthworms	25	25	25	25	25	24.5	24.5	24.5	24.0	0	0	0.2	0.2	0.2	0.2	0.2	0.2	0.2

(a) Concentrations estimated to the nearest 0.2 $ng.ml^{-1}$

Figure 8.9

Uptake of ∝ chlordane from aqueous solution by E.fetida , measured using GLC

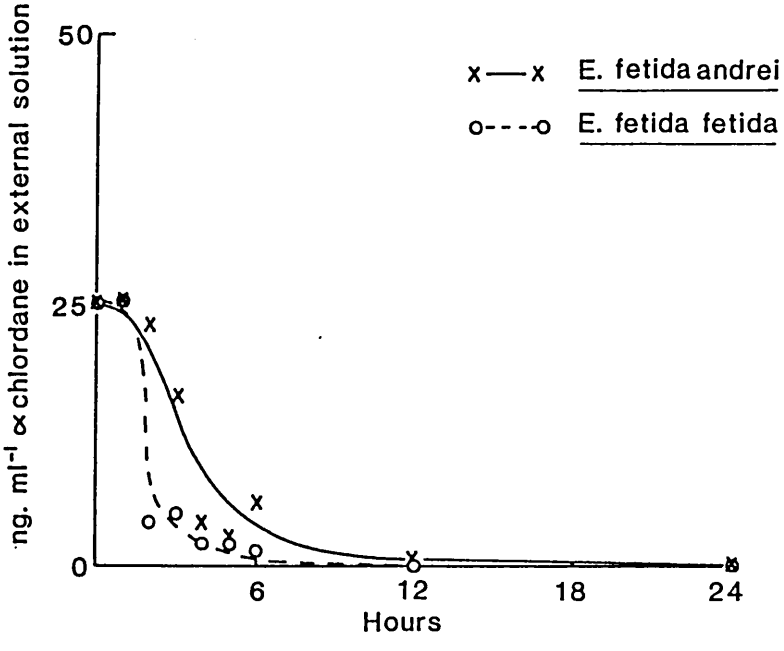


Table 8.5. The concentration of alpha-chlordane in the tissues of earthworms after 24 hours in the uptake (alpha-chlordane) or release (water) solutions, measured using gas-liquid chromatography.

	UPTAKE		RELEASE	
Species	Body weight (g)	alpha-chlordane concentration $ng.worm^{-1}$ ($ng.g^{-1}$ worm)	Body weight (g)	alpha-chlordane concentration $ng.worm^{-1}$ ($ng.g^{-1}$ worm)
E. fetida andrei	0.40	507 (1268)	0.41	320 (781)
E. fetida fetida	0.39	760 (1949)	0.40	410 (1025)

8.2.8 The uptake, release and metabolism of ^{14}C- alpha-chlordane and ^{14}C-iodoacetamide by E. fetida andrei and E. fetida fetida in aqueous solution, analysed by liquid scintillation counting and thin-layer chromatography

Methods

All the glassware, with the exception of that used for the extraction procedures that involved organic solvents, was pre-soaked for 24 hours in an aqueous solution of alpha-chlordane or iodoacetamide as appropriate to the experiment.

A saturated solution of alpha-chlordane, used to pre-treat the glassware, was produced by swirling 5 mg alpha-chlordane dissolved in 10 ml acetone, around a one litre glass conical flask and then gently evaporating the solvent to dryness with a jet of compressed air. One litre of distilled water was then added to this flask which was stoppered and shaken mechanically for 36 hours at 20°C in darkness. (The maximum solubility of alpha-chlordane in distilled water at 20°C was 0.9 $\mu g.ml^{-1}$). Iodoacetamide was water-soluble and a solution that contained 1 $\mu g.ml^{-1}$ was used to pre-treat the glassware.

A solution of (4,5,6,7,8-^{14}C)alpha-chlordane (specific activity 28.1 $mCi.mmol^{-1}$ [68.5 $\mu Ci.mg^{-1}$] and a molecular weight at this specific activity of 410) was made up in acetone and an aliquot of this, equivalent to 0.03645 mg (or 5.5×10^6 dpm), was added to one litre of distilled water to give a final concentration of 36.45 $ng.ml^{-1}$.

Iodo(1-^{14}C)acetamide (specific activity of 54.0 $mCi.mmol^{-1}$ [288 $\mu Ci.mg^{-1}$] and a molecular weight at this specific activity of 187) was dissolved in diethyl-ether and an aliquot of this solution, equivalent to 0.03472 mg (or 2.2×10^7 dpm), was added to one litre of an aqueous solution of 1 $\mu g.ml^{-1}$ unlabelled iodoacetamide. The final concentration of the solution was 1034.72 $ng.ml^{-1}$ iodoacetamide (34.72 $ng.ml^{-1}$ ^{14}C-iodoacetamide and 1000 $ng.ml^{-1}$ iodoacetamide).

Alpha-chlordane and iodoacetamide were extracted from aqueous solution using similar methods, but were soluble in different solvents. These chemicals were extracted using n-hexane or dichloromethane respectively. One ml samples of the aqueous solution used during the uptake phase and 5 ml samples of the solution used in the release phase were extracted by shaking each with 5 ml of solvent for two minutes in a separating funnel. The organic solvent phase was retained and the ^{14}C-activity of the samples, taken in duplicate, was estimated using liquid scintillation counting.

Similar methods were used to extract both alpha-chlordane and iodoacetamide from the tissues of the earthworms, but again these chemicals required different solvents and for which n-hexane and dichloromethane were used respectively. The earthworms were removed from the solution of water or test chemical, gently blotted dry, weighed and dropped into a boiling tube containing 40 ml solvent and 1 g anhydrous Na_2SO_4. The tissue was homogenised in this mixture for 30 seconds, using a Silverson tissue macerator (Gallenkamp and Co. Ltd) and allowed to settle for two hours. The supernatant was collected and the ^{14}C-activity within it measured by liquid scintillation counting. These extracts were also analysed by thin-layer chromatography.

Duplicated one ml samples of the aqueous solutions, duplicated one ml aliquots of the unduplicated extracts of the aqueous solutions in solvent, and unduplicated samples of the thin-layer chromatography gels were placed directly into scintillation vials for measurement of the ^{14}C-activity. The 5 ml samples of the extracts of the tissue of the earthworms in dichloromethane (the solvent used for iodoacetamide), were evaporated to approximately one ml under a gentle airstream and then placed in a scintillation vial. This was to avoid the excessive quenching that can occur with this solvent. The 5 ml extracts of alpha-chlordane in n-hexane did not suffer from quenching due to the solvent and were not evaporated.

This was advantageous because ^{14}C-alpha-chlordane was volatile and could have been lost during the drying process.

These samples were held in tightly sealed scintillation vials and were mixed vigorously with 10 ml of the standard scintillant described earlier. The samples were kept in darkness for 48 hours prior to counting to avoid residual fluorescence, although some of the samples that contained extracts of the tissue of the earthworms showed chemiluminescence. The ^{14}C-activity of the samples was corrected automatically for quenching by the scintillation counter and in all subsequent calculations a background count of 40 dpm was assumed.

Both alpha-chlordane and iodoacetamide were analysed using a similar thin-layer chromatography technique, although a different solvent system was used for each chemical. Chlordane was run using a solvent system of n-hexane, ethyl acetate and toluene (18:3:1 v/v), whilst iodoacetamide was run with diethyl ether and acetone (4:1 v/v). The chromatography plates were glass measuring 20 cm x 12 cm and were covered with silica gel (GF 254: Merck, Sharp and Dohme Ltd.) that was marked into 2 cm wide lanes. The plates were held in a one litre chromatography tank (Shandon Ltd.). Extracts of the aqueous solutions and of the tissues of the earthworms dissolved in solvent were evaporated gently under a jet of compressed air to reduce the volume of the samples from 2 ml and 4 ml, to 0.2 ml and 0.4 ml respectively. The replicated samples of the tissue extracts of the earthworms were combined and, together with the samples extracted from the aqueous solutions and reference samples of alpha-chlordane and iodoacetamide, were spotted onto the gel. The spots of sample were allowed to dry and then the plate was placed vertically for 15 minutes in the chromatography tank with the appropriate solvent system. When the run was complete, the plate was removed and dried in an airstream, and the positions of any products that had separated were visualised using ultra-violet light (254 nm). The distance that the product had moved from

the baseline was measured and then the gel was scraped from each lane into a series of scintillation vials. The lanes were divided in such a way that the obvious and discreet deposits of separated material were contained within a single vial. These unduplicated samples were analysed for ^{14}C-activity using liquid scintillation counting.

The method for studying the uptake, release and metabolism of ^{14}C-alpha-chlordane and ^{14}C-iodoacetamide in aqueous solution by E. fetida andrei and E. fetida fetida was adapted from that of a previous study (Lord et al., 1980), and in this modified procedure four E. fetida andrei and four E. fetida fetida with unligatured guts were placed singly into eight 250 ml stoppered Pyrex conical flasks that contained 100 ml of the labelled solution. The flasks were gently and regularly agitated by hand and maintained at 25°C in the light.

One ml samples of the solution in which the earthworms were immersed were taken before the earthworms were introduced. This solution was then sampled at hourly intervals for 7 or 8 hours for ^{14}C-alpha-chlordane or ^{14}C-iodoacetamide respectively. The flasks were sampled again after 12 and 24 hours. The solutions that were used during the uptake and release phases were sampled in a similar manner and the ^{14}C-activity of each was estimated using liquid scintillation counting. In addition to these samples, one ml aliquots of the aqueous solution used in the uptake phase and 5 ml aliquots of the aqueous solution used in the release phase were taken initially, and then after 6, 12 and 24 hours. These samples were extracted using organic solvents by the method that was described earlier and the amount of ^{14}C-activity present was estimated using liquid scintillation counting. The extract taken after 24 hours from the aqueous solution used during the uptake period was also analysed using thin-layer chromatography. The earthworms that survived the uptake period were removed, washed briefly in distilled water and then immersed in 100 ml distilled water for a further 24 hours.

At the end of the 24 hour uptake and release periods, two earthworms of each subspecies were killed and the tissues extracted with solvents as described earlier. The ^{14}C-activity in these extracts was estimated using liquid scintillation counting and also analysed by thin-layer chromatography.

The control flasks did not hold any earthworms but contained radiolabelled solutions or distilled water during the uptake and release periods respectively. Such controls accompanied the experiments with both chemicals. These controls were, in all other respects, treated in a similar way to those flasks that contained earthworms.

Results

The uptake, release and metabolism of ^{14}C-alpha-chlordane by E. fetida andrei and E. fetida fetida in aqueous solution

The glass surfaces that had been pre-treated with alpha-chlordane did not appear to adsorb ^{14}C-alpha-chlordane from solution, or allow alpha-chlordane to desorb subsequently into distilled water.

E. fetida andrei and E. fetida fetida accumulated ^{14}C-alpha-chlordane from solution strongly at rates that were similar. The mean of the data for both subspecies indicated that 73% of the ^{14}C-alpha-chlordane calculated to be present initially in the solution used during the uptake period was removed (Figure 8.10). As one of the E. fetida fetida began to die during the uptake period, the amount of ^{14}C-alpha-chlordane that remained in solution decreased quickly. E. fetida andrei was not affected adversely by this concentration of ^{14}C-alpha-chlordane.

Figure 8.10

Uptake of ^{14}C ∝ chlordane from aqueous solution

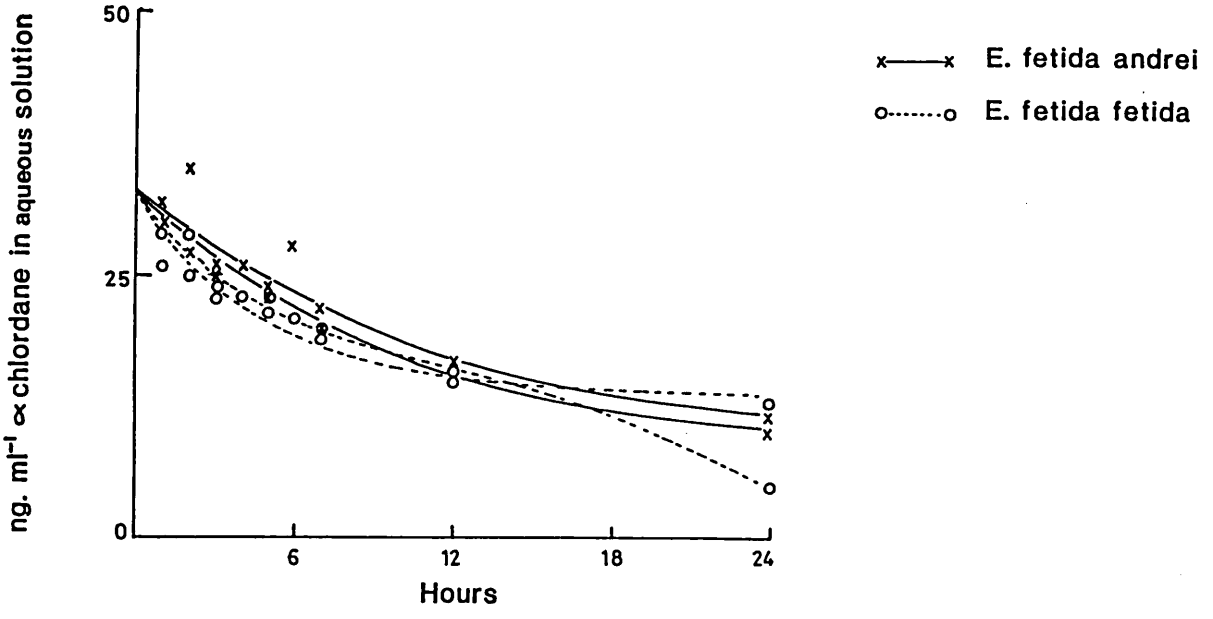


Figure 8.11

Release of ^{14}C ∝ chlordane into aqueous solution

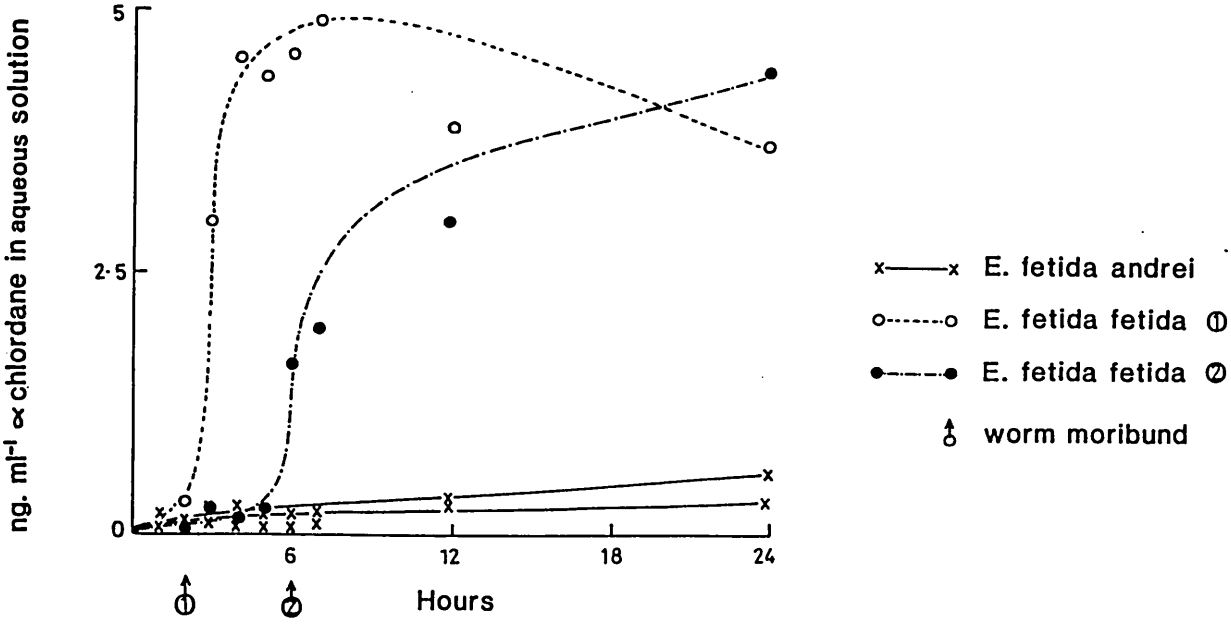
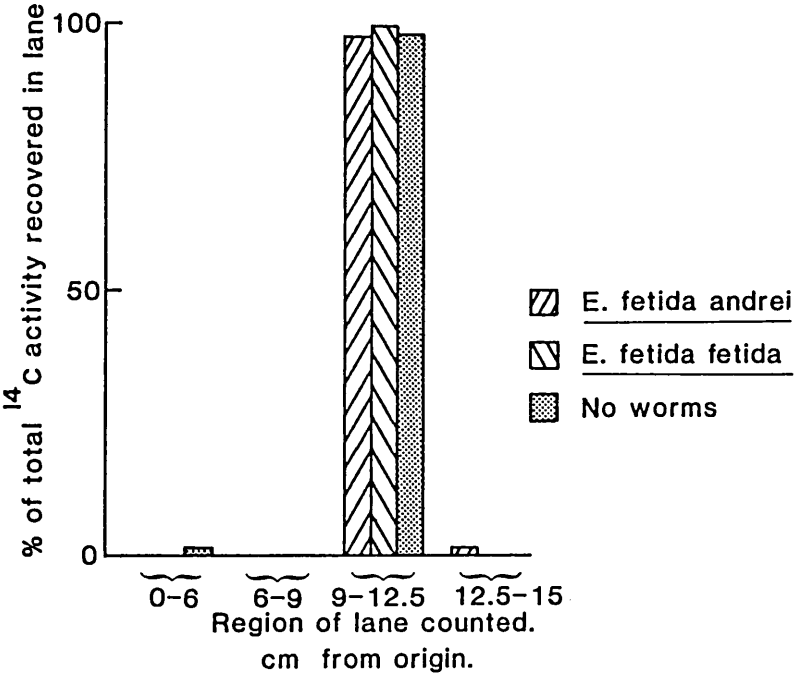


Figure 8.12

Thin layer chromatography of ^{14}C ∝ chlordane in extracts of the aqueous uptake solution taken after 24 hours.



Thin layer chromatography of ^{14}C ∝ chlordane
in worm extracts

Figure 8.13

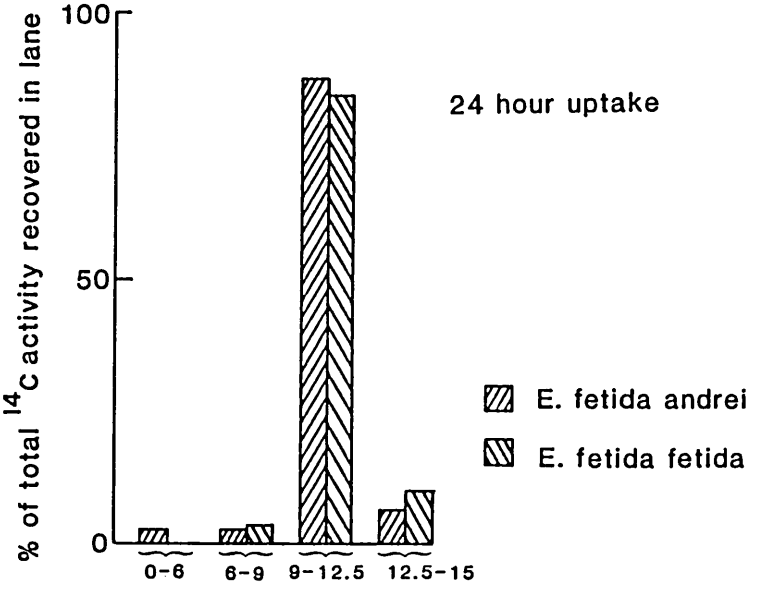
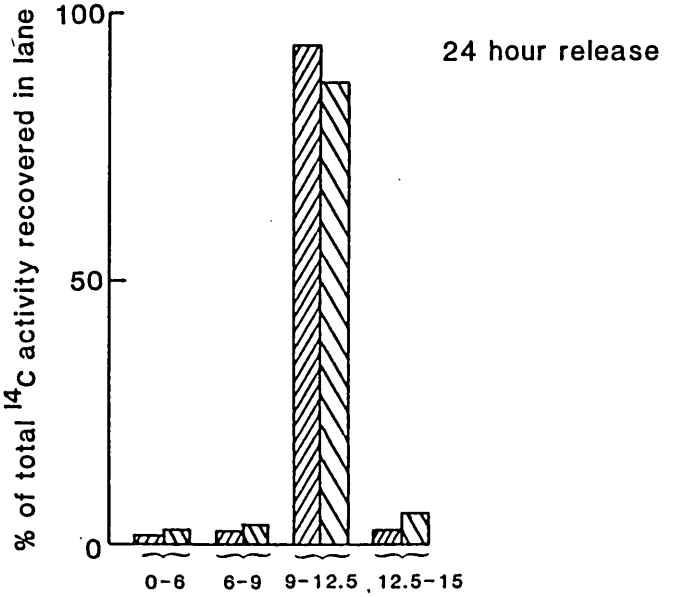


Figure 8.14



E. fetida andrei continued to remain healthy during the subsequent 24 hour period of immersion in distilled water, and released very little ^{14}C-alpha-chlordane into aqueous solution. In contrast to this observation, E. fetida fetida began to die after 3-6 hours in the distilled water. As the earthworms died, they began to haemorrhage and the amount of ^{14}C-alpha-chlordane in the aqueous solution increased quickly, although the rate of loss of ^{14}C-alpha-chlordane from the tissues of healthy individuals appeared to be minimal and similar for both subspecies (Figure 8.11).

The amount of ^{14}C-alpha-chlordane that accumulated in the tissues of the earthworms was 2.20 $\mu g.worm^{-1}$ (5.4 $\mu g.g^{-1}$ worm) for E. fetida andrei and 2.47 $\mu g.worm^{-1}$ (6.2 $\mu g.g^{-1}$ worm) for E. fetida fetida, and these estimates agreed closely with those infered from the measurements of the amount of ^{14}C-alpha-chlordane remaining in solution. These data also confirmed that E. fetida andrei did not release any ^{14}C-alpha-chlordane back into aqueous solution, and while E. fetida fetida was seen to release 20% of that which had been accumulated during the uptake period, this release only occurred after the earthworms had died. The amount of ^{14}C-alpha-chlordane accumulated by the individuals of the two subspecies of E. fetida in this study was similar to that seen in the previous experiment measured using gas-liquid chromatography.

Most of the ^{14}C-labelled material extracted from the aqueous solution of ^{14}C-alpha-chlordane used during the uptake period appeared to move on the thin-layer chromatography plate with a speed similar to that of alpha-chlordane (Figure 8.12). The extracts of the tissues of the earthworms taken after the uptake and the release periods also contained ^{14}C-labelled material that moved with the same speed as alpha-chlordane (rf 0.72). Small amounts of other ^{14}C-labelled compounds were also separated which had faster (rf 0.92) and slower (rf 0.50 and 0.20) running speeds than alpha-chlordane (Figures 8.13 and 8.14). E. fetida

fetida contained slightly more of these fast and slow running materials than E. fetida andrei.

The estimates of the concentration of ^{14}C-alpha-chlordane in aqueous solution obtained by counting the unextracted samples, the samples that had been extracted with n-hexane and the samples that were separated using thin-layer chromatography were similar.

The uptake, release and metabolism of ^{14}C-iodoacetamide by E. fetida andrei and E. fetida fetida in aqueous solution

The glass surfaces that had been pre-treated with iodoacetamide did not adsorb ^{14}C-iodoacetamide in significant amounts, or allow iodoacetamide to desorb subsequently into distilled water.

The concentration of ^{14}C-iodoacetamide in aqueous solution decreased slightly after 24 hours and by a similar amount in the flasks that contained E. fetida andrei and E. fetida fetida (Figure 8.15). The mean of the data for both subspecies indicated that of the ^{14}C-iodoacetamide calculated to be present initially in the solution used during the uptake period, 7% was removed from solution. Very little ^{14}C-labelled material was released back into solution by individuals of either subspecies of E. fetida, although the amount released by E. fetida fetida was slightly greater than that released by E. fetida andrei (Figure 8.16).

The amount of ^{14}C-iodoacetamide that accumulated in the tissues of the earthworms was 0.22 $\mu g.worm^{-1}$ (0.55 $\mu g.g^{-1}$ worm) for E. fetida andrei and 0.20 $\mu g.worm^{-1}$ (0.48 $\mu g.g^{-1}$ worm) for E. fetida fetida, and these estimates agreed closely with those infered from the measurements of the amount of ^{14}C-iodoacetamide remaining in solution. These data also confirmed that the amount of ^{14}C-labelled material which was released from E. fetida andrei and E. fetida fetida after the 24 hour period of immersion in distilled water was 18% and 35% respectively of that accumulated previously.

Figure 8.15

Uptake of ^{14}C iodoacetamide from aqueous solution

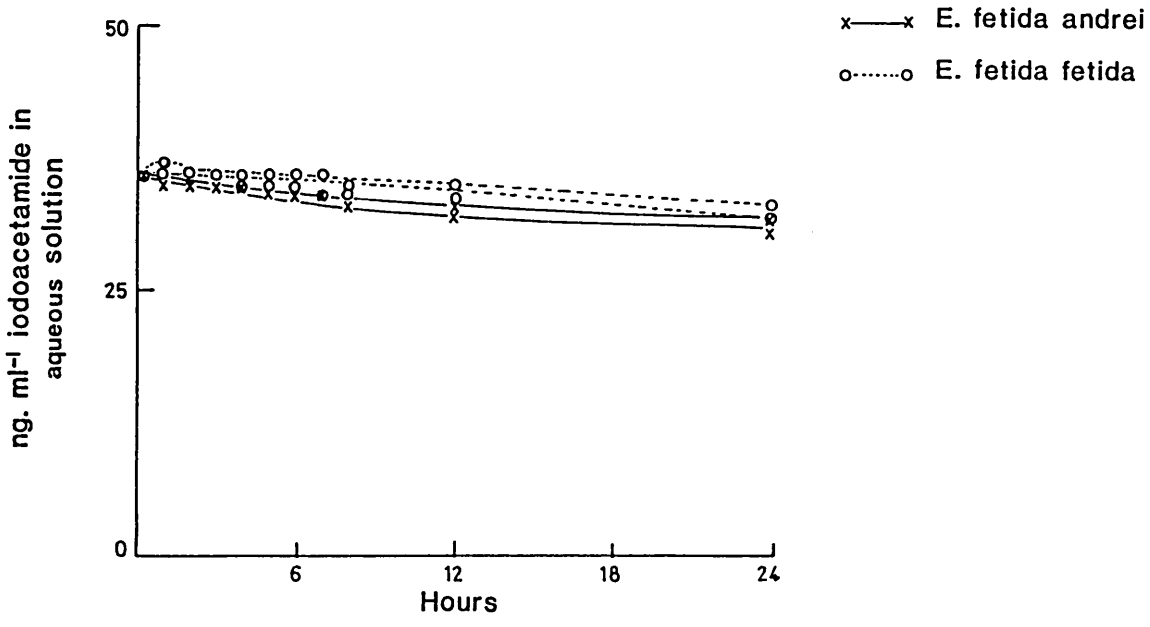


Figure 8.16

Release of ^{14}C iodoacetamide into aqueous solution

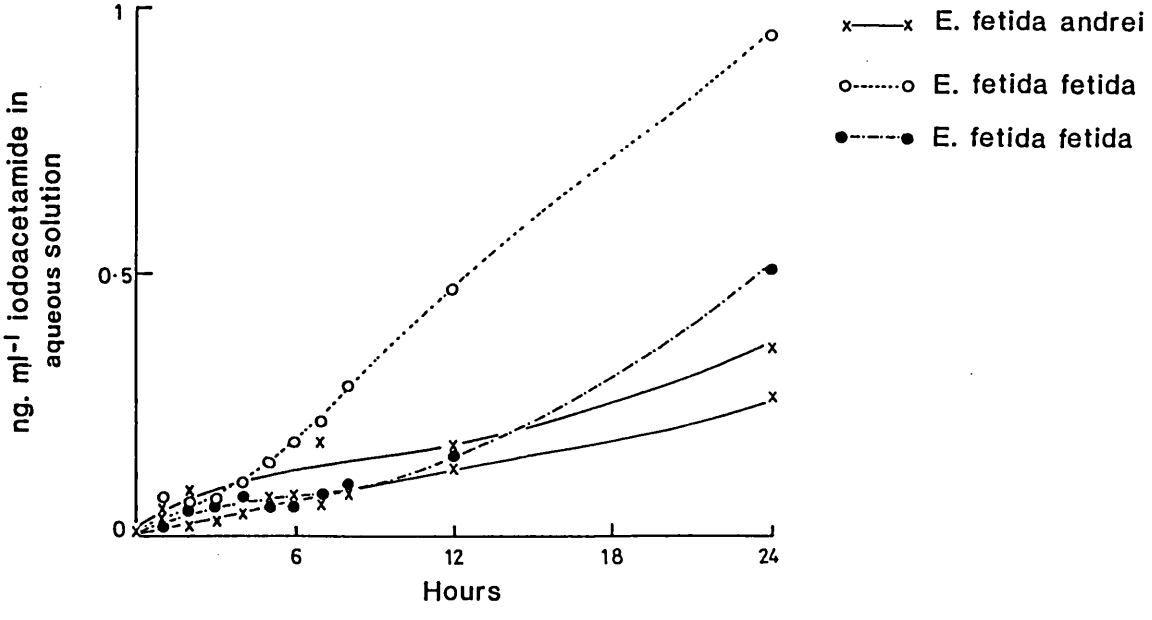
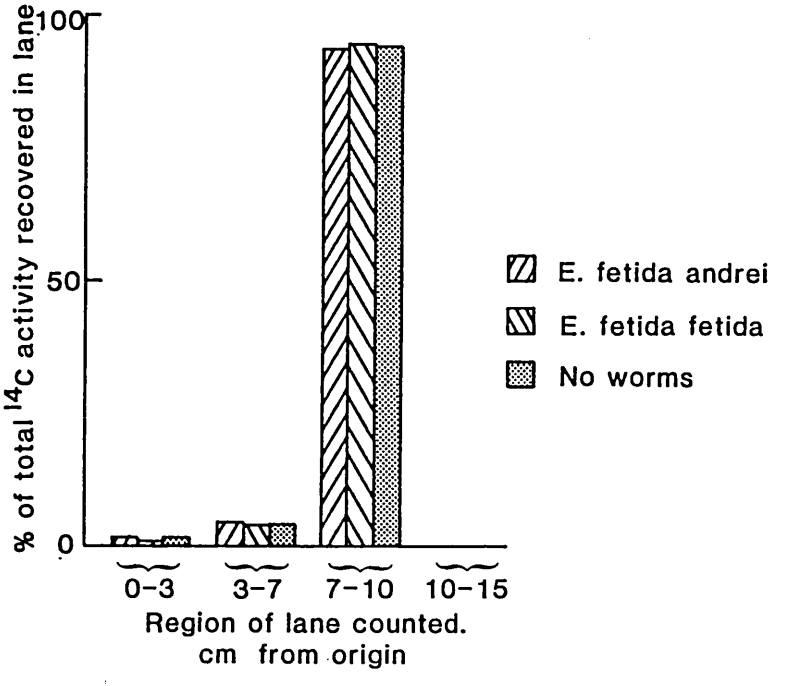


Figure 8.17

Thin layer chromatography of ^{14}C iodoacetamide in extracts of the aqueous uptake solution taken after 24 hours.



Thin layer chromatography of ^{14}C iodoacetamide in worm extracts

Figure 8.18

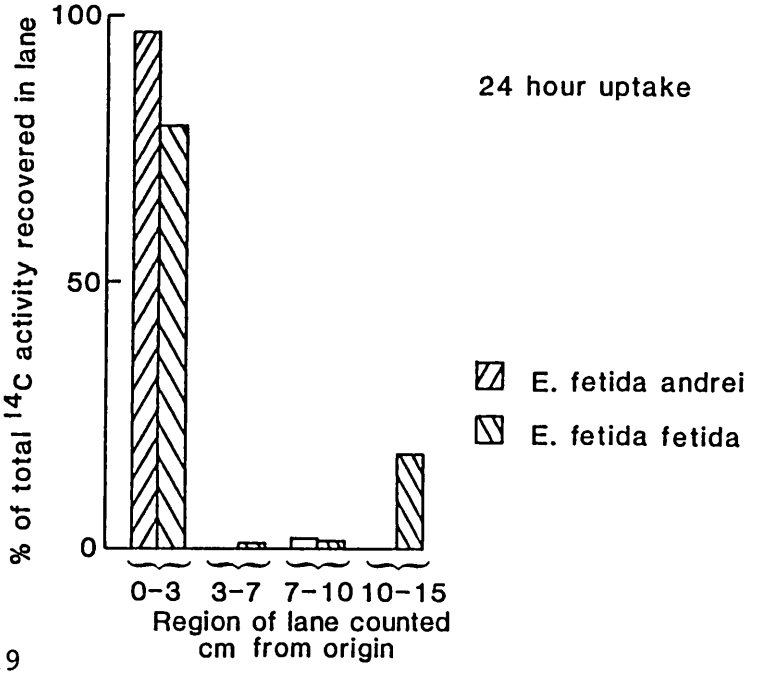
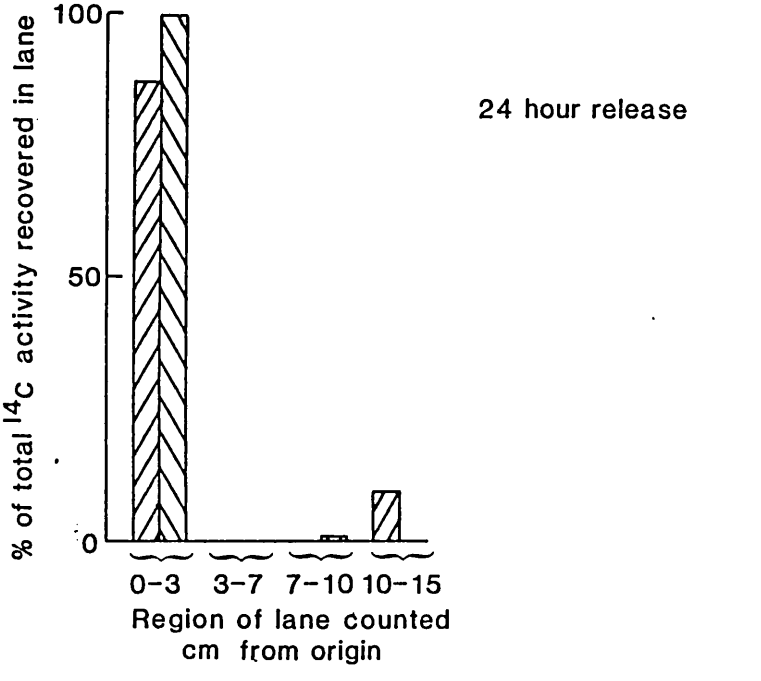


Figure 8.19



Most of the ^{14}C-labelled material extracted from the aqueous solution of ^{14}C-iodoacetamide used during the uptake period moved on the thin-layer chromatography plate at a speed similar (rf 0.57) to that of iodoacetamide (Figure 8.17). By contrast, the majority of the ^{14}C-labelled material in the extracts of the tissues of the earthworms moved much more slowly (rf 0.1) than iodoacetamide (Figures 8.18 and 8.19), although a fast running component (rf 0.83) was also present, and occurred in larger amounts in the samples taken from E. fetida fetida after the 24 hour uptake period, and in the samples taken from E. fetida andrei after the 24 hour release period.

The estimates of the concentration of ^{14}C-iodoacetamide in aqueous solution obtained by counting the unextracted samples, the samples that had been extracted with dichloromethane and the samples that were separated using thin-layer chromatography were similar.

8.3. Discussion

The earthworms used in the tests for the toxicity of chemicals were selected to have individual body weights that fell within a narrow range. The heaviest earthworms had a body weight that approximated to the mean weight for individuals collected at random from a natural population. Thus the inter-specific differences in the susceptibility to chemicals that were observed in the tests conducted in the laboratory, could be compared directly with field studies without further consideration of body weight. The body weight of the lightest earthworms that were used in the experiments approximated to the mean individual body weight of earthworms from a natural population, less the amount of weight lost during one month of culture in the laboratory. This ensured a maximal supply of earthworms for the experiments.

The samples of earthworms collected at random from the field indicated that L. terrestris had the largest mean body size followed, in a descending order of mean body weight, variation of body weight about the mean body weight, mean body length, diameter and volume by A. longa, A. caliginosa and (from cultures in the laboratory) E. fetida. The mean body sizes and weights of L. terrestris and A. longa were similar, as were the mean sizes and weights of A. caliginosa and E. fetida, although the dimensions of the two groups were dissimilar. Mean body length and diameter differed less between the species of earthworm than the mean body volume.

The artificial soil test indicated that L. terrestris and A. longa had a similar susceptibility to chlordane, pentachlorophenol and chloroacetamide, which was generally lower than that shown by A. caliginosa. However, field studies have shown that L. terrestris is often more susceptible to pesticides than either A. longa or A. caliginosa (Stringer and Lyons, 1974, 1977), although such differences were thought to arise mainly from the variety of feeding and burrowing strategies that were adopted by these species.

E. fetida andrei was less susceptible to chlordane than any of the other species of earthworm. The 14 day LC_{50} of 11.11 $mg.kg^{-1}$ obtained for this species using the artificial soil test was similar to the 4 day LC_{50} estimated previously for chlordane with E. fetida in a loam soil of 2.5 $mg.kg^{-1}$ (Hopkins and Kirk, 1957). However, the 14 day LC_{50} for L. terrestris exposed to chlordane in the artificial soil of 3.00 $mg.kg^{-1}$ did not compare well with other reports which indicated that the 3 day LC_{50} for chlordane with L. terrestris in a potting compost was >16 $mg.kg^{-1}$ (Ruppel and Laughlin, 1977).

My results show that E. fetida andrei was less susceptible than A. caliginosa to pentachlorophenol, which supports the observation that earthworms containing a large amount of the enzyme glutathione

S-transferase are less susceptible to this class of chemicals. These enzymes are capable of conjugating pentachloronitrobenzene, which is closely related to PCP (Stenersen and Øien, 1981) and occur in the tissues of E. fetida at a concentration twice that found in L. terrestris, A. longa or A. caliginosa (Stenersen, Guthenberg and Mannervik, 1979).

My observations that indicate E. fetida is relatively tolerant of toxic chemicals are also supported by other studies in which E. fetida was apparently less susceptible to the cholinesterase inhibiting pesticides than other species of earthworm (Gilman and Vardanis, 1974; Stenersen, 1979a).

The susceptibility of E. fetida andrei to chloroacetamide was greater than that of the other species of earthworm. E. fetida andrei gave a 14 day LC_{50} in the artificial soil of 8.88 $mg.kg^{-1}$, compared with an LC_{50} of 38.00 $mg.kg^{-1}$ for L. terrestris and 30.12 $mg.kg^{-1}$ for A. caliginosa. These data compare well with those from other reports in which the 14 day LC_{50} for this chemical tested using soil in the laboratory, was determined with E. fetida, L. terrestris and A. caliginosa to be 29.5, 21.5 and 15.5 $mg.kg^{-1}$ respectively (Edwards, 1985).

Some of the differences in the susceptibility to chemicals that were seen between species may be related to body size. Species of earthworm that have a large body also have a higher volume:surface area ratio and a greater metabolic and excretory capacity than species with a small body. Thus, large earthworms would probably need to be exposed to a chemical for a longer period of time and at a higher concentration than a smaller earthworm in order to achieve a toxic concentration in the tissues.

The results of this study support the generally accepted opinion that A. caliginosa is very susceptible to chemicals, and is followed in a decreasing order of susceptibility, by L. terrestris and E. fetida andrei (Dean-Ross, 1983). The results obtained with chloroacetamide were probably anomalous.

The reproducibility of the results of the tests for toxicity (inferred from a chi-squared test for parallelism and the position of the Y-intercept of the probit lines calculated for individual replicates) did not appear to be related to the species of earthworm used, although some chemicals gave more reproducible results than others. The replicates of chloroacetamide did not differ significantly when tested with each of the species of earthworm, which supports the choice of this chemical as the reference compound for statutory tests of toxicity to earthworms (Inglesfield, 1984).

The gradient of the probit lines calculated from the experiments with E. fetida andrei were generally shallow, which indicated that E. fetida andrei was susceptible to the chemicals over a wider range of concentrations than other species. The gradients of the probit lines calculated for A. caliginosa were, by contrast, generally steep and thus the susceptibility of this species was restricted to a relatively narrow range of concentrations of the test chemicals. Thus, assessments of the toxicity of chemicals made with A. caliginosa should be more reproducible than those made with other species, which indicates that A. caliginosa also is suitable for testing toxicity as suggested in previous reports (Edwards, 1982; Lofs-Holmin, 1982b).

The filter paper contact test was used to investigate further the differences in susceptibility that were seen between A. caliginosa and E. fetida. The subspecies of E. fetida were used separately because E. fetida fetida was seen to survive in more putrid conditions within a compost heap than E. fetida andrei and would possibly show a different response to toxic chemicals. The recent electrophoretic studies of Jaenike (1982), and latterly those of Øien and Stenersen (1984) have suggested that E. fetida andrei and E. fetida fetida are reproductively isolated and therefore separate species. These workers propose that the new species be named E. unicolor (Andre) and E. fetida (Savigny) respectively. This division has yet to be adopted formally in the taxonomy of the Lumbricidae

and thus, I have retained the classification that was in use during the period of my studies.

The filter paper contact test showed that A. caliginosa was considerably more susceptible to chlordane, slightly more susceptible to pentachlorophenol and iodoacetamide, and less susceptible to chloroacetamide than E. fetida. These results are supported by previous observations (Edwards, 1985; Stenersen, 1979a). The two subspecies of E. fetida had a similar susceptibility to pentachlorophenol and to chloroacetamide, although E. fetida andrei was more susceptible to chlordane and iodoacetamide than E. fetida fetida. In general, the gradients of the probit lines for the chemicals tested with A. caliginosa and E. fetida andrei were steep, although chlordane was the exception to this. This species and subspecies of earthworm were therefore susceptible to these test chemicals over a narrower range of concentrations than E. fetida fetida. Thus, the response of E. fetida fetida probably caused the shallow gradients of the probit lines seen when a mixed population of the two subspecies of E. fetida were used in the experiments with the artificial soil.

Further studies with the filter paper contact test showed that E. fetida fetida was also less susceptible than E. fetida andrei to iodoacetamide when measured as an LT_{50}, and to chlordane measured as an LC_{50}. The LT_{50} for chlordane was similar for both subspecies of E. fetida using the filter paper contact and artificial soil tests, and thus the difference in the susceptibility to this chemical would seem dependent upon concentration and not upon time. The high concentration of chlordane that was present during the experiments to determine an LC_{50} may be more detrimental to E. fetida andrei than to the other subspecies. The radiochemical studies described later confirmed that such differences in metabolism did exist between these subspecies.

Several studies have compared the susceptibility of different species of earthworm to toxic chemicals (Edwards, 1985; Gilman and Vardanis, 1974; Stenersen, 1979a), although the differences between the responses shown by more closely related groups remained unexplored. The preceding assessments of the toxicity of chemicals using the two subspecies of E. fetida, together with the survey of the distribution of these subspecies within compost heaps, suggested that an investigation of the uptake, release and metabolism of certain chemicals might reveal the nature of the differences that exist between them.

The components of the tissues of earthworms from both subspecies of E. fetida, extracted using n-hexane:acetone (and analysed subsequently by gas-liquid chromatography) were of similar type and quantity. Thus, the difference that was seen subsequently between the amount of chemical accumulated by earthworms of these two subspecies was probably not related to the amount of lipid present in the tissues. Fortunately, the retention time of alpha-chlordane in the chromatograph was discrete from that of the other substances that were extracted from the tissues of the earthworms, and it was thus possible to use this analytical technique to estimate the amount of alpha-chlordane taken up from an aqueous solution by individuals of both subspecies of E. fetida.

Analyses by gas-liquid chromatography showed that the earthworms had removed 99% of the alpha-chlordane that was in solution within 24 hours, and released subsequently only a very small amount of this compound into distilled water. Alpha-chlordane accumulated more rapidly in E. fetida fetida than E. fetida andrei, although the concentration of alpha-chlordane that remained in aqueous solution after 24 hours was similar for both subspecies.

The earthworms that were used in the experiments with radiolabelled chemicals were not ligatured at the mouth and anus, because this tended to cause necrosis and in any case the ligature did not affect the uptake of

chemicals (Lord et al., 1980). Healthy individuals of the subspecies E. fetida andrei and E. fetida fetida removed approximately 73% of the ^{14}C-alpha-chlordane that was available in solution. This value was somewhat lower than that of the mean of both subspecies seen during the experiments measured using gas-liquid chromatography.

An analysis of the tissues of the earthworms showed that there was little difference in the amount of ^{14}C-alpha-chlordane that was present within E. fetida andrei and E. fetida fetida after the 24 hour uptake period.

^{14}C-alpha-chlordane was not released back into distilled water in appreciable amounts by healthy individuals of either subspecies of E. fetida, and E. fetida andrei retained almost all of the ^{14}C-alpha-chlordane that had been accumulated previously, during a 24 hour immersion in distilled water. E. fetida fetida retained only 80% of that taken up, but died during the course of the experiment.

These results contrast with those from previous tests for toxicity, because E. fetida fetida (which was seen previously to be less susceptible to the toxic effects of alpha-chlordane than E. fetida) was killed by the concentration of 36.5 ng.ml^{-1} ^{14}C-alpha-chlordane present in aqueous solution. This concentration had no effect upon E. fetida andrei. As earthworms of the subspecies E. fetida fetida began to die, coelomic fluid and blood was lost into the surrounding medium. Coincidentally to this, a sudden influx of ^{14}C-alpha-chlordane into, or efflux from, the tissues of the earthworm occurred during the periods of uptake and release respectively. Although water can move across the cuticle easily (Pieарce, 1981), the regulation of the volume of the coelomic fluid in L. terrestris is an active process (Carley, Caracciolo and Mason, 1983). Chemicals such as dieldrin (which is closely related to chlordane) can disrupt the neurohormonal regulation of the ion and water balance in E. fetida (Drewes and Vining, 1984) and cause a loss of body fluid. My results suggest that

an appreciable amount of chlordane was held within the coelomic fluid of the earthworm as a solution in water or lipid. Chlordane probably interfered with the regulation of the active processes controlling the movement of materials across the body wall and, as the earthworms began to die, these substances became redistributed according to the prevailing concentration gradients.

Other studies of the accumulation of organochlorine chemicals by earthworms have not only failed to find differences between sub-species, but also between species. For instance, the amount of dieldrin that was accumulated from aqueous solution by L. terrestris and A. longa did not differ significantly (Lord et al., 1980).

Thin-layer chromatography confirmed that the ^{14}C-label was still alpha-chlordane, because most of the ^{14}C-activity in the samples moved at a similar speed to alpha-chlordane (rf 0.72), although some materials had slower (rf 0.20 and 0.50) or faster (rf 0.92) running speeds. Previous reports described a similar chromatography system used to study alpha-chlordane that had been injected into L. terrestris. This was extracted mainly as unchanged chemical (rf 0.75) although small amounts of chlordane chlorohydrin (rf 0.21), an unidentified metabolite (rf 0.59) and oxychlordane (rf 0.86) were also found (Chio and Sanborn, 1976). The results of my studies support this investigation, because I found that the majority of the ^{14}C-alpha-chlordane remained unchanged although a significant amount of oxychlordane, and a smaller amount of chlordane chlorohydrin were probably also present.

The earthworm tissue:water partition coefficient was calculated from the mean concentration of alpha-chlordane present in the tissues in healthy individuals of both subspecies (and assuming that the earthworms contained 15% dry matter [Edwards and Lofty, 1977]) and the mean concentration of alpha-chlordane that remained in aqueous solution after the 24 hour uptake period. The earthworm tissue:water partition

coefficient of alpha-chlordane was approximately 3850, as predicted from the high octan-1-ol:water partition coefficient of this (Briggs, 1982) and of other strongly lipophilic chemicals (Chiou et al., 1977). However, this value was somewhat lower than the octan-1-ol:water partition coefficients reported for other organochlorine pesticides in similar experiments with L. terrestris (Lord et al., 1980).

The uptake of polar chemicals by earthworms proceeded in a manner dissimilar to that seen for lipophilic chemicals. Polar molecules are accumulated poorly by earthworms (Briggs and Lord, 1983; Lord et al., 1980) and slugs (Haque and Ebing, 1983b). Chemicals such as carbofuran, which is water-soluble and polar, were taken up by L. terrestris in amounts that were proportional to the external concentration of the chemical alone (Stenersen et al., 1973).

Iodoacetamide was used to study the behaviour of a polar compound in a similar experimental system to that used previously with alpha-chlordane. Very little ^{14}C-iodoacetamide was accumulated by E. fetida andrei or E. fetida fetida from aqueous solution, as predicted by previous work (Briggs and Lord, 1983; Lord et al., 1980), although the rate at which this process took place was similar for each subspecies. The extracts of the distilled water that had been exposed to E. fetida fetida during the release period contained low levels of ^{14}C-activity, whilst those exposed to E. fetida andrei contained somewhat less.

The tissues of E. fetida andrei and E. fetida fetida accumulated very little of the ^{14}C-iodoacetamide that was available in solution, and after the 24 hour release period E. fetida andrei retained more ^{14}C-iodoacetamide accumulated previously than did E. fetida fetida. E. fetida fetida seemed to release marginally more ^{14}C-iodoacetamide into aqueous solution than E. fetida andrei, although the quantities involved were very much smaller than those seen with alpha-chlordane.

An analysis of the extracts in organic solvent taken at the end of the uptake period from the aqueous solution of ^{14}C-iodoacetamide, gave a slightly lower estimate of the ^{14}C-activity than that obtained by counting the unextracted solution. This suggested that a small proportion of the labelled compound was no longer iodoacetamide.

An analysis of the extracts of the aqueous solutions made using thin-layer chromatography also indicated that most of the ^{14}C-labelled compound in the aqueous solution was still iodoacetamide after the 24 hour uptake period. However, the ^{14}C-labelled compound within the extracts of the tissues of the earthworms was metabolised into substances with faster and slower running speeds than iodoacetamide. The concentration of the fast running component was higher in E. fetida fetida after 24 hours and only became apparent in E. fetida andrei after 48 hours, which suggested that the detoxification processes of the latter subspecies were somewhat slower.

The earthworm tissue:water partition coefficient was determined in the same way as that described earlier for alpha-chlordane. The value of 104 was higher than that expected from the octan-1-ol:water partition coefficient which was 0.6 (Briggs, 1982), but approximated the values determined for similar polar and water soluble chemicals (Chiou et al., 1977).

Thus, species and subspecies of earthworm differed in susceptibility to toxic chemicals. Large earthworms like L. terrestris and A. longa were generally less susceptible to such chemicals than smaller earthworms, even when behavioural factors were controlled. A. caliginosa was the species that was most susceptible to the effects of chemicals, and this sensitivity occurred over a narrow range of concentration. Were it not for the ease with which E. fetida could be cultured and handled in the laboratory, A. caliginosa would appear better suited to testing toxicity.

E. fetida appeared to be generally less susceptible to toxic chemicals than the other species of earthworm that were examined, although other assesments of the toxicity of chemicals to E. fetida correlated well with those made with more sensitive species like L. terrestris (Heimbach, 1985a, 1985b). Thus, E. fetida is able to indicate adequately the toxicity of chemicals to earthworms that live in arable soil.

E. fetida fetida appeared to be less susceptible than E. fetida andrei to most of the chemicals that were tested. E. fetida andrei is therefore better suited to testing the toxicity of chemicals to earthworms than E. fetida fetida. These differences in the susceptibility to chemicals seen between E. fetida andrei and E. fetida fetida may explain why the latter subspecies is more tolerant of the putrid conditions that occur within compost heaps.

CHAPTER 9

GENERAL DISCUSSION

This thesis includes a complete review of the effects of chemicals upon earthworms, which supersedes that of other similar works (Davey, 1963; Edwards and Thompson, 1973; Satchell, 1955a) and extends considerably the limited reviews of Blackshaw (1980), Clutterbuck (1973) and Dean-Ross (1983). My review is original in its use of an objective system for categorising according to toxicity to earthworms chemicals that were assessed using a variety of testing methods (Section 1.5).

The chemicals that have the most severe toxicity to earthworms include some of the inorganic salts (calcium arsenate, lead arsenate, mercuric chloride) and aromatic or organochlorine compounds (chlordane, endosulphan, endrin), the organophosphorus insecticides (ethoprophos, phorate, turbufos), carbamate insecticides and fungicides (bufencarb, carbaryl, carbofuran, benomyl, carbendazim, thiophanate-methyl) together with some of the nematicides (chloropicrin, dazomet, formaldehyde). Many other organochlorine compounds, fungicides and most herbicides have a low toxicity to earthworms. The conclusions drawn from my review agree with many of the findings of previous studies (Bauer, 1964; Davey, 1963; Dean-Ross, 1983; Edwards and Thompson, 1973; Escritt and Arthur, 1948; Gough, 1945; Satchell, 1955a; Thompson and Edwards, 1974).

Species of earthworm differ in their susceptibility to chemicals and also in their behaviour. These factors can influence the assessments of toxicity that are made using earthworms. Thus, it was important to choose a species for use in the tests for toxicity that was relatively sensitive to chemicals, easy to culture in the laboratory and free of undesirable behavioural characteristics.

The tests for toxicity that I developed minimised the influence of the behaviour of the earthworms upon the assessments of toxicity, by limiting the amount of space available to the earthworms. Behavioural differences often cause the variation in the susceptibility to chemicals seen between species of earthworm in the field. For instance, earthworms that burrow deep into the soil such as L. terrestris and A. longa (Edwards and Lofty, 1977), avoid chemicals that contaminate the surface of the soil, although L. terrestris may be exposed to a high concentration of chemicals when moving on the soil surface at night in search of food. Other species, including A. longa and A. caliginosa aestivate during unfavourable conditions and are therefore exposed less to soil pollutants (Edwards and Lofty, 1977, 1978; Stringer and Lyons, 1974, 1977).

Differences in susceptibility to chemicals between species of earthworm that arise from metabolic or physiological causes cannot always be restricted by a toxicity test. I have demonstrated that A. caliginosa is particularly sensitive to toxic chemicals and, whilst L. terrestris and A. longa are moderately susceptible, E. fetida is the least susceptible of these species. These results were obtained using the filter paper contact and artificial soil tests, and are supported by the findings of previous studies (Davey, 1963; Dean-Ross, 1983; Edwards, 1985; Gilman and Vardanis, 1974; Haque and Ebing, 1983a; Stenersen, 1979a).

Although A. caliginosa was very sensitive to chemicals and has been proposed previously as a species suitable for testing toxicity (Edwards, 1982; Lofs-Holmin, 1982b), it was less easy to culture under laboratory conditions than E. fetida (Tomlin, 1977). In contrast to this, E. fetida breeds rapidly in the laboratory using animal dung as a culture medium (Neale and Edwards, 1982). I found that it achieved a body weight that was suitable for testing toxicity (0.4-0.6 g) within 60 days of hatching from the cocoon, a conclusion which was supported by the data of Neale

(1984). Whilst E. fetida was slightly less susceptible to toxic chemicals than other species of earthworm, it was able to withstand the diverse environmental conditions that were present within the various tests for toxicity which I evaluated experimentally. This ability to tolerate environmental change was also beneficial in the assessment of toxicity using the filter paper contact and artificial soil tests, because a small fluctuation in the experimental conditions used within, or between, laboratories had little effect upon the results. The consistency of the results that were obtained using the artificial soil test was clearly due in part to this tolerance displayed by E. fetida, which was verified by Heimbach and Edwards (1983).

The decision to adopt E. fetida as the test species was taken in consultation with an EEC committee. This choice of species was criticised by Lofs-Holmin (1982b), who considered that E. fetida could not indicate the toxicity of chemicals to the earthworms that live in arable soils, because it was found mainly in compost and dung heaps. This criticism was answered when Heimbach (1985a, 1985b) demonstrated that assessments of toxicity with E. fetida correlated closely (r=0.81) with those obtained using L. terrestris. Other toxicity tests using soil in the laboratory have shown that L. terrestris and A. caliginosa respond to chemicals in a similar manner to E. fetida (Edwards, 1985), and the choice of E. fetida as the test species has been supported subsequently by several recent studies (Reinecke and Venter, 1985; Rhett et al., 1985; Roberts and Dorough, 1984).

I have also shown that E. fetida andrei is more susceptible to the effects of toxic chemicals than E. fetida fetida, and it is therefore best to use the former subspecies of E. fetida in tests for toxicity. This conclusion was confirmed by the initial experiments which were done with E. fetida using the artificial soil and filter paper contact tests. The results from these experiments were compared with those that were

collected subsequently using E. fetida andrei, and showed clearly that the reproducibility of the tests was improved considerably by choosing this subspecies.

Experiments with the filter paper contact test indicated that the susceptibility of E. fetida to toxic chemicals was related inversely to the body weight of the earthworm, thus supporting the observations that were made previously with aquatic organisms (Wilson, 1975). Lebrun et al. (1981) reported a similar response with L. terrestris, although other studies with E. fetida have failed to find a relationship between the body weight of earthworms and their susceptibility to toxic substances (Heimbach and Edwards, 1983). I also discovered that the earthworms became more susceptible to the effects of chemicals as they began to mature sexually at a body weight of 0.3 - 0.4 g. Earthworms within this range of body weights reacted less predictably to chemicals in the toxicity tests, and the use of such earthworms should be avoided if possible. Large earthworms are easier to handle, and E. fetida that weighed between 0.4 - 0.6 g seemed to be the most suitable for use in the development of the toxicity tests. Furthermore, E. fetida within this weight range have been used in similar tests for toxicity (Roberts and Dorough, 1984). The EEC committee coordinating the development of these tests suggested initially that earthworms with a lower body weight should be used (0.3 - 0.6 g), because these could be cultured more rapidly in the laboratory and were of similar weight to the E. fetida used in other studies of toxicity (Bouche, 1982; Bouche, 1984a; Haque and Ebing, 1983a; Heimbach, 1985a, 1985b).

The first stage in the process that led to the development of methods for testing toxicity to earthworms that were suitable for general ecotoxicological applications, was to evaluate the efficiency of existing toxicity tests. Field experiments have many advantages when estimating the effect of chemicals upon natural populations of soil organisms, but within the context of statutory testing for toxicity to earthworms, this

type of test had several undesirable features. The soil environment can fluctuate according to the season and influence the activity and longevity of chemicals within it, as well as the behaviour and distribution of the earthworms (Evans and Guild, 1947, 1948; Gerard, 1967). Field experiments are often conducted over a long period of time and require a large area of land. The size of the trial site is dictated by the number of treatments that are used, the degree of replication and the minimum size of plot that is necessary to minimise the effect of earthworms migrating from one plot to another (Edwards and Brown, 1982). Thus, field experiments are expensive to conduct and give results from which it is difficult to predict the effects of a chemical upon another species of earthworm, or upon the same species of earthworm on a different type of soil. Experiments in the field are more difficult to standardise than those done in the laboratory, and ill-conceived attempts to achieve uniform conditions in a series of field trials may restrict the usefulness of such tests by allowing the unexpected effects of a chemical to pass unnoticed (Stanley, 1983). On the other hand, tests for toxicity in the laboratory may be standardised readily, although such methods can rarely account for all the environmental influences that affect the toxicity of chemicals in the field (Kenaga, 1975b).

The tests for toxicity to earthworms that were suitable for general use had to be rapid and inexpensive to operate without requiring special expertise. Such tests also needed to have the characteristics of a good bioassay and be sensitive, reproducible, simple to use and give a reliable indication of the relative toxicity of different chemicals to earthworms in the field. Most of these qualities were not present in the methods used to test toxicity to earthworms in the field directly, or in the methods that used a sublethal end-point or measured bioaccumulation. However, these qualities were found with acute LC_{50} tests for toxicity that had a well defined end-point and were conducted in the laboratory.

I reviewed the methods for testing the acute toxicity of chemicals to earthworms that existed before my own work. Many of these methods had been adapted from studies of toxicity using insects such as the cockroach, housefly and flour beetle. Such techniques included the application of the test chemical topically using a paintbrush or microapplicator, by dipping the insect in a solution of chemical, and by shooting droplets or spraying the chemical onto the insect in a spray tower or aerosol chamber. Insects have also been exposed to chemicals deposited on a variety of surfaces, mixed with food that was either ingested voluntarily or force fed, or dissolved in water in which the insect was immersed. Toxicity tests by immersion were considered to be suitable only for arthropods with an aquatic stage in their life history (Busvine, 1957).

Several methods for testing toxicity to earthworms possessed serious disadvantages. These methods were rejected without being evaluated experimentally. The rejected methods included those in which the test chemical was applied to the earthworm topically using a paintbrush (Aspock and An Der Lan, 1963), microapplicator (Fisher, 1984) or spray, or by dipping the earthworm in a solution of the test chemical (Stenersen, 1979a; Stringer and Wright, 1973), because chemicals applied topically were carried off the surface of the earthworm in a layer of mucus. The injection of chemicals into the body of the earthworm (Gilman and Vardanis, 1974; Nakatsugawa and Nelsen, 1972; Stenersen et al., 1973) caused direct injury and it was also difficult to place the test chemical accurately within the body (Stenersen, 1981). The disadvantage of studies that relied upon voluntary feeding (Stringer and Wright, 1973; Wright, 1977) lay in the difficulty with which antifeedant effects were distinguished from toxic effects. An electrophysiological method was developed to measure changes in the velocity with which the giant axons of earthworms conducted impulses when exposed to low concentrations of pesticides (Drewes and Callahan, 1985; Drewes, Vining and Callahan,

1984). Unfortunately this method required an elaborate apparatus and in common with the foregoing methods for testing toxicity, gave results that were hard to relate to the effects of chemicals upon earthworms in the field.

The more promising methods for testing toxicity to earthworms were evaluated experimentally. Such methods included an immersion test, in which the earthworms were placed in a solution of the test chemical, and contact tests where the test chemical was applied to a glass or filter paper surface over which the earthworms crawled. Other methods used media which were treated with chemicals and within which the earthworms could move. These media included sand, small glass beads (antibumping granules), a natural soil and a paste of fine silica and water supported on a matrix of glass balls. Chemicals were also fed forcibly to the earthworms.

The concentration-mortality data obtained using these methods for testing toxicity to earthworms, and also that collected subsequently from all the experiments which gave concentration- or time-mortality results, were processed by a computed probit analysis (Finney, 1971). These calculations allowed the LC_{50} (or LT_{50}), fiducial limits of the LC_{50} (or LT_{50}) and the gradient of the probit line to be determined. The gradient of the probit line indicated the range of concentrations of, or periods of exposure to, the test chemical within which a proportion of the population of the earthworms used in the test responded to that chemical. The gradient also provided an indication of the way in which chemicals act. These analyses allowed estimates of the toxicity of chemicals to earthworms obtained using the tests to be compared, although the techniques for testing toxicity to earthworms that appeared to be most suitable for further development were selected mainly from practical considerations. Suitable methods were required to give a well defined end-point with any chemical in a rapid and economical manner, and to be simple to use by

operators without previous experience of testing toxicity to earthworms.

I found that the immersion test gave a rapid and reproducible estimate of toxicity to earthworms, although it was difficult to produce stable and long-lived suspensions of many non-water soluble chemicals in water without using a formulating agent. This problem was encountered previously when similar types of toxicity test were used with insects (Busvine, 1957), although other reports that describe immersion tests with earthworms do not mention this difficulty (Ghabbour and Imam, 1967; Lebrun et al., 1981; Martin and Wiggans, 1959). Non-water soluble chemicals can be dispersed in water using formulating agents such as Teepol (a detergent), although my results indicated that the toxicity of this wetter to earthworms was, in part, additive to that of benomyl. To avoid the unpredictable effect that formulation can have upon the toxicity of chemicals to earthworms, I used technical or analytical grade chemicals in all the experiments and developed another method to disperse chemicals that would not dissolve in water. This method was adapted from that of McIntosh et al. (1981) and gave a stable dispersion of chemicals in water, by using a very small amount of an organic solvent and a detergent which had no measurable effect upon the earthworms. Although this method was valuable during the experimental evaluation of the efficiency of the toxicity tests, it was not included as a part of the tests for toxicity using earthworms that were adopted by the EEC.

The test method in which the chemical was applied to a glass surface over which the earthworms crawled did not work well. This type of test was developed originally to estimate the toxicity of oil-based formulations of insecticides to walking insects (Busvine, 1957); however the brittle deposits of chemicals which I used became redistributed within the vial by the movement of the earthworms. The redistributed test chemicals collected in a narrow band at the base of the vial, were less available to the earthworms and therefore appeared to have a lower toxicity than was indicated by other testing methods.

Supplementary experiments showed that the uptake of hexachlorobenzene by E. fetida was more rapid and complete from a deposit on glass than from one on filter paper. However, the benefits of using a filter paper surface which could be treated uniformly and easily with test chemical to give a deposit that remained stable, outweighed the disadvantage that, for certain chemicals, the assessment of toxicity was slightly less reproducible than that achieved on a glass surface.

My experiments showed that E. fetida died in media that consisted of sand or small glass pellets (antibumping granules), and so the assessment of the toxicity of chemicals dispersed on these materials was unreliable. Similar observations were reported by Ferriere et al. (1981), who found that E. fetida died rapidly in a matrix of glass balls, although further studies by this worker indicated that E. fetida would live in gravel for at least eleven days.

The pesticide-free sandy clay loam soil that was collected from Geescroft Field (Rothamsted Experimental Station), and used in the natural soil test also appeared to produce conditions that were unsuitable for E. fetida. Individual E. fetida lost weight rapidly during a seven day exposure to this natural soil, whilst L. terrestris, A. longa and A. caliginosa maintained or increased their body weight. Fayolle (1979) supported the opinion that E. fetida could not be used satisfactorily to test for toxicity in a natural soil, although other workers (Edwards, 1985; Ferriere et al., 1981) report that E. fetida can be used in this type of test by selecting a suitable soil. This incompatibility between E. fetida and the natural soil from Geescroft Field caused the chemicals that were tested in this medium to appear more toxic than when tested using other methods (e.g. the silica paste-glass ball test).

Despite the numerous studies in which toxicity to earthworms has been tested in natural soils (Caseley and Eno, 1966; Edwards and Jeffs, 1974; Edwards, 1985; Martin, 1982; Ruppel and Laughlin, 1977), none

produced results that could be compared directly with my own, because different species of earthworm, periods of exposure or test chemicals were used.

The advantage of using a natural soil as the medium for testing toxicity to earthworms in the laboratory rests in the ability of such a soil to reproduce aspects of the physico-chemical environment that are found in the field. Thus, chemicals incorporated into a natural soil should behave in a manner similar to that seen in field experiments on the same type of soil, and allow the effect upon natural populations of earthworms to be predicted accurately. The disadvantage of such tests is that it is difficult to standardise the type of soil that is used (Dean-Ross, 1983).

A test consisting of a fine silica powder mixed with a solution of test chemical and supported upon a matrix of 2 cm diameter glass balls (Bouche, 1982; Ferriere et al., 1981) gave sensitive and reproducible estimates of toxicity using E. fetida. The medium had an adsorptive capacity which was similar to that of an arable soil and was tolerated well by E. fetida. I concluded that this method had considerable potential for testing toxicity to earthworms. Recent studies have shown that this test can be been used successfully to estimate the toxicity of chemicals to earthworms, as a part of wider studies in ecotoxicology (Bouche, 1984a). However, this method had several disadvantages which prevented further development within the context of my studies. The test was laborious to perform and allowed only a single assessment of mortality, because the earthworms were separated from the medium at the end of the test by washing the silica through a sieve. In contrast to this, mortality in my artificial soil test could be assessed many times, which increased the likelihood that the resultant data would be suitable for probit analysis. Furthermore, the silica paste tended to acidify due to the accumulation of carbon dioxide released by the earthworms. This acidification occurred because the silica paste was unbuffered. The artificial soil contained

enough calcium carbonate to limit this effect and to allow the neutralisation of acidic test chemicals in a manner similar to that which occurs in the field.

Force feeding earthworms with an agar-agar gel containing the test chemical (Stringer and Wright, 1976) was a slow and difficult procedure, particularly after I had modified the apparatus for use with E. fetida, which is a small species of earthworm. A dilute solution of ethanol was used to anaesthetise the earthworms before feeding, but the anaesthetic affected the earthworms adversely and thus influenced the assessments of toxicity. This method was also unable to test the toxicity of thermolabile chemicals, because the agar-agar gel which acted as a vehicle for the test chemical was prepared by heating the ingredients to 60°C. This type of test has only been used to assess toxicity to earthworms by the authors who described it (Stringer and Wright, 1976; Wright, 1977) - an eloquent comment upon its practicality. This supported my decision not to develop the method further. However, this technique does offer certain qualities not found in the other tests. The forced feeding test gave an assessment of mortality based upon an individual dose of chemical, and was the only method in which toxicity could be assumed with confidence to arise from uptake over the gut alone.

These methods for testing toxicity to earthworms gave results that were necessarily expressed in a variety of units of concentration and were difficult to compare directly with each other. This difficulty is apparent when one attempts to interpret the existing literature that is concerned with the toxicity of chemicals to earthworms. I overcame this problem by comparing the order in which each test ranked the LC_{50} of the test chemicals. Those methods which gave a reproducible estimate of toxicity after a short period of exposure to the test chemical using a chemically inert medium (e.g. glass contact, filter paper contact, sand, glass granules and immersion in water), ranked the LC_{50}'s in a similar manner.

This ranking was dissimilar to that obtained using the tests of longer duration in which adsorptive or chemically reactive materials were used (e.g. natural soil, artificial soil, silica paste-glass balls). The latter tests were shown subsequently to give estimates of toxicity to earthworms which predicted accurately the effects of chemicals upon earthworms in the field. The reproducibility of the tests from both groups was similar, which indicated that each had been conducted to a uniform and high standard of laboratory practice.

Apart from the natural soil and silica paste-glass ball tests, the further development of which gave rise to the artificial soil test, the most useful method for testing toxicity to earthworms was that in which the earthworms were exposed to a deposit of the test compound supported on a chemically inert surface. Thus, a contact test for toxicity to earthworms was developed using filter paper.

The filter paper contact method consisted of a small glass vial, lined internally with a moist filter paper treated with test chemical, containing one E. fetida and sealed with an airtight lid. I found that this method was capable of testing the toxicity of any chemical to earthworms and gave a sensitive and reproducible result rapidly. My decision to develop this method further was supported by others who used this type of test for toxicity successfully with earthworms (Reinecke and Nash, 1984; Roberts and Dorough, 1984) and insects (e.g. Busvine, 1957; Busvine and Nash, 1953; Yokoyama et al., 1984).

The filter paper contact test seemed unlikely to be able to predict accurately the toxicity of chemicals to earthworms in the field. This deficiency was overcome by developing another test that could be used in parallel with the filter paper contact test.

The complementary method combined the advantages of the silica paste-glass ball test (i.e. a standardised medium that was acceptable to E. fetida) with those of a natural soil test (which could reproduce

approximately the route of exposure [Fisher, 1984] and conditions found in an arable soil). An artificial soil was designed that showed many of the characteristics of an arable sandy loam soil (including similar mineral components, pH and cation exchange capacity) and contained fine quartz sand, kaolinitic clay, peat, calcium carbonate and water. The artificial soil supported E. fetida for an extended period of time when held in a transparent plastic flask with a non-airtight lid, although a relatively high content of organic matter and, therefore, of moisture in the soil was necessary to keep E. fetida healthy. The artificial soil test was designed to give an estimate of the toxicity of chemicals to earthworms in the field.

The filter paper contact and artificial soil tests were developed in the same way, by a process in which aspects of the experimental procedure of a prototype method were varied individually. The optimum conditions for testing toxicity to earthworms defined by this process were then incorporated into the design of the test.

I found that the assessments of the toxicity of chemicals to earthworms made using the filter paper contact test were affected by the temperature, period of exposure and type of filter paper used (together with the amount of moisture held within it), the ventilation and illumination of the glass vial and the use of formulating agents. The area of filter paper that was used to line vials of various sizes had no effect upon the assessment of toxicity, and the number of replicates used at each concentration of the test chemical had little influence upon the magnitude of an LC_{50}, but did affect its reproducibility.

The results obtained using the artificial soil test were affected by the temperature and period of exposure of the test, together with the moisture and organic matter content of the soil. The toxicity of chemicals was unaffected by the pH and clay content of the soil, the amount of soil used in each test vessel, and the method by which the test chemicals were applied to the soil. The number of replicates affected the reproducibility of

an LC_{50}, but not its magnitude. I concluded that the artificial soil test gave an estimate of the toxicity of chemicals to earthworms that was unaffected by most changes in the environment of the test and subsequent studies using this method, conducted by other workers, have supported this conclusion (Heimbach and Edwards, 1983).

Before discussing in greater detail those aspects of the experimental methods that did influence the assessment of toxicity in these tests, I will consider briefly the conditions that had little or no effect upon the estimation of an LC_{50}. In each case, I will consider the condition within the filter paper contact test together with the equivalent condition from the artificial soil test, in order to draw a comparison between them.

The area of filter paper that lined the glass vial used in the filter paper contact test and the quantity of soil held in each test vessel used in the artificial soil test did not affect the assessment of toxicity, although a large area of filter paper and a large volume of soil were more difficult to treat uniformly with the test chemicals than a smaller area or volume. For the filter paper contact test I decided to use 75 cm^2 of filter paper, because this held one ml distilled water when saturated to capacity, humidifed the air in the vial adequately and fitted the internal side walls of a type of vial that was available easily within Europe. The quantity of artificial soil used in each test vessel had to be sufficient to allow each of the earthworms within the medium to die independently (so that the results could be processed by probit analysis [Finney, 1971]). Since the test chemical could be metabolised within the gut of the earthworm, the proportion of the total amount of soil within each container that was ingested by the earthworms during the period of the test was kept to a minimum. The quantity of soil ingested was calculated using the estimates of gut load and gut transit time reported by Hartenstein et al. (1981) and Morgan (1984). These limitations were overcome by using 750 g (wet weight) of artificial soil in each container. This amount of soil could be

treated uniformly with test chemical and handled easily, thus minimising the cost of conducting the test.

A standardised amount of water, that almost saturated the filter paper to capacity, ensured maximal contact between the earthworm and the test chemical, and gave a reproducible rate of volatilization of the test chemical from the moisture film that surrounded the filter paper (Briggs, 1981; Gantz and Slife, 1960). Similarly, the artificial soil was moistened almost to field capacity. Standing water was undesirable in both tests because this allowed water soluble chemicals to become distributed unevenly within the test medium and caused mortality in the controls.

The estimate of an LC_{50} of a test chemical became more precise as the number of replicates used at each concentration of the chemical increased. These estimates became considerably more accurate when ten or more replicates (each containing one E. fetida) were used in the filter paper contact test, and four or more replicates (each containing ten E. fetida) were used in the artificial soil test. These levels of replication seemed to represent the minimum that was compatible with obtaining an accurate estimate of the LC_{50}, and since additional replication would make the test more expensive to operate, these numbers of replicates were apparently optimum.

The toxicity of chemicals to earthworms was found to be correlated positively with temperature in the filter paper contact and artificial soil tests, and these observations were supported by previous studies (Harris, 1972a; Hoffman and Lindquist, 1949: Wilson, 1975). This effect can be explained in terms of the decreased adsorption of the test chemical onto soil particles, increased volatilization from the moisture film surrounding these particles and increased activity of the earthworms (leading to greater exposure to the test chemical [Busvine, 1957]) which occur at higher temperatures. High temperatures can affect the earthworms adversely (Kapan et al., 1980) and hasten the decomposition of the test chemical,

whilst low temperatures can also have a detrimental effect upon the earthworms and necessitate a long period of exposure in order to obtain a reproducible assessment of toxicity. As an effective compromise between these considerations, the filter paper contact and artificial soil tests were done at 20° ± 2°C. This temperature allowed a reliable end-point to be obtained within a convenient and short period of exposure, and was similar to the temperature chosen subsequently for testing toxicity to earthworms in other studies (Anon., 1981c; Anon., 1983; Haque and Ebing, 1983a; Heimbach, 1984).

The period of exposure that was used in the filter paper contact and artificial soil tests was correlated positively with the toxicity of chemicals to earthworms. The optimum duration for the filter paper contact test was 48 hours, which minimised the variability of the results, avoided mortality in the controls and prevented the test from becoming uneconomic. This test duration was somewhat shorter than that recommended for similar acute toxicity tests with aquatic organisms (Besch, 1976b), but falls within the range of the periods of exposure used frequently to test for toxicity to insects, which extends from 24 hours (Morrison, 1950) to 140 hours (Parkin, 1951). The artificial soil test proved to have an optimum period of exposure of 14 days, because the assessments of toxicity to earthworms that were obtained using a test duration of less than 14 days were highly variable, and seven day tests with earthworms using soil in the laboratory were found to be unreliable (Fayolle, 1979; Martin, 1982). Long periods of exposure are undesirable because the tests become expensive to conduct, allow the test chemical to decompose and often cause mortality in the controls. Such mortality can be avoided in soil for up to 28 days by adding food (Anon., 1981c; Haque and Ebing, 1983a; Heimbach, 1984; Karnak and Hamelink, 1982), although such foods often consist of unstandardised materials such as animal dung which can make artificial media less easy to standardise and the results more variable. A 14 day

period of exposure was chosen, because this avoided the need to provide food, minimized the problems that were associated with very short or long duration tests, and fitted conveniently into a weekly working schedule.

Other factors that affected the assessment of toxicity using the filter paper contact and artificial soil tests included the illumination and ventilation of the test vessels. The filter paper contact test appeared to give a more sensitive assessment of toxicity when done in darkness, because this stimulated the activity of the earthworms and increased the contact that they made with the test chemical. This effect has also been observed with insects (Busvine, 1957), and as darkness minimized the photodecomposition of the test chemical and avoided the need for a constant environment cabinet fitted with uniform lighting, an unilluminated test was recommended. The artificial soil test performed best when illuminated, because the negative phototaxism displayed by E. fetida overcame the repellent effect that some test chemicals had upon the earthworms.

My results showed that the earthworms were slightly more susceptible to the effects of chemicals in ventilated, rather than airtight vials, although other workers report that a limited amount of oxygen increased the susceptibility of organisms to chemicals (Besch, 1976b; Stenersen, 1981). E. fetida is known to become less active under adverse conditions, and because of this can appear to be more tolerant of them (Satchell and Dottie, 1984). Thus the conditions within the airtight vials may limit the activity of the earthworms to cause the decrease in susceptibility that I observed. By stimulating the activity of the earthworms in an airtight vial, results similar to those of Stenersen (1981) may be achieved. Results obtained using the filter paper contact test can probably be used to predict the effects that chemicals have upon earthworms after a longer period of exposure, in a manner similar to that reported for other tests for toxicity in which oxygen was restricted (Stephenson, 1984).

Furthermore, an airtight vial allowed the toxicity to earthworms of volatile chemicals to be tested reproducibly. It was not possible to use a sealed test vessel for the artificial soil test, because this caused significant mortality in the controls. The minimal loss of volatile test chemical that possibly occurred through the semi-airtight lid did not seem to affect the results.

An experimental variable specific to the filter paper contact test was the choice of material from which the filter paper was made. Glass fibre and cellulose filter papers gave estimates of toxicity to earthworms which appeared to correlate negatively with the capacity of the paper to absorb water. The least absorbent paper that was tested (Whatman No.1) gave the most sensitive assessment of toxicity, which was due presumably to the increased contact that occurred between the earthworm and the test chemical.

Certain aspects of the experimental method using the artificial soil similarly had no equivalent in the filter paper contact test. These included the characteristics of the soil, such as the pH, the amount of organic matter and kaolinitic clay present and the moisture content of the soil, as well as the method by which the test chemical was incorporated into the soil.

The pH of the soil had very little effect upon the assessment of toxicity to earthworms. This was unexpected because previous work had shown that pH influenced the adsorptive capacity of soils (Edwards, 1974), the ionisation of chemicals within the soil (Bailey and White, 1964), the rate at which earthworms accumulated chemicals (Lofs-Holmin, 1981; Stringer and Wright, 1976) and the ability of micro-organisms in the soil to break down chemicals (Torstensson, 1975). Thus the pH of the artificial soil could be selected using criteria other than the effect that it had upon the physico-chemical behaviour of the test compound. A soil with a pH of 6.5 ± 0.5 was chosen (with an optimum pH of 6.8), because this was most

acceptable to E. fetida (Kaplan et al., 1980) and could be produced by using a small amount (1% dry weight) of calcium carbonate. (A large amount of calcium carbonate was undesirable, as supplementary experiments showed that this compound could react chemically with acidic test compounds to give materials that were less toxic to earthworms.) A soil within this range of pH resembled that found in many arable soils, and limited the effect of extreme acidity or alkalinity upon the toxicity of chemicals to earthworms.

My results showed that several test chemicals became less toxic to earthworms as the amount of organic matter in the soil was increased. This effect has been reported in other studies of toxicity using earthworms (Caseley and Eno, 1966), and was probably due to the adsorption of these chemicals onto the organic matter (Edwards, 1974; Harris, 1966; Harris, 1972a; Hassall, 1982). To maximise the sensitivity of the test, I chose the minimum amount of organic matter (8% dry weight) that was necessary to prevent mortality of E. fetida in the controls during the 14 day period of exposure.

The capacity of the soil to retain water was largely a function of the amount of organic matter present, and measurements clearly demonstrated that this capacity was not affected appreciably by the presence of kaolinitic clay. E. fetida lost body weight rapidly through dessication in soils that contained less than 10% water and appeared to grow best in a soil that contained more than 40% water. Chemicals that were water soluble were less toxic to earthworms in soils that contained a small amount of water. This observation was supported by other work showing that many chemicals adsorb strongly onto dry soil (Bailey and White, 1964; Harris, 1964) and become unavailable to earthworms. The toxicity to earthworms of the non-water soluble chemicals appeared to be relatively unaffected by the amount of moisture in the soil and, therefore, the optimum conditions for testing toxicity to earthworms, which maximised the contact

between the earthworm and the test chemical, seemed to be produced by a soil that was almost saturated and contained 48% water. (The maximum water holding capacity of the soil was 50%).

The amount of kaolinitic clay (a non-expanding clay) present in the soil had little influence upon the assessments of toxicity to earthworms. This type of clay was used in the artificial soil because it had a low adsorptive capacity (Weber and Weed, 1974). However, clays (of an unspecified type) have been reported to reduce the toxicity of chemicals to earthworms in other studies using soil (Lofs-Holmin, 1980). The low adsorptive capacity of kaolinite was an especially useful property, because the organic matter in the soil alone had an adsorptive capacity equal to that of many natural arable soils. Montmorillonitic clays are more common in European soils than kaolinites (Brown, 1974), but montmorillonites had a cation exchange capacity which was sufficiently high (Bailey and White, 1964; White, 1976) to preclude their use in an artificial soil that contained a lot of organic matter. Kaolinitic clay constituted 20% (dry weight) of the artificial soil, which reproduced approximately the amount of clay present in a natural sandy loam soil (Avery, 1980).

Three methods for applying the test chemical to the artificial soil were evaluated. These included the application of a solution of the chemical to the soil using a spray or a drench followed by the incorporation of the resultant deposit manually, or the incorporation of the chemical into the soil manually without using solvents. A suitable method was one capable of distributing the test chemical within the soil uniformly in a rapid, simple and economical manner. My experiments with these techniques were done under rigorous conditions and the results indicated that the method of application of the test chemical did not affect the assessment of toxicity significantly. However, the use of these methods under less rigorous conditions would be likely to show differences between them in the reproducibility of the results.

The method of application of the test chemical to the soil was selected using aspects of its performance other than those mentioned above. The test chemicals often needed to be dissolved in an organic solvent before they were applied to the soil as a spray (Edwards and Jeffs, 1974) or a drench (Davis, 1971; Heimbach, 1984). Such solvents are difficult to remove from the soil, can increase the amount of chemical adsorbed onto the soil (Wiese, 1964) and may interact directly with the test chemical (Burrell and Corke, 1980). These difficulties were minimised by using a spray generated by a chromatographic atomiser. The spray consisted of a small volume of organic solvent that did not penetrate the superficial layers of the soil and had, therefore, a limited interaction with it. Furthermore, the removal of solvents applied to the soil in this way was achieved rapidly and efficiently by placing the treated soil in a gentle airflow for five minutes. This technique of application had to be used carefully to prevent the loss of test chemical through misapplication or spray drift, but it was easy to use and capable of giving reproducible results. Chemicals that were drenched into the soil were more likely to be affected by the interactions between the organic solvent and the soil than were chemicals applied as a spray. Organic solvents drenched into the soil were subsequently removed with difficulty, although this type of method was used successfully to apply the chemicals to the filter paper in the contact test. The incorporation of chemicals into the soil without using solvents was laborious, particularly when these chemicals were viscous liquids such as technical chlordane. This method had the advantage of permitting the application of chemicals for which a suitable solvent could not be found, and had been used successfully in previous tests for toxicity to earthworms in soil (Anon., 1981c; Haque and Ebing, 1983a; Heimbach, 1984; Hopkins and Kirk, 1957). The most appropriate method for use in the artificial soil test was that in which the chemical was applied as a spray, although the option of incorporating the chemical into

the soil directly was retained for those compounds that would not dissolve in suitable solvents. The use of additional chemicals to disperse the test materials in water interfered with the assessments of toxicity to earthworms that were made using the filter paper contact test. Therefore, the use of formulating agents was avoided in the methods of application used in both the filter paper contact and the artificial soil tests.

These investigations made it possible to design a methodology for the filter paper contact and artificial soil tests which optimised each aspect of the experimental procedure.

The optimum method for the filter paper contact test using E. fetida consisted of a transparent soda glass vial measuring 8 cm x 3 cm diameter, the side walls of which were lined internally with moist Whatman No.1 cellulose filter paper. The filter paper was treated by applying the test chemical in water or a suitable volatile organic solvent. These organic solvents were evaporated using compressed air, and the deposit of the test chemical that remained on the filter paper was moistened with one ml distilled water. Each vial held one earthworm and a minimum of ten vials were used at each concentration of the test chemical. The vials were stored in the dark at 20° ± 2°C for 48 hours, after which mortality was assessed mechanically by prodding the earthworm with a spatula and recording death in the absence of a twitch. This filter paper contact test produced sensitive and reproducible estimates of the toxicity of chemicals to earthworms quickly and cheaply, and the results that I obtained agreed closely with those reported from other studies that used my method (Roberts and Dorough, 1984). The filter paper contact test is also finding applications in research (Drewes et al., 1984).

The best methodology for the artificial soil test used a soil which consisted of 71% fine quartz sand, 20% kaolinitic clay, 8% finely milled and airdried sphagnum peat with 1% calcium carbonate. This soil had a pH of 6.8 and a cation exchange capacity of 162 meq.kg^{-1} at a moisture content

of 48%. Four replicates were used at each concentration of the test chemical and each replicate consisted of ten earthworms held in 750 g (wet weight) soil contained in a one litre plastic flask with a ventilated lid. The soil was treated with the test chemical by spraying a solution onto the soil and incorporating the deposit manually, or by incorporating the chemical manually without the use of solvents. The number of earthworms that had died was estimated after 14 days and the exposure took place at 20° ± 2°C in the light. This method is now used extensively to test for the toxicity of chemicals to earthworms (Anon., 1983; Edwards, 1985; Heimbach, 1984, 1985a, 1985b; Inglesfield, 1984).

Confirmation that my methods for testing toxicity to earthworms were suitable for general ecotoxicological purposes came after they had been evaluated critically by many independent, collaborating laboratories worldwide. Two surveys were done in which the testing protocol, three unidentified chemicals and one reference compound were circulated to each participant, who was asked to estimate the LC_{50}'s of the chemicals. The assessments of toxicity, advice and criticism that were received from the participants allowed the methods to be refined into the form that was adopted by the EEC to test the toxicity of industrial chemicals to earthworms. These improvements to the methods included the use of E. fetida andrei rather than E. fetida (E. fetida andrei is usually more susceptible to the effects of chemicals than E. fetida fetida), and that the artificial soil should contain less organic matter (decreased from 10% to 8% dry weight), less calcium carbonate (decreased from 2% to 1% dry weight), a lower pH (decreased from 7 to 6.5 ± 0.5) and a higher moisture content (increased from 35% to 48% wet weight). The improved methods, which were used in a second survey, gave results that were less varied than those obtained during the first study. Furthermore, the reproducibility of the results that were produced by the filter paper contact test was better than that obtained from either the artificial soil or the silica paste-glass

ball tests, although both the filter paper contact and artificial soil tests proved to be sufficiently accurate to test for toxicity to earthworms reliably.

Originally these tests for the toxicity of chemicals to earthworms were designed to be used in series with the filter paper contact test used as a preliminary screen. During the process of development it became clear that the filter paper contact test was unable to indicate all those chemicals which were potentially toxic to earthworms and which should be tested further with the artificial soil test. This difficulty arose because the filter paper contact test gave estimates of the toxicity of chemicals to earthworms that were quite dissimilar to those obtained using the artificial soil test. Thus, the filter paper contact test was unable to estimate the toxicity of a chemical in terms of a 'threshold' LC_{50} which, if exceeded by the LC_{50} of a test chemical, would indicate that this particular chemical should be tested further in the artificial soil test. Furthermore, the concepts of 'threshold' values and 'safe' concentrations in the assessment of toxicity have been found to be unsatisfactory in other studies (Wilson, 1975).

The differences between the assessments of the toxicity of chemicals to earthworms obtained from the filter paper contact and artificial soil tests arose from the differences that existed between the conditions within each test (the media, ventilation of the test vessel and illumination) and the period of exposure.

The interaction of the test chemicals with the media used in the tests affected the toxicity of those chemicals to earthworms. Such an interaction was seen clearly with copper sulphate and trichloroacetic acid, which were very toxic to earthworms in the filter paper contact test, but of only low toxicity in the artificial soil test. Copper ions can be adsorbed onto the soil particles (McLaren et al., 1981; Stiff, 1971) and become unavailable to the earthworms, whilst trichloroacetic acid reacts with alkaline elements in the soil, to form products that are less toxic to earthworms than the original material.

The period of exposure can also affect the assessment of the toxicity of chemicals to earthworms. This was shown clearly when I tested the toxicity of the benzimidazole fungicides to E. fetida. These fungicides act slowly upon earthworms (Stringer and Wright, 1976) and were much more toxic in the long duration artificial soil test (and the silica paste-glass ball test) than in the shorter duration filter paper contact test (and immersion test).

The results from the artificial soil test and the silica paste-glass ball test were found to be similar, yet unlike those obtained using the filter paper contact test. Subsequent work (Heimbach, 1984, 1985b) has supported these observations and shown that the artificial soil and the silica paste-glass ball tests give results that correlate highly (r=0.91), but that these assessments of toxicity correlate poorly (r=0.55 and 0.48 respectively) with those obtained using the filter paper contact test. This evidence confirmed that the filter paper contact and artificial soil tests should be used in parallel to give complementary assessments of toxicity, rather than in series.

A test for the toxicity of chemicals to earthworms used in the laboratory should give results from which the effect of a chemical upon populations of earthworm in the field can be predicted. The effect of a chemical in the field can be influenced by the behaviour of the earthworms and their ability to reproduce (species living near the surface of the soil often reproduce rapidly to compensate for the high mortality they experience due to predation [Bouche, 1984a]), the season of the year (season affects the abundance and activity of earthworms [Edwards and Lofty, 1977; Evans and Guild, 1947, 1948]) and the physico-chemical properties and micro-flora of the soil (chemicals may be adsorbed and inactivated on organic matter and clay [Edwards, 1974], leached or evaporated from the soil [Weber, Weed and Sheets, 1972] and decomposed by micro-organisms or radiation [Weber and Weed, 1974]). The efficiency

with which tests for the toxicity of chemicals to earthworms in the laboratory predicted toxicity to earthworms in the field was evaluated by comparing the results obtained from tests done in the laboratory with those from field experiments using the same chemicals.

Two field experiments were set up at Rothamsted and Sittingbourne on soils containing 5.1% and 6.9% organic matter. The chemicals had been tested for toxicity to earthworms previously in the laboratory and included chlordane, carbaryl, thiophanate-methyl, triazophos and pentachlorophenol. Each chemical was applied at two rates and the effect of these treatments upon the population of earthworms was assessed using the formalin expellant method after one and six months post-treatment. Sampling methods using baits (Lofs-Holmin, 1979), handsorting or an electric current (Edwards and Lofty, 1975) were inadequate for this type of experiment. The results were processed using a computed analysis of covariance.

The two field experiments showed that the largest number of earthworms was collected during the autumn and that L. terrestris was active at both of the sampling dates, whilst A. longa and A. caliginosa were found infrequently in the untreated plots during late spring. These observations were supported by previous studies of earthworm ecology (Edwards and Lofty, 1977; Evans and Guild, 1947, 1948; Gerard, 1967).

Occasionally the weights of the earthworms in a plot showed a different response to the treatments from that seen for the numbers of earthworms. This effect may have been caused by the adult earthworms responding to the chemicals in a different way from the juvenile earthworms. Juvenile earthworms can be more susceptible than adults to certain chemicals in the filter paper contact test, and these results were confirmed by other workers (Lebrun et al., 1981).

The numbers of earthworms in plots that had been treated with large doses of toxic chemicals sometimes exceeded those in the untreated plots.

Several explanations for this anomalous observation exist. Chemicals may have a herbicidal effect which increases the amount of rotting vegetation available to the earthworms as food, and which compensates for the toxic effect of the treatment (Edwards, Lofty and Stafford, 1971). Other workers (Edwards and Brown, 1982) have suggested that an increase in the number of earthworms could be due to young earthworms that survive the application of the treatment as a cocoon hatching subsequently into an environment in which they face little competition. Furthermore, earthworms may immigrate from the soil which is adjacent to the treated area once the toxic chemical has been degraded, and also exploit the non-competitive environment that exists (Hoogerkamp et al., 1983).

Chlordane decreased the number of earthworms severely at both field sites and was very toxic to earthworms, which agrees with the conclusions of other studies (Doane, 1962; Legg, 1968; Lidgate, 1966). Carbaryl was also extremely toxic to earthworms in these experiments, which confirmed the observations of Edwards and Thompson (1969), Legg (1968) and Thompson (1971). Chlordane and carbaryl are sold commercially as vermicides. The action of chlordane was more limited in the soil which contained the most organic matter at Sittingbourne. The decrease caused by carbaryl in the numbers of earthworms lasted for one month, and such a non-persistent action was also reported by Legg (1968). It is unclear whether the rapid return of the population of earthworms treated with carbaryl to a size that was similar to that of the untreated population was due to a rapid immigration of earthworms, or to the recovery of the earthworms in the treated area from the paralysis that carbaryl can induce at low concentrations in the soil (Fisher, 1984; Stenersen, 1979a). Such paralysis could account for these observations in the field, but this speculation must be treated with caution. Chlordane continued to kill earthworms for six months (confirmed by Legg, 1968), due to the long half life of this chemical in the soil (Hassall, 1982).

My results showed that thiophanate-methyl and triazophos were moderately toxic to earthworms in the field, although earthworms were almost unaffected by pentachlorophenol. These observations contrast with those from other field studies which claim that thiophanate-methyl was very toxic to earthworms in the field and the laboratory (King and Dale, 1977; Stringer and Lyons, 1974; Stringer and Wright, 1973; Wright and Stringer, 1973) and that triazophos (untested in the field) was also very toxic to earthworms when tested in soil in the laboratory (Haque and Ebing 1983a). Studies of the effect of pentachlorophenol upon earthworms in a woodland soil (Rombke, 1984) have given results that agree with my own and indicate a low toxicity of this chemical to earthworms.

The toxicity of thiophanate-methyl, triazophos and to a lesser extent pentachlorophenol to earthworms persisted for six months, although in the absence of analyses for pesticide residues in the soil, it is not possible to determine whether this was due to the mortality suffered by the earthworms initially, or whether the chemicals persisted for six months in the soil.

The experiments at Rothamsted and Sittingbourne showed that A. caliginosa was the most susceptible species to the test chemicals and that A longa and L. terrestris were less sensitive. Such results agree with those reported from other field experiments (Lofs-Holmin, 1982a; Stringer and Lyons, 1974, 1977). These species are ranked in an order of susceptibility to toxic chemicals which is usually the same as that found in experiments done in the laboratory by me and other independent workers (Edwards, 1985).

Few quantitative comparisons have been made between the assessments of the toxicity of chemicals to earthworms made in the laboratory and in the field. One way of relating the results from several dissimilar types of toxicity test conducted in the laboratory, with assessments of the toxicity of chemicals to earthworms made in the field, is by using the order in

which the LC_{50}'s of the test chemicals are ranked by the tests in the laboratory, and the ratio of these LC_{50}'s to that of chlordane. Chlordane was chosen as the reference chemical because it had a toxicity to earthworms that was consistent, whether assessed using tests in the laboratory or in the field. I did not use chloroacetamide as a reference compound although this chemical has been used previously as a standard in tests for toxicity to earthworms (Inglesfield, 1984), because this chemical decomposes very rapidly in field soils (Heimbach, 1982) and does not therefore, have a reproducible effect upon populations of earthworms. The assessments of toxicity to earthworms interpreted in this way show that the artificial soil, silica paste-glass ball and fine quartz sand tests predicted correctly the severe toxicity of chlordane and carbaryl to earthworms in the field, and the low toxicity of pentachlorophenol. These results therefore confirm my hypothesis, and the subsequent speculation of Dean-Ross (1983), Fisher (1984) and Heimbach and Edwards (1983), that a test for the toxicity of chemicals to earthworms using an artificial soil was the most likely to be capable of predicting accurately the toxicity of chemicals to earthworms in the field. The immersion test (in which the earthworms were immersed in a solution of test chemical) and the filter paper contact test identified correctly the toxicity of most of the test chemicals to earthworms in the field, but underestimated the toxicity of certain chemicals. These observations were also confirmed by those of Dean-Ross (1983), from which it follows that the latter methods have only a limited application to general programmes for testing toxicity to earthworms. The tests using a natural soil or forced feeding in the laboratory gave the poorest prediction of toxicity to earthworms in the field. This deficiency of the natural soil test arose from the inability of E. fetida to tolerate the sandy clay loam that was used. The forced feeding test failed to predict accurately the toxicity of chemicals to

earthworms in the field, as very few species are exposed naturally to chemicals at the surface of the gut alone. The forced feeding test was designed for, and is used most appropriately with, L. terrestris which feeds upon litter (Stringer and Wright, 1976) and is therefore likely to take up chemicals naturally from the gut.

These data can be presented by an alternative method which clarifies the relationship between assessments of the toxicity of chemicals to earthworms made in the laboratory and the field. The LC_{50}'s of the test chemicals and the ranking of the toxicity of these chemicals estimated using laboratory tests for toxicity (Table 9.1), were compared with an index of toxicity constructed from the data for all the species of earthworm tested using the same chemicals in the field (Table 9.2).

Those chemicals that were very toxic to earthworms after six months in the field had LC_{50}'s that were estimated to be less than 50 $mg.kg^{-1}$ by the artificial soil and silica paste-glass ball tests. Carbaryl was very toxic to earthworms in the field after one month at the highest rate of application, but would only be included in the category that contained the toxic chemicals by adopting an upper LC_{50} for this category of approximately 90 $mg.kg^{-1}$ for the artificial soil test or 120 $mg.kg^{-1}$ for the silica paste-glass ball test. Chemicals with an LC_{50} greater than 90 $mg.kg^{-1}$ or 120 $mg.kg^{-1}$ respectively in these tests can, on the basis of these data, be assumed to have no detrimental effects upon earthworms in the field.

Table 9.1. The toxicity of chemicals to earthworms assessed using tests done in the laboratory.

Method and units in which the LC_{50} is expressed	Toxicity of chemicals to earthworms expressed as a ranking of toxicity and (in brackets) as the LC_{50}						
	Exposure period	Chlordane	Carbaryl	Pentachloro-phenol	Chloro-acetamide	Thiophanate-methyl	Triazophos
Immersion $mg.kg^{-1}$ (Test chemical:water)	1 day	2 (8.6)	3 (25.2)	1 (0.7)	4 (81.8)	6 (754.7)	5 (109.0)
Filter paper contact $\mu g.cm^{-2}$ (Test chemical:filter paper surface)	2 days	1 (0.6)	5 (24.2)	3 (5.3)	2 (2.9)	6 (539.5)	4 (16.9)
Silica paste-glass ball $mg.kg^{-1}$ (Test chemical:dry weight of silica paste)	14 days	1 (23.9)	3 (112.3)	5 (277.0)	4 (183.5)	2 (44.6)	-
Sand $mg.kg^{-1}$ (Test chemical:dry weight of sand)	2 days	1 (97.0)	-	3 (882.7)	-	2 (118.0)	-
Artificial soil $mg.kg^{-1}$ (Test chemical:dry weight of soil)	14 days	4 (27.0)	6 (85.7)	5 (45.5)	2 (18.4)	3 (20.1)	1 (10.5)
Natural soil $mg.kg^{-1}$ (Test chemical:dry weight of soil)	14 days	1 (0.01)	4 (43.1)	2 (0.01)	5 (43.30)	3 (0.95)	-
Forced feeding $mg.kg^{-1}$ (Test chemical:wet weight of agar-agar gel	6 days	1 (4.9)	-	-	-	2 (93.0)	-

Table 9.2. The toxicity of chemicals to earthworms assessed using tests done in the field

		Toxicity of chemicals to earthworms expressed as a toxicity index. 1 = very toxic; 4 = non-toxic									
Chemical		Chlordane		Carbaryl		Pentachlorophenol		Chloro-acetamide	Thiophanate-methyl		Triazophos	
Rate of Application $kg.ha^{-1}$		5	10	2.5	25	12.5	75	50	3	6	3	6
	Exposure period											
Effect upon the number of earthworms	1 month	4	2	3	1	4	3	-	3	3	3	3
	6 months	3	1	3	4	4	4	4(a)	4	4	4	3

(a) Data obtained from Edwards (1985), the other data were collected from the field experiments described previously at Rothamsted and Sittingbourne.

The artificial soil test gave LC_{50}'s for the test chemicals that were lower than those obtained using the silica paste-glass ball test. Thus the artificial soil test provided the most sensitive indication of the toxicity of chemicals to earthworms, and reduced the risk of a toxic chemical falling into the category of substances classified as harmless to earthworms.

The data obtained with the filter paper contact test are less easy to interpret in terms of an index of toxicity to earthworms in the field. This test indicated that most of the chemicals showing a detrimental effect upon earthworms in the field gave LC_{50}'s that were less than 25 $\mu g.cm^{-2}$. The exception to this was thiophanate-methyl which gave an LC_{50} of 755 $\mu g.cm^{-2}$ and was moderately toxic to natural populations of earthworms. Thus, it would seem unwise to propose that chemicals with an LC_{50} of greater than 25 $\mu g.cm^{-2}$ in this test would be harmless to earthworms in the field.

Whilst guidelines such as these can be applied to the data that are generated by the artificial soil and silica paste-glass ball tests, such an interpretation of the assessments of toxicity to earthworms for chemicals that are used in the field at high rates of application should be considered with caution, because the statement of Paracelcus in the 16th Century that 'it is only the dose that makes the poison' remains valid.

The ratio between the LC_{50}'s for test chemicals estimated using the artificial soil test, and the minimum (toxic) concentration (MTC) that has a detrimental effect (>toxicity index 3) upon earthworms after one month post-treatment in the field (MTC/LC_{50}), falls between 0.03-0.82 (if the extreme value of 0.82 [that occurs when these calculations include the data obtained with pentachlorophenol, which had very little effect upon earthworms when applied in the field at 75 $kg.ha^{-1}$] is discounted, the ratios fall within the range 0.02-0.19). This evidence suggests that the range of the rates of application at which chemicals become toxic to earthworms in the field can probably be predicted by multiplying the LC_{50}

of that chemical, estimated using the artificial soil test, by 0.03 and 0.82. These calculations remain an approximation and the ratios are smaller than those obtained from previous studies with insects. The estimates of an ED_{90} (the 'effective' dose, i.e. that dose which has an effect upon 90% of the test organisms) made in the laboratory using insects were multiplied by 3 (Williams, 1973) or by 4-12 (Haverty and Robertson, 1982) to obtain the rate of application required for effective control of these pests in the field. The amount of chemical that was needed to control insects in the field was higher than that required in the laboratory (the converse of the observations made using earthworms), because much of the chemical did not reach the insect (the larvae were sheltered from the spray, or the spray ran off the leaf surface) and the deposit was washed off the plants by rain or decomposed by sunlight. However, some chemicals persist in soil for a long time in a form that is available to earthworms, and thus can have an effect at a lower concentration than that used in short duration tests in the laboratory.

A mathematical relationship exists between the toxicity ratios (the ratio between the effective concentration of a test chemical and that of a standard chemical) calculated from the results of assessments of toxicity made in the laboratory, and for those made in the field. This technique was used successfully to process the extensive toxicity data that were available for certain insects (Sun, 1950, 1966), but could not be applied to the more limited information available for earthworms.

The methods of testing the toxicity of chemicals to earthworms by the filter paper contact and artificial soil tests have been published (Goats, 1983; Goats and Edwards, 1982, 1983) or are in press (Goats and Edwards, 1985), and allow a standardised data base to be formed from independent studies of toxicity using earthworms. Previously, such studies have been of limited use because the data were collected using a variety of inadequate methods for testing the toxicity of chemicals to

earthworms, and also the standard of the experimental procedures and the subsequent reporting was poor (Lofs-Holmin and Bostrom, 1985). The methods which I developed have been adopted in slightly different forms by the EEC and OECD, and both the filter paper contact and artificial soil tests (or slightly modified versions of them) are now used widely to test for toxicity to earthworms (Anon., 1981c; Anon., 1983; Edwards, 1985; Haque and Ebing, 1983a; Heimbach, 1984, 1985a, 1985b; Inglesfield, 1984; Roberts and Dorough, 1984).

Although the assessment of chronic toxicity to earthworms was not within the scope of this thesis, it is possible that the results from tests for acute toxicity would be complemented by the measurement of chronic toxicity and indicate the effects of a chemical upon earthworms more comprehensively (Lofs-Holmin, 1982b; Ma, 1984; Wilson, 1975). The artificial soil is a very adaptable medium which can support E. fetida for a considerable period without causing the earthworms to lose a lot of weight. Therefore this testing method can probably be modified to measure the sublethal toxic effects of a chemical upon earthworms, and to provide data to support the increasing number of studies in which earthworms are being used to monitor the pollution of the soil with persistent chemicals (Ebing et al., 1984; Fanelli et al., 1980a, 1980b; Marquenie and Simmers, 1985; Rhett et al., 1985). The filter paper contact and artificial soil tests may also have an important role in identifying toxic substances present in the substrates used to produce earthworms commercially, or in those materials (such as animal waste and sewage cake) that can be processed by earthworms into horticultural composts (Neuhauser and Edwards, 1985).

I used the filter paper contact and artificial soil tests as research tools to investigate inter- and intra-specific differences in the susceptibility of earthworms to toxic chemicals. I also made a brief study of a population of earthworms that seemed to have developed a natural

resistance to pesticides affecting cholinesterase and will consider this investigation first.

I collected samples from a population of earthworms that lived in an experimental orchard at the Shell (UK) Research Centre at Sittingbourne. This orchard had been sprayed regularly and frequently with organophosphorus and carbamate insecticides for at least 15 years. Carboxylesterases (which show increased polymorphism in populations of insects that are resistant to pesticides which inhibit cholinesterase [Pasteur and Sinegre, 1975]) were extracted from the tissues of earthworms found in this orchard and at a site untreated by pesticides (the margin of Park Grass Field, Rothamsted Experimental Station). These enzymes were then separated using gel electrophoresis. The banding pattern of the carboxylesterases from A. longa collected in the orchard appeared to show an increased polymorphism when compared with those extracted from A. longa sampled at the control site. L. terrestris did not show any increased polymorphism. Thus the population of A. longa from the site treated regularly with pesticides that affected cholinesterase had possibly developed resistance to these compounds.

L. terrestris is known to possess only one cholinesterase (Silver, 1974), whereas E. fetida contains two such enzymes (Stenersen, 1980a). Since both of these enzymes would need to be inactivated for E. fetida to die (Stenersen, 1979c), the presence of this additional cholinesterase (which can also show polymorphism) could increase the possibility that resistance to pesticides with an anti-cholinesterase effect might arise in this species. Thus, earthworms that contain such polymorphic enzymes may be less susceptible to chemicals than those that do not, and the use of these less susceptible earthworms in tests for toxicity should be avoided.

The differences in the susceptibility to chemicals between L. terrestris, A. longa, A. caliginosa and E. fetida, and between E. fetida

andrei and E. fetida fetida were estimated using the filter paper contact and artificial soil tests. A. caliginosa was more susceptible to chemicals than either L. terrestris or A. longa, whilst E. fetida was the least susceptible species. This ranking of susceptibility was similar to that reported in other studies (Edwards, 1985; Gilman and Vardanis, 1974; Stenersen, 1979a). E. fetida fetida was generally less susceptible to chemicals than E. fetida andrei. This was the first time that a difference in the response to chemicals had been seen between two subspecies of earthworm (although recent work has suggested that these subspecies of E. fetida may be a separate species [Jaenike, 1982; Øien and Stenersen, 1984]), and supported the decision to use E. fetida andrei in the tests for the toxicity of chemicals to earthworms that were intended for general use. These observations may help to explain why E. fetida fetida is found in the wet and putrid layers within a compost heap and is apparently more able than E. fetida andrei to tolerate conditions that are anaerobic and waterlogged (Edwards and Neale, 1983).

Further studies of the differences in susceptibility to chemicals between E. fetida andrei and E. fetida fetida were made using chromatographic and radiolabelling techniques. These experiments investigated the uptake, release and metabolism of chlordane and iodoacetamide.

The tissues of E. fetida andrei and E. fetida fetida were extracted using organic solvents, and the quantity and type of lipid present was analysed by gas-liquid chromatography. Slight differences were seen between the lipids that were present in the tissues taken from earthworms of each subspecies, although these differences were very small and unlikely to have influenced the capacity of the earthworms to accumulate chemicals from aqueous solution (Briggs, 1982).

Gas-liquid chromatography was used to measure the amount of alpha-chlordane that was accumulated from aqueous solution by earthworms

of both subspecies of E. fetida. E. fetida fetida accumulated alpha-chlordane more quickly than E. fetida andrei, although the concentrations of alpha-chlordane achieved within the tissues of earthworms of each subspecies were similar. A negligible amount of alpha-chlordane accumulated previously by earthworms of either subspecies was released back into aqueous solution. These observations were confirmed by subsequent experiments using radiolabelled alpha-chlordane, which could be measured at a lower concentration than the unlabelled chlordane detected using gas-liquid chromatography.

A small amount of alpha-chlordane was probably metabolised by E. fetida to oxychlordane and chlordane chlorohydrin. These products have been identified in similar experiments using L. terrestris (Chio and Sanborn, 1976) and it would therefore seem likely that the processes of detoxification of alpha-chlordane in E. fetida and L. terrestris are similar.

In contrast to the results that were obtained using a lipophilic compound such as alpha-chlordane, the hydrophilic chemical iodoacetamide was accumulated poorly from aqueous solution by both E. fetida andrei and E. fetida fetida. Radiolabelled iodoacetamide was metabolised rapidly by earthworms of both subspecies of E. fetida to give products that could not be identified. This process appeared to be more rapid and complete in E. fetida fetida and suggested that this subspecies probably possessed a more efficient metabolism for detoxifying such chemicals.

The earthworms accumulated alpha-chlordane (a lipophilic compound) to a much higher concentration than iodoacetamide (a hydrophilic compound), and the earthworm tissue:water partition coefficient for alpha-chlordane was 3850, compared with 104 for iodoacetamide. These values were similar to the octan-1-ol:water partition coefficients for alpha-chlordane and iodoacetamide. Such results support the conclusions drawn from previous studies with L. terrestris and A. longa (Briggs and Lord, 1983; Lord et al., 1980), that the octan-1-ol:water partition

coefficient of a chemical (together with the soil organic matter:water partition coefficient) can be used to predict the equilibrium concentration for that chemical in the tissues of earthworms in the soil.

Some E. fetida fetida died in the aqueous solutions used in these experiments which contained a low concentration of alpha-chlordane. As these earthworms began to die, alpha-chlordane was seen to move rapidly across the body wall in a direction that was dictated by the prevailing concentration gradient for that chemical. The concentration of alpha-chlordane within the earthworms would therefore seem to be regulated by an active process. Whilst active transport mechanisms exist for substances such as Na^+, Cl^-, amino acids, sugars and fatty acids (Prusch and Otter, 1977; Richards and Arme, 1982), the movement of alpha-chlordane is more likely to be a function of an association of this chemical with the lipids present in the coelomic fluid (these lipids occur in aqueous suspension and as a component of the membranes of cells that float freely in the fluid). When the earthworms died and lost the ability to regulate the movement of coelomic fluid, alpha-chlordane was redistributed rapidly between the tissues of the earthworm and the external medium.

APPENDIX 1

THE EFFECTS OF PESTICIDES AND INDUSTRIAL CHEMICALS UPON EARTHWORMS: A REVIEW OF THE LITERATURE

Key: - Detrimental effect upon earthworms.
0 No effect upon earthworms.
+ Encouragement of earthworms.
* Data represent an LC_{50} or an LD_{50}.
-- Data not available

Notes

(1) Rates of application in the field that were quoted originally in non-metric units were, where possible, converted to $kg.ha^{-1}$

(2) The concentrations of the test chemicals used in the laboratory tests were, where possible, converted to $mg.kg^{-1}$ by assuming that a rate of application in the field of 2 $kg.ha^{-1}$ was equivalent to a concentration in the soil of 1 $mg.kg^{-1}$ (Dean-Ross, 1983; Thompson and Troeh, 1973). Other units of concentration are specified separately as they occur.

APPENDIX 1

Table A1.1. THE EFFECTS OF PESTICIDES AND INDUSTRIAL CHEMICALS UPON EARTHWORMS. A REVIEW OF THE LITERATURE

"Sola dosis fecit venum..." (It is only the dose that makes the poison). Paracelsus (16th Century).

INORGANIC CHEMICALS

Chemical	Application rate tested in the field $kg.ha^{-1}$ (1)	Concentration tested in the laboratory $mg.kg^{-1}$ (2)	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
Calcium arsenate	508	--	6 years	-	Turf	Escritt 1955
	424	--	5 years	-	Turf	Lidgate 1966
Calcium Cyanamide	--	129*	14 days	- Lumbricus terrestris	Laboratory soil	Haque and Ebing 1983a
	--	120*	14 days	- Eisenia fetida		
Copper chloride	--	370	42 days	- Lumbricus rubellus	Laboratory soil (Growth inhibition)	Ma 1984
Copper Oxychloride	--	98*	14 days	- L. terrestris	Laboratory soil	Haque and Ebing 1983a
	--	80	45 days	- Aporrectodea caliginosa	Laboratory soil (Reproduction depressed)	Van Rhee 1969

Chemical	Application rate tested in the field $kg.ha^{-1}$	Concentration tested in the laboratory $mg.kg^{-1}$	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
(With benzimidazole fungidicdes)	Heavy application	--	2 years	- L. terrestris - A. caliginosa - Aporrectodea rosea	Orchard	Niklas and Kennel 1978
	108	--	-	-	Turf	Escritt and Arthur 1948
Copper Sulphate	--	1500-2000	-	0 L. terrestris	Laboratory soil	Raw and Lofty 1962
	--	1500-2000	-	- Other Lumbricidae	(Contaminated leaf litter)	
(As Bordeaux Mixture)	430	--	31 years	0 Lumbricus castaneus	Orchard	Raw 1961
	430	--	31 years	- Other Lumbricidae		
Lead arsenate	508-678	--	5 years	-	Turf	Escritt 1955
	678	--	5 years	-	Turf	Escritt and Arthur 1948
	678	--	5 years	-	Turf	Lidgate 1966
	244	--	1.6 years	0	Field	Polivka 1951
	732	--	1.6 years	-		
	500	--	5 years	-		
	436	--	4 years	0	Field	Polivka 1953 (from Davey 1963)
	436	--	5 years	-		
	871	--	4 years	0		
	871	--	5 years	-		
	1307	--	4 years	-		
	1307	--	5 years	-		

Chemical	Application rate tested in the field $kg.ha^{-1}$	Concentration tested in the laboratory $mg.kg^{-1}$	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
Sulphur	678	--	--	-	Turf	Escritt and Arthur 1948
Mercuric chloride	8.5	--	--	-	Turf	Escritt and Arthur 1948
Potassium bromide	--	393*	28 days	- E. fetida	Laboratory soil	Heimbach 1984
Potassium permanganate	169	--	Rapid	- Expellant	Turf	Escritt and Arthur 1948
	376	--	Rapid	- Expellant (Two applications)	Turf	Raw 1959
Sodium chlorate	150-400	--	1 year	0 Lumbricidae	Field	Bouche and Beugnot 1978
	--	)750*	14 days	- L. terrestris	Laboratory soil	Haque and Ebing 1983a
	--	)750*	14 days	- E. fetida		
	5700	--	210 days	-	Field	Malone and Reichle 1973

Chemical	Application rate tested in the field $kg.ha^{-1}$	Concentration tested in the laboratory $mg.kg^{-1}$	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
Enterobacterin	--	2000-5000	110 days	- A. caliginosa	Laboratory soil (Straw disintegration)	Atlavinyte, Galvelis, Daciulyte and Lugauskas 1982
	0.6-60	--	63 days	0	Forest soil	Benz and Altwegg 1975
	--	($600\ g.m^{-2}$)	63 days	- L. terrestris	Laboratory soil	Smirnoff and Heimpel 1961
Mowrah meal	--	--	--	-	Turf	Bingley 1949 (from Davey 1963)
	1017-2034	--	1 day	- Expellant	Turf	Dawson, Boyns and Shorrock 1938
	1356-2712	--	Rapid	- Expellant	Turf	Escritt and Arthur 1948
	--	--	--	- Expellant	Turf	Jefferson 1955 (from Davey 1963)
Rotenone	--	--	--	- Expellant	Turf	Bingley 1949 (from Davey 1963)
	170-508	--	1 day	- Slow expellant	Turf	Dawson, Boyns and Shorrock 1938
	6.8	--	--	-	Turf	Escritt and Arthur 1948
	6.8	--	1 day	- Expellant	Turf	Harris 1949 (from Davey 1963)
Mustard	--	--	--	0	Turf	Escritt and Arthur 1948

Chemical	Application rate tested in the field $kg.ha^{-1}$	Concentration tested in the laboratory $mg.kg^{-1}$	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
Aldrin	1.5	--	3 years	+	Field	Bigger and Decker 1966
	--	62*	42 days	- L. terrestris	Laboratory soil substitute	Cathey 1982
	--	--	--	0	-	Edwards 1965
	4.0	--	1 year	0	Field	Edwards and Arnold 1963
	4.7	--	1 year	0	Field	Edwards, Dennis and Empson 1967
	4.5	--	--	0	Field	Edwards and Jeffs 1965
	--	--	--	-	Laboratory soil	Edwards and Lofty 1973
	--	--	--	0	Field	Edwards and Lofty 1973
	--	--	--	0	Field	Edwards, Lofty, Whiting and Jeffs 1971
	2.25	--	180 days	0 L. terrestris	Field	Griffiths, Raw and Lofty 1967
	2.25	--	180 days	0 Aporrectodea longa		
	2.25	--	180 days	+ A. caliginosa		
	0.2	--	60 days	- Lumbricidae	Field	Heungens 1969
	--	2.13*	4 days	- E. fetida	Laboratory soil	Hopkins and Kirk 1957

Chemical	Application rate tested in the field kg.ha^{-1}	Concentration tested in the laboratory mg.kg^{-1}	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
Aldrin	--	0.09	60 days	0 *E. fetida*	Laboratory soil	Hopkins and Kirk 1957
	4.5	--	85 days	-	Turf	Legg 1968
	15.3	--	Rapid	-	Nursery soil	Patel 1960
	5.0	--	60 days	0 *Pheretima hupeiensis*	Turf	Schread 1952
	33.0	--	150 days	- *P. hupeiensis*		
(With chlordane and phorate)	22.5	--	7 years	-	Field (Repeated applications)	Clements and Henderson 1977
(With phorate)	8.9	--	2.5 years	-	Field	Clements, Henderson and Bentley 1982
Aramite	--	9.76	7 days	0	Laboratory soil	Hyche 1956
Chlordane	10.0	--	1.5 years	- *L. terrestris*	Field	Doane 1962
	10.0	--	1.5 years	- *A. caliginosa*		
	--	--	--	-	Field	Edwards 1965
	--	--	--	-	Laboratory soil	Edwards and Lofty 1973
	--	--	--	-	Field	Edwards, Lofty, Whiting and Jeffs 1971
	10.0	--	180 days	- *L. terrestris*	Field	Goats 1983
	10.0	--	180 days	- *A. longa*		
	10.0	--	180 days	- *A. caliginosa*		

Chemical	Application rate tested in the field kg.ha^{-1}	Concentration tested in the laboratory mg.kg^{-1}	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
Chlordane	--	26.96*	14 days	- E. fetida	Laboratory soil	Goats and Edwards 1985
	10.0	--	180 days	- L. terrestris	Field	Goats and Edwards 1985
	10.0	--	180 days	- A. longa		
	--	42*	28 days	- E. fetida	Laboratory soil	Heimbach 1984
	--	2.5*	4 days	- E. fetida	Laboratory soil	Hopkins and Kirk 1957
	4.5	--	85 days	-	Turf	Legg 1968
	9.0-18.0	--	5 years	-	Turf	Lidgate 1966
	--	)16*	3 days	- L. terrestris	Laboratory soil	Ruppel and Laughlin 1977
	9.6-19.2	--	120 days	- P. hupeiensis	Turf	Schread 1952
(With aldrin and phorate)	11.2	--	7 years	-	Field (Repeated application)	Clements and Henderson 1977
DDE	--	61*	42 days	- L. terrestris	Laboratory soil substitute	Cathey 1982
DDT	0.25% (Tree spray)	--	270 days	-	Forest	Baker 1946
	--	--	4 years	0 Eudrilus sp.	Field	Cook, Critchley, Critchley, Perfect and Yeadon 1980
	--	--	4 years	0 Hyperiodrilus		

Chemical	Application rate tested in the field $kg.ha^{-1}$	Concentration tested in the laboratory $mg.kg^{-1}$	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
DDT	25	--	1.5 years	- L. terrestris	Field	Doane 1962
	25	--	1.5 years	- A. caliginosa		
	2.8	--	1.5 years	0		
	--	--	--	0	-	Edwards 1965
	60	--	1 year	0	Field	Edwards and Arnold 1963
	12.5	--	1 year	0 Lumbricidae	Field	Edwards, Dennis and Empson 1967
	--	--	--	0	Field	Edwards, Lofty, Whiting and Jeffs 1971
	2.5	--	--	0 P. hupeiensis	Turf	Fleming and Hadley 1945
	--	2000	7 days	0	Laboratory soil	Goffart 1949 (from Satchell 1955a)
	--	50	30 days	-	Laboratory soil	Greenwood 1945
	--	0.75	60 days	0 E. fetida	Laboratory soil	Hopkins and Kirk 1957
	--	40	56 days	0 A. longa	Laboratory soil	Hoy 1955
	--	40	56 days	0 L. rubellus		
	--	80	56 days	- A. longa		
	--	80	56 days	- L. rubellus		
	30	--	1.5 years	0	Field	Hoy 1955
	60	--	1.5 years	-		
	2.24	--	35 days	0 A. caliginosa	Field	Martin 1976
	2.24	--	35 days	0		

Chemical	Application rate tested in the field $kg.ha^{-1}$	Concentration tested in the laboratory $mg.kg^{-1}$	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
DDT	--	100-1000*	26 hours	- L. terrestris	Laboratory immersion 2 hours and inert media 24 hours	Martin and Wiggans 1959
	12.5	--	5 years	- (Slight effect)	Field	Polivka 1951
	37.5	--	5 years	-	Field	
	2.2-3.3	--	--	0	Orchard	Stringer and Pickard 1963 (from Edwards and Thompson 1973)
	5.0	--	21 days	- Lumbricidae	Field	Thompson 1971
	5.6	--	21 days	-	Field	Thompson and Sans 1974
Dieldrin	10	--	1.5 years	- L. terrestris	Field	Doane 1962
	10	--	1.5 years	- A. caliginosa		
	--	--	--	-	Laboratory soil	Edwards and Lofty 1973
	--	--	--	0	Field	Edwards and Lofty 1973
	--	--	--	0	Field	Edwards, Lofty, Whiting and Jeffs 1971
	--	1.37*	4 days	- E. fetida	Laboratory soil	Hopkins and Kirk 1957
	--	0.038	60 days	0 E. fetida		
	2.25	--	85 days	-	Turf	Legg 1968
	3.3	--	--	0	Field	Luckman and Decker 1960

Chemical	Application rate tested in the field kg.ha^{-1}	Concentration tested in the laboratory mg.kg^{-1}	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
Dieldrin	80	--	180 days	-	Turf	Polivka 1953 (from Davey 1963)
	25	--	42 days	0	Turf	
	44	--	1 year	-	Turf	Schread 1952
DNOC	--	--	--	-	Orchard	Van der Drift 1963 (from Edwards and Thompson 1973)
	--	13.1*	7 days	0 Allolobophora chlorotica	Laboratory soil	Fayolle 1979
	--	21*	28 days	- E. fetida	Laboratory soil	Heimbach 1984
Endosulphan	--	(10	7 days	- E. fetida	Laboratory soil substitute	Bouche 1984a
	2.2	--	--	0	Field	Edwards, Lofty and Stafford 1974
	--	8.96*	14 days	- L. terrestris	Laboratory soil	Haque and Ebing 1983a
	--	6.7*	28 days	- E. fetida	Laboratory soil	Heimbach 1984
	--	24* µg.cm^{-2}	2 days	- E. fetida	Laboratory contact test	Roberts and Dorough 1984
Endrin	--	66*	42 days	- L. terrestris	Laboratory soil substitute	Cathey 1982
	8.0	--	150 days	-	Field	Edwards, Lofty and Stafford 1972
	9.0	--	--	-	Field	Edwards, Lofty and Stafford 1974

Chemical	Application rate tested in the field kg.ha^{-1}	Concentration tested in the laboratory mg.kg^{-1}	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
Endrin	--	--	--	-	Field	Edwards, Lofty, Whiting and Jeffs 1971
	--	0.05	60 days	0 E. fetida	Laboratory soil	Hopkins and Kirk 1957
	3.05	--	Rapid	-	Nursery soil	Patel 1960
	1.0	--	21 days	- Lumbricidae	Field	Thompson 1971
	1.12	--	21 days	-	Field	Thompson and Sans 1974
HCH	--	275*	7 days	- E. fetida	Laboratory soil substitute	Bouche 1984a
	--	--	180 days	-	Field	DeMedts 1981
	--	--	--	0	Field	Edwards 1965
	--	--	--	-	Laboratory soil	Edwards and Lofty 1973
	--	--	--	0	Field	Edwards and Lofty 1973
	--	--	--	0	Field	Edwards, Lofty, Whiting and Jeffs 1971
	--	49.8*	7 days	0 A. chlorotica	Laboratory soil	Fayolle 1979
	30.0	--	90 days	+	Field	Grigoreva 1952
	500	--	60 days	0	Field	Gunthart 1947 (from Satchell 1955a)

Chemical	Application rate tested in the field $kg.ha^{-1}$	Concentration tested in the laboratory $mg.kg^{-1}$	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
HCH	--	113*	14 days	- L. terrestris	Laboratory soil	Haque and Ebing 1983a
	--	135*	14 days	- E. fetida		
	--	0.9*	4 days	- E. fetida	Laboratory soil	Hopkins and Kirk 1957
	--	0.075	60 days	0 E. fetida		
	--	10	56 days	0 A. caliginosa		
	--	10	56 days	0 L. rubellus		
	--	40	56 days	0 A. caliginosa		
	--	40	56 days	0 L. rubellus		
	30	--	1.5 years	-	Field	Hoy 1955
	60	--	1.5 years	-		
	1.0-2.88	--	1.5 years	+	Field	Lipa 1958
	5.76	--	1.5 years	0		
	--	50	8 days	0 Helodulus roseus	Laboratory soil	Morrison 1950
	--	100	8 days	- H. roseus		
	24.0	--	--	0	Field	Prisyazhnyuk 1950 (from Satchell 1955a)
	0.24	--	--	0	Forest soil	Richter 1953 (from Satchell 1955a)
	--	400-800*	80 days	- A. caliginosa	Laboratory immersion	Stenersen 1979a
	--	400-800*	80 days	- A. chlorotica	80 days	
	--	400-800*	80 days	- L. rubellus	30 min and observation	
	--	400-800*	80 days	- E. fetida		

Chemical	Application rate tested in the field $kg.ha^{-1}$	Concentration tested in the laboratory $mg.kg^{-1}$	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
HCH	--	64	30 days	0 A. caliginosa	Laboratory soil	Stenersen 1979a
	--	64	30 days	0 A. chlorotica		
	--	64	30 days	0 L. rubellus		
	--	64	30 days	0 E. fetida		
Heptachlor	1.5	--	3 years	+	Field	Bigger and Decker 1966
	--	--	--	-	-	Edwards 1965
	8.0	--	--	-	Field	Edwards and Arnold 1966
	--	--	--	-	Field	Edwards, Lofty, Whiting and Jeffs 1971
	--	2.03*	4 days	- E. fetida	Laboratory soil	Hopkins and Kirk 1957
	--	0.12	60 days	0 E. fetida		
	1.4	--	--	-	Aerial spray	Rhoades 1963 (from Edwards and Thompson 1973)
Isobenzan	--	--	--	0	Field	Edwards 1965
	--	--	--	0	Field	Edwards, Lofty, Whiting and Jeffs 1971
	2.2	--	--	-	Field	Kelsey and Arlidge 1968
	2.25	--	126 days	- Lumbricidae	Field	Moedd 1975

Chemical	Application rate tested in the field $kg.ha^{-1}$	Concentration tested in the laboratory $mg.kg^{-1}$	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
Napthalene	--	6000 (Distributed in soil)	150 days	- A. caliginosa	Laboratory straw disintegration	Atlavinyte 1975
	--	--	--	-	Laboratory straw disintegration	Atlavinyte, Daciulyte and Lugauskas 1974
1-Naphthol	--	17.8* $\mu g.cm^{-2}$	2 days	- E. fetida	Laboratory contact test	Roberts and Dorough 1984
Tetradifon	2.2	--	--	0	Field	Edwards, Lofty and Stafford 1974
Toxaphene	--	3.0	60 days	- E. fetida	Laboratory soil	Hopkins and Kirk 1957
	10.8	--	85 days	-	Turf	Legg 1968

Chemical	Application rate tested in the field $kg.ha^{-1}$	Concentration tested in the laboratory $mg.kg^{-1}$	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
Acephate	--	692* $\mu g.cm^{-2}$	48 hours	- L. rubellus	Laboratory contact test	Roberts and Dorough 1984
	--	851* $\mu g.cm^{-2}$	48 hours	- E. fetida		
Azinphos-methyl	--	0.15*	4 days	- E. fetida	Laboratory soil	Hopkins and Kirk 1957
	--	0.06	60 days	0 E. fetida		
Bromophos	--	270*	7 days	- E. fetida	Laboratory soil substitute	Bouche 1984a
Carbophenothion	--	--	--	0	Field	Edwards, Lofty, Whiting and Jeffs 1971
Chlorfenvinphos	--	--	--	0	Field	Edwards, Lofty, Whiting and Jeffs 1971
	4.0	--	--	-	Field	Edwards and Thompson 1969
	4.5	--	--	-	Field	Edwards, Thompson and Benyon 196
(With trifluralin, nitrofen and propachlor)	1.9	--	150 days	- Lumbricidae	Field	Finlayson, Campbell and Roberts, 1975

Chemical	Application rate tested in the field $kg.ha^{-1}$	Concentration tested in the laboratory $mg.kg^{-1}$	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
Chlormephos	--	41*	14 days	- E. fetida	Laboratory soil substitute	Bouche 1984a
Chlorpyriphos	0.45	--	7 days	-	Field	Kring 1969
	--	1.71* $ug.cm^{-2}$	2 days	- L. rubellus	Laboratory contact test	Roberts and Dorough 1984
	--	15.6* $\mu g.cm^{-2}$	2 days	- E. fetida		
	--	)16*	3 days	- L. terrestris	Laboratory soil	Ruppel and Laughlin 1977
	4.0	--	13 days	0 L. terrestris	Field	Ruppel and Laughlin 1977
	2.0	--	21 days	- Lumbricidae	Field	Thompson 1971
	2.24	--	21 days	0	Field	Thompson and Sans 1974
(With diazinon)	--	0.55	--	0	Laboratory soil	Whitney 1967 (from Edwards and Thompson 1973)
Chlorpyriphos-ethyl	--	205*	14 days	- E. fetida	Laboratory soil substitute	Bouche 1984a
Demeton-S-methyl	--	--	180 days	0	Field	DeMedts 1981
Dialifos	--	133*	28 days	- E. fetida	Laboratory soil	Heimbach 1984

Chemical	Application rate tested in the field kg.ha^{-1}	Concentration tested in the laboratory mg.kg^{-1}	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
Diazinon	--	--	--	0	Laboratory soil	Edwards and Lofty 1973
	--	--	--	0	Field	Edwards, Lofty, Whiting and Jeffs 1971
	4.0	--	--	-	Field	Edwards and Thompson 1969
	8.0	--	1 year	0	Field	Edwards, Thompson and Lofty 1968
	4.48	--	7 days	0 L. terrestris	Field	Kring 1969
	--	8.49	--	- A. caliginosa	Laboratory soil (Zero growth)	Martin 1983
	2.25	--	126 days	- Lumbricidae	Field	Moedd 1975
	--	)16*	3 days	- L. terrestris	Laboratory soil	Ruppel and Laughlin 1977
(With chlorpyriphos)	--	1.1	--	0	Laboratory soil	Whitney 1967 (from Edwards and Thompson 1973)
Dimethoate	--	--	--	-	Laboratory soil	Atlavinyte 1981
	--	18.2*	7 days	0 A. chlorotica	Laboratory soil	Fayolle 1979
Disulfoton	--	--	--	0	Laboratory soil	Edwards and Lofty 1973
	--	--	--	0	Field	Edwards, Lofty, Whiting and Jeffs 1971

Chemical	Application rate tested in the field $kg.ha^{-1}$	Concentration tested in the laboratory $mg.kg^{-1}$	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
Disulfoton	4.0	--	--	-	Field	Edwards and Thompson 1969
	8.0	--	1 year	0	Field	Edwards, Thompson and Lofty 1958
	4.48	--	7 days	0 L. terrestris	Field	Kring 1969
	--	)16*	3 days	- L. terrestris	Laboratory soil	Ruppel and Laughlin 1977
Ethoprophos	--	187.5	112 days	- E. fetida	Laboratory soil	Milne and du Toit 1976
	--	2.75*	3 days	- L. terrestris	Laboratory soil	Ruppel and Laughlin 1977
	4.0	--	13 days	- L. terrestris	Field	Ruppel and Laughlin 1977
	--	--	--	- L. terrestris	Field	Ruppell, Laughlin and Fogg 1973
Ethyl-parathion	--	80.1*	7 days	- A. chlorotica	Laboratory soil	Fayolle 1979
Fenamiphos	--	10.2	--	- A. caliginosa	Laboratory soil (Zero growth)	Martin 1983
	18.6	--	90 days	- A. caliginosa caliginosa	Field (Pretreated with benomyl)	McColl 1984
	18.6	--	90 days	- A. caliginosa trapezoides		
	18.6	--	90 days	- Eiseniella tetrahedra tetrahedra		

Chemical	Application rate tested in the field kg.ha^{-1}	Concentration tested in the laboratory mg.kg^{-1}	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
Fenamiphos	--	187.5	112 days	- E. fetida	Laboratory soil	Milne and du Toit 1976
	--	)16*	3 days	- L. terrestris	Laboratory soil	Ruppel and Laughlin 1977
	8.0	--	13 days	0 L. terrestris	Field	Ruppel and Laughlin 1977
Fenitrothion	--	--	--	0	Laboratory soil	Edwards and Lofty 1973
	--	--	--	0	Field	Edwards, Lofty, Whiting and Jeffs 1971
	4.0	--	--	0	Field	Edwards and Thompson 1969
	1.5	--	180 days	0 L. terrestris	Field	Griffiths, Raw and Lofty 1967
	1.5	--	180 days	0 A. longa		
	1.5	--	180 days	+ A. caliginosa		
	2.24	--	35 days	0 A. caliginosa	Field	Martin 1976
Fensulfothion	2.0	--	60 days	0	Field	Fox 1974
	4.48	--	7 days	0 L. terrestris	Field	Kring 1969
	2.24	--	35 days	0 A. caliginosa	Field	Martin 1976
	--	5.1	--	- A. caliginosa	Laboratory soil (Zero growth)	Martin 1983

Chemical	Application rate tested in the field $kg.ha^{-1}$	Concentration tested in the laboratory $mg.kg^{-1}$	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
Fensulfothion	2.25	--	126 days	- Lumbricidae	Field	Moedd 1975
	--	)16*	3 days	- L. terrestris	Laboratory soil	Ruppel and Laughlin 1977
	3.0	--	21 days	- Lumbricidae	Field	Thompson 1971
	3.36	--	21 days	-	Field	Thompson and Sans 1974
Fonofos	--	--	--	-	Laboratory soil	Edwards and Lofty 1973
	--	--	--	0	Field	Edwards, Lofty, Whiting and Jeffs 1971
	--	0.04* $\mu g.cm^{-2}$	2 days	- L. rubellus	Laboratory contact test	Roberts and Dorough 1984
	--	1.1* $\mu g.cm^{-2}$	2 days	- E. fetida		
	--	12.5*	3 days	- L. terrestris	Laboratory soil	Ruppel and Laughlin 1977
Formothion	--	--	--	-	Laboratory soil	Atlavinyte 1981
	--	200	60 days	- A. caliginosa	Laboratory soil	Atlavinyte, Lugauskas and Kilikevicius 1980
Isazophos	--	0.71	--	- A. caliginosa	Laboratory soil (Zero growth)	Martin 1983

Chemical	Application rate tested in the field $kg.ha^{-1}$	Concentration tested in the laboratory $mg.kg^{-1}$	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
Isofenphos	0.45	--	180 days	+	Field	Finlayson, Campbell and Roberts 1975
Leptophos	3.4	--	21 days	- Lumbricidae (slight effect)	Field	Tomlin and Gore 1974
Malathion	--	--	--	- A. caliginosa	Field	Galvyalis and Lugauskas 1978
	--	815.24	7 days	-	Laboratory soil	Hyche 1956
	--	0.1-1.0*	26 hours	- L. terrestris	Laboratory immersion 2 hours and inert media 24 hours	Martin and Wiggins 1959
	--	0.27* $\mu g.cm^{-2}$	2 days	- L. rubellus	Laboratory contact test	Roberts and Dorough 1984
	--	13.5* $\mu g.cm^{-2}$	2 days	- E. fetida		
Menazon	--	--	--	0	Field	Edwards, Lofty, Whiting and Jeffs 1971
	--	1.0	90 days	0 L. terrestris	Laboratory soil	Raw 1965
	--	1.0	90 days	0 A. caliginosa		
	--	1.0	90 days	0 A. chlorotica		
	--	100	90 days	0 A. caliginosa	Laboratory soil	Raw 1965

Chemical	Application rate tested in the field $kg.ha^{-1}$	Concentration tested in the laboratory $mg.kg^{-1}$	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
Menazon	--	1.0	90 days	0 L. terrestris	Laboratory soil	Raw and Lofty 1964
	--	1.0	90 days	0 A. caliginosa		
	--	1.0	90 days	0 A. chlorotica		
	--	100	90 days	- A. caliginosa	Laboratory soil	Raw and Lofty 1964
	--	1.0	90 days	- A. chlorotica	(Reproduction depressed)	
	--	250	--	-	Small plots (Temporary decrease in activity)	Way and Scopes 1965
	--	250	7 days	0	Small plots	Way and Scopes 1968
Methamidophos	--	109*	14 days	- L. terrestris	Laboratory soil	Haque and Ebing 1983a
	--	17.3*	14 days	- E. fetida		
Methaphenamiphos	--	318*	28 days	- E. fetida	Laboratory soil	Heimbach 1984
Methidathion	--	3.6*	28 days	- E. fetida	Laboratory soil	Heimbach 1984
Methyl-parathion	--	20.37	7 days	0	Laboratory soil	Hyche 1956
Monocrotophos	--	--	--	0	Laboratory soil	Edwards and Lofty 1973
	--	--	--	0	Field	Edwards, Lofty and Stafford 1974

Chemical	Application rate tested in the field kg.ha^{-1}	Concentration tested in the laboratory mg.kg^{-1}	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
Paraoxon	--	100-200*	80 days	- E. fetida	Laboratory immersion 30 mins and observation 80 days	Stenersen 1979a
	--	32	30 days	- A. caliginosa	Laboratory soil	Stenersen 1979a
	--	32	30 days	- A. chlorotica		
	--	64	30 days	- E. fetida		
Parathion	--	44*	42 days	- L. terrestris	Laboratory soil substitute	Cathey 1982
	--	--	180 days	0	Field	DeMedts 1981
	4.0	--	1 year	-	Field	Edwards, Arnold and Thompson 1966
	--	--	--	0	Field	Edwards, Lofty, Whiting and Jeffs 1971
	4.0	--	--	0	Field	Edwards and Thompson 1969
	8.0	--	1 year	0	Field	Edwards, Thompson and Lofty 1968
	--	10	3 days	-	Laboratory immersion 10 mins and observation 3 days	Goffart 1949 (from Satchell 1955a)
	80.0	--	--	-	Forest soil	Heungens 1966 (from Edwards and Thompson 1973)
	--	10.18 e.c.	7 days	-	Laboratory soil	Hyche 1956

Chemical	Application rate tested in the field $kg.ha^{-1}$	Concentration tested in the laboratory $mg.kg^{-1}$	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
Parathion	--	9.76 w.p.	7 days	0	Laboratory soil	Hyche 1956
	9.17	--	Short	-	Nursery soil	Patel 1960
	--	1.12* $\mu g.cm^{-2}$	2 days	- L. rubellus	Laboratory contact test	Roberts and Dorough 1984
	--	14.8* $\mu g.cm^{-2}$	2 days	- E. fetida		
	21.5-32.0	--	14 days	- P. hupeiensis	Turf	Schread 1952
	--	400-800*	80 days	- E. fetida	Laboratory immersion 30 min and observation 80 days	Stenersen 1979a
	--	64	30 days	0 A. caliginosa	Laboratory soil	Stenersen 1979a
	--	64	30 days	0 A. chlorotica		
	--	64	30 days	0 L. rubellus		
	--	64	30 days	0 E. fetida		
	7.8	--	21 days	-	Field	Weber 1953 (from Edwards and Thompson 1973)
Phorate	9.0	--	--	-	Field	Edwards, Lofty and Stafford 1974
	--	--	--	-	Field	Edwards, Lofty, Whiting and Jeffs 1971
	4.0	--	--	0	Field	Edwards and Thompson 1969
	4.0	--	1 year	-	Field	Edwards, Thompson and Lofty 1968

Chemical	Application rate tested in the field $kg.ha^{-1}$	Concentration tested in the laboratory $mg.kg^{-1}$	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
Phorate	44.8	--	210 days	-	Field	Malone and Reichle 1973
	--	0.27	--	- A. caliginosa	Laboratory soil (Zero growth)	Martin 1983
	--	15*	3 days	- L. terrestris	Laboratory soil	Ruppel and Laughlin 1977
	1.0	--	1 year	- A. caliginosa	Field	Saunders and Forgie 1977
	1.0	--	1 year	- A. rosea		
	1.0	--	1 year	- L. rubellus (Slight effect)		
	2.0	--	1 year	- A. caliginosa	Field	Saunders and Forgie 1977
	2.0	--	1 year	- A. rosea		
	2.0	--	1 year	- L. rubellus		
	3.4	--	21 days	- Lumbricidae	Field	Tomlin and Gore 1974
	--	10	--	-	Small plots	Way and Scopes 1965
	--	250	7 days	-	Small plots	Way and Scopes 1968
(With aldrin and chlordane)	3.3	--	7 years	-	Field (Repeated application)	Clements and Henderson 1977
(With aldrin)	2.7	--	2.5 years	-	Field	Clements, Henderson and Bentley 1982

Chemical	Application rate tested in the field kg.ha^{-1}	Concentration tested in the laboratory mg.kg^{-1}	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
Phosalone	--	--	--	-	Laboratory soil	Atlavinyte 1981
	--	150	75 days	- A. caliginosa	Laboratory soil	Atlavinyte, Lugauskas and Kilikevicius 1980
	--	61*	7 days	- E. fetida	Laboratory soil substitute	Bouche 1984a
	--	)16*	3 days	- L. terrestris	Laboratory soil	Ruppel and Laughlin 1977
Phosphamidon	--	16.53*	1 day	- Lampeto mauritii	Laboratory immersion test	Bharathi and Subba Rao 1984
	--	26.95*	1 day	- L. mauritii	Laboratory soil substitute	Bharathi and Subba Rao 1984
Terbufos	--	13*	14 days	- E. fetida	Laboratory soil substitute	Bouche 1984a
	--	4.6*	14 days	- L. terrestris	Laboratory soil	Haque and Ebing 1983a
	--	6.62*	14 days	- E. fetida		
	--	)16.0*	3 days	- L. terrestris	Laboratory soil	Ruppel and Laughlin 1977
	2.8	--	13 days	- L. terrestris	Field	Ruppel and Laughlin 1977

Chemical	Application rate tested in the field $kg.ha^{-1}$	Concentration tested in the laboratory $mg.kg^{-1}$	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
Tetrachlorvinphos	--	--	--	0	Laboratory soil	Edwards and Lofty 1973
	8.0	--	150 days	0	Field	Edwards, Lofty and Stafford 1972
	--	--	--	0	Laboratory soil	Edwards, Lofty and Stafford 1974
Thionazin	--	--	--	-	Laboratory soil	Edwards and Lofty 1973
	--	--	--	0	Field	Edwards and Lofty 1973
	--	--	--	0	Field	Edwards, Lofty, Whiting and Jeffs 1971
	4.0	--	--	0	Field	Edwards and Thompson 1969
	1.5	--	180 days	+ L. terrestris	Field	Griffiths, Raw and Lofty 1967
	1.5	--	180 days	0 A. longa		
	1.5	--	180 days	+ A. caliginosa		
	--	10	--	-	Small plots	Way and Scopes 1965
Triazophos	--	210*	14 days	- L. terrestris	Laboratory soil	Haque and Ebing 1983a
Trichloronate	2.0	--	60 days	0	Field	Fox 1964
	8.36	--	21 days	0	Field	Thompson and Sans 1974

Chemical	Application rate tested in the field $kg.ha^{-1}$	Concentration tested in the laboratory $mg.kg^{-1}$	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
Trichoronate	--	800*	80 days	- E. fetida	Laboratory immersion 30 min and observation 80 days	Stenersen 1979a
	--	64	30 days	0 A. caliginosa	Laboratory soil	Stenersen 1979a
	--	64	30 days	0 A. chlorotica		
	--	64	30 days	0 L. rubellus		
	--	64	30 days	0 E. fetida		
Trichlorphon	--	--	--	-	Laboratory straw disintegration	Atlavinyte, Daciulyte and Lugauskas 1974
	--	80	75 days	- A. caliginosa	Laboratory soil	Atlavinyte, Lugauskas and Kilikevicius 1980
	--	--	--	0	Laboratory soil	Edwards and Lofty 1973
	4.0	--	--	-	Field	Edwards and Thompson 1969
	--	--	--	- A. caliginosa	Field	Galvyalis and Lugauskas 1978
	-	1000(Distributed in soil)	104 days	- A. caliginosa (Slight effect)	Laboratory straw disintegration	Atlavinyte 1975
	--	--	--	-	Laboratory soil	Atlavinyte 1981

CARBAMATE INSECTICIDES

Chemical	Application rate tested in the field $kg.ha^{-1}$	Concentration tested in the laboratory $mg.kg^{-1}$	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
Aldicarb	--	--	180 days	-	Field	DeMedts 1981
	11.2	--	150 days	0 Lumbricidae	Field	Edwards and Lofty 1971
	6.8	--	150 days	0 L. terrestris	Field	Edwards, Lofty and Stafford 1972
	--	3.5*	7 days	- A. chlorotica	Laboratory soil	Fayolle 1979
	--	26*	14 days	- L. terrestris	Laboratory soil	Haque and Ebing 1983a
	--	3.0*	14 days	- E. fetida	Laboratory soil	Haque and Ebing 1983a
	--	0.29*	29 hours	- L. terrestris	Laboratory immersion 5 hours and observation 24 hours	Lebrun, DeMedts and Wauthy 1981
	--	0.07	--	- A. caliginosa	Laboratory soil (Zero growth)	Martin 1983
	--	0.02* $\mu g.cm^{-2}$	2 days	- L. rubellus	Laboratory contact test	Roberts and Dorough 1984
	--	3.2* $\mu g.cm^{-2}$	2 days	- E. fetida		
	--	2.75*	3 days	- L. terrestris	Laboratory soil	Ruppel and Laughlin 1977
	4.0	--	13 days	- L. terrestris	Field	Ruppel and Laughlin 1977
	--	--	--	- L. terrestris	Field	Ruppel, Laughlin and Fogg 1973

Chemical	Application rate tested in the field $kg.ha^{-1}$	Concentration tested in the laboratory $mg.kg^{-1}$	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
Aldicarb	--	3.1-6.2*	80 days	- A. caliginosa	Laboratory immersion	Stenersen 1979a
	--	12.5-25*	80 days	- L. rubellus	30 min and observation	
	--	25-50*	80 days	- E. fetida	80 days	
	--	4	30 days	- A. caliginosa	Laboratory soil	Stenersen 1979a
	--	4	30 days	- A. chlorotica		
	--	4	30 days	- L. rubellus		
	--	16	30 days	- E. fetida		
Aminocarb	0.18	--	60 days	-	Forest soil (Aerial application)	Bracher and Bider 1982
Bufencarb	4.48	--	7 days	- L. terrestris	Field	Kring 1969
	--	4.5*	3 days	- L. terrestris	Laboratory soil	Ruppel and Laughlin 1977
	1.0	--	21 days	- Lumbricidae	Field	Thompson 1971
	1.12	--	21 days	-	Field	Thompson and Sans 1974
Carbaryl	--	200(Distributed in soil)	71 days	- A. caliginosa	Laboratory straw disintegration	Atlavinyte 1975
	--	--	--	-	Laboratory straw disintegration	Atlavinyte, Daciulyte and Lugauskas 1974
	--	25*	14 days	- E. fetida	Laboratory soil substitute	Bouche 1984a

Chemical	Application rate tested in the field $kg.ha^{-1}$	Concentration tested in the laboratory $mg.kg^{-1}$	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
Carbaryl	--	33*	42 days	- L. terrestris	Laboratory soil substitute	Cathey 1982
	--	200	1 day	- Pheretima posthuma	Laboratory soil	Dikshith and Gupta 1981
	4.0	--	--	-	Field	Edwards and Thompson 1969
	--	--	--	-	Laboratory soil	Edwards and Lofty 1973
	--	--	--	-	Field	Edwards, Lofty, Whiting and Jeffs 1971
	--	26.66*	14 days	- L. terrestris	Laboratory immersion 30 min and observation 14 days	Fisher 1984
	--	26.42*	14 days	- L. terrestris	Laboratory soil	Fisher 1984
	--	70.81*$\mu g.worm^{-1}$	14 days	- L. terrestris	Laboratory topical application	Fisher 1984
	25.0	--	180 days	- L. terrestris	Field	Goats 1983
	25.0	--	180 days	- A. longa		
	25.0	--	180 days	- A. caliginosa		
	--	85.74*	14 days	- E. fetida	Laboratory soil	Goats and Edwards 1985
	25.0	--	180 days	- L. terrestris	Field	Goats and Edwards 1985
	25.0	--	180 days	- A. longa		
	--	174*	28 days	- E. fetida	Laboratory soil	Heimbach 1984

Chemical	Application rate tested in the field $kg.ha^{-1}$	Concentration tested in the laboratory $mg.kg^{-1}$	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
Carbaryl	0.2	--	60 days	- Lumbricidae	Field	Heungens 1969
	--	319	30 days	- Pontoscolex corethrurus	Laboratory soil	Kale and Krishnamoorthy 1979
	--	250	30 days	- P. corethrurus	Laboratory soil	Kale and Krisnamoorthy 1982
	5.0	--	85 days	-	Turf	Legg 1968
	--	0.28* $\mu g.cm^{-2}$	2 days	- L. rubellus	Laboratory contact test	Roberts and Dorough 1984
	--	9.0* $\mu g.cm^{-2}$	2 days	- E. fetida		
	--	3.1-6.3*	80 days	- A. caliginosa	Laboratory immersion	Stenersen 1979a
	--	200*	80 days	- A. chlorotica	30 min and observation	
	--	25-50*	80 days	- L. rubellus	80 days	
	--	200-800*	80 days	- E. fetida		
	--	4	30 days	- A. caliginosa	Laboratory soil	Stenersen 1979a
	--	4	30 days	- A. chlorotica		
	--	4	30 days	- L. rubellus		
	--	64	30 days	0 E. fetida		
	2.0	--	21 days	- Lumbricidae	Field	Thompson 1971
	2.24	--	21 days	-	Field	Thompson and Sans 1974
Carbofuran	--	57*	14 days	- E. fetida	Laboratory soil substitute	Bouche 1984a
	1.1 (In row)	--	154 days	0	Field	Broadbent and Tomlin 1982
	1.1 (Broadcast)	--	154 days	-	Field	Broadbent and Tomlin 1982

Chemical	Application rate tested in the field $kg.ha^{-1}$	Concentration tested in the laboratory $mg.kg^{-1}$	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
Carbofuran	2.2	--	--	-	Field	Edwards, Lofty and Stafford 1974
	--	--	52 hours	-	Field	Flickinger, King, Stout and Mohn 1980
	--	13.2*	5 days	- L. terrestris	Laboratory soil	Gilman and Vardanis 1974
	--	24.5*	5 days	- E.fetida	Laboratory soil	Gilman and Vardanis 1974
	--	5.0*	14 days	- L. terrestris	Laboratory soil	Haque and Ebing 1983a
	--	28*	14 days	- E. fetida		
	--	0.5*	29 hours	- E. fetida	Laboratory immersion 5 hours and observation 24 hours	Houpert, Jenot and Lardier 1982
	4.48	--	7 days	- L. terrestris	Field	Kring 1969
	--	0.52*	29 hours	- L. terrestris	Laboratory immersion 5 hours and observation 24 hours	Lebrun, DeMedts and Wauthy 1981
	2.24	--	35 days	- A. caliginosa	Field	Martin 1976
	--	0.31* $\mu g.cm^{-2}$	2 days	- L. rubellus	Laboratory contact test	Roberts and Dorough 1984
	--	0.3* $\mu g.cm^{-2}$	2 days	- E. fetida		
	--	4.0*	3 days	- L. terrestris	Laboratory soil	Ruppel and Laughlin 1977
	4.0	--	13 days	- L. terrestris	Field	Ruppel and Laughlin 1977
	--	--	--	- L. terrestris	Field	Ruppel, Laughlin and Fogg 1973

Chemical	Application rate tested in the field $kg.ha^{-1}$	Concentration tested in the laboratory $mg.kg^{-1}$	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
Carbofuran	--	0.39-0.78*	80 days	- A. caliginosa	Laboratory immersion 30 min and observation 80 days	Stenersen 1979a
	--	5-10*	80 days	- A. chlorotica		
	--	5-10*	80 days	- L. rubellus		
	--	400-800*	80 days	- E. fetida		
	--	4	30 days	- A. caliginosa	Laboratory soil	Stenersen 1979a
	--	4	30 days	- A. chlorotica		
	--	4	30 days	- L. rubellus		
	--	64	30 days	0 E. fetida		
	--	1.3*	--	- L. terrestris	Laboratory injection into body	Stenersen, Gilman and Vardanis 1973
	--	12.2*	5 days	- L. terrestris	Laboratory soil	Stenersen, Gilman and Vardanis 1973
	4.0	--	21 days	- Lumbricidae	Field	Thompson 1971
	4.48	--	21 days	-	Field	Thompson and Sans 1974
	3.4	--	21 days	- Lumbricidae	Field	Tomlin and Gore 1974
	5.6	--	21 days	- Lumbricidae		
(With trifluralin, nitrofen and propachlor)	2.39	--	150 days	- Lumbricidae	Field	Finlayson, Campbell and Roberts 1975
(With atrazine)	--	0.4*	29 hours	- E. fetida	Laboratory immersion 5 hours and observation 24 hours	Houpert, Jenot and Lardier 1982

Chemical	Application rate tested in the field $kg.ha^{-1}$	Concentration tested in the laboratory $mg.kg^{-1}$	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
Ethiofencarb	--	262*	28 days	- E. fetida	Laboratory soil	Heimbach 1984
Methiocarb	1.0	--	8 days	- A. caliginosa	Field (Spray)	Barker 1982
	1.0	--	8 days	- L. rubellus		
	1.0	--	8 days	0 A. caliginosa	Field (Bait)	Barker 1982
	1.0	--	8 days	- L. rubellus		
	--	42*	7 days	- E. fetida	Laboratory soil substitute	Bouche 1984a
	--	--	--	0	Laboratory soil	Edwards and Lofty 1973
	--	--	--	0	Laboratory soil	Edwards, Lofty and Stafford 1974
	--	8.1*	7 days	- A. chlorotica	Laboratory soil	Fayolle 1979
	--	129*	28 days	- E. fetida	Laboratory soil	Heimbach 1984
	--	1.64 $\mu g.g^{-1}$ worm	8-11 days	- L. terrestris	Laboratory forced feeding	Stringer and Wright 1980
Methomyl	11.2	--	150 days	0	Field	Edwards and Lofty 1971
	--	--	--	-	Laboratory soil	Edwards and Lofty 1973
	11.2	--	150 days	0 Lumbricidae	Field	Edwards, Lofty and Stafford 1972
	--	0.36	-	- A. caliginosa	Laboratory soil (Zero growth)	Martin 1983

Chemical	Application rate tested in the field $kg.ha^{-1}$	Concentration tested in the laboratory $mg.kg^{-1}$	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
Methomyl	--	0.08* $\mu g.cm^{-2}$	2 days	- L. rubellus	Laboratory contact test	Roberts and Dorough 1984
	--	2.0* $\mu g.cm^{-2}$	2 days	- E. fetida		
	--	5.2*	3 days	- L. terrestris	Laboratory soil	Ruppel and Laughlin 1977
	3.4	--	21 days	- Lumbricidae	Field	Tomlin and Gore 1974
Oxamyl	--	0.63	--	- A. caliginosa	Laboratory soil (Zero growth)	Martin 1983
	12.7	--	90 days	- A. caliginosa caliginosa	Field (Pretreated with triadimefon and iprodione)	McColl 1984
	12.7	--	90 days	- A. caliginosa trapezoides		
	12.7	--	90 days	- Eiseniella tetrahedra tetrahedra		
	--	6.5*	3 days	- L. terrestris	Laboratory soil	Ruppel and Laughlin 1977
	--	)200*	80 days	- A. caliginosa	Laboratory immersion 30 min and observation 80 days	Stenersen 1979a
	--	)200*	80 days	- A. chlorotica		
	--	)200*	80 days	- L. rubellus		
	--	)200*	80 days	- E. fetida		
	--	64	30 days	0 A. caliginosa	Laboratory soil	Stenersen 1979a
	--	64	30 days	0 A. chlorotica		
	--	64	30 days	0 L. rubellus		
	--	64	30 days	0 E. fetida		
Promecarb	--	)16*	3 days	- L. terrestris	Laboratory soil	Ruppel and Laughlin 1977

Chemical	Application rate tested in the field $kg.ha^{-1}$	Concentration tested in the laboratory $mg.kg^{-1}$	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
Propoxur	2.0	--	60 days	-	Field	Fox 1964
	--	10*	28 days	- E. fetida	Laboratory soil	Heimbach 1984
	--	0.71* $\mu g.cm^{-2}$	2 days	- L. rubellus	Laboratory contact test	Roberts and Dorough 1984
	--	5.3* $\mu g.cm^{-2}$	2 days	- E. fetida		
	--	9.0*	3 days	- L. terrestris	Laboratory soil	Ruppel and Laughlin 1977
Thiofanox	--	67*	7 days	- E. fetida	Laboratory soil substitute	Bouche 1984a
	--	2.58*	29 hours	- L. terrestris	Laboratory immersion 5 hours and observation 24 hours	Lebrun, DeMedts and Wauthy 1981
	--	3.5*	29 hours	- E. fetida	Laboratory immersion 5 hours and observation 24 hours	Houpert, Jenot and Lardier 1982
(With atrazine)	--	2.4*	29 hours	- E. fetida	Laboratory immersion 5 hours and observation 24 hours	Houpert, Jenot and Lardier 1982

SYNTHETIC PYRETHROID INSECTICIDES AND INHIBITORS OF CHITIN FORMATION

Chemical	Application rate tested in the field $kg.ha^{-1}$	Concentration tested in the laboratory $mg.kg^{-1}$	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
Alphamethrin	--	100	14 days	0 *E. fetida*	Laboratory soil	Inglesfield 1984
Cypermethrin	--	100	14 days	0 *E. fetida*	Laboratory soil	Inglesfield 1984
	--	26.9* $\mu g.cm^{-2}$	2 days	- *E. fetida*	Laboratory contact	Roberts and Dorough 1984
Diflubenzuron	--	--	--	0	Laboratory soil	Edwards and Lofty 1976
	--	--	--	0	Field	Edwards and Lofty 1976
Fenvalerate	0.1	--	90 days	0 *L. terrestris*	Field (Mixed spray programme)	Lofs-Holmin 1982a
	0.1	--	90 days	0 *A. caliginosa*		
	0.1	--	90 days	0 *A. rosea*		
	--	74.1* $\mu g.cm^{-2}$	2 days	- *E. fetida*	Laboratory contact test	Roberts and Dorough 1984
Permethrin	--	1000* $\mu g.cm^{-2}$	2 days	- *E. fetida*	Laboratory contact test	Roberts and Dorough 1984
Pyrethrins (unspecified)	--	5*	14 days	- *E. fetida*	Laboratory soil substitute	Bouche 1984a

Chemical	Application rate tested in the field kg.ha^{-1}	Concentration tested in the laboratory mg.kg^{-1}	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
Chloropicrin	4.5	--	30 days	- A. caliginosa	Glasshouse soil	Blankwaardt and Van der Drift 1961
	4.5	--	30 days	- A. chlorotica		
	4.5	--	30 days	- Dendrobaena rubida		
	--	--	1 year	- A. caliginosa	Glasshouse soil	Van Rhee 1969
Dazomet	--	97*	14 days	- E. fetida	Laboratory soil substitute	Bouche 1984a
	364	--	150 days	- Lumbricidae	Field	Edwards and Lofty 1971
	--	--	--	-	Laboratory soil	Edwards and Lofty 1973
	--	--	--	0	Field	Edwards and Lofty 1973
	364	--	150 days	- L. terrestris	Field	Edwards, Lofty and Stafford 1972
1,2-Dibromo-3 -chloropropane (DBCP)	0.57	--	90 days	0 Lumbricidae	Field	Heungens 1968
	0.75	--	60 days	0 Lumbricidae	Field	Heungens 1969
	--	46.86	112 days	0 E. fetida	Laboratory soil	Milne and du Toit 1976
Dibromo-ethene (Ethylene dibromide)	--	66.25	112 days	- E. fetida	Laboratory soil	Milne and du Toit 1976

Chemical	Application rate tested in the field $kg.ha^{-1}$	Concentration tested in the laboratory $mg.kg^{-1}$	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
1,3-Dichloropropene	--	26 $\mu g.cm^{-3}.day^{-1}$ (Soil water)	1 day	- E. fetida	Field	McKenry and Naylor 1975
1,2-Dichloropropane + 1,3-dichloropropene	400	--	135 days	-	Glasshouse soil	Buahin and Edwards 1963
	--	--	--	-	Field	Edwards, Lofty, Whiting and Jeffs 1971
(DD)	600	--	5 years	0 Lumbricidae	Field (5 annual applications)	Van den Brande and Heungens 1969
	--	2500	112 days	0 E. fetida	Laboratory soil	Milne and du Toit 1976
Formaldehyde	60	--	30 days	- A. caliginosa	Glasshouse soil	Blankwaardt and Van der Drift 1961
	60	--	30 days	- A. chlorotica		
	60	--	30 days	- D. rubida		
	5050	--	--	0	Field	Edwards and Lofty 1973
	538	--	Short	- Expellant (Two applications)	Turf	Raw 1959
Metham Sodium	--	--	--	-	Field	Edwards, Lofty, Whiting and Jeffs 1971
Methyl bromide	--	--	--	-	Field	Edwards, Lofty, Whiting and Jeffs 1971

FUNGICIDES

Chemical	Application rate tested in the field $kg.ha^{-1}$	Concentration tested in the laboratory $mg.kg^{-1}$	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
2-Aminobutane	--	40 $\mu g.worm^{-1}$	10 days	0 L. terrestris	Laboratory forced feeding	Stringer and Wright 1976
Aniyaline	85.4	--	1 year	-	Turf	King and Dale 1977
	--	89.95	64 days	- E. fetida	Laboratory soil	Roark and Dale 1979
Benomyl	180-360	-	90 days	- Lumbricus sp.	Soil injection	Black and Neely 1975a
	180-360	--	90 days	- Octolasion sp.		
	180-360	--	90 days	- Helodrilus sp.		
	180-360	--	90 days	- Diplocardia sp.		
	180-360	--	1 year	0 Lumbricidae	Soil injection	Black and Neely 1975a
	360	--	1 year	0 Lumbricidae	Soil injection	Black and Neely 1975b
	0.5	--	1 year	0 L. terrestris	Field	Blackshaw 1980
	0.5	--	1 year	0 A. longa		
	0.5	--	1 year	0 A. caliginosa		
	0.5	--	1 year	0 A. chlorotica		
	2.0	--	1 year	- L. terrestris	Field	Blackshaw 1980
	2.0	--	1 year	- A. longa		
	2.0	--	1 year	- A. caliginosa		
	2.0	--	1 year	- A. chlorotica		
	--	132*	14 days	- E. fetida	Laboratory soil Substitute	Bouche 1984a

Chemical	Application rate tested in the field kg.ha^{-1}	Concentration tested in the laboratory mg.kg^{-1}	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
Benomyl	Orchard rate	--	1 year	- Lumbricidae	Orchard	Cook and Swait 1975
	--	2*	14 days	- L. terrestris	Laboratory soil	Edwards 1985
	--	2*	14 days	- A. caliginosa		
	--	2*	14 days	- E. fetida		
	--	3.5	14 days	- E. fetida	Laboratory soil	Edwards 1985
	2.0	--	180 days	- L. terrestris	Field	Edwards 1985
	2.0	--	180 days	- A. caliginosa		
	--	--	--	-	Laboratory soil	Edwards and Lofty 1973
	10.0	--	--	-	Field	Edwards and Lofty 1973
	11.2	--	--	-	Field	Edwards, Lofty and Stafford 1974
	5.0	-	1 year	- L. terrestris	Field	Edwards and Brown 1982
	5.0	--	1 year	+ Lumbricus festivus (Transient increase)		
	--	8.0*	7 days	- A. chlorotica	Laboratory soil	Fayolle 1979
	--	0.42*	14 days	- L. terrestris	Laboratory soil	Karnak and Hamelink 1982
	0.56	--	--	-	Field (Activity)	Keogh and Whitehead 1975
	9.0	--	1 year	-	Turf	King and Dale 1977

Chemical	Application rate tested in the field kg.ha^{-1}	Concentration tested in the laboratory mg.kg^{-1}	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
Benomyl	--	3.5*	14 days	- L. terrestris	Laboratory soil	Haque and Ebing 1983a
	--	27*	14 days	- E. fetida		
	--	22*	28 days	- E. fetida	Laboratory soil	Heimbach 1984
	--	1.0	90 days	- L. terrestris	Laboratory soil	Lofs-Holmin 1980
	--	1.0	90 days	0 A. caliginosa		
	--	1.0	90 days	0 A. chlorotica		
	--	1.0	90 days	- A. rosea		
	2.0	--	180 days	- Lumbricidae	Field	Lofs-Holmin 1981
	0.15	--	270 days	0 L. terrestris	Field	Lofs-Holmin 1982a
	0.15	--	270 days	0 A. caliginosa	(Mixed spray programme)	
	0.15	--	270 days	0 A. rosea		
	0.25	--	90 days	0 L. terrestris	Field	Lofs-Holmin 1982a
	0.25	--	90 days	0 A. caliginosa	(Mixed spray programme)	
	0.25	--	90 days	0 A. rosea		
	--	0.5	26 days	- A. caliginosa	Laboratory soil (Sub-lethal)	Lofs-Holmin 1982b
	--	15.0	64 days	- E. fetida	Laboratory soil	Roark and Dale 1979
	--	9.1* µg.cm^{-2}	2 days	- E. fetida	Laboratory contact test	Roberts and Dorough 1984
	0.28	--	3 years	- Lumbricidae	Orchard (Multiple application)	Stringer and Lyons 1974

Chemical	Application rate tested in the field kg.ha^{-1}	Concentration tested in the laboratory mg.kg^{-1}	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
Benomyl	5.04 (HV) 0.50 (ULV)	-- --	180 days 180 days	- L. terrestris 0 L. terrestris	Orchard (Multiple application)	Stringer and Lyons 1977
	5.04 (HV) 0.50 (ULV)	-- --	180 days 180 days	0 A. longa 0 A. longa	Orchard (Multiple application)	Stringer and Lyons 1977
	--	1.75 µg.cm^{-2}	2 days	- L. terrestris	Laboratory leaf disc (Antifeedant)	Stringer and Wright 1973
	--	500-5000	27 days	- L. terrestris	Laboratory immersion 1 min and observation 27 days	Stringer and Wright 1973
	1.55-38.75	--	14 days	- L. terrestris	Laboratory soil	Stringer and Wright 1973
	0.28	--	2 years	- Lumbricidae	Orchard (Multiple application)	Stringer and Wright 1973
	--	13.9* µg.worm^{-1}	10 days	- L. terrestris	Laboratory forced feeding	Stringer and Wright 1976
	7.8	--	21 days	- Lumbricidae	Turf	Tomlin and Gore 1974
	2.24	--	1 year	- L. terrestris	Turf	Tomlin, Tolman and Thorn 1981
	--	1.75 µg.cm^{-2}	14 days	- L. terrestris	Laboratory leaf disc (Antifeedant)	Wright 1977
	--	10.0* µg.worm^{-1}	--	- L. terrestris	Laboratory forced feeding	Wright 1977

Chemical	Application rate tested in the field $kg.ha^{-1}$	Concentration tested in the laboratory $mg.kg^{-1}$	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
Benomyl	0.28	--	2 years	- Lumbricidae	Orchard (14 sprays)	Wright 1977
	--	5000	14 days	- L. terrestris	Laboratory immersion 1 min and observation 14 days	Wright and Stringer 1973
(With copper oxychloride)	Heavy application	--	2 years	- L. terrestris - A. caliginosa - A. rosea	Orchard	Nicklas and Kennel 1978
Bupirimate	--	338*	28 days	- E. fetida	Laboratory soil	Heimbach 1984
Captafol	--	800*	14 days	- L. terrestris	Laboratory soil	Haque and Ebing 1983a
	--	496*	28 days	- E. fetida	Laboratory soil	Heimbach 1984
Captan	Orchard rate	--	1 year	0 Lumbricidae	Orchard	Cook and Swait 1975
	9.0	--	--	0	Field	Edwards and Lofty 1973
	--	237*	14 days	- L. terrestris	Laboratory soil	Haque and Ebing 1983a
	--	625*	28 days	- E. fetida	Laboratory soil	Heimbach 1984
	--	4.54	42 days	0 L. terrestris	Laboratory soil	Leger and Millette 1977

Chemical	Application rate tested in the field kg.ha^{-1}	Concentration tested in the laboratory mg.kg^{-1}	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
Captan	--	4.54	42 days	0 A. caliginosa	Laboratory soil	Leger and Millette 1977
	--	10-100*	26 hours	- L. terrestris	Laboratory immersion 2 hours and inert media 24 hours	Martin and Wiggins 1959
	4.73-18.9	--	180 days	0 L. terrestris	Orchard (Multiple application)	Stringer and Lyons 1977
	4.73-18.9	--	180 days	0 A. longa (Multiple application)	Orchard	Stringer and Lyons 1977
Carbendazim	Orchard rate	--	3 years	-	Orchard	Cook and Swait 1975
	0.56	--	--	-	Field (Activity)	Keogh and Whitehead 1975
	0.15	--	180 days	- Lumbricidae	Field	Lofs-Holmin 1981
	--	1.75 µg.cm^{-2}	2 days	- L. terrestris	Laboratory leaf disc (Antifeedant)	Stringer and Wright 1973
	--	10.2* µg.worm^{-1}	10 days	- L. terrestris	Laboratory forced feeding	Stringer and Wright 1976
	--	10.0* µg.worm^{-1}	--	- L. terrestris	Laboratory forced feeding	Wright 1977

Chemical	Application rate tested in the field $kg.ha^{-1}$	Concentration tested in the laboratory $mg.kg^{-1}$	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
Carbendazim	--	1.75 $\mu g.cm^{-2}$	14 days	- L. terrestris	Laboratory leaf disc (Antifeedant)	Wright 1977
	--	5000	14 days	- L. terrestris	Laboratory immersion 1 min and observation 14 days	Wright and Stringer 1973
Chlorthalonil	64.0	--	1 year	-	Turf	King and Dale 1977
	1.25	--	90 days	0 L. terrestris	Field (Mixed spray programme)	Lofs-Holmin 1982a
	1.25	--	90 days	0 A. caliginosa		
	1.25	--	90 days	0 A. rosea		
	--	134.9	64 days	- E. fetida	Laboratory soil	Roark and Dale 1979
Dichloran	2.0	--	--	0	Field	Edwards and Lofty 1973
Dinocap	--	2.59	64 days	0 E. fetida	Laboratory soil	Roark and Dale 1979
Ethazole	--	41.97	64 days	- E. fetida	Laboratory soil	Roark and Dale 1979
Fenaminosulf	--	83.93	64 days	- E. fetida	Laboratory soil	Roark and Dale 1979

Chemical	Application rate tested in the field $kg.ha^{-1}$	Concentration tested in the laboratory $mg.kg^{-1}$	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
Folpet	--	459*	14 days	- L. terrestris	Laboratory soil	Haque and Ebing 1983a
	--	338*	14 days	- E. fetida		
Fuberidazole	--	10.6* $\mu g.worm^{-1}$	10 days	- L. terrestris	Laboratory forced feeding	Stringer and Wright 1976
Mancozeb	136.5	--	1 year	-	Turf	King and Dale 1977
	--	95.92	64 days	- E. fetida	Laboratory soil	Roark and Dale 1979
Maneb	--	43.9*	7 days	0 A. chlorotica	Laboratory soil	Fayolle 1979
Quintozene	5.6	--	--	0	Field	Edwards and Lofty 1973
Thiabendazole	--	15.87	64 days	- E. fetida	Laboratory soil	Roark and Dale 1979
	--	1.75 $\mu g.cm^{-2}$	2 days	0 L. terrestris	Laboratory leaf disc (Antifeedant)	Stringer and Wright 1973
	--	13.7* $\mu g.worm^{-1}$	10 days	- L. terrestris	Laboratory forced feeding	Stringer and Wright 1976
	--	1.75 $\mu g.cm^{-2}$	14 days	- L. terrestris	Laboratory leaf disc (Antifeedant)	Wright 1977

Chemical	Application rate tested in the field kg.ha^{-1}	Concentration tested in the laboratory mg.kg^{-1}	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
Thiabendazole	--	10.0* µg.worm^{-1}	--	- L. terrestris	Laboratory forced feeding	Wright 1977
	--	5000	14 days	- L. terrestris	Laboratory immersion 1 min and observation 14 days	Wright and Stringer 1973
Thiophanate-methyl	--	995*	7 days	- E. fetida	Laboratory soil substitute	Bouche 1984a
	Orchard rate	--	1 year	- Lumbricidae	Orchard	Cook and Swait 1975
	--	20.06*	14 days	- E. fetida	Laboratory soil	Goats and Edwards 1985
	6.0	--	180 days	- L. terrestris (Slight effect)	Field	Goats and Edwards 1985
	6.0	--	30 days	- A. longa	Field	Goats and Edwards 1985
	9.0	--	1 year	-	Turf	King and Dale 1977
	--	15.0	64 days	- E. fetida	Laboratory soil	Roark and Dale 1979
	0.78	--	3 years	- Lumbricidae	Orchard (Multiple application)	Stringer and Lyons 1974
	--	1.75 µg.cm^{-2}	2 days	- L. terrestris	Laboratory leaf disc (Antifeedant)	Stringer and Wright 1973
	0.78	--	1 year	- L. terrestris	Orchard (7 applications)	Stringer and Wright 1973
	0.78	--	1 year	- A. longa		
	0.78	--	1 year	- A. chlorotica		
	0.78	--	1 year	- L. rubellus		

Chemical	Application rate tested in the field $kg.ha^{-1}$	Concentration tested in the laboratory $mg.kg^{-1}$	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
Thiophanate-methyl	--	1.75 $\mu g.cm^{-2}$	14 days	- L. terrestris	Laboratory leaf disc (Antifeedant)	Wright 1977
	--	10.0* $\mu g.worm^{-1}$	--	- L. terrestris	Laboratory forced feeding	Wright 1977
	0.78	--	2 years	- Lumbricidae	Orchard (14 sprays)	Wright 1977
	--	5000	14 days	- L. terrestris	Laboratory immersion 1 min and observation 14 days	Wright and Stringer 1973
Thiram	--	292*	14 days	- E. fetida	Laboratory soil substitute	Bouche 1984a
	6.7	--	--	0	Field	Edwards and Lofty 1973
	--	67.65	64 days	- E. fetida	Laboratory soil	Roark and Dale 1979
Triadimefon	--	)250*	14 days	0 L. terrestris	Laboratory soil	Haque and Ebing 1983a
	--	)250*	14 days	0 E. fetida		
	0.125	--	120 days	0 L. terrestris	Field (Mixed spray programme)	Lofs-Holmin 1982a
	0.125	--	120 days	0 A. caliginosa		
	0.125	--	120 days	0 A. rosea		
Triforine	--	--	--	0 E. fetida	Laboratory feeding trial (Weight gain)	Drandarevski, Eichler and Domsch 1977

Chemical	Application rate tested in the field $kg.ha^{-1}$	Concentration tested in the laboratory $mg.kg^{-1}$	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
Ziram	--	169*	14 days	- E. fetida	Laboratory soil substitute	Bouche 1984a

Chemical	Application rate tested in the field kg.ha^{-1}	Concentration tested in the laboratory mg.kg^{-1}	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
3-Aminotriazol	--	64	32 days	- L. terrestris (Probably L. rubellus; after Martin 1982)	Laboratory soil	Caseley and Eno 1966
	--	64	32 days	- Eudrilus eugenae		
	--	100	6 days	- A. caliginosa	Laboratory immersion and observation in inert media	Ghabbour and Imam 1967
	--	100	4 days	- Pheretima californica		
	--	100	4 days	- Alma sp.		
	--	100	7 days	0 A. caliginosa	Laboratory soil	Martin 1982
Asulam	--	100	7 days	0 A. caliginosa	Laboratory soil	Martin 1982
	--	100	7 days	- A. caliginosa (Growth reduced)		
Atrazine	--	64	32 days	0 L. terrestris	Laboratory soil	Caseley and Eno 1966
	--	64	32 days	- E. eugenae		
	8.0	--	1.2 years	-	Turf	Fox 1964
	--	100	4 days	- A. caliginosa	Laboratory immersion and observation in inert media	Ghabbour and Imam 1967
	--	100	1 day	- P. californica		
	--	100	2 days	- Alma sp.		
	--	444*	14 days	- L. terrestris	Laboratory soil	Haque and Ebing 1983a

Chemical	Application rate tested in the field $kg.ha^{-1}$	Concentration tested in the laboratory $mg.kg^{-1}$	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
Atrazine	--	130*	14 days	- E. fetida	Laboratory soil	Haque and Ebing 1983a
	--	100	7 days	0 A. caliginosa	Laboratory soil	Martin 1982
Aziprotryne	--	100	7 days	0 A. caliginosa	Laboratory soil	Martin 1982
	--	1.0	7 days	- A. caliginosa (Growth reduced)		
Bromacil	--	64	32 days	- L. terrestris	Laboratory soil	Caseley and Eno 1966
	--	16	32 days	- E. eugenae		
	--	100	7 days	0 A. caliginosa	Laboratory soil	Martin 1982
	--	100	7 days	- A. caliginosa (Growth reduced)		
	--	--	--	0 Pheretima divergens	Orchard	Takahashii and Sakai 1982
Chloroacetamide	--	21.5*	14 days	- L. terrestris	Laboratory soil	Edwards 1985
	--	15.5*	14 days	- A. caliginosa		
	--	29.5*	14 days	- E. fetida		
	--	26*	14 days	- E. fetida	Laboratory soil	Edwards 1985
	50	--	180 days	0 L. terrestris	Field	Edwards 1985

Chemical	Application rate tested in the field $kg.ha^{-1}$	Concentration tested in the laboratory $mg.kg^{-1}$	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
Chloroacetamide	50	--	180 days	0 A. caliginosa	Field	Edwards 1985
	--	24*	28 days	- E. fetida	Laboratory soil	Heimbach 1984
	--	30*	14 days	- E. fetida	Laboratory soil	Inglesfield 1984
Chlorpropham	--	1.0	--	-	Laboratory soil	Bauer 1964
	--	32	32 days	- L. terrestris	Laboratory soil	Caseley and Eno 1966
	--	64	32 days	- E. eugenae		
Chlorthiamid	16.0	--	5 years	-	Field (Several applications)	Edwards and Stafford 1976
	8.96	--	15 years	-	Field (Several applications)	Edwards and Stafford 1979
Chlortoluron	--	6100*	14 days	- E. fetida	Laboratory soil substitute	Bouche 1984a
Cyanazine	2.0	--	--	+	Field	Edwards, Lofty and Stafford 1971
	4.0	--	--	+		
	4.0	--	150 days	+ L. terrestris	Field	Edwards, Lofty and Stafford 1972
	--	--	--	0	Field	Edwards, Lofty and Stafford 1974

Chemical	Application rate tested in the field $kg.ha^{-1}$	Concentration tested in the laboratory $mg.kg^{-1}$	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
Cycloate	--	3.0	--	0	Laboratory soil	Lhoste 1975
2,4-D	--	100	11 days	- A. caliginosa	Laboratory immersion and observation in inert media	Chabbour and Imam 1967
	--	100	1 days	- P. californica		
	--	100	2 days	- Alma sp.		
	--	100	7 days	0 A. caliginosa	Laboratory soil	Martin 1982
	--	10	7 days	- A. caliginosa (Growth reduced)		
	--	100-1000*	26 hours	- L. terrestris	Laboratory immersion 2 hours and observation in inert media 24 hours	Martin and Wiggans 1959
	--	61.6* $\mu g.cm^{-2}$	2 days	- E. fetida	Laboratory contact test	Roberts and Dorough 1984
Dalapon	--	--	--	-	Laboratory soil	Atlavinyte 1981
	--	64	32 days	0 L. terrestris	Laboratory soil	Caseley and Eno 1966
	--	8.0	32 days	- E. eugenae	Laboratory soil	Caseley and Eno 1966
	--	--	--	0	Field	Edwards, Dennis and Empson 1967
	20.0	--	1.2 years	0	Turf	Fox 1964
	--	100	7 days	0 A. caliginosa	Laboratory soil	Martin 1982

Chemical	Application rate tested in the field $kg.ha^{-1}$	Concentration tested in the laboratory $mg.kg^{-1}$	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
Di-allate	--	1.4	--	0	Laboratory soil	Lhoste 1975
Dinoseb	--	8.3*	7 days	- A. chlorotica	Laboratory soil	Fayolle 1979
	Recommended rate	--	5 days	- L. terrestris	Field (Cane fruit)	White 1980
Diphenamid	--	32	32 days	- L. terrestris	Laboratory soil	Caseley and Eno 1966
	--	16	32 days	- E. eugenae		
Diquat	--	258*	7 days	- E. fetida	Laboratory soil substitute	Bouche 1984a
Diuron	--	64	32 days	- L. terrestris	Laboratory soil	Caseley and Eno 1966
	--	16	32 days	- E. eugenae		
	--	100	7 days	0 A. caliginosa	Laboratory soil	Martin 1982
	--	100	7 days	- A. caliginosa (Growth reduced)		
Endothal	--	64	32 days	0 L. terrestris	Laboratory soil	Caseley and Eno 1966
	--	64	32 days	- E. eugenae		

Chemical	Application rate tested in the field $kg.ha^{-1}$	Concentration tested in the laboratory $mg.kg^{-1}$	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
Glyphosate	--	100	7 days	0 A. caliginosa	Laboratory soil	Martin 1982
	--	1.0	7 days	- A. caliginosa (Growth reduced)		
	--	--	--	0 P. divergens	Orchard	Takahashi and Sakai 1982
Hexazinone	--	100	7 days	0 A. caliginosa	Laboratory soil	Martin 1982
	--	100	7 days	- A. caliginosa (Growth reduced)		
Lenacil	--	4.0	--	0	Laboratory soil	Lhoste 1975
Linuron	0.84	--	1 year	0	Field	Edwards 1970
	4.5	--	1 year	0	Field	Edwards and Arnold 1964
	--	100	7 days	0 A. caliginosa	Laboratory soil	Martin 1982
	--	100	7 days	- A. caliginosa (Growth reduced)		
MCPA	1.68	--	1 year	0	Field	Edwards 1970
	3.0	--	1 year	0	Field	Edwards and Arnold 1964
	--	100	7 days	0 A. caliginosa	Laboratory soil	Martin 1982

Chemical	Application rate tested in the field kg.ha^{-1}	Concentration tested in the laboratory mg.kg^{-1}	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
MCPB	--	100	7 days	0 A. caliginosa	Laboratory soil	Martin 1982
	--	100	7 days	- A. caliginosa (Growth reduced)		
Mecoprop	--	--	--	0	Field	Edwards, Dennis and Empson 1967
Methabenzthiazuron	--	214.8*	7 days	- A. chlorotica	Laboratory soil	Fayolle 1979
	--	100	7 days	0 A. caliginosa	Laboratory soil	Martin 1982
	--	100	7 days	- A. caliginosa (Growth reduced)		
Metribuzin	--	100	7 days	0 A. caliginosa	Laboratory soil	Martin 1982
	--	100	7 days	- A. caliginosa (Growth reduced)		
Monolinuron	--	288*	14 days	- L. terrestris	Laboratory soil	Haque and Ebing 1983a
Monuron	10.0	--	1.2 years	-	Turf	Fox 1964
	--	100	13 days	0 A. caliginosa	Laboratory immersion and observed in inert media	Ghabbour and Imam 1967
	--	100	11 days	0 P. californica		
	--	100	12 days	0 Alma sp.		

Chemical	Application rate tested in the field $kg.ha^{-1}$	Concentration tested in the laboratory $mg.kg^{-1}$	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
Monuron	--	10-100*	26 hours	- L. terrestris	Laboratory immersion 2 hours and observed in inert media 24 hours	Martin and Wiggans 1959
Nitrofen	--	166.8*	7 days	0 A. chlorotica	Laboratory soil	Fayolle 1979
Oxadiazon	--	100	7 days	0 A. caliginosa	Laboratory soil	Martin 1982
	--	100	7 days	- A. caliginosa (Growth reduced)		
Paraquat	--	64	32 days	0 L. terrestris	Laboratory soil	Caseley and Eno 1966
	--	64	32 days	0 E. eugenae		
	11.4	--	2 years	+	Field	Edwards 1970
	--	--	6 years	0 L. terrestris	Field	Edwards, Lofty and Whiting 1972
	Recommended rate for grass control	--	1.5 years	- A. caliginosa (Transient increase)	Field	Edwards and Brown 1982
	--	650*	14 days	- L. terrestris	Laboratory soil	Edwards 1985
	--	370*	14 days	- A. caliginosa		
	--	2000*	14 days	- E. fetida		
	--	1000*	14 days	- E. fetida	Laboratory soil	Edwards 1985
	200	--	180 days	- L. terrestris	Field	Edwards 1985
	200	--	180 days	- A. caliginosa	Field	Edwards 1985

Chemical	Application rate tested in the field $kg.ha^{-1}$	Concentration tested in the laboratory $mg.kg^{-1}$	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
Paraquat	--	)200*	14 days	- L. terrestris	Laboratory soil	Haque and Ebing 1983a
	--	)200*	14 days	- E. fetida		
	--	500* $\mu g.cm^{-2}$	2 days	- E. fetida	Laboratory contact test	Roberts and Dorough 1984
	--	--	--	0 P. divergens	Orchard	Takahashi and Sakai 1982
Pentachlorophenol	75.0	--	30 days	- L. terrestris	Field	Goats 1983
	--	45.53*	14 days	- E. fetida	Laboratory soil	Goats and Edwards 1985
	12.5	--	180 days	- L. terrestris (Slight effect)	Field	Goats and Edwards 1985
	75.0	--	180 days	0 A. longa		
	--	87*	28 days	- E. fetida	Laboratory soil	Heimbach 1984
	10-50	--	--	-	Forest soil	Rombke 1984
Phenmedipham	--	1.0	--	0	Laboratory soil	Lhoste 1975
Prometryn	--	100	7 days	0 A. caliginosa	Laboratory soil	Martin 1982
	--	100	7 days	- A. caliginosa (Growth reduced)		

Chemical	Application rate tested in the field $kg.ha^{-1}$	Concentration tested in the laboratory $mg.kg^{-1}$	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
Propazine	--	100	7 days	0 A. caliginosa	Laboratory soil	Martin 1982
	--	10	7 days	- A. caliginosa (Growth reduced)	Laboratory soil	Martin 1982
Propham	--	1.9	--	-	Laboratory soil	Bauer 1964
Pyrazone	--	47.8*	7 days	- A. chlorotica	Laboratory soil	Fayolle 1979
	--	16.0	--	0	Laboratory soil	Lhoste 1975
Sesone	--	64	32 days	- L. terrestris	Laboratory soil	Caseley and Eno 1966
	--	64	32 days	- E. eugenae		
Simazine	--	--	--	0	Laboratory soil	Atlavinyte, Daciulyte and Lugauskas 1977
	1.68	--	1 year	0	Field	Edwards 1970
	3.0	--	1 year	-	Field	Edwards and Arnold 1964
	--	--	--	- A. caliginosa	Field	Galvyalis and Lugauskas 1978
	--	100	8 days	- A. caliginosa	Laboratory immersion and observation in inert media	Ghabbour and Imam 1967
	--	100	7 days	- P. californica		
	--	100	7 days	- Alma sp.		

Chemical	Application rate tested in the field $kg.ha^{-1}$	Concentration tested in the laboratory $mg.kg^{-1}$	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
Simazine	--	--	--	0	Field	Ilijin 1969
	--	100	7 days	0 A. caliginosa	Laboratory soil	Martin 1982
	--	1.0	7 days	- A. caliginosa (Growth reduced)		
Sodium trichloroacetate	--	400 (Distributed in soil)	71 days	0 A. caliginosa	Laboratory straw disintegration	Atlavinyte 1975
	--	--	--	+	Laboratory straw disintegration	Atlavinyte, Daciulyte and Lugauskas 1974
	--	--	--	- A. caliginosa	Field	Galvyalis and Lugauskas 1978
2,4,5-T	--	45.7* $\mu g.cm^{-2}$	2 days	- E. fetida	Laboratory contact test	Roberts and Dorough 1984
2,3,6-TBA	--	64	32 days	- L. terrestris	Laboratory soil	Caseley and Eno 1966
	--	64	32 days	- E. eugenae		
Terbacil	--	100	7 days	0 A. caliginosa	Laboratory soil	Martin 1982
	--	100	7 days	- A. caliginosa (Growth reduced)		
(With diuron)	--	--	--	0 P. divergens	Orchard	Takahashi and Sakai 1982

Chemical	Application rate tested in the field $kg.ha^{-1}$	Concentration tested in the laboratory $mg.kg^{-1}$	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
Trichloroacetic acid	--	656 (Distributed in soil	170 days	-	Laboratory straw disintegration	Atlavinyte, Daciulyte and Lugauskas 1978
	80.0	--	1.2 years	-	Turf	Fox 1964
	--	40	90 days	0 L. terrestris	Laboratory soil	Lofs-Holmin 1980
	--	40	90 days	- A. caliginosa		
	--	40	90 days	0 A. chlorotca		
	--	40	90 days	- A. rosea		
Tri-allate	--	183*	7 days	- E. fetida	Laboratory soil substitute	Bouche 1984a
	1.68	--	1 year	0	Field	Edwards 1970
	3.0	--	1 year	0	Field	Edwards and Arnold 1964
	--	7.0	--	0	Laboratory soil	Lhoste 1975
Trifluralin	--	1480*	14 days	- E. fetida	Laboratory soil substitute	Bouche 1984a
	--	100	7 days	- A. caliginosa	Laboratory soil	Martin 1982

MISCELLANEOUS COMPOUNDS

Chemical	Application rate tested in the field $kg.ha^{-1}$	Concentration tested in the laboratory $mg.kg^{-1}$	Period of Exposure	Effect upon individual earthworms or populations of earthworms	Exposure conditions and end-point used, if not mortality.	Reference
Chlormequat chloride	--	460*	14 days	- L. terrestris	Laboratory soil	Haque and Ebing 1983a
	--	460*	14 days	- E. fetida		
2,4-Dichlorophenol	--	4.4* $\mu g.cm^{-2}$	2 days	- E. fetida	Laboratory contact test	Roberts and Dorough 1984
Hexoestrol	--	16.7	56 days	0 L. terrestris	Laboratory soil	Raw 1960a
	--	16.7	56 days	0 A. caliginosa		
	--	16.7	56 days	0 A. chlorotica		
Maleic Hydrazide	6.4	--	60 days	0	Field	Lyons, Milsom, Morgan and Stringer 1972
(With 2,4-D)	6.4	--	6 years	0	Field	Lyons, Milsom, Morgan and Stringer 1972
p-Nitrophenol	--	2.4* $\mu g.cm^{-2}$	2 days	- E. fetida	Laboratory contact test	Roberts and Dorough 1984
TCDD ('Dioxin')	0.01	--	5 years	- A. rosea	Field	Martinucci, Crespi, Omodeo, Osella and Traldi 1983
	--	5.0	85 days	0 A. caliginosa	Laboratory soil	Reinecke and Nash 1984
	--	5.0	85 days	0 L. rubellus		
2,4,5-Trichlorophenol	--	2.4* $\mu g.cm^{-2}$	2 days	- E. fetida	Laboratory contact test	Roberts and Dorough 1984

APPENDIX 2

THE UPTAKE BY EARTHWORMS OF HEXACHLOROBENZENE FROM DEPOSITS ON GLASS AND FILTER PAPER

A2.1. Introduction

Cellulose filter paper was non-adsorptive but possessed absorbent qualities which could affect the amount of test chemical that was in contact with the earthworm. To investigate this phenomenon, the uptake of hexachlorobenzene into earthworms from deposits on glass and filter paper was studied. Hexachlorobenzene is a lipophilic compound and is practically insoluble in water.

A2.2. Materials and methods

A2.2.1. Conditions under which the earthworms were exposed to hexachlorobenzene

The glass contact test (Section 2.2.4) and filter paper contact test (Section 3.2.3) were used in this study, and all the reagents were of analytical grade. One ml of a 0.1 $g.litre^{-1}$ solution of hexachlorobenzene in n-hexane was pipetted into 8 cm x 3 cm diameter glass vials. One series of vials had the end and side walls lined with Whatman No. 1 cellulose filter paper, whilst the other series were unlined. The solution was swirled to give a uniform deposit and the solvent evaporated gently with compressed air, taking care not to volatilise the hexachlorobenzene which had a high vapour pressure (approximately $1.1x10^{-5}$mm Hg at 20°C). The area of surface that was treated was 82.5 cm^2 and held 100 µg hexachlorobenzene. The vials that were lined with filter paper were moistened by adding 1 ml distilled water, whilst the unlined vials were humidified by a plug of damp cotton wool held in the cap. The caps of both series of vials were airtight. Each vial contained one earthworm

(sexually mature E. fetida andrei, cultured in cow manure and weighing 0.4 ± 0.01 g) and was stored on its side for a maximum of 6 days at 16°C in the dark. The amount of hexachlorobenzene remaining as a surface deposit or accumulated by the earthworms, was estimated by extracting the contents of one vial of each type at regular intervals.

A2.2.2. Extraction of hexachlorobenzene from the surface deposits

After removing the earthworm from the vial, 5 ml of n-hexane was added to each vial and swirled for 30 seconds. The aqueous and organic phases separated, whereafter aliquots of the latter phase were analysed by gas-liquid chromatography.

A2.2.3. Extraction of hexachlorobenzene from the earthworms

Hexachlorobenzene was extracted from the earthworms using the method of Edwards and Jeffs (1974). The earthworms were ground with anhydrous sodium sulphate (1:4 live weight:Na_2SO_4), mixed with 100 ml acetone:n-hexane (1:1) and shaken for 2 hours. This mixture was filtered through Whatman No.1 filter paper in a Buchner funnel, and shaken for a further 30 seconds with 100ml of 2% aqueous Na_2SO_4 solution in a separating funnel. The organic phase was collected and stored in an airtight flask containing 1 g anhydrous Na_2SO_4 at 4°C prior to analysis by gas-liquid chromatography.

A2.2.4. Gas-liquid chromatography

The analysis was conducted using a Pye series 104 gas chromatograph fitted with an electron capture detector. The column contained 5% SE30 Chromosorb W, flushed with 4.5 litres.min^{-1} nitrogen, whilst the oven and detector temperatures were 160° and 300°C respectively.

A2.3. Results

The amount of hexachlorobenzene in the surface deposit and in the tissue of the earthworms was determined during a 6 day period of exposure (Table A2.1, Figure A2.1). The total amount of hexachlorobenzene that was recovered after 6 days from the filter paper and glass surfaces was 32.8 and 58.4 µg hexachlorobenzene respectively, following an application of 100 $\mu g.vial^{-1}$. The concentration of hexachlorobenzene in the surface deposit decreased with time whilst that in the earthworms increased. The amount that was present in the deposit on the filter paper surface after 6 days was slightly smaller, and showed more variability between replicates, than that collected from the unlined vials, although the rate at which hexachlorobenzene was lost from each type of surface deposit was similar. The earthworms accumulated 28.1 and 51.9 µg hexachlorobenzene.$worm^{-1}$ (equivalent to 70.25 and 129.75 µg hexachlorobenzene.g^{-1} worm) from the lined and unlined vials after 6 days respectively. The rate of accumulation of hexachlorobenzene by earthworms was similar from each type of surface and was particularly rapid during the initial 24 hours.

A2.4. Discussion

Hexachlorobenzene may have evaporated during the process of application or during the 6 day period of exposure, resulting in the small amount that was collected at the end of the experiment. The amount of hexachlorobenzene that was present initially in the vials was not assessed and it was therefore difficult to relate these results to those of previous work. The equilibrium concentration of hexachlorobenzene was not reached in the earthworms after 6 days, although this was unlikely to be due to the metabolism of hexachlorobenzene, as slugs were reported to contain 99.6% unchanged hexachlorobenzene after 10 days had elapsed following exposure to this chemical (Haque and Ebing, 1983b).

Table A2.1

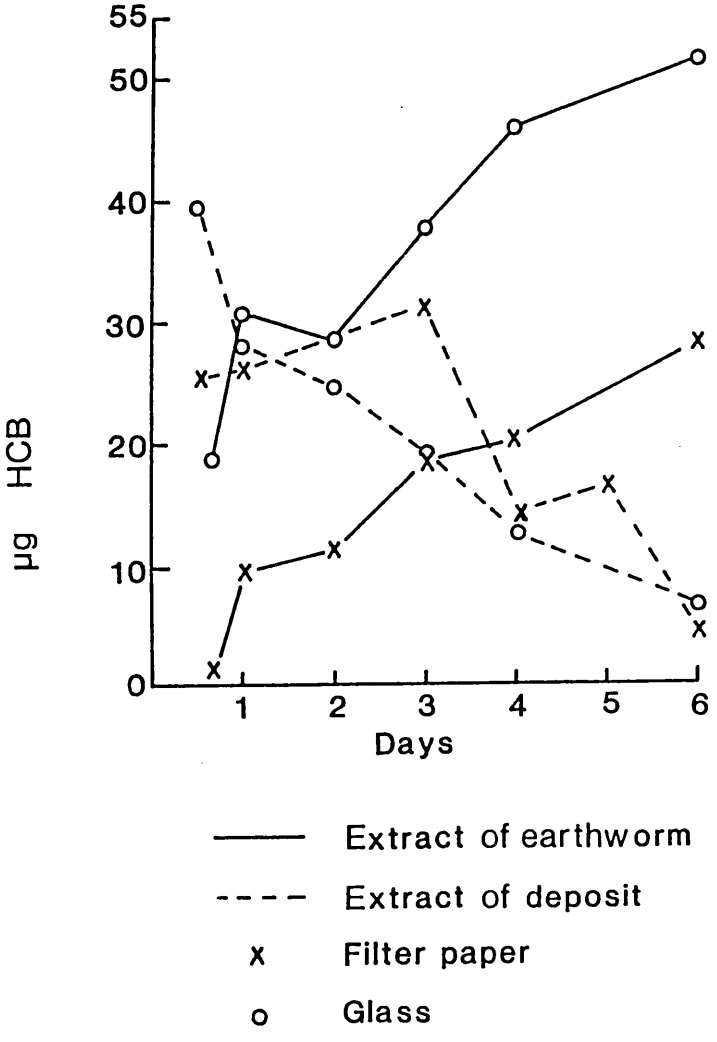
The uptake by earthworms of hexachlorobenzene (HCB) from deposits on a glass and a filter paper surface

Exposure period (days)	-	0.5	1	2	3	4	6
Glass surface(1)	Amount of HCB in the deposit $\mu g.vial^{-1}$	39.8	28.1	24.7	19.3	12.7	6.5
	Amount of HCB in the earthworm $\mu g.worm^{-1}$	18.7	30.7	28.3	37.8	45.9	51.9
Filter paper surface(1)	Amount of HCB in the deposit $\mu g.vial^{-1}$	25.3	26.3	31.1	14.3	16.5	4.7
	Amount of HCB in the earthworm $\mu g.worm^{-1}$	1.2	9.7	11.3	18.9	20.1	28.1

(1) Data are the mean values of two replicates and show the amount of HCB extracted from the earthworms and the vials.

Figure A2.1

Uptake of Hexachlorobenzene by E. fetida from deposits on filter paper and glass surfaces



Assuming that slugs and earthworms take up such chemicals equally (Lord et al., 1980), the results of this study suggest that hexachlorobenzene was accumulated more efficiently from the deposits upon filter paper and glass, than from soil. This conclusion follows from the observation that slugs exposed to a soil that contained 1 $mg.kg^{-1}$ hexachlorobenzene achieved an equilibrium after 18 days with a tissue concentration of 1.4 $\mu g.g^{-1}$ live weight (Haque and Ebing, 1983b). This concentration was much lower than that found in E. fetida during the present study.

Thus the uptake of chemicals from a glass surface seems to be more efficient and predictable than from a filter paper surface, and hexachlorobenzene was accumulated most rapidly during the initial 24 hours. The design of contact methods for testing the toxicity of chemicals to earthworms should ensure that the period of exposure is greater than 24 hours and that highly absorbent surfaces are not used to support the deposit of the test chemical.

APPENDIX 3

THE TOXICITY OF TRICHLOROACETIC ACID TO EARTHWORMS

A3.1. Introduction

Trichloroacetic acid is a strong acid (pKa 0.7) and was found to be highly toxic to earthworms in the filter paper contact test, but much less toxic when tested in a laboratory soil. To ascertain whether trichloroacetic acid reacted with alkaline material in the soil to give a product that was less toxic to earthworms, the toxicity of trichloroacetic acid, neutralised trichloroacetic acid, sodium chloroacetate and hydrochloric acid to E. fetida andrei was assessed using the filter paper contact test.

A3.2. Materials and methods

The filter paper contact test (Section 3.2.3) was used to test the toxicity of the following analytical grade chemicals: a) trichloroacetic acid, b) trichloroacetic acid titrated with 3M $NaHCO_3$ solution to pH 7, c) sodium chloroacetate and d) hydrochloric acid. Ten replicates were used at five concentrations of each chemical in a logarithmic dilution series and the mortality of E. fetida andrei was assessed after 48 hours.

A3.3. Results

The results (Table A3.1) would not allow a probit analysis, but indicated that trichloroacetic acid and hydrochloric acid were very toxic to E. fetida andrei. This toxicity was probably due to the acid conditions produced by these chemicals. Sodium chloroacetate and neutralised trichloroacetic acid (sodium trichloroacetate) were less toxic to the earthworms.

Table A3.1. The toxicity of trichloroacetic acid, sodium trichloroacetate, sodium chloroacetate and hydrochloric acid to E. fetida andrei in the filter paper contact test.

Concentration ($g.cm^{-2}$) (2)	Number of earthworms alive after 48 hours(1)			
	Trichloroacetic acid	Trichloroacetic acid neutralised with $NaHCO_3$	Sodium chloroacetate	Hydrochloric acid
1.0×10^{-3}	0	0	0	0
1.0×10^{-4}	3	8	10	0
1.0×10^{-5}	10	10	10	10
1.0×10^{-6}	10	10	10	10
1.0×10^{7}	10	10	10	10

(1) Ten earthworms were used at each concentration of the test chemical.

(2) One $ml.cm^{-2}$ of $1.4 \times 10^{-2} g.ml^{-1}$ (0.39 M) hydrochloric acid is equivalent in concentration to 1.0×10^{-3} $g.cm^{-2}$ trichloroacetic acid

A3.4. Discussion

The results suggest that in alkaline soils, trichloroacetic acid will react with basic compounds to produce salts that are less toxic to earthworms than the parent material. Previously, trichloroacetic acid was found to be toxic to earthworms in the field (Fox, 1964) and also in experiments in which toxicity to earthworms was tested using soil in the laboratory (Lofs-Holmin, 1980). Such results do not necessarily conflict with those of my study, because toxicity to earthworms was observed only when trichloroacetic acid was applied to the soil at a very high rate.

APPENDIX 4

ELECTROPHORESIS OF ESTERASES FROM EARTHWORMS

A4.1. Introduction

There is some evidence that earthworms exposed to pesticides may show an increased tolerance to them, since earthworms collected from field plots treated repeatedly over a ten year period with the benzoic acid herbicides chloramben and dicamba, appear to be better able to metabolise these compounds than earthworms collected from non-treated plots (Chio and Sanborn, 1978). It is thus possible that earthworms exposed to organophosphorus or carbamate insecticides over a long period may show tolerance to these compounds.

Such pesticides act by blocking the action of acetylcholinesterase, the enzyme responsible for the rapid destruction of the neurotransmitter acetylcholine following its release from the presynaptic neuron (Silver, 1974). There is, as yet, little information available on earthworm esterases. Cholinesterase substrate and inhibitor studies (Stenersen, 1980a) using the naturally-occurring carbamate compound eserine, identified two cholinesterases in E. fetida, which were later characterised by ion exchange chromatography (Stenersen, 1980b). Both enzymes appear to hydrolyse organophosphorus compounds and it has been suggested by Stenersen (1979c) that both would have to be inactivated for the earthworm to die. Histochemical studies indicate that L. terrestris may possess only a single cholinesterase (Silver, 1974).

In this present work, the possibility that lumbricid earthworms show altered cholinesterase activity following long term exposure to choline esterase inhibiting pesticides was investigated. It is now well established that in some insects, for instance in the aphid Myzus persicae (Devonshire, 1977), increased activity of certain esterases

(carboxylesterases) confers resistance to insecticides. When these enzymes are separated electrophoretically, they show as bands of enhanced staining compared with enzymes taken from susceptible insects. Hence, this study investigated whether esterase conferred resistance occurred in earthworms exposed to high concentrations of pesticides. Esterases of two species of earthworm from arable land, L. terrestris and A. longa, were studied using electrophoresis. The earthworms were collected from field sites which (a) had been treated for several years with carbaryl, a compound which inhibits the activity of cholinesterase or (b) had remained pesticide free.

Previous studies on earthworm cholinesterases separated by electrophoresis (Stenersen, 1980b) have used homogenates from whole earthworms. In this study, by contrast, either the gizzard or the whole anterior part of the body (segments 1-5) was used. These tissues were chosen because the gut is in close contact with contaminated soil and was observed to accumulate high concentrations of pesticide (Barker, 1958), whilst the anterior segments of the earthworm contain much nervous tissue and may, as a consequence, show modified esterase activity.

A4.2. Materials and methods

A4.2.1. Earthworms

Worms were collected from the trial sites by formalin extraction (Raw, 1959). They were washed thoroughly in a large volume of water, removed and then stored in a pesticide-free clay loam soil at 10°C in the dark for 7 days. Before use, the earthworms were placed overnight on damp tissue paper to void the contents of the gut.

A4.2.2. Field sites

The treated area was an experimental orchard at the Shell (UK) Research Centre, Sittingbourne. This had been treated with

organophosphorus and carbamate insecticides, at the manufacturers' recommended rates, for at least 15 years. The untreated site was the margin of Park Grass Field, Rothamsted Experimental station and was an area known to have always been free of pesticides. The earthworm populations at both sites were high.

A4.2.3. Chemicals

All the reagents used were of analytical grade.

A4.2.4. Sample preparation

Tissue was dissected from unanaesthetised earthworms and homogenised by hand, in unbuffered 15% sucrose solution (1:15 tissue wet weight to sucrose solution), using a small ground-glass tissue grinder (Gallenkamp and Company Ltd.).

A4.2.5 Electrophoretic running procedure

The methods used were those of Loxdale, Castanera and Brookes (1983). Electrophoretic separations were performed on slab polyacrylamide gels using standard vertical equipment (Genetic Research Instrumentation Ltd.) and a discontinuous buffer (Electrode buffer: 8.3 mM TRIS/30 mM diethyl barbituric acid at pH 7.45; Gel buffer: 70.6 mM TRIS/56.7 mM Cl^- at pH 7.8). Each sample was duplicated and run simultaneously on separate gels between 5-10°C at constant 150v, 40 $mA.gel^{-1}$ for 2 hours.

A4.2.6 Staining procedure

Gels were stained for esterase activity using a diazo-coupled stain reaction mixture (2.5 ml 0.5 M TRIS/HCl at pH 7.1, 25 mg fast blue RR salt, 0.75 ml 1% 1-(alpha)naphthyl and 2-(beta)naphthyl acetate in 50% (v/v) acetone:distilled water). The green-white precipitate formed was filtered before use. The stain was divided equally and eserine sulphate

added to give a final concentration of 1 μM in one portion. Only samples from untreated plots were stained with an eserine containing reaction mixture in order to identify the type of esterase present in the control population. After about 30 minutes, the black-red bands formed were fixed in 7% acetic acid for 5 minutes, whereafter gels were stored refrigerated at 4°C.

A4.3. Results

The esterase banding patterns from the anterior segments and gizzard of L. terrestris and A. longa (Figure A4.1) were similar for any one species; the patterns obtained from both types of tissue were identical, with the gizzard usually staining with greater intensity. L. terrestris consistently showed four to six black staining bands ("alpha-activity"), whereas A. longa had a maximum of seven black bands. These bands may be the product of separate loci and have arbitrarily been assigned to such here. For each esterase separated, slow and fast mobility variants were found, suggesting a single locus, two allele system to be present, alleles coding for monomeric allozymes. Some individuals were found to be in either a homozygous (slow and fast bands only) or heterozygous (both slow and fast together) condition (Ferguson, 1980). Only in A. longa was non-homologous banding seen. This species showed more bands in both the anterior and gizzard tissue respectively than were seen in those from L. terrestris. With both species, gels representing samples taken from pesticide free plots stained with the eserine containing reaction mixture showed a slight diminution of staining intensity (Figure A4.2).

A4.4. Discussion

The reduction in staining intensity of some bands stained in the presence of eserine indicates cholinesterase activity (EC 3.1.1.7 and 3.1.1.8, [Silver, 1974]). The remaining bands probably reflect the

Figure A4.1

GEL ELECTROPHORESIS OF ESTERASES FROM TWO EARTHWORM SPECIES TAKEN FROM PLOTS TREATED WITH COMPOUNDS THAT AFFECT CHOLINESTERASE

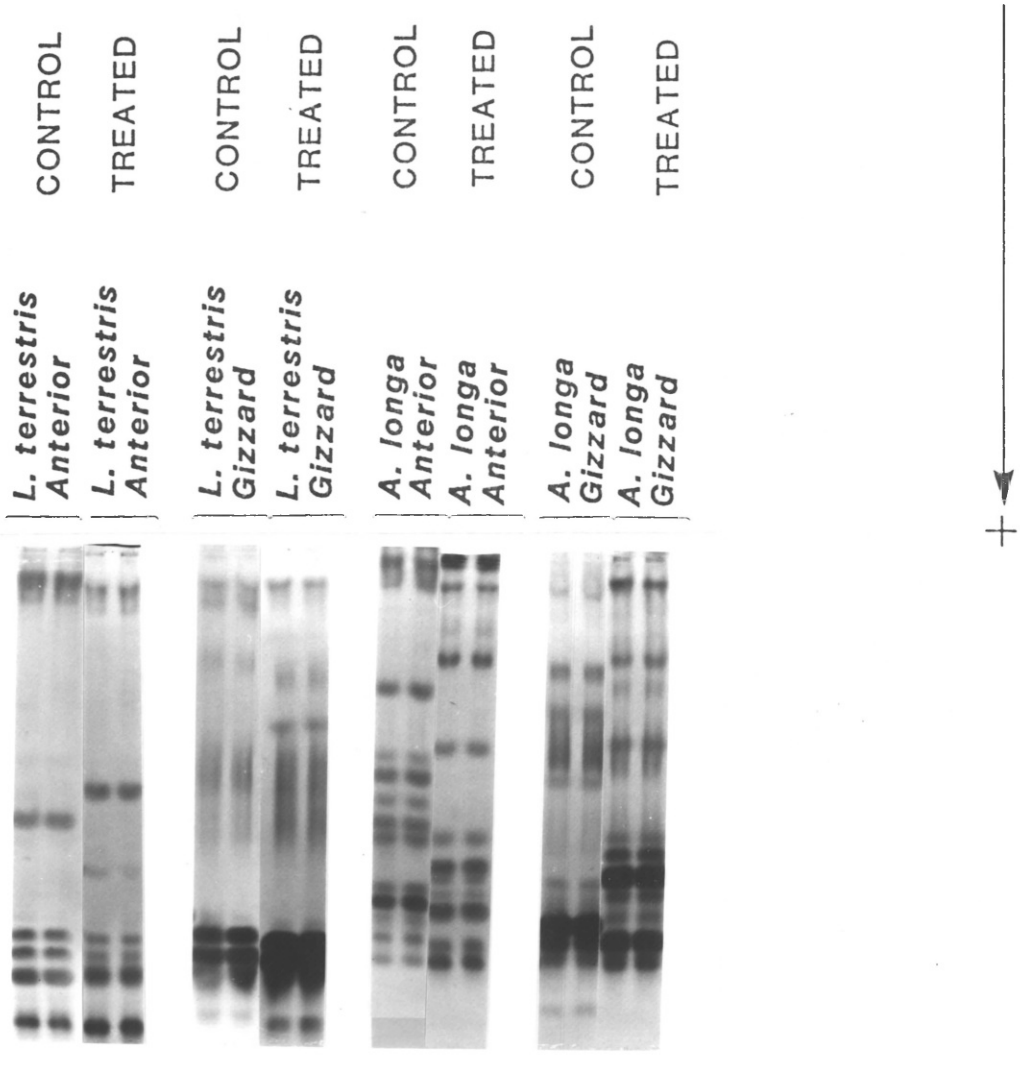
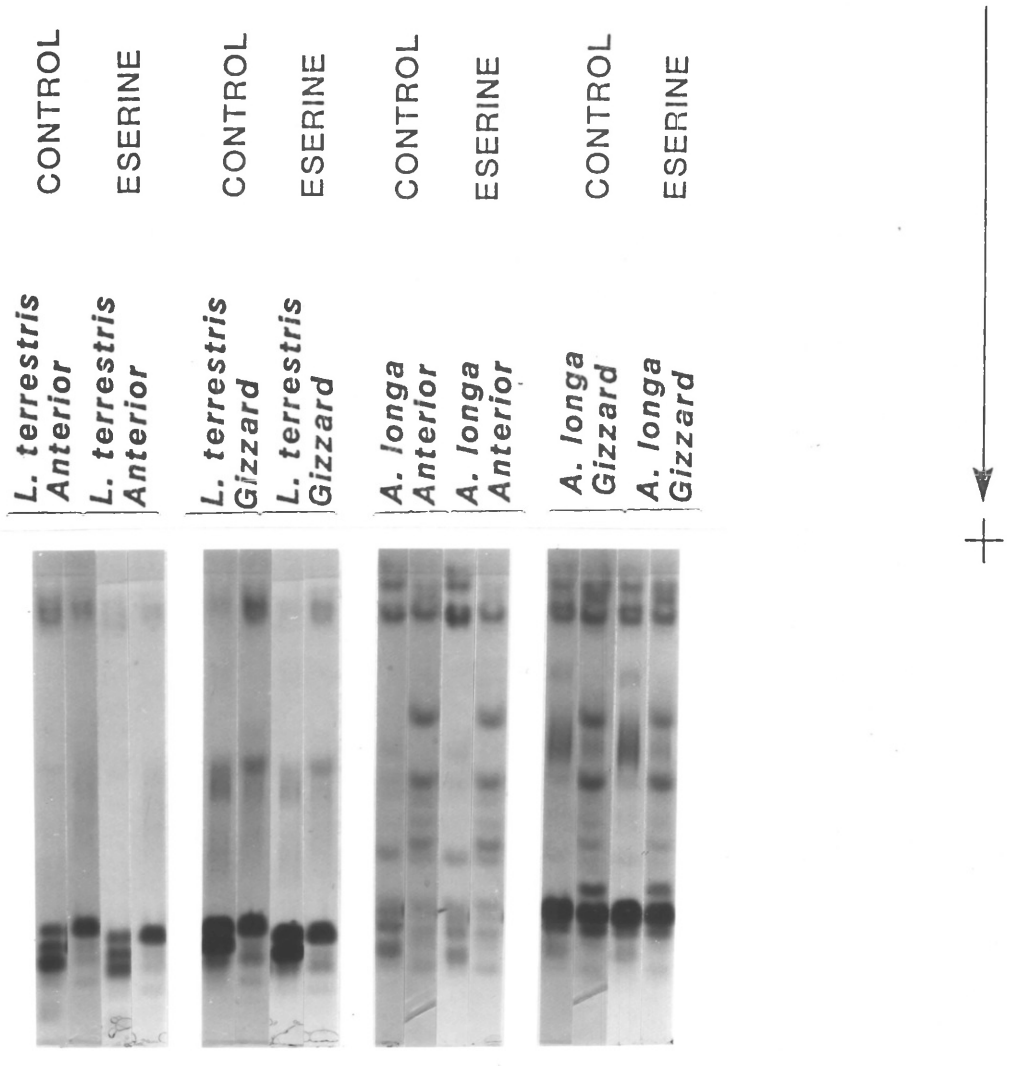


Figure A4.2

GEL ELECTROPHORESIS OF ESTERASES FROM TWO EARTHWORM SPECIES TAKEN FROM PESTICIDE FREE PLOTS. THE EFFECT OF ESERINE



activity of carboxylesterases (EC 3.1.1.1) which, in some insects, for example aphids (Devonshire, 1977) and mosquitos (Pasteur and Sinegre, 1975), are responsible for the degradation of organophosphorus pesticides. All the bands separated display some degree of polymorphism, both in terms of mobility, number and, in some cases, band activity as indicated by the staining intensity. In the mosquito, Culex pipiens, esterase polymorphism has been correlated with decreased sensitivity to chlorpyriphos (Pasteur and Sinegre, 1975). However, in the present work, the populations of earthworms investigated were very small. Whilst it is possible that the increased number of bands in the treated A. longa samples indicate the enhanced esterase activity that was associated with pesticide resistance, the apparent association of such phenotypes with tolerance to chemicals remains unconfirmed for earthworms. In contrast, L. terrestris showed no such band variation.

In conclusion, this preliminary examination of lumbricid earthworm populations in untreated field plots and those treated with pesticides affecting cholinesterase, points to the possibility of pesticide resistance being enhanced in A. longa but not L. terrestris.

APPENDIX 5

SPECIFICIATIONS OF THE APPARATUS, OTHER MATERIALS AND TEST CHEMICALS USED IN THE FILTER PAPER CONTACT AND ARTIFICIAL SOIL TESTS

Table A5.1. Filter paper contact test: apparatus.

Materials	Specification	Manufacturer or Supplier
Glass vial with plastic cap	Transparent, colourless soda glass vial with polypropylene cap. Dimensions: 8 cm x 3 cm diameter	FKB Trident Ltd. Bristol, Avon
Filter paper	Whatman Grade No.1 Qualitative cellulose filter paper sheet.	Whatman Ltd., Maistone, Kent

Table A5.2. Artificial soil test: apparatus.

Materials	Specification	Manufacturer or Supplier
Plastic flask	1 litre transparent, colourless, plastic flask	Norvale Perry Ltd. Maulden, Bedfordshire
Flask lid	90mm diameter disposable plastic Petri dish (lower section)	Sterilin Ltd. Teddington, Middlesex
Spray unit	Glass chromatographic sprayer with a 100ml flask	Gallenkamp and Co. Ltd. London EC2

Table A5.3. Artificial soil test: soil components

Materials	Specification	Manufacturer or Supplier
Sand	British Industrial Sand 110 grade fine washed quartz sand Particle size distribution Sieve size: μ — % Retained 38 — 1.7 45 — 9.3 63 — 29.0 90 — 34.3 125 — 20.8 180 — 4.0 250 — 0.7	British Industrial Sand Ltd., Redhill, Surrey
Clay	Watts, Blake and Bearne Airflo R kaolinitic clay Composition % Kaolinite 51 Potash mica 17 Soda mica 4 Quartz 25 pH 5.1 Surface area 19-20 $m^2.g^{-1}$	Watts, Blake, Bearne and Company Ltd., Newton Abbot, Devon
Peat	Irish Peat Development Authority "Shamrock" Irish moss peat. Sphagnum moss peat. pH 3.7-4.2	Irish Peat Development Authority, Dublin, Ireland.

Table A5.4. The chemicals tested for toxicity to earthworms.

Common name	Chemical name (1)	Solvent	Trade name	% a.i.	Formulation (2)	Manufacturer or Supplier
Benomyl	Methyl 1-(butylcarbamoyl) benzimidazol-2 yl carbamate	chloroform	Benlate	50	w.p.	E.I. DuPont de Nemours and Company
Cadmium acetate	Cadmium acetate	water	-	-	-	Sigma Chemical Company
Carbaryl	1-naphthyl methylcarbamate	chloroform	Sevin	85	w.p.	Union Carbide Corporation
Chlordane	1,2,3,4,5,6,7,8,8-octa-chloro 2,3,3a,4,7,7a-hexahydro-4,7-methanoindene	acetone	Chlordane 25	25	e.c.	Velsicol Chemical Corporation Supplied by Synchemicals Ltd.
Chloroacetamide	Chloroacetamide	water	-	-	-	Sigma Chemical Company
Copper sulphate	Copper sulphate	water	-	-	-	Sigma Chemical Company
Dieldrin	(1R,4S,4aS,5R,6R,7S,8S, 8aR)-1,2,3,4,10,10-hexachloro 1,4,4a,5,6, 7,8,8a-octahydro 6,7-epoxy 1,4:5,8-dimethano-napthalene	acetone	Dieldrex	20	e.c.	Shell International Chemical Company
Iodoacetamide	Iodoacetamide	water	-	-	-	Sigma Chemical Company

Table A5.4. The chemicals tested for toxicity to earthworms - continuation

Common name	Chemical name (1)	Solvent	Trade name	% a.i.	Formulation (2)	Manufacturer or Supplier
Lead acetate	Lead acetate	water	-	-	-	Sigma Chemical Company
Pentachlorophenol	Pentachlorophenol	acetone	Witophen P	100	Flakes	Dynamit Nobel A.G.
Potassium bromide	Potassium bromide	water	-	-	-	Sigma Chemical Company
Thiophanate-methyl	Dimethyl 4,4'-(0-phenylene) bis (3-thioallophante)	chloroform	Cercobin	50	w.p.	Nippon Soda Company Ltd. Supplied by May & Baker Ltd.
Triazophos	0-0-diethyl 0-1-phenyl H1,2,4-triazol 3-yl phosphorothioate	acetone	Hostathion	40	e.c.	Hoechst A.G.
Trichloroacetic Acid	Trichloroacetic acid	water	-	-	-	Sigma Chemical Company

1) Chemical names conform to the IUPAC system.

2) Formulations: w.p. wettable powder

e.c. emulsion concentrate.

APPENDIX 6

ASSESSMENTS OF THE TOXICITY OF CHEMICALS TO EARTHWORMS OBTAINED USING THE FILTER PAPER CONTACT, ARTIFICIAL SOIL AND SILICA PASTE-GLASS BALL TESTS IN SEVERAL LABORATORIES

Table A6.1. Assessments of toxicity to earthworms using the filter paper contact test. First multi-laboratory exercise.

Laboratory (1)	Pentachlorophenol		Carbaryl		Trichloroacetic acid		Copper sulphate	
	LC_{50} $\mu g.cm^{-2}$	95% C.L. or F.L. (2)	LC_{50} $\mu g.cm^{-2}$	95% C.L. or F.L.	LC_{50} $\mu g.cm^{-2}$	95% C.L. or F.L.	LC_{50} $\mu g.cm^{-2}$	95% C.L. or F.L.
1	2.7	2.0-3.7	3.6	1.7-7.9	119.0	74.0-193.0	10.7	0.7-19.2
2	6.0	-	2.0	-	60.0	-	15.0	-
3	15.4	11.0-23.0	1.7	1.2-2.7	100.0	70.0-150.0	25.6	19.0-37.0
4	3.2	2.6-3.8	3.3	1.4-5.2	46.0	19.0-74.0	25.0	4.0-42.0
5*	7.2	4.4-8.3	35.0	19.4-65.3	97.8	73.9-135.0	62.0	35.8-85.8
6	6.1	4.3-8.4	1.5	0.8-2.5	98.9	73.0-129.0	30.0	20.0-43.0
7	8.7	-	12.0	-	93.0	-	10.5	-
8	2.0	-	5.0	-	80.0	-	14.0	-
9	3.4	-	2.7	-	130.0	-	21.0	-
10	-	-	6.2	-	130.0	-	25.0	-
11	5.4	-	3.0	-	166.0	-	8.5	-
12	1.6	1.1-2.4	0.9	0.4-2.0	18.0	10.0-30.0	33.0	20.0-43.0
13	4.2	3.2-5.2	3.0	0.6-10.9	85.0	57.0-125.0	24.7	19.0-32.0
14	2.0	1.4-2.9	1.5	0.7-3.0	130.0	100.0-160.0	23.0	15.0-33.0
15	1.6	-	0.3	6.0-14.0	10.0	-	3.4	-
16	13.0	5.0-32.0	9.0	-	280.0	-	80.0	10.0-100.0
17	5.0	-	1.5	-	30.0	-	10.0	-
18	3.0	-	40	-	87.0	-	23.0	-

Table A6.1 - continued

Laboratory (1)	Pentachlorophenol		Carbaryl		Trichloroacetic acid		Copper sulphate	
	LC_{50} $\mu g.cm^{-2}$	95% C.L. or F.L. (2)	LC_{50} $\mu g.cm^{-2}$	95% C.L. or F.L.	LC_{50} $\mu g.cm^{-2}$	95% C.L. or F.L.	LC_{50} $\mu g.cm^{-2}$	95% C.L. or F.L.
19	1.5	-	1.3	-	17.5	-	2.7	-
20	1.5	-	0.1	-	58.0	-	0.3	-
21	2.5	-	1.5	-	45.0	-	5.0	-
22	1.2	-	0.1	-	-	-	4.0	-
23	1.1	7.1-15.9	23.2	12.7-49.7	108.8	30.0-144.0	13.0	10.0-17.0
24	30.0	20.0-45.0	-	-	170.0	120.0-250.0	58.0	40.0-85.0
25	60.0	44.0-85.0	4.0	2.8-5.8	70.0	58.0-90.0	18.0	14.0-26.0
26	4.1	3.4-5.0	3.5	-	43.0	39.0-47.0	5.4	4.9-6.0
27	4.2	-	33.0	-	230.0	-	30.0	-
28	2.5	-	4.4	-	100.0	-	107.0	-
29	1.6	-	2.1	-	150.0	-	27.0	-
30	1.0	-	0.1	-	100.0	-	10.0	-
31	12.0	-	4.5	-	48.0	-	7.0	-
32	0.4	-	14.0	-	205.0	140.0-280.0	90.0	12.0-2.0
33	7.0	5.6-9.0	3.6	2.6-5.0	39.0	20.0-52.0	16.0	9.5-27.0
34	9.5	7.6-12.0	7.5	3.0-18.0	130.0	72.0-220.0	26.0	17.0-36.0

*Data collected at Rothamsted.

1) Laboratory code numbers are not the same as in the second multi-laboratory exercise.

2) C.L. and F.L. = Confidence and Fiducial limits.

Table A6.2. Assessments of toxicity to earthworms using the artificial soil test. First multi-laboratory exercise.

Laboratory (1)	Pentachlorophenol		Carbaryl		Trichloroacetic acid		Copper sulphate	
	LC_{50} $mg.kg^{-1}$	95% C.L. or F.L. (2)	LC_{50} $mg.kg^{-1}$	95% C.L. or F.L.	LC_{50} $mg.kg^{-1}$	95% C.L. or F.L.	LC_{50} $mg.kg^{-1}$	95% C.L. or F.L.
4	50.0	39.0-61.0	13.3	12.5-14.0	5.1	3.6-6.6	0.9	0.7-1.1
5*	49.9	37.8-65.5	113.5	87.0-146.6	11029	9556-12925	5544	4907-6308
6	133.0	116.0-153.0	72.0	55.0-114.0	>1000	-	4299	3163-5869
7	12.9	3.6-47.5	111.0	-	>1000	-	1066	432.0-3000
12	25.0	-	32.0	-	>1000	-	>1000	-
14	68.0	48.0-92.0	22.0	10.0-42.0	>1000	-	>1000	-
15	73.0	-	7.3	-	1333	-	733	-
16	80.0	-	75.0	-	>1000	-	>1000	-
17	60.0	-	10.0	-	>1000	-	>1000	-
18	120.0	-	160.0	-	>5000	-	1400	-
19	50.0	-	500.0	-	>1000	-	>1000	-
20	300.0	-	280.0	-	700.0	-	250.0	-
21	50.0	-	50.0	-	>1000	-	550.0	-
23	50.0	-	25.0	-	>1000	-	2000	-
25	43.7	40.2-47.6	7.3	3.9-9.0	>1000	-	>1000	-
27	250.0	-	27.4	-	>1250	-	>1250	-
28	39.4	27.2-57.0	15.2	-	>1000	-	>1000	-

Table A6.2 - continued

Laboratory (1)	Pentachlorophenol		Carbaryl		Trichloroacetic acid		Copper sulphate	
	LC_{50} $mg.kg^{-1}$	95% C.L. or F.L. (2)	LC_{50} $mg.kg^{-1}$	95% C.L. or F.L.	LC_{50} $mg.kg^{-1}$	95% C.L. or F.L.	LC_{50} $mg.kg^{-1}$	95% C.L. or F.L.
30	20.1	-	0.1	-	>1000	-	>1000	-
31	50.0	-	150.0	-	1000	-	1000	-
32	8.0	3.9-16.1	4.7	1.4-98.9	>1000	-	>1000	-
33	82.0	68.0-98.0	170.0	118.0-240.0	>1000	-	>1000	-
34	54.0	42.0-70.0	65.0	36.0-110.0	>1000	-	>1000	-

*Data collected at Rothamsted.

1) Laboratory code numbers are not the same as in the second multi-laboratory exercise.

2) C.L. and F.L. = Confidence and Fiducial limits.

Table A6.3. Assessments of toxicity to earthworms using the filter paper contact test. Second multi-laboratory exercise.

Laboratory (1)	Potassium bromide		Pentachlorophenol		Chlordane		Chloroacetamide	
	LC_{50} $\mu g.cm^{-2}$	95% C.L. or F.L. (2)	LC_{50} $\mu g.cm^{-2}$	95% C.L. or F.L.	LC_{50} $\mu g.cm^{-2}$	95% C.L. or F.L.	LC_{50} $\mu g.cm^{-2}$	95% C.L. or F.L.
1	470.0	350.0-630-0	2.4	1.7-3.4	0.9	-	4.0	3.0-6.0
2	560.0	450.0-700.0	5.8	4.2-7.9	4.0	2.8-5.8	4.5	3.6-5.6
3	350.0	180.0-580.0	1.1	0.6-1.6	0.8	4.0-1.2	1.7	1.3-2.2
4	400.0	320.0-520.0	5.9	3.8-9.8	2.3	1.7-3.0	2.0	1.0-3.2
5	650.0	-	4.2	-	4.2	-	2.5	-
6	370.0	250.0-500.0	6.2	6.0-10.0	3.7	2.5-5.0	2.5	0.9-5.0
7	450.0	300.0-600.0	2.4	1.0-5.0	2.8	1.0-5.0	2.9	1.0-4.0
8	1330.0	800.0-2000.0	2.6	2.0-4.0	1.9	1.0-2.0	3.0	2.0-5.0
9	530.0	430.0-640.0	2.6	1.6-3.7	1.3	0.4-2.0	2.4	2.1-2.8
10	90.0	-	3.2	-	1.2	-	0.9	-
11	450.0	-	6.2	-	1.5	-	5.5	-
12	120.0	87.0-320.0	4.0	1.6-7.2	2.7	1.7-4.3	0.5	0.3-0.8
13	480.0	-	6.0	-	3.0	-	2.5	-
14	900.0	-	3.7	-	2.5	-	6.0	-
15	200.0	-	2.5	-	1.8	-	1.5	-
16*	155.0	97.4-263.0	5.0	3.7-6.3	0.9	0.7-1.2	3.5	2.8-4.4
17	350.0	-	6.0	-	6.0	-	1.6	1.2-2.1
18	660.0	500.0-850.0	3.4	2.3-5.2	1.3	0.8-1.9	2.6	1.8-3.6
19	450.0	360.0-560.0	4.7	3.4-6.2	1.7	1.3-2.4	1.4	1.1-1.8
20	250.0	-	1.0	-	0.7	-	1.8	-

*Data collected at Rothamsted.

1) Laboratory code numbers are not the same as in the first multi-laboratory exercise.

2) C.L. and F.L. = Confidence and Fiducial limits.

Table A6.4. Assessments of toxicity to earthworms using the artificial soil test. Second multi-laboratory exercise.

Laboratory (1)	Potassium bromide		Pentachlorophenol		Chlordane		Chloroacetamide	
	LC_{50} $mg.kg^{-1}$	95% C.L. or F.L. (2)	LC_{50} $mg.kg^{-1}$	95% C.L. or F.L.	LC_{50} $mg.kg^{-1}$	95% C.L. or F.L.	LC_{50} $mg.kg^{-1}$	95% C.L. or F.L.
1	160.0	110.0-215.0	43.0	38.0-49.0	45.0	-	18.0	8.0-50.0
2	94.7	67.9-132.4	26.2	18.8-36.6	28.4	21.0-38.3	15.6	12.4-19.8
3	-	-	492.0	346.0-476.0	122.0	104.0-139.0	80.0	-
4	150.0	128.0-175.0	74.0	56.0-100.0	48.0	44.0-53.0	74.0	56.0-100.0
5	-	-	60.0	-	48.0	-	12.0	-
6	-	-	29.0	18.0-35.0	29.0	18.0-35.0	20.0	12.0-25.0
7	10.0	0.5-20.0	110.0	100.0-400.0	129.0	75.0-500.0	13.0	7.0-20.0
9	393.0	213.0-1306	80.5	63.3-103.7	42.1	-	26.0	18.0-32.0
10	230.0	-	75.0	-	27.0	-	15.0	-
11	1000	-	185.0	125.0-250.0	46.5	62.5-31.3	37.5	25.0-50.0
12	157.0	124.0-439.0	123.3	30.2-321.3	56.8	42.9-70.9	39.0	16.0-98.0
13	120.0	-	120.0	-	200.0	-	50.0	-
14	100.0	-	30.0	-	35.0	-	40.0	-
15	150.0	-	80.0	-	50.0	-	70.0	-
16*	460.5	369.0-576.1	41.8	33.0-53.0	24.6	17.9-34.1	19.1	15.5-24.4
18	125.0	74.0-200.0	94.0	71.0-128.0	35.0	22.0-53.0	15.0	11.0-23.0
19	300.0	205.0-480.0	55.0	38.0-82.0	50.0	41.0-59.0	32.0	25.0-40.0
20	200.0	-	60.0	-	30.0	-	30.0	-
21	76.0	-	50.0	-	-	-	-	-

*Data collected at Rothamsted.
1) Laboratory code numbers are not the same as in the first multi-laboratory exercise.
2) C.L. and F.L. = Confidence and Fiducial limits.

Table A6.5. Assesments of toxicity to earthworms using the silica paste and glass-ball ("Artisol") test. Second multi-laboratory exercise.

Laboratory (1)	Potassium bromide		Pentachlorophenol		Chlordane		Chloroacetamide	
	LC_{50} $mg.kg^{-1}$	95% C.L. or F.L. (2)	LC_{50} $mg.kg^{-1}$	95% C.L. or F.L.	LC_{50} $mg.kg^{-1}$	95% C.L. or F.L.	LC_{50} $mg.kg^{-1}$	95% C.L. or F.L.
5	-	-	56.0	-	20.0	-	34.0	-
6	-	-	35.0	20.0-60.0	14.0	10.0-20.0	45.0	25.0-50.0
7	3500	-	50.0	20.0-100.0	39.5	10.0-90.0	51.0	20.0-100.0
9	1000	-	206.0	69.0-347.0	15.4	12.7-18.0	80.0	56.0-100.0
10	1000	-	10.0	-	1000	-	100.0	-
11	1000	-	66.9	-	16.0	-	72.2	-
12	-	-	-	-	31.0	-	25.0	-
16*	2115	1966-2301	266.4	239.5-294.1	20.2	15.8-25.9	188.5	172.9-205.9

*Data collected at Rothamsted.

1) Laboratory code numbers are not the same as in the first multi-laboratory exercise.

2) C.L. and F.L. = Confidence and Fiducial limits.

APPENDIX 7

SUMMARISED PROBIT ANALYSES OF MORTALITY DATA FROM TESTS FOR THE TOXICITY OF CHEMICALS TO EARTHWORMS

KEY

X The parallelism and/or position of the Y-intercept of individual probits lines did not differ significantly when tested by χ^2 and the data were combined to give a single probit line.

χ^2 Chi-squared test results presented as:
χ^2 value (degrees of freedom) significance of χ^2
(significance of F)
An F test was done to calculate the significance of the differences between the parallelism and position of the Y-intercept of individual probit lines, when the χ^2 for total heterogeneity for the data was significant.

\- Data not obtained or analysed.

\+ Data not suitable for analysis.

*** $P<0.001$

R Results obtained by the author during the multilaboratory evaluation of the methods for testing toxicity to earthworms.

APPENDIX 7

Summarised probit analyses of mortality data from tests for the toxicity of chemicals using earthworms

Table A7.1 Immersion test: 24 hour LC50 ($mg.kg^{-1}$ test chemical: water)

		Individual LC50 estimates								Comparison of repeated LC50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LC50 (log scale)	Adjusted S.E. of LC50 (log scale)	Gradient (log scale)	LC50 (log scale)	LC50 (antilog scale)	Fiducial limits of LC50 (log scale)	Fiducial limits of LC50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Chlordane	24h	11.248(6)NS	0.05939	-	4.65	1.08725	12.23	0.9217-1.1967	8.35-15.73	11.405(11)NS	0.565(1)NS	14.180(1)***
Chlordane	24h	0.158(5)NS	0.05293	-	6.36	0.78447	6.09	0.6244-0.8936	4.21-7.83			
X	X	-	-	-	5.19	-	-	-	-			
Carbaryl	24h	2.411(5)NS	0.09705	-	3.07	1.50756	32.18	1.2780-1.7128	18.97-51.61	4.531(10)NS	1.033(1)NS	1.957(1)NS
Carbaryl	24h	2.120(5)NS	0.11291	-	2.18	1.29471	19.71	1.0512-1.5479	11.25-35.27			
X	X	7.521(12)NS	0.07587	-	2.42	1.39353	24.75	1.2386-1.5523	17.32-35.67			
Pentachloro-phenol	24h	0.586(5)NS	0.09438	-	3.42	-0.29428	0.51	-0.5357--0.0919	0.29-0.81	1.420(9)NS	0.066(1)NS	3.912(1)*
Pentachloro-phenol	24h	0.834(4)NS	0.09488	-	3.07	-0.03046	0.93	-0.2925-0.1828	0.51-1.52			
X	X	-	-	-	3.26	-	-	-	-			

		Individual LC50 estimates								Comparison of repeated LC50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LC50 (log scale)	Adjusted S.E. of LC50 (log scale)	Gradient (log scale)	LC50 (log scale)	LC50 (antilog scale)	Fiducial limits of LC50 (log scale)	Fiducial limits of LC50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Chloro-acetamide	24h	0.141(5)NS	0.03734	-	9.30	2.02925	106.97	1.8700-2.0995	75.34-125.75	6.587(10)NS	4.197(1)NS	1.438(1)*
Chloro-acetamide	24h	6.446(5)NS	0.09102	-	3.25	1.79606	62.53	1.5398-1.9589	34.66-90.98			
X	X	-	-	-	3.93	-	-	-	-			
Thiophanate-methyl	24h	0.153(5)NS	0.05116	-	6.37	2.90816	809.39	2.7822-3.0336	605.59-1080.48	0.249(10)NS	0.039(1)NS	0.649(1)NS
Thiophanate-methyl	24h	0.096(5)NS	0.05451	-	5.84	2.84735	703.64	2.6985-2.9751	499.44-944.37			
X	X	-	-	-	6.01	-	-	-	-			
Triazophos	24h	0.290(4)NS	0.07948	-	3.26	1.98158	95.85	1.7865-2.1506	61.16-141.46	1.259(8)NS	1.087(1)NS	1.154(1)NS
Triazophos	24h	0.969(4)NS	0.06392	-	4.83	2.09346	124.01	1.9491-2.2440	88.94-175.37			
X	X	3.500(10)NS	0.05090	-	3.80	2.03741	109.00	1.9298-2.1437	85.08-139.23			
Chlordane 25	24h	6.079(4)NS	0.15471	-	1.60	-0.31881	0.48	-0.6425-0.0196	0.23-1.05	-	-	-

		Individual LC50 estimates								Comparison of repeated LC50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LC50 (log scale)	Adjusted S.E. of LC50 (log scale)	Gradient (log scale)	LC50 (log scale)	LC50 (antilog scale)	Fiducial limits of LC50 (log scale)	Fiducial limits of LC50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Cercobin	24h	5.358(4)NS	0.07984	-	2.50	2.52996	338.82	2.3516-2.6983	224.71-499.28	-	-	-
Hostathion	24h	4.738(4)NS	0.11467	-	2.40	1.46391	29.10	1.1635-1.6847	14.57-48.38	-	-	-

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Table A7.2 Glass deposit contact test: 24 and 48 hour LC50 ($g.cm^{-2}$ test chemical: glass surface)

		Individual LC50 estimates								Comparison of repeated LC50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LC50 (log scale)	Adjusted S.E. of LC50 (log scale)	Gradient (log scale)	LC50 (log scale)	LC50 (antilog scale)	Fiducial limits of LC50 (log scale)	Fiducial limits of LC50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Chlordane	24h	-	-	-	-	-	No LC50	-	-	-	-	-
Chlordane	24h	-	-	-	-	-	No LC50	-	-	-	-	-
Chlordane	48h	1.194(3)NS	0.66243	-	0.79	-7.49126	3.23×10^{-8}	-27.4569--6.6939	3.49×10^{-28}-2.02×10^{-7}	1.213(6)NS	2.071(1)NS	0.928(1)NS
Chlordane	48h	0.019(3)NS	0.18333	-	1.83	-6.71040	1.95×10^{-7}	-7.3096--6.2932	4.90×10^{-8}-5.09×10^{-7}			
X	X	4.212(8)NS	0.21981	-	1.20	-6.96596	1.08×10^{-7}	-7.7493--6.6137	1.7811×10^{-8}-2.4339×10^{-7}			
Thiophanate-methyl	24h	-	-	-	-	-	No LC50	-	-	-	-	-
Thiophanate-methyl	24h	-	-	-	-	-	No LC50	-	-			
Thiophanate-methyl	48h	0.026(5)NS	0.08162	-	3.61	-3.14965	7.09×10^{-4}	-3.5279--2.6652	2.97×10^{-4}-2.16×10^{-3}	0.026(10)NS	0.055(1)NS	2.071(1)NS
Thiophanate-methyl	48h	0.000(5)NS	0.09263	-	4.26	-3.00011	1.00×10^{-3}	-	-			

		Individual LC50 estimates								Comparison of repeated LC50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LC50 (log scale)	Adjusted S.E. of LC50 (log scale)	Gradient (log scale)	LC50 (log scale)	LC50 (antilog scale)	Fiducial limits of LC50 (log scale)	Fiducial limits of LC50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
X	X	2.152(12)NS	0.06200	-	3.68	-3.07022	8.51×10^{-4}	-3.1893- -2.8242	6.47×10^{-4} - 1.50×10^{-3}			
Benomyl	24h	-	-	-	-	-	No LC50	-	-	-	-	-
Benomyl	24h	-	-	-	-	-	No LC50	-	-	-	-	-
Benomyl	48h	0.309(4)NS	0.11826	-	2.83	-3.80428	1.57×10^{-4}	-4.1440- -3.5654	7.18×10^{-5} - 2.72×10^{-4}	1.362(8)NS	0.136(1)NS	0.174(1)NS
Benomyl	48h	1.052(4)NS	0.12672	-	2.43	-3.75241	1.77×10^{-4}	-4.1198- -3.5070	7.59×10^{-5} - 3.11×10^{-4}			
X	X	1.672(10)NS	0.08720	-	2.59	-3.77779	1.67×10^{-4}	-3.9893- -3.6132	1.02×10^{-4} - 2.44×10^{-4}			

Table A7.3 Filter paper contact test in vials: 24, 48 and 72 hr LC50 ($g.cm^{-2}$ test chemical: filter paper surface)

		Individual LC50 estimates								Comparison of repeated LC50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LC50 (log scale)	Adjusted S.E. of LC50 (log scale)	Gradient (log scale)	LC50 (log scale)	LC50 (antilog scale)	Fiducial limits of LC50 (log scale)	Fiducial limits of LC50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Chlordane	24h	5.162(4)NS	0.06683	-	3.94	-4.30774	4.92×10^{-5}	-4.4723- -4.1538	3.37×10^{-5} = 7.02×10^{-5}	-	-	-
Chlordane	48h	0.602(4)NS	0.08854	-	2.95	-5.12580	7.49×10^{-6}	-5.3746- -4.9476	4.22×10^{-6} = 1.23×10^{-5}	-	-	-
Pentachloro-phenol	24h	-	-	-	-	-	No LC50	-	-	-	-	-
Pentachloro-phenol	48h	1.488(4)NS	0.05484	-	5.43	-4.22262	5.99×10^{-5}	-4.3502- -4.0728	4.46×10^{-5} = 8.46×10^{-5}	-	-	-
Thiophanate-methyl	24h	-	-	-	-	-	No LC50	-	-	-	-	-
Thiophanate-methyl	48h	1.188(4)NS	0.14841	-	2.23	-3.52738	2.97×10^{-4}	-3.7987- -2.9955	1.59×10^{-4} = 1.01×10^{-3}	-	-	-

		Individual LC50 estimates								Comparison of repeated LC50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LC50 (log scale)	Adjusted S.E. of LC50 (log scale)	Gradient (log scale)	LC50 (log scale)	LC50 (antilog scale)	Fiducial limits of LC50 (log scale)	Fiducial limits of LC50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Thiophanate-methyl	72h	0.093(4)NS	0.09455	-	4.00	-3.97858	1.05×10^{-4}	-4.150- -3.3129	7.08×10^{-5} - 4.87×10^{-4}	-	-	-
Benomyl	24h	-	-	-	-	-	No LC50	-	-	-	-	-
Benomyl	48h	2.304(4)NS	0.14654	-	2.45	-3.90914	1.23×10^{-4}	-4.1203- -3.0006	7.58×10^{-5} - 9.99×10^{-4}	-	-	-
Benomyl	72h	6.085(4)NS	0.21935	-	1.07	-4.15852	6.94×10^{-5}	-4.5017- -3.0504	3.15×10^{-5} - 8.90×10^{-4}	-	-	-
Triazophos	24h	-	-	-	-	-4.7932- -4.4089	1.61×10^{-5} - 3.90×10^{-5}	-	-	-	-	-
Triazophos	48h	0.254(4)NS	0.06186	-	5.63	-4.64439	2.27×10^{-5}	-4.7908- -4.5027	1.62×10^{-5} - 3.14×10^{-5}	-	-	-

Table A7.4 Filter paper contact test in Petri dishes: 24, 48 and 72 hour LC50 ($g.cm^{-2}$ test chemical: filter paper surface)

		Individual LC50 estimates								Comparison of repeated LC50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LC50 (log scale)	Adjusted S.E. of LC50 (log scale)	Gradient (log scale)	LC50 (log scale)	LC50 (antilog scale)	Fiducial limits of LC50 (log scale)	Fiducial limits of LC50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Chlordane	24h	-	-	-	-	-4.4089- -4.1911	3.90×10^{-5} = 6.44×10^{-5}	-	-	-	-	-
Chlordane	48h	2.328(4)NS	0.07446	-	3.61	-4.89073	1.29×10^{-5}	-5.06 6- -4.72 96	8.68×10^{-6} = 1.86×10^{-5}	-	-	-
Pentachloro-phenol	24h	-	-	-	-	-	No LC50	-	-	-	-	-
Pentachloro-phenol	48h	0.619(4)NS	0.29377	-	2.68	-3.63335	2.33×10^{-4}	+	+	-	-	-
Thiophanate-methyl	24h	-	-	-	-	-	No LC50	-	-	-	-	-
Thiophanate-methyl	48h	-	-	-	-	-	No LC50	-	-	-	-	-
Thiophanate-methyl	72h	0.212(4)NS	0.12162	-	2.55	-3.75696	1.75×10^{-4}	-3.9962- -3.4064	1.01×10^{-4} = 3.92×10^{-4}	-	-	-

		Individual LC50 estimates								Comparison of repeated LC50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LC50 (log scale)	Adjusted S.E. of LC50 (log scale)	Gradient (log scale)	LC50 (log scale)	LC50 (antilog scale)	Fiducial limits of LC50 (log scale)	Fiducial limits of LC50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Benomyl	24h	-	-	-	-	-	No LC50	-	-	-	-	-
Benomyl	48h	-	-	-	-	-	No LC50	-	-	-	-	-
Benomyl	72h	2.130(4)NS	0.29498	-	1.72	-3.66561	2.16×10^{-4}	-4.0138- -1.4212	9.69×10^{-5} - 3.79×10^{-2}	-	-	-
Triazophos	24h	-	-	-	-	-	No LC50	-	-	-	-	-
Triazophos	48h	2.330(4)NS	0.21369	-	1.01	-4.23697	5.79×10^{-5}	-4.6080- -3.3272	2.47×10^{-5} - 4.71×10^{-4}	-	-	-

Table A7.5 Sand test. 24 and 48 hour LC50 ($mg.kg^{-1}$ test chemical: dry weight of silica sand)

		Individual LC50 estimates								Comparison of repeated LC50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LC50 (log scale)	Adjusted S.E. of LC50 (log scale)	Gradient (log scale)	LC50 (log scale)	LC50 (antilog scale)	Fiducial limits of LC50 (log scale)	Fiducial limits of LC50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Chlordane	24h	-	-	-	-	-	No LC50	-	-	-	-	-
Chlordane	48h	5.202(2)NS	0.24340	-	1.45	-2.31459	0.00485	-3.5826- -1.9744	0.0003- 0.0106	-	-	-
Pentachloro-phenol	24h	-	-	-	-	-	No LC50	-	-	-	-	-
Pentachloro-phenol	48h	0.527(2)NS	0.04764	-	6.13	-1.35522	0.04413	-1.4641- -1.2422	0.0343- 0.0573	-	-	-
Thiophanate-methyl	24h	-	-	-	-	-	No LC50	-	-	-	-	-
Thiophanate-methyl	48h	3.196(10)NS	0.06138	-	3.69	-2.22919	0.00590	-2.3594- -2.0736	4.37×10^{-3}- 8.44×10^{-3}	-	-	-

Table A7.6 Antibumping granule test: 48 and 96 hour LC50 ($mg.kg^{-1}$ test chemical: dry weight of antibumping granules)

		Individual LC50 estimates								Comparison of repeated LC50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LC50 (log scale)	Adjusted S.E. of LC50 (log scale)	Gradient (log scale)	LC50 (log scale)	LC50 (antilog scale)	Fiducial limits of LC50 (log scale)	Fiducial limits of LC50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Chlordane	48h	-	-	-	-	-	No LC50	-	-	-	-	-
Chlordane	48h	-	-	-	-	-	No LC50	-	-	-	-	-
Chlordane	96h	3.166(3)NS	0.17055	-	2.20	1.51730	32.91	0.8751-1.9351	7.50-86.13	4.208(6)NS	0.302(1)NS	0.206(1)NS
Chlordane	96h	1.042(3)NS	0.20577	-	1.64	1.60257	40.05	0.8267-2.0465	6.71-111.31			
X	X	4.716(8)NS	0.13500	-	1.85	1.56081	36.38	1.2015-1.8238	15.90-67.43			
Pentachloro-phenol	48h	-	-	-	-	-	No LC50	-	-	-	-	-
Pentachloro-phenol	48h	-	-	-	-	-	No LC50	-	-	-	-	-
Pentachloro-phenol	96h	1.752(4)NS	0.21553	-	1.14	-2.25795	5.52×10^{-3}	-2.7329--1.7459	1.85×10^{-3}-1.80×10^{-2}	3.248(8)NS	1.079(1)NS	0.036(1)NS
Pentachloro-phenol	96h	1.496(4)NS	0.30079	-	0.77	-2.11375	7.70×10^{-3}	-2.6742-	2.12×10^{-3}-			

		Individual LC50 estimates								Comparison of repeated LC50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LC50 (log scale)	Adjusted S.E. of LC50 (log scale)	Gradient (log scale)	LC50 (log scale)	LC50 (antilog scale)	Fiducial limits of LC50 (log scale)	Fiducial limits of LC50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
X	X	4.363(10)NS	0.18040	-	0.91	-2.20036	6.30×10^{-3}	-1.2530 -2.5494- -1.7381	5.46×10^{-2} 2.82×10^{-3} - 1.63×10^{-2}			
Thiophanate-methyl	48h	-	-	-	-	-	No LC50	-	-	-	-	-
Thiophanate-methyl	48h	-	-	-	-	-	No LC50	-	-	-	-	-
Thiophanate-methyl	96h	-	-	-	-	-	No LC50	-	-	-	-	-
Thiophanate-methyl	96h	-	-	-	-	-	No LC50	-	-	-	-	-

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Table A7.7 Silica paste and glass ball test: 14 day LC50 ($mg.kg^{-1}$ test chemical: dry weight of amorphous silica)

		Individual LC50 estimates								Comparison of repeated LC50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LC50 (log scale)	Adjusted S.E. of LC50 (log scale)	Gradient (log scale)	LC50 (log scale)	LC50 (antilog scale)	Fiducial limits of LC50 (log scale)	Fiducial limits of LC50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Chlordane	14 day	8.316(3)*	0.02739	0.04560	5.25	1.45127	28.27	1.3931-1.5037	24.72-31.90	15.586(6)* (-)	4.245(1)* (NS)	6.226(1)- (NS)
Chlordane	14 day	7.269(3)NS	0.05218	-	3.50	1.30532	20.20	1.1984-1.4139	15.79-25.93			
Carbaryl	14 day	15.189(4)**	0.04057	0.07906	2.78	2.10523	127.42	2.0224-2.1856	105.29-153.32	28.912(8)*** (-)	4.703(1)* (NS)	1.353(1)- (NS)
Carbaryl	14 day	13.723(4)**	0.07651	0.14171	1.85	1.99578	99.03	1.8308-2.1456	67.74-140.16			
Pentachloro-phenol	14 day	1.911(3)NS	0.02690	-	5.65	2.40062	251.55	2.3443-2.4533	220.94-283.98	2.283(6)NS	0.288(1)NS	2.837(1)NS
Pentachloro-phenol	14 day	0.372(3)NS	0.03642	-	6.45	2.48428	304.99	2.3937-2.5557	250.45-359.51			
X	X	5.408(8)NS	0.02221	-	5.69	2.42551	266.39	2.3794-2.4684	239.53-294.07			
Chloro-acetamide	14 day	0.906(3)NS	0.02155	-	8.36	2.28357	192.12	2.2409-2.3284	174.13-213.03	0.948(6)NS	0.715(1)NS	0.703(1)NS

		Individual LC50 estimates								Comparison of repeated LC50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LC50 (log scale)	Adjusted S.E. of LC50 (log scale)	Gradient (log scale)	LC50 (log scale)	LC50 (antilog scale)	Fiducial limits of LC50 (log scale)	Fiducial limits of LC50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Chloro-acetamide	14 day	0.042(3)NS	0.04095	-	6.80	2.24352	175.19	2.1506-2.3266	141.43-212.14			
X	X	2.365(8)NS	0.01888	-	7.91	2.27534	188.51	2.2377-2.3136	172.88-205.87			
Thiophanate-methyl	14day	11.524(3)**	0.07916	0.15515	2.44	1.55396	35.81	1.3761-1.7127	23.77-51.61	22.662(6)*** (-)	0.961(1)- (NS)	3.103(1)- (NS)
Thiophanate-methyl	14day	11.138(3)*	0.06376	0.12285	3.27	1.74419	55.49	1.5995-1.8790	39.76-75.68			
Potassium bromide	14day	4.793(3)NS	0.02039	-	8.49	3.32507	2113.81	3.2889-3.3740	1944.76-2365.97	10.256(6)NS	2.459(1)NS	0.579(1)NS
Potassium bromide	14day	5.463(3)NS	0.03147	-	5.87	3.31507	2065.73	3.2534-3.3858	1792.31-2430.83			
X	X	13.293(8)NS	0.01698	-	7.21	3.32540	2115.45	3.2936-3.3621	1966.28-2301.82			

Table A7.8 Natural soil test: 14 day LC50 ($mg.kg^{-1}$ test chemical: dry weight of natural soil)

		Individual LC50 estimates								Comparison of repeated LC50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LC50 (log scale)	Adjusted S.E. of LC50 (log scale)	Gradient (log scale)	LC50 (log scale)	LC50 (antilog scale)	Fiducial limits of LC50 (log scale)	Fiducial limits of LC50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Chlordane	14day	0.005(3)NS	0.07203	-	2.89	-2.32293	0.00475	-2.4834- -2.1841	0.0033- 0.0065			
Chlordane	14day	0.117(3)NS	0.07884	-	2.23	-2.34685	0.00450	-2.5104- -2.1858	0.0031- 0.0065	0.122(6)NS	1.345(1)NS	0.013(1)NS
X	X	1.480(8)NS	0.05302	-	2.50	-2.33918	0.00458	-2.4474- -2.2345	0.0036- 0.0058			
Carbaryl	14day	7.316(4)NS	0.04298	-	2.68	1.62025	41.71	1.5345- 1.7074	34.43- 50.98			
Carbaryl	14day	23.465(4)***	0.07616	0.18446	1.24	1.64900	44.57	1.5011- 1.8092	31.70- 64.45	30.781(8)*** (-)	20.484(1)*** (*)	0.004(1)- (-)
Pentachloro-phenol	14day	1.562(5)NS	0.07041	-	2.54	-2.04015	0.00912	-2.2027- -1.9107	0.0063- 0.0123			
Pentachloro-phenol	14day	2.760(5)NS	0.06824	-	2.36	-1.88598	0.01300	-2.0402- -1.7598	0.0091- 0.0174	4.322(10)NS	0.120(1)NS	3.209(1)NS
X	X	7.651(12)NS	0.04950	-	2.40	-1.96290	0.01089	-2.0695- -1.8707	0.0085- 0.0135			

		Individual LC50 estimates							Comparison of repeated LC50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LC50 (log scale)	Adjusted S.E. of LC50 (log scale)	Gradient (log scale)	LC50 (log scale)	LC50 (antilog scale)	Fiducial limits of LC50 (log scale)	Fiducial limits of LC50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Chloro-acetamide	14day	0.009(3)NS	0.07330	-	2.69	1.61331	41.05	1.4583-1.7595	28.73-57.48			
Chloro-acetamide	14day	3.957(5)NS	0.03203	-	5.89	1.69449	49.49	1.6231-1.7537	41.99-56.72	24.452(13)NS	16.999(2)***	1.018(2)-
Chloro-acetamide	14day	20.487(5)**	0.04047	0.08192	3.20	1.60163	39.96	1.5190-1.6821	33.04-48.09			
Thiophanate-methyl	14day	0.680(4)NS	0.08925	-	1.71	0.07774	1.20	-0.1074-0.2548	0.78-1.80	4.802(8)NS	1.806(1)NS	3.014(1)NS
Thiophanate-methyl	14day	4.122(4)NS	0.07905	-	2.27	-0.11870	0.76	-0.2893-0.0388	0.51-1.09			
X	X	9.621(10)NS	0.06086	-	1.87	-0.01933	0.96	-0.1430-0.1004	0.72-1.26			

Table A7.9. Forced feeding test: 48, 72 and 144 hour LC50 ($mg.kg^{-1}$ test chemical: agar-agar gel. 5µl of gel force fed per worm)

		Individual LC50 estimates								Comparison of repeated LC50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LC50 (log scale)	Adjusted S.E. of LC50 (log scale)	Gradient (log scale)	LC50 (log scale)	LC50 (antilog scale)	Fiducial limits of LC50 (log scale)	Fiducial limits of LC50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Chlordane	48 h	-	-	-	-	-	No LC50	-	-	-	-	-
Chlordane	48 h	-	-	-	-	-	No LC50	-	-	-	-	-
Chlordane	72 h	0.024(3)NS	0.08294	-	4.39	0.99593	9.91	0.8447-1.6715	6.99-46.93	0.761(6)NS	0.221(1)NS	4.401(1)*
Chlordane	72 h	0.737(3)NS	0.09544	-	3.49	1.28900	19.45	1.0879-1.5103	12.24-32.38			
X	X	-	-	-	3.68	-	-	-	-			
Chlordane	144 h	0.585(3)NS	0.12051	-	2.08	0.68291	4.82	0.3830-0.9361	2.42-8.63	0.609(6)NS	2.336(1)NS	0.058(1)NS
Chlordane	144 h	0.024(3)NS	0.08302	-	4.39	0.70301	5.05	0.0174-0.8543	1.04-7.15			
X	X	3.003(8)NS	0.07502	-	2.58	0.67609	4.74	0.5048-0.8263	3.20-6.70			
Thiophanate-methyl	48 h	-	-	-	-	-	No LC50	-	-	-	-	-
Thiophanate-methyl	48 h	-	-	-	-	-	No LC50	-	-	-	-	-

		Individual LC50 estimates								Comparison of repeated LC50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LC50 (log scale)	Adjusted S.E. of LC50 (log scale)	Gradient (log scale)	LC50 (log scale)	LC50 (antilog scale)	Fiducial limits of LC50 (log scale)	Fiducial limits of LC50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Thiophanate-methyl	72 h	2.728(4)NS	0.21448	-	1.18	2.75450	568.20	2.4013-3.5371	251.94-3444.04	5.096(8)NS	5.467(1)*	1.632(1)-
Thiophanate-methyl	72 h	2.368(4)NS	0.10056	-	2.80	2.40062	251.55	2.1781-2.6195	150.71-416.43			
Thiophanate-methyl	144 h	0.882(4)NS	0.13489	-	1.70	1.78381	60.79	1.4628-2.0585	29.02-114.42	3.240(8)NS	0.648(1)NS	4.161(1)*
Thiophanate-methyl	144 h	2.358(4)NS	0.11167	-	2.22	2.15284	142.18	1.9047-2.3970	80.29-249.45			
X	X	-	-	-	1.92	-	-	-	-			
Benomyl	48 h	-	-	-	-	-	No LC50	-	-	-	-	-
Benomyl	48 h	-	-	-	-	-	No LC50	-	-	-	-	-
Benomyl	72 h	3.567(4)NS	0.12534	-	1.09	2.11239	129.54	1.8551-2.3778	71.63-238.68	5.017(8)NS	6.771(1)**	0.568(1)-
Benomyl	72 h	1.450(4)NS	0.10006	-	2.71	1.89412	78.36	1.6601-2.1631	45.72-145.59			

		Individual LC50 estimates								Comparison of repeated LC50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LC50 (log scale)	Adjusted S.E. of LC50 (log scale)	Gradient (log scale)	LC50 (log scale)	LC50 (antilog scale)	Fiducial limits of LC50 (log scale)	Fiducial limits of LC50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Benomyl	144 h	0.772(4)NS	0.08224	-	2.44	1.49573	31.31	1.3020-1.6522	20.04-44.90	2.677(8)NS	0.122(1)NS	14.649(1)***
Benomyl	144 h	1.905(4)NS	0.11430	-	2.19	0.91068	8.14	0.6390-1.1522	4.36-14.20			
X	X	-	-	-	2.34	-	-	-	-			

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Table A7.10.1 Earthworm body weight and sexual maturity. Chlordane LT50: hours ($1\mu g.cm^{-2}$ test chemical: filter paper surface)

		Individual LT50 estimates								Comparison of repeated LT50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LT50 (log scale)	Adjusted S.E. of LT50 (log scale)	Gradient (log scale)	LT50 (log scale)	LT50 (antilog scale)	Fiducial limits of LT50 (log scale)	Fiducial limits of LT50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Chlordane	0.1g	1.482(7)NS	0.02390	-	11.61	1.37719	23.83	1.3078-1.4257	20.31-26.65	3.200(10)NS	0.000(1)NS	2.249(1)NS
Chlordane	0.1g	1.718(3)NS	0.02766	-	11.61	1.31881	20.84	1.2627-1.3953	18.31-24.85			
X	X	5.449(12)NS	0.01929	-	10.33	1.35121	22.45	1.3093-1.3903	20.38-24.56			
Chlordane	0.2g	5.185(7)NS	0.03676	-	5.36	1.42029	26.32	1.3340-1.4911	21.58-30.98	7.245(10)NS	1.305(1)NS	1.529(1)NS
Chlordane	0.2g	2.060(3)NS	0.03165	-	7.65	1.48791	30.75	1.4152-1.5547	26.01-35.86			
X	X	10.078(12)NS	0.02448	-	6.10	1.45210	28.32	1.3996-1.5002	25.10-31.64			
Chlordane	0.3g	4.387(7)NS	0.03627	-	5.11	1.68906	48.87	1.6174-1.7724	41.44-59.20	4.767(10)NS	0.235(1)NS	0.474(1)NS
Chlordane	0.3g	0.380(3)NS	0.05666	-	6.42	1.70748	50.99	1.6274-2.1457	42.40-139.87			
X	X	5.476(12)NS	0.02926	-	5.45	1.70090	50.22	1.6474-1.7703	44.40-58.92			

		Individual LT50 estimates								Comparison of repeated LT50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LT50 (log scale)	Adjusted S.E. of LT50 (log scale)	Gradient (log scale)	LT50 (log scale)	LT50 (antilog scale)	Fiducial limits of LT50 (log scale)	Fiducial limits of LT50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Chlordane	0.4g	5.585(7)NS	0.03626	-	4.90	1.57007	37.16	1.4915-1.6449	31.01-44.15	8.351(10)NS	0.803(1)NS	0.183(1)NS
Chlordane	0.4g	2.766(3)NS	0.03447	-	6.59	1.54586	35.14	1.4686-1.6254	29.42-42.21			
X	X	9.337(12)NS	0.02615	-	5.32	1.56177	36.46	1.5083-1.6159	32.23-41.30			
Chlordane	0.5g	0.917(7)NS	0.02346	-	13.10	1.86715	73.65	1.8130-1.9214	65.02-83.45			
Chlordane	0.5g	-	-	-	-	-	No LT50	-	-	-	-	-

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Table A7.10.2. Earthworm body weight and sexual maturity. Chloroacetamide LT50: hours ($8\mu g.cm^{-2}$ test chemical: filter paper surface)

		Individual LT50 estimates								Comparison of repeated LT50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LT50 (log scale)	Adjusted S.E. of LT50 (log scale)	Gradient (log scale)	LT50 (log scale)	LT50 (antilog scale)	Fiducial limits of LT50 (log scale)	Fiducial limits of LT50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Chloro-acetamide	0.1g	2.074(8)NS	0.03004	-	7.08	0.87189	7.45	0.8131-0.9531	6.50-8.98	-	-	-
Chloro-acetamide	0.2g	2.514(8)NS	0.03420	-	8.34	1.05869	11.45	0.9935-1.1429	9.85-13.90	-	-	-
Chloro-acetamide	0.3g	0.077(8)NS	0.02458	-	14.78	1.32408	21.09	1.1903-1.3673	15.50-23.30	-	-	-
Chloro-acetamide	0.4g	0.003(8)NS	0.01501	-	22.94	1.35896	22.85	1.3258-1.3932	21.17-24.73			
Chloro-acetamide	0.5g	2.310(8)NS	0.03087	-	8.02	1.35922	22.87	1.2780-1.4195	18.97-26.27			

Table A7.10.3. Earthworm body weight and sexual maturity. Copper sulphate LT50: hours ($50\mu g.cm^{-2}$ test chemical: filter paper surface)

		Individual LT50 estimates								Comparison of repeated LT50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LT50 (log scale)	Adjusted S.E. of LT50 (log scale)	Gradient (log scale)	LT50 (log scale)	LT50 (antilog scale)	Fiducial limits of LT50 (log scale)	Fiducial limits of LT50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Copper sulphate	0.1g	2.354(3)NS	0.09073	-	3.78	1.25749	18.09	1.0239-1.4297	10.57-26.90	3.570(6)NS	0.021(1)NS	0.808(1)NS
Copper sulphate	0.1g	1.215(3)NS	0.08200	-	3.96	1.15273	14.21	0.9698-1.3234	9.33-21.07			
X	X	4.399(8)NS	0.06097	-	3.80	1.20108	15.89	1.0678-1.3189	11.69-20.84			
Copper sulphate	0.2g	0.560(3)NS	0.10891	-	6.51	1.46467	29.15	+	+	2.579(6)NS	0.616(1)NS	1.417(1)NS
Copper sulphate	0.2g	2.019(3)NS	0.08128	-	4.07	1.25529	18.00	1.0533-1.4080	11.31-25.59			
X	X	4.612(8)NS	0.06086	-	4.18	1.31877	20.83	1.1693-1.4266	14.77-26.71			
Copper sulphate	0.3g	2.354(3)NS	0.09073	-	3.78	1.25749	18.09	1.0239-1.4297	10.57-26.90	2.736(6)NS	1.467(1)NS	1.339(1)NS
Copper sulphate	0.3g	0.381(3)NS	0.07610	-	7.90	1.49563	31.31	+	+			
X	X	5.542(8)NS	0.06030	-	4.11	1.33771	21.76	1.1877-1.4438	15.41-27.79			

		Individual LT50 estimates								Comparison of repeated LT50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LT50 (log scale)	Adjusted S.E. of LT50 (log scale)	Gradient (log scale)	LT50 (log scale)	LT50 (antilog scale)	Fiducial limits of LT50 (log scale)	Fiducial limits of LT50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Copper sulphate	0.4g	0.025(3)NS	0.02145	-	18.66	1.61844	41.54	1.5615-1.6712	36.43-46.91	2.282(6)NS	3.025(1)*	2.851(1)*
Copper sulphate	0.4g	2.258(3)NS	0.03148	-	7.98	1.67549	47.37	1.5815-1.7596	38.15-57.49			
Copper sulphate	0.5g	0.560(3)NS	0.10891	-	6.51	1.46467	29.15	+	+	0.605(6)NS	0.012(1)NS	2.006(1)**
Copper sulphate	0.5g	0.045(3)NS	0.06395	-	5.96	1.55232	35.67	-0.4187-1.6386	0.38-43.51			
Copper sulphate	0.6g	1.259(3)NS	0.02329	-	14.67	1.67962	47.82	1.6095-1.7315	40.69-53.88	4.752(6)NS	0.141(1)NS	0.003(1)NS
Copper sulphate	0.6g	3.493(3)NS	0.02296	-	12.39	1.67941	47.80	1.6172-1.7294	41.42-53.63			
X	X	4.896(8)NS	0.01640	-	13.30	1.67950	47.81	1.6409-1.7126	43.75-51.59			

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Table A7.11 Temperature. 48 hour LC50. ($g.cm^{-2}$ test chemical: filter paper surface)

		Individual LC50 estimates								Comparison of repeated LC50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LC50 (log scale)	Adjusted S.E. of LC50 (log scale)	Gradient (log scale)	LC50 (log scale)	LC50 (antilog scale)	Fiducial limits of LC50 (log scale)	Fiducial limits of LC50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Chlordane	5°	6.576(5)NS	0.19264	-	1.02	-4.73930	1.82×10^{-5}	-5.1597- -4.3417	6.92×10^{-6} - 4.55×10^{-5}	6.972(10)NS	5.896(1)*	1.122(1)-
Chlordane	5°	0.396(5)NS	0.11415	-	2.64	-5.04573	9.0×10^{-6}	-5.2791- -4.4937	5.26×10^{-6} - 3.21×10^{-5}			
Chlordane	10°	0.671(5)NS	0.14134	-	2.33	-5.95643	1.11×10^{-6}	-6.3844- -5.6911	4.13×10^{-7} - 2.04×10^{-6}	0.837(10)NS	0.210(1)NS	12.439(1)***
Chlordane	10°	0.166(5)NS	0.13025	-	1.93	-5.30292	4.98×10^{-6}	-5.6144- -4.9869	2.43×10^{-6} - 1.03×10^{-5}			
X	X	-	-	-	2.13	-	-	-	-			
Chlordane	20°	2.121(5)NS	0.13106	-	2.20	-5.70244	1.98×10^{-6}	-6.0776- -5.4464	8.36×10^{-7} - 3.58×10^{-6}	3.984(10)NS	0.004(1)NS	4.313(1)*
Chlordane	20°	1.864(5)NS	0.15445	-	2.14	-6.10949	7.77×10^{-6}	-6.5529- -5.8220	2.80×10^{-7} - 1.51×10^{-6}			
X	X	-	-	-	2.17	-	-	-	-			
Chlordane	25°	3.490(5)NS	0.13394	-	2.04	-5.65206	2.23×10^{-6}	-6.0264- -5.3835	9.41×10^{-7} - 4.14×10^{-6}	4.604(10)NS	0.043(1)NS	4.194(1)*

		Individual LC50 estimates								Comparison of repeated LC50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LC50 (log scale)	Adjusted S.E. of LC50 (log scale)	Gradient (log scale)	LC50 (log scale)	LC50 (antilog scale)	Fiducial limits of LC50 (log scale)	Fiducial limits of LC50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Chlordane	25°	1.114(5)NS	0.14876	-	2.21	-6.03305	9.27×10^{-7}	-6.4707- -5.7557	3.38×10^{-7} - 1.76×10^{-6}			
X	X	-	-	-	2.12	-	-	-	-			
Chlordane	30°	6.074(5)NS	0.16443	-	1.45	-5.72057	1.90×10^{-6}	-6.1108- -5.3792	7.75×10^{-7} - 4.18×10^{-6}	6.444(10)NS	0.477(1)NS	11.707(1)***
Chlordane	30°	0.370(5)NS	0.16981	-	1.87	-6.52069	3.02×10^{-7}	-6.9726- -6.1716	1.07×10^{-7} - 6.74×10^{-7}			
X	X	-	-	-	1.60	-	-	-	-			
Carbaryl	5°	5.195(3)NS	0.44554	-	0.57	-3.61333	2.44×10^{-4}	-4.3443- -1.9168	4.53×10^{-5} - 1.21×10^{-2}			
Carbaryl	5°	5.233(3)NS	0.32431	-	0.69	-4.08389	8.24×10^{-5}	-4.7075- -3.1798	1.96×10^{-5} - 6.61×10^{-4}	10.428(6)NS	0.225(1)NS	0.571(1)NS
X	X	11.224(8)NS	0.26434	-	0.63	-3.86487	1.36×10^{-4}	-4.3353- -3.1828	4.62×10^{-5} - 6.56×10^{-4}			
Carbaryl	10°	5.446(3)NS	0.29927	-	0.73	-4.46321	3.44×10^{-5}	-5.0693- -3.7375	8.53×10^{-6} - 1.83×10^{-4}			
Carbaryl	10°	4.234(3)NS	0.25053	-	0.93	-4.44128	3.62×10^{-5}	-4.9882- -3.8785	1.03×10^{-5} - 1.32×10^{-4}	9.681(6)NS	0.521(1)NS	0.017(1)NS
X	X	10.218(8)NS	0.19483	-	0.81	-4.45856	3.48×10^{-5}	-4.8470- -4.0389	1.42×10^{-5} - 9.14×10^{-5}			

		Individual LC50 estimates								Comparison of repeated LC50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LC50 (log scale)	Adjusted S.E. of LC50 (log scale)	Gradient (log scale)	LC50 (log scale)	LC50 (antilog scale)	Fiducial limits of LC50 (log scale)	Fiducial limits of LC50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Carbaryl	20°	4.012(3)NS	0.30282	-	0.69	-4.65757	2.20×10^{-5}	-5.3026- -3.9333	4.98×10^{-6} - 1.17×10^{-4}	7.201(6)NS	1.174(1)NS	0.103(1)NS
Carbaryl	20°	3.189(3)NS	0.23774	-	1.00	-4.55931	2.76×10^{-5}	-5.0858- -4.0332	8.21×10^{-6} - 9.26×10^{-5}			
X	X	8.478(8)NS	0.19054	-	0.82	-4.61486	2.43×10^{-5}	-5.0014- -4.2093	9.97×10^{-6} - 6.18×10^{-5}			
Carbaryl	25°	2.514(3)NS	0.40160	-	0.48	-4.97320	1.00×10^{-5}	-5.9634- -4.0168	1.09×10^{-6} - 9.62×10^{-5}	4.342(6)NS	2.285(1)NS	0.001(1)NS
Carbaryl	25°	1.827(3)NS	0.26534	-	0.83	-4.96573	1.08×10^{-5}	-5.5615- -4.3954	2.74×10^{-6} - 4.02×10^{-5}			
X	X	6.628(8)NS	0.22962	-	0.62	-4.96981	1.07×10^{-5}	-5.4564- -4.4944	3.50×10^{-6} - 3.20×10^{-5}			
Carbaryl	30°	3.189(3)NS	0.23774	-	1.00	-5.44070	3.62×10^{-6}	-5.9668- -4.9142	1.08×10^{-6} - 1.22×10^{-5}	5.170(6)NS	0.019(1)NS	0.079(1)NS
Carbaryl	30°	1.981(3)NS	0.23213	-	1.05	-5.53372	2.92×10^{-6}	-6.0530- -5.0211	8.85×10^{-7} - 9.53×10^{-6}			
X	X	5.268(8)NS	0.16623	-	1.02	-5.48712	3.26×10^{-6}	-5.8332- -5.1462	1.47×10^{-6} - 7.20×10^{-6}			

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Table A7.12.1 Filter paper area (side wall). 48 hour LC50. ($g.cm^{-2}$ test chemical: filter paper surface)

		Individual LC50 estimates								Comparison of repeated LC50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LC50 (log scale)	Adjusted S.E. of LC50 (log scale)	Gradient (log scale)	LC50 (log scale)	LC50 (antilog scale)	Fiducial limits of LC50 (log scale)	Fiducial limits of LC50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Chlordane	75 cm^2	1.857(4)NS	0.14824	-	2.01	-5.90413	1.25×10^{-6}	-6.3294- -5.6267	4.68×10^{-7} - 2.36×10^{-6}	2.971(8)NS	0.062(1)NS	0.554(1)NS
Chlordane	75 cm^2	1.114(4)NS	0.14877	-	2.21	-6.03305	9.27×10^{-7}	-6.4707- -5.7557	3.38×10^{-7} - 1.76×10^{-6}			
X	X	3.587(10)NS	0.10554	-	2.08	-5.96817	1.08×10^{-6}	-6.2258- -5.7744	5.95×10^{-7} - 1.68×10^{-6}			
Chlordane	100 cm^2	3.499(4)NS	0.15247	-	1.93	-6.10868	7.79×10^{-7}	-6.4848- -5.7978	3.27×10^{-7} - 1.59×10^{-6}	5.362(8)NS	0.076(1)NS	0.006(1)NS
Chlordane	100 cm^2	1.864(4)NS	0.15443	-	2.14	-6.10949	7.77×10^{-7}	-6.5529- -5.8220	2.80×10^{-7} - 1.51×10^{-6}			
X	X	5.445(10)NS	0.10779	-	2.03	-6.11209	7.73×10^{-7}	-6.3603- -5.9064	4.36×10^{-7} - 1.24×10^{-6}			
Chlordane	190 cm^2	1.857(4)NS	0.14824	-	2.01	-5.90413	1.25×10^{-6}	-6.3294- -5.6267	4.68×10^{-7} - 2.36×10^{-6}	3.683(8)NS	0.234(1)NS	0.254(1)NS
Chlordane	190 cm^2	1.826(4)NS	0.16159	-	1.68	-6.04104	9.10×10^{-7}	-6.4474- -5.7220	3.57×10^{-7} - 1.90×10^{-6}			
X	X	4.172(10)NS	0.11023	-	1.80	-5.97823	1.05×10^{-6}	-6.2319- -5.7692	5.86×10^{-7} - 1.70×10^{-6}			

Table A7.12.2. Filter paper area (lined end wall). 48 hour LC50. ($g.cm^{-2}$ test chemical: filter paper surface)

		Individual LC50 estimates								Comparison of repeated LC50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LC50 (log scale)	Adjusted S.E. of LC50 (log scale)	Gradient (log scale)	LC50 (log scale)	LC50 (antilog scale)	Fiducial limits of LC50 (log scale)	Fiducial limits of LC50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Chlordane	Unlined 75 cm^2	0.000(4)NS	0.06795	-	5.10	-6.04969	8.92×10^{-7}	-6.1947- -5.5824	6.39×10^{-7} - 2.62×10^{-6}	-	-	-
Chlordane	Lined 82.5 cm^2	0.000(4)NS	0.09263	-	4.26	-6.00011	1.00×10^{-6}	+	+	-	-	-
Copper sulphate	Unlined 75 cm^2	1.055(3)NS	0.19383	-	1.45	-4.49971	3.16×10^{-5}	-4.9446- -4.0518	1.14×10^{-5} - 8.88×10^{-5}	-	-	-
Copper sulphate	Lined 82.5 cm^2	0.543(3)NS	0.21510	-	1.19	-4.80727	1.56×10^{-5}	-5.2797- -4.3233	5.25×10^{-6} - 4.75×10^{-5}	-	-	-

Table A7.13 Filter paper grade (Whatman grades). 48 hour LC50. ($g.cm^{-2}$ test chemical: filter paper surface)

		Individual LC50 estimates								Comparison of repeated LC50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LC50 (log scale)	Adjusted S.E. of LC50 (log scale)	Gradient (log scale)	LC50 (log scale)	LC50 (antilog scale)	Fiducial limits of LC50 (log scale)	Fiducial limits of LC50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Chlordane	Grade 1	3.105(4)NS	0.15706	-	1.72	-5.97234	1.07×10^{-6}	-6.3603- -5.6578	1.36×10^{-7} - 2.20×10^{-6}	4.961(8)NS	0.183(1)NS	0.043(1)NS
Chlordane	Grade 1	1.857(4)NS	0.14824	-	2.01	-5.90413	1.25×10^{-6}	-6.3294- -5.6267	4.68×10^{-7} - 2.36×10^{-6}			
X	X	5.187(10)NS	0.10817	-	1.83	-5.94314	1.14×10^{-6}	-6.1912- -5.7372	6.44×10^{-7} - 1.83×10^{-6}			
Chlordane	Grade 3	1.289(4)NS	0.10282	-	3.12	-5.54848	2.83×10^{-6}	-5.8215- -5.3421	1.51×10^{-6} - 4.55×10^{-6}	3.547(8)NS	0.020(1)NS	0.134(1)NS
Chlordane	Grade 3	2.258(4)NS	0.10336	-	2.96	-5.50086	3.16×10^{-6}	-5.7768- -5.2900	1.67×10^{-6} - 5.13×10^{-6}			
X	X	3.701(10)NS	0.07295	-	3.03	-5.52446	2.99×10^{-6}	-5.6935- -5.3833	2.03×10^{-6} - 4.14×10^{-6}			
Chlordane	Grade 6	1.720(4)NS	0.11663	-	2.55	-5.62680	2.36×10^{-6}	-5.9480- -5.3933	1.13×10^{-6} - 4.04×10^{-6}	3.577(8)NS	0.366(1)NS	1.820(1)NS
Chlordane	Grade 6	1.857(4)NS	0.14824	-	2.01	-5.90413	1.25×10^{-6}	-6.3294- -5.6267	4.68×10^{-7} - 2.36×10^{-6}			
X	X	5.763(10)NS	0.09583	-	2.17	-5.76537	1.72×10^{-6}	-5.9985- -5.5865	1.00×10^{-6} - 2.59×10^{-6}			

Table A7.14 Filter paper material (Whatman materials). LT50: hours (78.5 μg.cm^{-2} test chemical: filter paper surface)

		Individual LT50 estimates								Comparison of repeated LT50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LT50 (log scale)	Adjusted S.E. of LT50 (log scale)	Gradient (log scale)	LT50 (log scale)	LT50 (antilog scale)	Fiducial limits of LT50 (log scale)	Fiducial limits of LT50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Chlordane	Cellulose Grade 1	1.114(1)NS	0.05064	-	5.49	1.51776	32.94	1.3782-1.6172	23.89-41.42	-	-	-
Chlordane	Glass fibre Grade GF/A	2.547(1)NS	0.04695	-	5.62	1.58392	38.36	1.4657-1.6816	29.22-48.04	-	-	-

Table A7.15 Period of exposure 24,48,72 and 96 hour LC50 ($g.cm^{-2}$ test chemical: filter paper surface)

		Individual LC50 estimates								Comparison of repeated LC50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LC50 (log scale)	Adjusted S.E. of LC50 (log scale)	Gradient (log scale)	LC50 (log scale)	LC50 (antilog scale)	Fiducial limits of LC50 (log scale)	Fiducial limits of LC50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Carbaryl	24 h	-	-	-	-	-	No LC50	-	-	-	-	-
Carbaryl	48 h	1.040(3)NS	0.11389	-	1.69	-4.65291	2.22×10^{-5}	-4.8932- -4.4165	1.28×10^{-5} = 3.83×10^{-5}	-	-	-
Carbaryl	72 h	3.430(3)NS	0.12022	-	1.53	-5.79972	1.59×10^{-6}	-6.0565- -5.5550	8.78×10^{-7} = 2.79×10^{-6}	-	-	-
Carbaryl	96 h	-	-	-	-	-	100% mortality	-	-	-	-	-

Table A7.16 Replication. 48 hour LC50 ($g.cm^{-2}$ test chemical: filter paper surface)

		Individual LC50 estimates								Comparison of repeated LC50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LC50 (log scale)	Adjusted S.E. of LC50 (log scale)	Gradient (log scale)	LC50 (log scale)	LC50 (antilog scale)	Fiducial limits of LC50 (log scale)	Fiducial limits of LC50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Carbaryl	5 replicates	0.086(3)NS	0.24622	-	1.81	-4.49999	3.16×10^{-5}	-5.3130- -3.6863	4.86×10^{-6} - 2.06×10^{-4}	-	-	-
Carbaryl	10 replicates	0.499(3)NS	0.18371	-	1.62	-4.59498	2.54×10^{-5}	-5.0284- -4.1690	9.37×10^{-6} - 6.78×10^{-5}	-	-	-
Carbaryl	15 replicates	0.548(3)NS	0.14770	-	1.67	-4.56373	2.73×10^{-5}	-4.8895- -4.2407	1.29×10^{-5} - 5.75×10^{-5}	-	-	-
Carbaryl	20 replicates	0.997(3)NS	0.12778	-	1.68	-4.69059	2.04×10^{-5}	-4.9659- -4.4217	1.08×10^{-5} - 3.79×10^{-5}	-	-	-
Carbaryl	25 replicates	1.040(3)NS	0.11389	-	1.69	-4.65291	2.22×10^{-5}	-4.8932- -4.4165	1.28×10^{-5} - 3.83×10^{-5}	-	-	-

Table A7.17 Ventilated and sealed vials. 48 hour LC_{50} ($g.cm^{-2}$ test chemical: filter paper surface)

		Individual LC50 estimates								Comparison of repeated LC50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LC50 (log scale)	Adjusted S.E. of LC50 (log scale)	Gradient (log scale)	LC50 (log scale)	LC50 (antilog scale)	Fiducial limits of LC50 (log scale)	Fiducial limits of LC50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Carbaryl	Ventilated	1.428(5)NS	0.16374	-	1.33	-4.46541	3.42×10^{-5}	-4.8662- -4.1483	1.36×10^{-5} - 7.11×10^{-5}	-	-	-
Carbaryl	Unventilated	2.290(5)NS	0.16150	-	1.30	-4.29229	5.10×10^{-5}	-4.6781- -3.9708	2.10×10^{-5} - 1.07×10^{-4}	-	-	-
Thio-phanate-methyl	Ventilated	3.507(4)NS	0.10360	-	2.84	-3.45432	3.51×10^{-4}	-3.7290- -3.2359	1.87×10^{-4} - 5.81×10^{-4}	5.875(8)NS	0.001(1)NS	0.980(1)NS
Thio-phanate-methyl	Ventilated	2.368(4)NS	0.10056	-	2.80	-3.59938	2.52×10^{-4}	-3.8219- -3.3805	1.51×10^{-4} - 4.16×10^{-4}			
X	X	6.856(10)NS	0.07376	-	2.73	-3.53053	2.95×10^{-4}	-3.6910- -3.3818	2.04×10^{-4} - 4.15×10^{-4}			
Thio-phanate-methyl	Unventilated	4.391(4)NS	0.12125	-	2.24	-3.52877	2.96×10^{-4}	-3.8564- -3.2681	1.39×10^{-4} - 5.39×10^{-4}	6.802(8)NS	0.690(1)NS	0.021(1)NS
Thio-phanate-methyl	Unventilated	2.411(4)NS	0.09706	-	3.07	-3.49244	3.22×10^{-4}	-3.7220- -3.2872	1.90×10^{-4} - 5.16×10^{-4}			
X	X	7.514(10)NS	0.07441	-	2.66	-3.50707	3.11×10^{-4}	-3.6684- -3.3559	2.15×10^{-4} - 4.41×10^{-4}			

		Individual LC50 estimates								Comparison of repeated LC50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LC50 (log scale)	Adjusted S.E. of LC50 (log scale)	Gradient (log scale)	LC50 (log scale)	LC50 (antilog scale)	Fiducial limits of LC50 (log scale)	Fiducial limits of LC50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Potassium bromide	Ventilated	0.583(4)NS	0.10167	-	3.37	-4.59894	2.52×10^{-5}	-4.8628- -4.3936	1.37×10^{-5} - 4.04×10^{-5}	1.119(9)NS	0.074(1)NS	1.955(1)NS
Potassium bromide	Ventilated	0.536(5)NS	0.09753	-	3.79	-4.39556	4.02×10^{-5}	-4.7969- -4.2269	1.60×10^{-5} - 5.93×10^{-5}			
X	X	3.149(11)NS	0.07153	-	3.30	-4.50304	3.14×10^{-5}	-4.6744- -4.3689	2.12×10^{-5} - 4.28×10^{-5}			
Potassium bromide	Unventi-lated	0.150(4)NS	0.13968	-	3.15	-3.85870	1.38×10^{-4}	-4.0628- -2.5459	8.65×10^{-5} - 2.85×10^{-3}	3.803(9)NS	0.017(1)NS	0.002(1)NS
Potassium bromide	Unventi-lated	3.653(5)NS	0.09772	-	2.95	-3.85364	1.40×10^{-4}	-4.0631- -3.6232	8.65×10^{-5} - 2.38×10^{-4}			
X	X	3.821(11)NS	0.07561	-	3.01	-3.85188	1.41×10^{-4}	-3.9929- -3.6659	1.02×10^{-4} - 2.16×10^{-4}			

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Table A7.18. Ventilation and illumination of vials. LT_{50}: hours (5 μg.cm^{-2} test chemical: filter paper surface)

		Individual LT50 estimates								Comparison of repeated LT50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LT50 (log scale)	Adjusted S.E. of LT50 (log scale)	Gradient (log scale)	LT50 (log scale)	LT50 (antilog scale)	Fiducial limits of LT50 (log scale)	Fiducial limits of LT50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Chlor-dane	Unventilated dark	1.259(8)NS	0.01584	-	19.83	1.30045	19.97	1.2578-1.3331	18.11-21.53	2.055(16)NS	0.004(1)NS	0.919(1)NS
Chlor-dane	Unventilated dark	0.797(8)NS	0.01607	-	19.37	1.27853	18.99	1.2392-1.3145	17.35-20.63			
X	X	2.973(18)NS	0.01147	-	18.96	1.28930	19.47	1.2637-1.3123	18.35-20.53			
Chlor-dane	Ventilated dark	3.171(8)NS	0.02125	-	12.17	1.23332	17.11	1.1856-1.2781	15.33-18.97	9.736(16)NS	5.280(1)*	5.254(1)-
Chlor-dane	Ventilated dark	6.565(8)NS	0.03171	-	6.22	1.13737	13.72	1.0673-1.2014	11.68-15.90			
Chlor-dane	Unventilated light	1.755(8)NS	0.02947	-	6.57	1.30947	20.39	1.2485-1.3758	17.72-23.76	4.066(16)NS	0.141(1)NS	0.112(1)NS
Chlor-dane	Unventilated light	2.311(8)NS	0.03184	-	5.88	1.29674	19.80	1.2321-1.3686	17.07-23.37			
X	X	4.319(18)NS	0.02176	-	6.18	1.30303	20.09	1.2599-1.3491	18.19-22.34			

Table A7.19 Formulating and wetting agents. LT50: hours (Benomyl and Benylate at 0.5 $\mu g.cm^{-2}$ a.i.; Teepol and xylene at 500 $\mu g.cm^{-2}$ a.i.)

		Individual LT50 estimates								Comparison of repeated LT50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LT50 (log scale)	Adjusted S.E. of LT50 (log scale)	Gradient (log scale)	LT50 (log scale)	LT50 (antilog scale)	Fiducial limits of LT50 (log scale)	Fiducial limits of LT50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Benomyl	Technical compound	0.140(2)NS	0.03261	-	9.65	1.70565	50.78	1.6000-1.7679	39.81-58.60	-	-	-
Benylate	Formulated compound w.p.	1.761(2)NS	0.04305	-	5.65	1.67663	47.49	1.5703-1.7610	37.18-57.67	-	-	-
Benomyl + Teepol	Technical compound + Wetter	0.556(2)NS	0.04088	-	7.59	1.57455	37.54	1.4736-1.6568	29.76-45.37	-	-	-
Teepol	Wetter	0.303(2)NS	0.02474	-	12.54	1.79663	62.61	1.7363-1.8492	54.49-70.66	-	-	-
Xylene	Solvent	-	-	-	-	0.00000-1.38021	1.00-24.00	-	-	-	-	-

Table A7.20. Assessments of toxicity : Filter paper contact test. 48 hour LC50 ($g.cm^{-2}$ test chemical: filter paper surface)

		Individual LC50 estimates								Comparison of repeated LC50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LC50 (log scale)	Adjusted S.E. of LC50 (log scale)	Gradient (log scale)	LC50 (log scale)	LC50 (antilog scale)	Fiducial limits of LC50 (log scale)	Fiducial limits of LC50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Chlordane	R2	1.839(5)NS	0.05631	-	3.95	-6.05161	8.9×10^{-7}	-6.1899- -5.9227	6.5×10^{-7} - 1.2×10^{-6}	5.485(8)NS	0.579(1)NS	10.108(1)*
Chlordane	-	3.646(3)NS	0.09798	-	2.94	-6.44710	3.57×10^{-7}	-6.6780- -6.2349	2.10×10^{-7} - 5.82×10^{-7}			
X	X	-	-	-	3.32	-	-	-	-			
Dieldrin	-	0.154(5)NS	0.16993	-	1.92	-2.69489	2.02×10^{-3}	-3.1113- -2.2765	7.74×10^{-4} - 5.29×10^{-3}			
Triazophos	-	1.034(4)NS	0.09009	-	3.41	-4.74205	1.81×10^{-5}	-4.9640- -4.5383	1.09×10^{-5} - 2.90×10^{-5}	2.526(8)NS	1.150(1)NS	0.149(1)NS
Triazophos	-	1.493(4)NS	0.10940	-	2.30	-4.80181	1.58×10^{-5}	-5.0395- -4.5595	9.13×10^{-6} - 2.76×10^{-5}			
X	X	3.825(10)NS	0.07281	-	2.64	-4.77693	1.67×10^{-5}	-4.9289- -4.6255	1.18×10^{-5} - 2.37×10^{-5}			
Carbaryl	R1	0.885(4)NS	0.12123	-	1.91	-4.45600	3.50×10^{-5}	-4.7125- -4.1854	1.94×10^{-5} - 6.53×10^{-5}	6.19(8)NS	0.386(1)NS	4.515(1)*

		Individual LC50 estimates								Comparison of repeated LC50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LC50 (log scale)	Adjusted S.E. of LC50 (log scale)	Gradient (log scale)	LC50 (log scale)	LC50 (antilog scale)	Fiducial limits of LC50 (log scale)	Fiducial limits of LC50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Carbaryl	-	5.306(4)NS	0.10470	-	2.32	-4.77728	1.67×10^{-5}	-5.0302--4.5756	9.33×10^{-6} - 2.66×10^{-5}			
X	X	-	-	-	2.10	-	-	-	-			
Benomyl	-	0.547(2)NS	0.32703	-	1.10	-4.06508	8.61×10^{-5}	-4.5820--2.0104	2.62×10^{-5} - 9.76×10^{-3}	7.260(6)NS	0.100(1)NS	3.498(1)NS
Benomyl	-	6.713(4)NS	0.15519	-	1.27	-3.47362	3.36×10^{-4}	-3.8036--3.1273	1.57×10^{-4} - 7.46×10^{-4}			
X	X	10.857(8)NS	0.16432	-	1.00	-3.61912	2.40×10^{-4}	-3.9333--3.2334	1.17×10^{-4} - 5.84×10^{-4}			
Thiophanate-methyl	-	0.398(3)NS	0.02788	-	10.56	-3.08882	8.15×10^{-4}	-3.1644--3.0184	6.85×10^{-4} - 9.59×10^{-4}	3.544(7)NS	2.915(1)NS	26.062(1)***
Thiophanate-methyl	-	3.146(4)NS	0.05602	-	4.90	-3.44720	3.57×10^{-4}	-3.5943--3.3392	2.55×10^{-4} - 4.58×10^{-4}			
Pentachloro-phenol	R1	0.154(5)NS	0.03931	-	9.58	-5.14453	7.20×10^{-6}	-5.3549--5.0789	4.40×10^{-6} - 8.34×10^{-6}	15.416(13)NS	5.740(2)NS	6.392(2)*
Pentachloro-phenol	R2	2.210(4)NS	0.05174	-	4.77	-5.30439	5.00×10^{-6}	-5.4377--5.2007	3.65×10^{-6} - 6.30×10^{-6}			
Pentachloro-phenol	-	13.052(4)*	0.06280	0.11344	3.65	-5.38251	4.14×10^{-6}	-5.5266--5.2519	2.97×10^{-6} - 5.60×10^{-6}			
X	X	-	-	-	4.73	-	-	-	-			

		Individual LC50 estimates								Comparison of repeated LC50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LC50 (log scale)	Adjusted S.E. of LC50 (log scale)	Gradient (log scale)	LC50 (log scale)	LC50 (antilog scale)	Fiducial limits of LC50 (log scale)	Fiducial limits of LC50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Trichloro-acetic acid	R1	1.822(4)NS	0.05525	-	5.52	-4.00956	9.78×10^{-5}	-4.1313- -3.8707	7.39×10^{-5} - 1.35×10^{-4}	5.929(9)NS	2.253(1)NS	5.091(1)*
Trichloro-acetic acid	-	4.108(5)NS	0.07243	-	3.22	-3.76957	1.70×10^{-4}	-3.9219- -3.6117	1.20×10^{-4} - 2.45×10^{-4}			
X	X	-	-	-	3.78	-	-	-	-			
Chloro-acetamide	R2	0.050(3)NS	0.04389	-	8.67	-5.45278	3.53×10^{-6}	-5.5593- -5.3545	2.80×10^{-6} - 4.42×10^{-6}	0.798(7)NS	4.139(1)*	3.499(1)-
Chloro-acetamide	-	0.748(4)NS	0.06831	-	4.04	-5.63812	2.30×10^{-6}	-5.8091- -5.5052	1.55×10^{-6} - 3.12×10^{-6}			
X	X	-	-	-	5.23	-	-	-	-			
Potassium bromide	R2	2.414(3)NS	0.09655	-	3.07	-3.80849	1.55×10^{-4}	-4.0115- -3.5803	9.74×10^{-5} - 2.63×10^{-4}	5.910(6)NS	0.315(1)NS	1.159(1)NS
Potassium bromide	-	3.497(3)NS	0.10440	-	2.55	-3.65021	2.24×10^{-4}	-3.8785- -3.4201	1.32×10^{-4} - 3.80×10^{-4}			
X	X	7.384(8)NS	0.07192	-	2.71	-3.72547	1.88×10^{-4}	-3.8731- -3.5757	1.34×10^{-4} - 2.67×10^{-4}			

		Individual LC50 estimates								Comparison of repeated LC50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LC50 (log scale)	Adjusted S.E. of LC50 (log scale)	Gradient (log scale)	LC50 (log scale)	LC50 (antilog scale)	Fiducial limits of LC50 (log scale)	Fiducial limits of LC50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Copper sulphate	R1	0.798(6)NS	0.07915	-	2.98	-4.20752	6.20×10^{-5}	-4.4459- -4.0667	3.58×10^{-5} - 8.58×10^{-5}	2.101(11)NS	2.116(1)NS	3.088(1)NS
Copper sulphate	-	1.303(5)NS	0.05003	-	5.19	-4.32243	4.76×10^{-5}	-4.4520- -4.2241	3.53×10^{-5} - 5.97×10^{-5}			
X	X	7.305(13)NS	0.04882	-	3.41	-4.26707	5.41×10^{-5}	-4.3837- -4.1758	4.13×10^{-5} - 6.67×10^{-5}			
Lead acetate	-	0.945(4)NS	0.19959	-	1.45	-2.67643	2.11×10^{-3}	-3.1336- -2.1613	7.35×10^{-4} - 6.90×10^{-3}	-	-	-
Cadmium acetate	-	0.000(4)NS	0.06795	-	5.10	-4.04969	8.92×10^{-5}	-4.1944- -3.5827	6.39×10^{-5} - 2.61×10^{-4}	-	-	-

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Table A7.21.1. Soil moisture content. Chlordane LT50: days (50 $mg.kg^{-1}$ test chemical: wet weight of artificial soil)

		Individual LT50 estimates								Comparison of repeated LT50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LT50 (log scale)	Adjusted S.E. of LT50 (log scale)	Gradient (log scale)	LT50 (log scale)	LT50 (antilog scale)	Fiducial limits of LT50 (log scale)	Fiducial limits of LT50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Chlordane	10%	1.776(6)NS	0.05823	-	5.14	0.13002	1.35	-0.0378-0.2361	0.92-1.72	2.305(12)NS	0.320(1)NS	0.000(1)NS
Chlordane	10%	0.528(6)NS	0.05001	-	6.37	0.14236	1.39	0.0093-0.2426	1.02-1.75			
X	X	2.625(14)NS	0.03814	-	5.67	0.13677	1.37	0.0458-0.2079	1.11-1.61			
Chlordane	20%	1.339(6)NS	0.08324	-	4.30	0.02466	1.06	-0.3063-0.1551	0.49-1.43	6.105(12)NS	0.063(1)NS	0.466(1)NS
Chlordane	20%	4.765(6)NS	0.08175	-	3.86	0.06998	1.17	-0.2106-0.1999	0.62-1.58			
X	X	6.634(14)NS	0.05916	-	4.01	0.04647	1.11	-0.1201-0.1432	0.76-1.39			
Chlordane	30%	0.019(7)NS	0.05518	-	6.08	-0.21385	0.61	-0.3937--0.0885	0.40-0.82	0.063(14)NS	0.077(1)NS	0.140(1)NS
Chlordane	30%	0.044(7)NS	0.06587	-	5.26	-0.25088	0.56	-0.5519--0.1217	0.28-0.76			
X	X	0.280(16)NS	0.04227	-	5.65	-0.23150	0.59	-0.3455--0.1498	0.45-0.71			

		Individual LT50 estimates								Comparison of repeated LT50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LT50 (log scale)	Adjusted S.E. of LT50 (log scale)	Gradient (log scale)	LT50 (log scale)	LT50 (antilog scale)	Fiducial limits of LT50 (log scale)	Fiducial limits of LT50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Chlordane	40%	0.718(7)NS	0.06579	-	4.50	-0.16140	0.69	-0.3582- -0.0295	0.44- 0.93	1.255(14)NS	0.744(1)NS	6.083(1)*
Chlordane	40%	0.537(7)NS	0.27330	-	2.67	-0.58075	0.26	+	+			
X	X	-	-	-	3.91	-	-	-	-			
Chlordane	50%	1.535(7)NS	0.05357	-	5.37	0.08695	1.22	-0.0448- 0.1941	0.90- 1.56	1.946(14)NS	0.199(1)NS	1.235(1)NS
Chlordane	50%	0.411(7)NS	0.05925	-	4.63	-0.00731	0.98	-0.1504- 0.1123	0.71- 1.30			
X	X	3.380(16)NS	0.04028	-	4.85	0.03854	1.09	-0.0499- 0.1170	0.89- 1.31			

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Table A7.21.2. Soil moisture content. Chloroacetamide LT50: days (50 mg.kg^{-1} test chemical: wet weight of artificial soil)

		Individual LT50 estimates								Comparison of repeated LT50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LT50 (log scale)	Adjusted S.E. of LT50 (log scale)	Gradient (log scale)	LT50 (log scale)	LT50 (antilog scale)	Fiducial limits of LT50 (log scale)	Fiducial limits of LT50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Chloro-acetamide	10%	-	-	-	-	-	No LT50	-	-	-	-	-
Chloro-acetamide	10%	-	-	-	-	-	No LT50	-	-	-	-	-
Chloro-acetamide	20%	-	-	-	-	-	No LT50	-	-	-	-	-
Chloro-acetamide	20%	-	-	-	-	-	No LT50	-	-	-	-	-
Chloro-acetamide	30%	2.491(6)NS	0.03822	-	7.00	0.40986	2.57	0.3068-0.4793	2.03-3.02	4.818(12)NS	0.893(1)NS	1.051(1)NS
Chloro-acetamide	30%	2.327(6)NS	0.04704	-	5.20	0.33009	2.14	0.2125-0.4165	1.63-2.61			
X	X	5.657(14)NS	0.02098	-	8.28	0.53205	3.40	0.4853-0.5717	3.07-3.73			
Chloro-acetamide	40%	-	-	-	-	0.00000-0.30103	1.00-2.00	-	-	-	-	-
Chloro-acetamide	40%	-	-	-	-	0.00000-0.30103	1.00-2.00	-	-	-	-	-

		Individual LT50 estimates								Comparison of repeated LT50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LT50 (log scale)	Adjusted S.E. of LT50 (log scale)	Gradient (log scale)	LT50 (log scale)	LT50 (antilog scale)	Fiducial limits of LT50 (log scale)	Fiducial limits of LT50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Chloro-acetamide	50%	-	-	-	-	0.00000-0.30103	1.00-2.00	-	-	-	-	-
Chloro-acetamide	50%	-	-	-	-	0.00000-0.30103	1.00-2.00	-	-	-	-	-

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Table A7.21.3. Soil moisture content. Pentachlorophenol LT50: days (100 mg.kg^{-1} test chemical: dry weight artificial soil)

		Individual LT50 estimates								Comparison of repeated LT50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LT50 (log scale)	Adjusted S.E. of LT50 (log scale)	Gradient (log scale)	LT50 (log scale)	LT50 (antilog scale)	Fiducial limits of LT50 (log scale)	Fiducial limits of LT50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Penta-chlorophenol	10%	-	-	-	-	-1.38021-0.00000	0.04-1.00	-	-	-	-	-
Penta-chlorophenol	10%	-	-	-	-	-1.38021-0.00000	0.04-1.00	-	-	-	-	-
Penta-chlorophenol	20%	0.765(6)NS	0.05694	-	5.53	0.10822	1.28	-0.0601-0.2146	0.87-1.64	1.068(12)NS	0.674(1)NS	0.612(1)NS
Penta-chlorophenol	20%	0.303(6)NS	0.04519	-	7.60	0.17624	1.50	0.0614-0.2701	1.15-1.86			
X	X	1.940(14)NS	0.02137	-	10.51	0.38583	2.43	0.3366-0.4282	2.17-2.68			
Penta-chlorophenol	30%	-	-	-	-	0.00000-0.30103	1.00-2.00	-	-	-	-	-
Penta-chlorophenol	30%	-	-	-	-	0.00000-0.30103	1.00-2.00	-	-	-	-	-
Penta-chlorophenol	40%	-	-	-	-	-1.38021-0.00000	0.04-1.00	-	-	-	-	-
Penta-chlorophenol	40%	-	-	-	-	-1.38021-0.00000	0.04-1.00	-	-	-	-	-

		Individual LT50 estimates								Comparison of repeated LT50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LT50 (log scale)	Adjusted S.E. of LT50 (log scale)	Gradient (log scale)	LT50 (log scale)	LT50 (antilog scale)	Fiducial limits of LT50 (log scale)	Fiducial limits of LT50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Penta-chlorophenol	50%	1.535(6)NS	0.05357	-	5.37	0.08695	1.22	-0.0448-0.1941	0.90-1.56	1.946(12)NS	0.199(1)NS	1.235(1)NS
Penta-chlorophenol	50%	0.411(6)NS	0.05925	-	4.63	-0.00731	0.98	-0.1504-0.1123	0.71-1.30			
X	X	3.380(14)NS	0.04028	-	4.85	0.03854	1.09	-0.0499-0.1170	0.89-1.31			

Table A7.22.1 Soil organic matter content. Chlordane LT50: days (50 mg.kg^{-1} test chemical: dry weight of artificial soil)

		Individual LT50 estimates								Comparison of repeated LT50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LT50 (log scale)	Adjusted S.E. of LT50 (log scale)	Gradient (log scale)	LT50 (log scale)	LT50 (antilog scale)	Fiducial limits of LT50 (log scale)	Fiducial limits of LT50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Chlordane	0%	0.211(7)NS	0.06258	-	4.99	-0.18775	0.65	-0.3887- -0.0576	0.41- 0.88	0.509(14)NS	0.521(1)NS	3.225(1)NS
Chlordane	0%	0.298(7)NS	0.17176	-	3.28	-0.44510	0.36	-7.2945- -0.2369	5.08×10^{-8}- 0.58			
X	X	4.254(16)NS	0.06460	-	4.05	-0.28958	0.51	-0.5056- -0.1850	0.31- 0.65			
Chlordane	5%	0.001(7)NS	0.04484	-	8.52	-0.15056	0.71	-0.2588- -0.0429	0.55- 0.91	1.352(14)NS	4.657(1)*	2.912(1)-
Chlordane	5%	1.351(7)NS	0.17129	-	2.82	-0.42984	0.37	-1.8194- -0.2081	0.02- 0.62			
Chlordane	10%	0.324(6)NS	0.03966	-	8.60	0.30933	2.04	0.1748- 0.3821	1.50- 2.41	2.453(12)NS	0.914(1)NS	0.026(1)NS
Chlordane	10%	2.129(6)NS	0.04577	-	5.99	0.28009	1.91	0.1651- 0.3670	1.46- 2.33			
X	X	3.393(14)NS	0.03071	-	6.79	0.29100	1.95	0.2198- 0.3484	1.66- 2.23			

		Individual LT50 estimates								Comparison of repeated LT50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LT50 (log scale)	Adjusted S.E. of LT50 (log scale)	Gradient (log scale)	LT50 (log scale)	LT50 (antilog scale)	Fiducial limits of LT50 (log scale)	Fiducial limits of LT50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Chlordane	20%	0.897(6)NS	0.03122	-	9.63	0.40004	2.51	0.3175-0.4638	2.08-2.91	1.400(12)NS	0.235(1)NS	0.165(1)NS
Chlordane	20%	0.504(6)NS	0.02782	-	11.62	0.42001	2.63	0.3496-0.4795	2.24-3.02			
X	X	1.800(14)NS	0.02083	-	10.49	0.40991	2.57	0.3629-0.4513	2.31-2.83			

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Table A7.22.2. Soil organic matter content. Chloroacetamide LT50: days (50 mg.kg^{-1} test chemical: dry weight of artificial soil)

		Individual LT50 estimates								Comparison of repeated LT50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LT50 (log scale)	Adjusted S.E. of LT50 (log scale)	Gradient (log scale)	LT50 (log scale)	LT50 (antilog scale)	Fiducial limits of LT50 (log scale)	Fiducial limits of LT50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Chloro-acetamide	0%	-	-	-	-	-1.38021-0.00000	0.04-1.00	-	-	-	-	-
Chloro-acetamide	0%	-	-	-	-	-1.38021-0.00000	0.04-1.00	-	-	-	-	-
Chloro-acetamide	5%	-	-	-	-	-1.38021-0.00000	0.04-1.00	-	-	-	-	-
Chloro-acetamide	5%	-	-	-	-	-1.38021-0.00000	0.04-1.00	-	-	-	-	-
Chloro-acetamide	10%	-	-	-	-	-	No LT50	-	-	-	-	-
Chloro-acetamide	10%	-	-	-	-	-	No LT50	-	-	-	-	-
Chloro-acetamide	20%	-	-	-	-	-	No LT50	-	-	-	-	-
Chloro-acetamide	20%	-	-	-	-	-	No LT50	-	-	-	-	-

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Table A7.22.3. Soil organic matter content. Pentachlorophenol LT50: days (100 mg.kg^{-1} test chemical: dry weight of artificial soil).

		Individual LT50 estimates								Comparison of repeated LT50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LT50 (log scale)	Adjusted S.E. of LT50 (log scale)	Gradient (log scale)	LT50 (log scale)	LT50 (antilog scale)	Fiducial limits of LT50 (log scale)	Fiducial limits of LT50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Penta-chlorophenol	0%	-	-	-	-	-1.38021-0.00000	0.00-1.00	-	-	-	-	-
Penta-chlorophenol	0%	-	-	-	-	-1.38021-0.00000	0.00-1.00	-	-	-	-	-
Penta-chlorophenol	5%	-	-	-	-	-1.38021-0.00000	0.00-1.00	-	-	-	-	-
Penta-chlorophenol	5%	-	-	-	-	-1.38021-0.00000	0.00-1.00	-	-	-	-	-
Penta-chlorophenol	10%	-	-	-	-	0.00000-0.30103	1.00-2.00	-	-	-	-	-
Penta-chlorophenol	10%	-	-	-	-	0.00000-0.30103	1.00-2.00	-	-	-	-	-
Penta-chlorophenol	20%	-	-	-	-	0.00000-0.30103	1.00-2.00	-	-	-	-	-
Penta-chlorophenol	20%	-	-	-	-	0.00000-0.30103	1.00-2.00	-	-	-	-	-

Table A7.23.1 Soil kaolinitic clay content. Chlordane LT50: days (50 $mg.kg^{-1}$ test chemical: dry weight of artificial soil)

		Individual LT50 estimates								Comparison of repeated LT50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LT50 (log scale)	Adjusted S.E. of LT50 (log scale)	Gradient (log scale)	LT50 (log scale)	LT50 (antilog scale)	Fiducial limits of LT50 (log scale)	Fiducial limits of LT50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Chlordane	0%	2.728(5)NS	0.04498	-	6.68	0.24178	1.74	0.1281-0.3305	1.34-2.14	4.032(10)NS	0.120(1)NS	1.171(1)NS
Chlordane	0%	1.304(5)NS	0.05102	-	5.91	0.16479	1.46	0.0296-0.2637	1.07-1.84			
X	X	5.323(12)NS	0.03406	-	6.15	0.20321	1.60	0.1257-0.2682	1.34-1.85			
Chlordane	10%	0.092(5)NS	0.03135	-	10.69	0.35158	2.25	0.2572-0.4181	1.81-2.62	2.390(10)NS	2.696(1)NS	0.197(1)NS
Chlordane	10%	2.298(5)NS	0.04657	-	5.61	0.29674	1.98	0.1796-0.3836	1.51-2.42			
X	X	5.283(12)NS	0.02984	-	6.73	0.31920	2.09	0.2505-0.3750	1.78-2.37			
Chlordane	20%	2.298(5)NS	0.04657	-	5.61	0.29674	1.98	0.1796-0.3836	1.51-2.42	2.464(10)NS	1.845(1)NS	0.013(1)NS
Chlordane	20%	0.166(5)NS	0.03572	-	9.46	0.33118	2.14	0.2098-0.4002	1.62-2.51			
X	X	4.322(12)NS	0.03048	-	6.60	0.30930	2.04	0.2389-0.3661	1.73-2.32			

		Individual LT50 estimates								Comparison of repeated LT50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LT50 (log scale)	Adjusted S.E. of LT50 (log scale)	Gradient (log scale)	LT50 (log scale)	LT50 (antilog scale)	Fiducial limits of LT50 (log scale)	Fiducial limits of LT50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Chlordane	30%	0.504(5)NS	0.02782	-	11.62	0.42001	2.63	0.3496-0.4795	2.24-3.02	2.384(10)NS	1.673(1)NS	4.244(1)*
Chlordane	30%	1.881(5)NS	0.04379	-	7.01	0.30175	2.00	0.1744-0.3807	1.49-2.40			
X	X	-	-	-	8.85	-	-	-	-			

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Table A7.23.2. Soil kaolinitic clay content. Chloroacetamide LT50: days (50 mg.kg^{-1} test chemical: dry weight of artificial soil)

		Individual LT50 estimates							Comparison of repeated LT50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LT50 (log scale)	Adjusted S.E. of LT50 (log scale)	Gradient (log scale)	LT50 (log scale)	LT50 (antilog scale)	Fiducial limits of LT50 (log scale)	Fiducial limits of LT50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Chloro-acetamide	0%	2.728(5)NS	0.04498	-	6.68	0.24178	1.74	0.1281-0.3305	1.34-2.14	8.541(10)NS	0.360(1)NS	0.006(1)NS
Chloro-acetamide	0%	5.813(5)NS	0.04961	-	5.46	0.22694	1.69	0.1021-0.3217	1.27-2.10			
X	X	8.906(12)NS	0.03345	-	5.97	0.23392	1.71	0.1586-0.2976	1.44-1.98			
Chloro-acetamide	10%	0.018(5)NS	0.02598	-	14.68	0.38878	2.45	0.3260-0.4489	2.12-2.81	0.067(10)NS	0.178(1)***	0.218(1)-
Chloro-acetamide	10%	0.048(5)NS	0.02810	-	12.31	0.37020	2.35	0.2974-0.4345	1.98-2.72			
Chloro-acetamide	20%	0.322(5)NS	0.03023	-	10.51	0.38583	2.43	0.3051-0.4495	2.02-2.82	0.340(10)NS	0.668(1)**	0.000(1)-
Chloro-acetamide	20%	0.018(5)NS	0.02598	-	14.68	0.38878	2.45	0.3260-0.4489	2.12-2.81			
Chloro-acetamide	30%	0.166(5)NS	0.03572	-	9.46	0.33118	2.14	0.2098-0.4002	1.62-2.51	1.814(10)NS	2.013(1)**	0.162(1)-
Chloro-acetamide	30%	1.648(5)NS	0.04830	-	5.48	0.27726	1.89	0.1538-0.3665	1.43-2.33			

Table A7.23.3. Soil kaolinitic clay content. Pentachlorophenol LT50: days (100 mg.kg^{-1} test chemical: dry weight of artificial soil)

		Individual LT50 estimates							Comparison of repeated LT50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LT50 (log scale)	Adjusted S.E. of LT50 (log scale)	Gradient (log scale)	LT50 (log scale)	LT50 (antilog scale)	Fiducial limits of LT50 (log scale)	Fiducial limits of LT50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Penta-chlorophenol	0%	0.104(6)NS	0.04722	-	7.32	0.11724	1.31	-0.0044-0.2208	0.99-1.66	0.207(12)NS	0.000(1)NS	0.000(1)NS
Penta-chlorophenol	0%	0.104(6)NS	0.04722	-	7.32	0.11724	1.31	-0.0044-0.2208	0.99-1.66			
X	X	0.207(14)NS	0.03339	-	7.32	0.11724	1.31	0.0421-0.1851	1.10-1.53			
Penta-chlorophenol	10%	0.765(6)NS	0.05694	-	5.53	0.10822	1.28	-0.0601-0.2146	0.87-1.64	2.541(12)NS	0.037(1)NS	0.132(1)NS
Penta-chlorophenol	10%	1.776(6)NS	0.05823	-	5.14	0.13002	1.35	-0.0378-0.2361	0.92-1.72			
X	X	2.710(14)NS	0.04089	-	5.30	0.11917	1.32	0.0186-0.1936	1.04-1.56			
Penta-chlorophenol	20%	0.819(6)NS	0.04565	-	7.08	0.19942	1.58	0.0824-0.2914	1.21-1.96	1.066(12)NS	0.306(1)NS	3.746(1)NS
Penta-chlorophenol	20%	0.247(6)NS	0.06178	-	5.63	0.04988	1.12	-0.1735-0.1625	0.67-1.45			
X	X	5.117(14)NS	0.03757	-	5.92	0.12560	1.34	0.0360-0.1964	1.09-1.57			

		Individual LT50 estimates								Comparison of repeated LT50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LT50 (log scale)	Adjusted S.E. of LT50 (log scale)	Gradient (log scale)	LT50 (log scale)	LT50 (antilog scale)	Fiducial limits of LT50 (log scale)	Fiducial limits of LT50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Penta-chlorophenol	30%	0.050(6)NS	0.04388	-	8.67	0.14928	1.41	0.0428-0.2475	1.10-1.77	2.357(12)NS	0.104(1)NS	2.383(1)NS
Penta-chlorophenol	30%	2.306(6)NS	0.04571	-	7.64	0.24244	1.75	0.1068-0.3255	1.28-2.12			
X	X	4.843(14)NS	0.03264	-	7.48	0.19690	1.57	0.1211-0.2589	1.32-1.81			

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Table A7.24.1 Soil acidity. Chlordane LT50: days (50 $mg.kg^{-1}$ test chemical: dry weight of artificial soil)

		Individual LT50 estimates							Comparison of repeated LT50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LT50 (log scale)	Adjusted S.E. of LT50 (log scale)	Gradient (log scale)	LT50 (log scale)	LT50 (antilog scale)	Fiducial limits of LT50 (log scale)	Fiducial limits of LT50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Chlordane	pH 3.7	0.765(6)NS	0.05694	-	5.53	0.10822	1.28	-0.0601-0.2146	0.87-1.64	1.294(12)NS	0.129(1)NS	0.134(1)NS
Chlordane	pH 3.7	0.528(6)NS	0.05001	-	6.37	0.14236	1.38	0.0093-0.2426	1.02-1.75			
X	X	1.556(14)NS	0.03757	-	5.92	0.12560	1.34	0.0360-0.1964	1.09-1.57			
Chlordane	pH 6.2	0.819(6)NS	0.04565	-	7.08	0.19942	1.58	0.0824-0.2914	1.21-1.96	3.998(12)NS	1.016(1)NS	0.150(1)NS
Chlordane	pH 6.2	3.179(6)NS	0.05808	-	4.93	0.15150	1.42	-0.0111-0.2567	0.97-1.81			
X	X	5.164(14)NS	0.03615	-	5.77	0.17556	1.50	0.0918-0.2437	1.24-1.75			
Chlordane	pH 7.7	4.765(6)NS	0.08175	-	3.86	0.06998	1.17	-0.2106-0.1999	0.62-1.58	6.541(12)NS	0.557(1)NS	0.079(1)NS
Chlordane	pH 7.7	1.776(6)NS	0.05823	-	5.14	0.13002	1.35	-0.0378-0.2361	0.92-1.72			
X	X	7.177(14)NS	0.04832	-	4.43	0.10258	1.27	-0.0222-0.1861	0.95-1.53			

		Individual LT50 estimates								Comparison of repeated LT50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LT50 (log scale)	Adjusted S.E. of LT50 (log scale)	Gradient (log scale)	LT50 (log scale)	LT50 (antilog scale)	Fiducial limits of LT50 (log scale)	Fiducial limits of LT50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Chlordane	pH 8.0	2.306(6)NS	0.04571	-	7.64	0.24244	1.75	0.1068-0.3255	1.29-2.12	2.961(12)NS	0.018(1)NS	0.520(1)NS
Chlordane	pH 8.0	0.655(6)NS	0.04238	-	8.07	0.28709	1.93	0.1507-0.3638	1.41-2.31			
X	X	3.499(14)NS	0.03131	-	7.77	0.26485	1.84	0.1857-0.3211	1.53-2.09			

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Table A7.24.2. Soil acidity. Chloroacetamide LT50: days (50 mg.kg^{-1} test chemical: dry weight of artificial soil)

		Individual LT50 estimates								Comparison of repeated LT50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LT50 (log scale)	Adjusted S.E. of LT50 (log scale)	Gradient (log scale)	LT50 (log scale)	LT50 (antilog scale)	Fiducial limits of LT50 (log scale)	Fiducial limits of LT50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Chloro-acetamide	pH 3.7	0.324(6)NS	0.03966	-	8.60	0.30933	2.04	0.1748-0.3821	1.50-2.41	0.416(12)NS	0.227(1)NS	0.530(1)NS
Chloro-acetamide	pH 3.7	0.092(6)NS	0.03135	-	10.69	0.35158	2.25	0.2572-0.4181	1.81-2.62			
X	X	1.233(14)NS	0.01706	-	13.56	0.49986	3.16	0.4583-0.5329	2.87-3.41			
Chloro-acetamide	pH 6.2	-	-	-	-	0.00000-0.30103	1.00-2.00	-	-	-	-	-
Chloro-acetamide	pH 6.2	-	-	-	-	-0.00000-0.30103	1.00-2.00	-	-	-	-	-
Chloro-acetamide	pH 7.7	-	-	-	-	-0.00000-0.30103	1.00-2.00	-	-	-	-	-
Chloro-acetamide	pH 7.7	-	-	-	-	-0.00000-0.30103	1.00-2.00	-	-	-	-	-
Chloro-acetamide	pH 8.0	-	-	-	-	-0.00000-0.30103	1.00-2.00	-	-	-	-	-
Chloro-acetamide	pH 8.0	-	-	-	-	-0.00000-0.30103	1.00-2.00	-	-	-	-	-

Table A7.24.3. Soil acidity. Pentachlorophenol LT50: days (100 mg.kg^{-1} test chemical: dry weight of artificial soil

		Individual LT50 estimates							Comparison of repeated LT50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LT50 (log scale)	Adjusted S.E. of LT50 (log scale)	Gradient (log scale)	LT50 (log scale)	LT50 (antilog scale)	Fiducial limits of LT50 (log scale)	Fiducial limits of LT50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Penta-chlorophenol	pH 3.7	0.754(6)NS	0.03942	-	7.14	0.37683	2.38	0.2678-0.4486	1.85-2.81	2.579(12)NS	0.017(1)NS	0.159(1)NS
Penta-chlorophenol	pH 3.7	1.825(6)NS	0.03883	-	7.49	0.35892	2.29	0.2477-0.4307	1.77-2.70			
X	X	2.909(14)NS	0.01906	-	10.19	0.52759	3.37	0.4838-0.5637	3.05-3.66			
Penta-chlorophenol	pH 6.2	0.655(6)NS	0.04238	-	8.07	0.28709	1.94	0.1507-0.3638	1.41-2.31	1.921(12)NS	0.009(1)NS	0.127(1)NS
Penta-chlorophenol	pH 6.2	1.266(6)NS	0.04428	-	7.77	0.26485	1.84	0.1288-0.3450	1.35-2.21			
X	X	2.841(14)NS	0.19320	-	11.87	0.46566	2.92	0.4192-0.5021	2.63-3.18			
Penta-chlorophenol	pH 7.7	0.303(6)NS	0.04519	-	7.60	0.17624	1.50	0.0614-0.2701	1.15-1.86	0.406(12)NS	0.009(1)NS	0.797(1)NS
Penta-chlorophenol	pH 7.7	0.104(6)NS	0.04722	-	7.32	0.11724	1.31	-0.0044-0.2208	0.99-1.66			
X	X	1.008(14)NS	0.01972	-	12.21	0.38777	2.44	0.3438-0.4282	2.21-2.68			

		Individual LT50 estimates								Comparison of repeated LT50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LT50 (log scale)	Adjusted S.E. of LT50 (log scale)	Gradient (log scale)	LT50 (log scale)	LT50 (antilog scale)	Fiducial limits of LT50 (log scale)	Fiducial limits of LT50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Penta-chlorophenol	pH 8.0	-	-	-	-	-1.38021-0.00000	0.00-1.00	-	-	-	-	-
Penta-chlorophenol	pH 8.0	-	-	-	-	-1.38021-0.00000	0.00-1.00	-	-	-	-	-

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Table A7.25 Temperature. 14 day LC50 ($mg.kg^{-1}$ test chemical: dry weight of artificial soil

		Individual LC50 estimates								Comparison of repeated LC50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LC50 (log scale)	Adjusted S.E. of LC50 (log scale)	Gradient (log scale)	LC50 (log scale)	LC50 (antilog scale)	Fiducial limits of LC50 (log scale)	Fiducial limits of LC50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Chlordane	5°	9.623(5)NS	0.09119	-	1.23	0.52506	3.35	0.3284-0.6957	2.13-4.96	10.389(10)NS	31.113(1)***	107.950(1)-
Chlordane	5°	0.765(5)NS	0.04545	-	3.80	1.67138	46.92	1.5569-1.7519	36.05-56.48			
Chlordane	10°	6.485(5)NS	0.06382	-	1.87	1.01073	10.25	0.8778-1.1357	7.55-13.67	8.867(10)NS	13.784(1)***	19.815(1)-
Chlordane	10°	2.383(5)NS	0.04772	-	3.53	1.36493	23.17	1.2683-1.4604	18.55-28.87			
Chlordane	20°	3.617(5)NS	0.04907	-	3.71	1.57337	37.44	1.4788-1.6766	30.12-47.49	27.540(10)** (-)	0.334(1)NS (NS)	23.889(1)*** (*)
Chlordane	20°	23.923(5)***	0.05112	0.11182	3.38	1.24004	17.38	1.1430-1.3490	13.90-22.33			
Chlordane	25°	8.395(5)NS	0.04909	-	2.95	1.31904	20.85	1.2256-1.4276	16.81-26.77	17.724(10)NS	3.966(1)*	0.259(1)-
Chlordane	25°	9.329(5)NS	0.05184	-	4.48	1.24003	17.38	1.1469-1.3607	14.03-22.95			

		Individual LC50 estimates								Comparison of repeated LC50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LC50 (log scale)	Adjusted S.E. of LC50 (log scale)	Gradient (log scale)	LC50 (log scale)	LC50 (antilog scale)	Fiducial limits of LC50 (log scale)	Fiducial limits of LC50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Chlordane	30°	4.102(5)NS	0.09781	-	1.33	0.12796	1.34	-0.0878-0.3077	0.82-2.03	11.931(10)NS	10.575(1)**	34.726(1)-
Chlordane	30°	7.828(5)NS	0.05513	-	2.34	0.81801	6.58	0.7061-0.9297	5.08-8.51			
Carbaryl	5°	3.388(3)NS	0.08947	-	1.73	2.13215	135.57	1.9527-2.3182	89.68-208.06	17.320(8)* (-)	1.232(1)NS (NS)	0.066(1)NS (NS)
Carbaryl	5°	13.932(5)*	0.05792	0.09668	2.14	2.11455	130.18	1.9880-2.2233	97.28-167.21			
Carbaryl	10°	14.973(3)**	0.11221	0.25068	1.18	2.08624	121.96	1.8683-2.3221	73.84-209.97	48.921(8)*** (-)	1.753(1)NS (NS)	1.014(1)NS (NS)
Carbaryl	10°	33.948(5)***	0.07530	0.19621	1.46	1.95566	90.29	1.7969-2.0996	62.65-125.78			
Carbaryl	20°	11.234(3)*	0.11495	0.22244	1.10	1.97126	93.60	1.7471-2.2118	55.86-162.84	44.638(8)*** (-)	0.976(1)NS (NS)	0.349(1)NS (NS)
Carbaryl	20°	33.404(5)***	0.08145	0.21053	1.30	1.89111	77.82	1.7200-2.0473	52.48-111.50			

		Individual LC50 estimates								Comparison of repeated LC50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LC50 (log scale)	Adjusted S.E. of LC50 (log scale)	Gradient (log scale)	LC50 (log scale)	LC50 (antilog scale)	Fiducial limits of LC50 (log scale)	Fiducial limits of LC50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Carbaryl	25°	16.405(3)***	0.13109	0.30655	0.91	1.95274	89.69	1.7015-2.2310	50.29-170.21	69.079(8)*** (-)	2.019(1)NS (NS)	0.079(1)NS (NS)
Carbaryl	25°	52.674(5)***	0.08767	0.28455	1.14	1.89581	78.67	1.7155-2.0671	51.94-116.71			
Carbaryl	30°	18.551(3)***	0.12818	0.31874	0.90	1.55221	35.66	1.3023-1.8175	20.06-65.69	46.842(8)*** (-)	0.071(1)NS (NS)	13.669(1)- (NS)
Carbaryl	30°	28.291(5)***	0.11534	0.27436	0.87	0.92498	8.41	0.6849-1.1452	4.84-13.97			

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Table A7.26 Quantity of soil per replicate. 14 day LC50 ($mg.kg^{-1}$ test chemical: dry weight of artificial soil

		Individual LC50 estimates								Comparison of repeated LC50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LC50 (log scale)	Adjusted S.E. of LC50 (log scale)	Gradient (log scale)	LC50 (log scale)	LC50 (antilog scale)	Fiducial limits of LC50 (log scale)	Fiducial limits of LC50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Chlordane	100 g	0.000(4)NS	0.03582	-	6.98	1.42505	26.61	1.3772-1.6425	23.83-43.90	0.025(8)NS	0.688(1)NS	0.549(1)NS
Chlordane	100 g	0.025(4)NS	0.05577	-	4.19	1.44038	27.57	1.3686-2.2297	23.37-169.71			
X	X	1.262(10)NS	0.03474	-	5.37	1.43309	27.11	1.3864-1.5995	24.34-39.76			
Chlordane	200 g	0.000(4)NS	0.02111	-	12.96	1.42766	26.77	1.3971-1.5126	24.95-32.55	0.066(8)NS	4.471(1)*	2.352(1)-
Chlordane	200 g	0.066(4)NS	0.06553	-	4.16	1.48477	30.53	1.4022-2.0094	25.25-102.19			
Chlordane	400 g	0.000(4)NS	0.02111	-	12.96	1.42766	26.77	1.3971-1.5126	24.95-32.55	0.000(8)NS	0.267(1)NS	3.630(1)NS
Chlordane	400 g	0.000(4)NS	0.01878	-	10.55	1.39794	25.00	1.3676-1.4585	23.31-28.74			
X	X	3.897(10)NS	0.01479	-	11.07	1.41502	26.00	1.3913-1.4565	24.62-28.61			

		Individual LC50 estimates								Comparison of repeated LC50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LC50 (log scale)	Adjusted S.E. of LC50 (log scale)	Gradient (log scale)	LC50 (log scale)	LC50 (antilog scale)	Fiducial limits of LC50 (log scale)	Fiducial limits of LC50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Chlordane	800 g	0.000(4)NS	0.01915	-	11.98	1.41372	25.93	1.3842-1.4794	24.22-30.16	0.000(8)NS	0.506(1)NS	0.227(1)NS
Chlordane	800 g	0.000(4)NS	0.02653	-	8.99	1.41897	26.24	1.3807-1.5300	24.03-33.89			
X	X	0.734(10)NS	0.01606	-	10.29	1.41631	26.08	1.3909-1.4625	24.60-29.01			
Chlordane	1000 g	0.000(4)NS	0.01833	-	11.33	1.40347	25.32	1.374-1.4626	23.67-29.01	5.724(8)NS	9.349(1)**	0.003(1)-
Chlordane	1000 g	5.724(4)NS	0.05295	-	2.91	1.53118	33.98	1.4346-1.6587	27.20-45.57			

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Table A7.27. Period of exposure 7, 14, 28, 40 and 50 day LC50 ($mg.kg^{-1}$ test chemical: dry weight of artificial soil)

		Individual LC50 estimates								Comparison of repeated LC50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LC50 (log scale)	Adjusted S.E. of LC50 (log scale)	Gradient (log scale)	LC50 (log scale)	LC50 (antilog scale)	Fiducial limits of LC50 (log scale)	Fiducial limits of LC50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Carbaryl	7 day	15.754(3)**	0.06631	0.15195	1.28	1.92792	84.71	1.7982-2.0613	62.83-115.15	-	-	-
Carbaryl	14 day	18.446(3)***	0.07116	0.17645	1.12	1.83167	67.87	1.6927-1.9749	49.29-94.39	-	-	-
Carbaryl	28 day	23.845(3)***	0.08010	0.22582	0.89	1.25959	18.18	1.1022-1.4194	12.65-26.27	-	-	-
Carbaryl	40 day	25.250(3)***	0.08202	0.23795	0.89	0.54967	3.55	0.3838-0.7093	2.42-5.12	-	-	-
Carbaryl	50 day	10.341(3)*	0.08446	0.15681	0.93	0.18124	1.52	0.0058-0.3419	1.01-2.20	-	-	-

Table A7.28 Replication. 14 day LC50 ($mg.kg^{-1}$ test chemical: dry weight of artifical soil)

		Individual LC50 estimates								Comparison of repeated LC50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LC50 (log scale)	Adjusted S.E. of LC50 (log scale)	Gradient (log scale)	LC50 (log scale)	LC50 (antilog scale)	Fiducial limits of LC50 (log scale)	Fiducial limits of LC50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Carbaryl	20 replicates	4.102(3)NS	0.16059	-	1.09	1.59995	39.81	1.2758-1.9420	18.87-87.50	-	-	-
Carbaryl	40 replicates	9.755(3)*	0.11269	0.20320	1.13	1.84430	69.87	1.6231-2.0779	41.99-119.65	-	-	-
Carbaryl	60 replicates	13.768(3)**	0.09264	0.19846	1.11	1.76295	57.94	1.5816-1.9516	38.16-89.46	-	-	-
Carbaryl	80 replicates	15.394(3)**	0.07879	0.17848	1.15	1.88927	77.49	1.7353-2.0488	54.37-111.90	-	-	-
Carbaryl	100 replicates	18.446(3)***	0.07116	0.17645	1.12	1.83167	67.87	1.6927-1.9749	49.29-94.39	-	-	-

Table A7.29 Method of application of the test chemical. 14 day LC50 ($mg.kg^{-1}$ test chemical: dry weight of artificial soil)

		Individual LC50 estimates								Comparison of repeated LC50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LC50 (log scale)	Adjusted S.E. of LC50 (log scale)	Gradient (log scale)	LC50 (log scale)	LC50 (antilog scale)	Fiducial limits of LC50 (log scale)	Fiducial limits of LC50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Chlordane	Spray	0.472(3)NS	0.04255	-	3.54	0.92923	8.50	0.8468-1.0291	7.03-10.69	7.527(6)NS	4.558(1)*	6.923(1)-
Chlordane	Spray	7.055(3)NS	0.05626	-	2.22	1.14155	13.85	1.0301-1.2563	10.72-18.04			
Chlordane	Soil drench	0.343(3)NS	0.04703	-	3.61	1.12428	13.31	1.0372-1.2305	10.89-17.00	0.402(6)NS	0.925(1)NS	2.155(1)NS
Chlordane	Soil drench	0.060(3)NS	0.04147	-	4.67	1.01087	10.25	0.9409-1.1284	8.73-13.44			
X	X	3.483(8)NS	0.03243	-	3.84	1.07314	11.83	1.0140-1.1454	10.33-13.98			
Chlordane	Solid incorporation	0.473(3)NS	0.04231	-	3.42	0.86960	7.41	0.7814-0.9620	6.04-9.16	5.529(6)NS	0.588(1)NS	0.623(1)NS
Chlordane	Solid incorporation	5.056(3)NS	0.04873	-	2.81	0.92459	8.41	0.8270-1.0279	6.71-10.66			
X	X	6.740(8)NS	0.03269	-	3.04	0.89683	7.89	0.8321-0.9642	6.79-9.21			

		Individual LC50 estimates								Comparison of repeated LC50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LC50 (log scale)	Adjusted S.E. of LC50 (log scale)	Gradient (log scale)	LC50 (log scale)	LC50 (antilog scale)	Fiducial limits of LC50 (log scale)	Fiducial limits of LC50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Chloro-acetamide	Spray	1.843(3)NS	0.06010	-	2.02	1.09766	12.52	0.9740-1.2168	9.42-16.47	3.618(6)NS	1.098(1)NS	0.956(1)NS
Chloro-acetamide	Spray	1.775(3)NS	0.05331	-	2.43	1.02190	10.52	0.9144-1.1300	8.21-13.49			
X	X	5.672(8)NS	0.04019	-	2.19	1.06094	11.51	0.9804-1.1404	9.56-13.82			
Chloro-acetamide	Soil drench	4.956(3)NS	0.06322	-	1.91	0.98798	9.73	0.8555-1.1115	7.17-12.93	5.139(6)NS	8.318(1)**	1.737(1)-
Chloro-acetamide	Soil drench	0.183(3)NS	0.03996	-	3.72	0.88820	7.73	0.8070-0.9800	6.41-9.55			
Chloro-acetamide	Solid incorp-oration	6.179(3)NS	0.05762	-	2.14	1.14341	13.91	1.0249-1.2582	10.59-18.12	8.258(6)NS	1.144(1)NS	5.557(1)*
Chloro-acetamide	Solid incorp-oration	2.079(3)NS	0.05122	-	2.61	0.96208	9.16	0.8582-1.0666	7.22-11.66			
X	X	-	-	-	2.35	-	-	-	-			

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Table A7.30 Assessments of toxicity: Artificial soil test. 14 day LC50 ($mg.kg^{-1}$ test chemical: dry weight of artificial soil)

		Individual LC50 estimates								Comparison of repeated LC50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LC50 (log scale)	Adjusted S.E. of LC50 (log scale)	Gradient (log scale)	LC50 (log scale)	LC50 (antilog scale)	Fiducial limits of LC50 (log scale)	Fiducial limits of LC50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Chlordane	R2	74.229(6)***	0.06953	0.24456	1.24	1.39138	24.63	1.2534-1.5322	17.92-34.06			
Chlordane	-	15.296(4)**	0.04473	0.08747	3.07	1.45690	28.64	1.3707-1.5527	23.48-35.70			
										138.167(20)*** (-)	99.541(3)*** (*)	10.672(3)** (-)
Chlordane	-	3.029(5)NS	0.02798	-	6.28	1.37392	23.65	1.3129-1.4286	20.56-26.83			
Chlordane	-	45.614(5)***	0.03673	0.11094	3.15	1.50099	31.70	1.4247-1.5719	26.59-37.32			
Triazophos	-	52.821(3)***	0.07016	0.29440	1.59	1.26055	18.22	1.1026-1.3891	12.66-24.50			
										68.712(7)*** (-)	0.302(1)NS (NS)	25.657(1)*** (NS)
Triazophos	-	15.891(4)**	0.07297	0.14544	1.75	0.78391	6.08	0.6264-0.9202	4.23-8.32			
Carbaryl	R1	71.160(7)***	0.05659	0.18043	1.50	2.05514	113.54	1.9395-2.1660	87.00-146.55			
										74.032(11)*** (-)	4.671(1)* (NS)	7.714(1)** (NS)
Carbaryl	-	2.872(4)NS	0.04896	-	2.15	1.81126	64.75	1.7159-1.9140	51.99-82.04			

		Individual LC50 estimates								Comparison of repeated LC50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LC50 (log scale)	Adjusted S.E. of LC50 (log scale)	Gradient (log scale)	LC50 (log scale)	LC50 (antilog scale)	Fiducial limits of LC50 (log scale)	Fiducial limits of LC50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Benomyl	-	12.343(5)*	0.08832	0.13877	1.53	0.58307	3.83	0.3976-0.7532	2.50-5.67	37.381(10)*** (-)	0.004(1)NS (NS)	18.502(1)*** (NS)
Benomyl	-	25.038(5)***	0.07175	0.16056	1.54	1.07188	11.80	0.9182-1.2080	8.28-16.14			
Thiophanate-methyl	-	38.360(5)***	0.05733	0.15879	1.74	1.38087	24.04	1.2630-1.4925	18.33-31.08	48.381(10)*** (-)	8.881(1)** (NS)	10.635(1)** (NS)
Thiophanate-methyl	-	10.021(5)NS	0.04657	-	2.88	1.22376	16.74	1.1181-1.3080	13.13-20.32			
Penta-chlorophenol	R1	59.724(5)***	0.05926	0.20481	1.85	1.69765	49.85	1.5774-1.8160	37.79-65.47	185.228(19)*** (-)	25.027(3)*** (NS)	5.497(3)- (NS)
Penta-chlorophenol	R2	16.832(4)**	0.05055	0.10370	2.74	1.62158	41.83	1.5190-1.7242	33.03-52.99			
Penta-chlorophenol	-	53.785(5)***	0.06227	0.20423	1.60	1.73750	54.64	1.6212-1.8767	41.80-75.28			
Penta-chlorophenol	-	54.887(5)***	0.08137	0.26960	1.11	1.57660	37.72	1.4196-1.7503	26.28-56.58			

		Individual LC50 estimates								Comparison of repeated LC50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LC50 (log scale)	Adjusted S.E. of LC50 (log scale)	Gradient (log scale)	LC50 (log scale)	LC50 (antilog scale)	Fiducial limits of LC50 (log scale)	Fiducial limits of LC50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Trichloro-acetic acid	R1	29.342(4)***	0.03235	0.08762	4.09	4.04257	11029.86	3.9803-4.115	9556.65-12925.81	53.580(9)*** (-)	2.250(1)NS (NS)	4.176(1)* (NS)
Trichloro-acetic acid	-	24.238(5)***	0.01981	0.04362	5.26	3.96130	9147.45	3.9220-4.0016	8356.09-10035.97			
Chloro-acetamide	R2	9.396(5)NS	0.04813	-	3.12	1.28043	19.07	1.1889-1.3867	15.45-24.36	13.132(10)NS	0.015(1)NS	0.237(1)NS
Chloro-acetamide	-	3.736(5)NS	0.04261	-	3.19	1.24968	17.77	1.1547-1.3278	14.28-21.27			
X	X	13.383(12)NS	0.02988	-	3.20	1.26202	18.28	1.2011-1.3202	15.89-20.90			
Potassium bromide	R2	18.385(6)**	0.04849	0.08488	2.05	2.66319	460.46	2.5671-2.7609	369.06-576.65	23.383(10)** (-)	7.810(1)** (NS)	0.205(1)- (NS)
Potassium bromide	-	4.998(4)NS	0.03092	-	3.56	2.58172	381.69	2.5253-2.6537	335.23-450.47			
Copper sulphate	R1	6.788(4)NS	0.02696	-	5.64	3.74380	5543.77	3.6908-3.7999	4907.25-6307.89	7.544(8)NS	10.355(1)**	0.418(1)-
Copper sulphate	-	0.756(4)NS	0.01184	-	9.99	3.75581	5699.12	3.7321-3.7799	5396.30-6023.57			

Table A7.31 Inter-specific variation in susceptibility of earthworms to test chemicals. 14 day LC50 ($mg.kg^{-1}$ test chemical: dry weight of artificial soil)

		Individual LC50 estimates								Comparison of repeated LC50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LC50 (log scale)	Adjusted S.E. of LC50 (log scale)	Gradient (log scale)	LC50 (log scale)	LC50 (antilog scale)	Fiducial limits of LC50 (log scale)	Fiducial limits of LC50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Chlor-dane	L.terrestris	0.607(6)NS	0.05674	-	5.21	0.59133	3.90	0.4593-0.7148	2.88-5.19			
Chlor-dane	L.terrestris	0.505(4)NS	0.09903	-	2.92	0.55933	3.63	0.3692-0.8169	2.34-6.56	1.277(15)NS	2.812(2)NS	8.847(2)*
Chlor-dane	L.terrestris	0.165(5)NS	0.08000	-	3.67	0.28249	1.92	0.1250-0.6002	1.33-3.98			
X	X	-	-	-	3.71	-	-	-	-			
Chlordane	A.longa	1.132(6)NS	0.05746	-	4.99	0.61807	4.15	0.4854-0.7440	3.06-5.55			
Chlordane	A.longa	0.106(4)NS	0.08999	-	4.06	0.65531	4.52	0.4638-0.8654	2.91-7.33	1.732(15)NS	0.809(2)NS	11.979(2)**
Chlordane	A.longa	0.494(5)NS	0.08424	-	3.47	0.32091	2.09	0.1600-0.6058	1.45-4.04			
X	X	-	-	-	4.23	-	-	-	-			
Chlor-dane	A.caliginosa	1.839(6)NS	0.05412	-	4.87	-0.05852	0.87	-0.1837-0.0664	0.66-1.17			
Chlor-dane	A.caliginosa	3.176(4)NS	0.14672	-	1.45	0.34650	2.22	0.0384-0.6848	1.09-4.84	5.015(15)NS	13.595(2)**	9.940(2)-

		Individual LC50 estimates							Comparison of repeated LC50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LC50 (log scale)	Adjusted S.E. of LC50 (log scale)	Gradient (log scale)	LC50 (log scale)	LC50 (antilog scale)	Fiducial limits of LC50 (log scale)	Fiducial limits of LC50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Chlor-dane	A.caliginosa	0.000(5)NS	0.06120	-	5.29	0.03975	1.10	-0.2672-0.1602	0.54-1.45			
Chlor-dane	E.fetida	0.527(6)NS	0.08650	-	3.28	1.05715	11.41	0.9244-1.3839	8.40-24.21			
Chlor-dane	E.fetida	3.468(4)NS	0.10742	-	2.46	1.21785	16.51	1.0190-1.4837	10.45-30.46	11.304(15)NS	1.868(2)NS	10.187(2)**
Chlor-dane	E.fetida	7.308(5)NS	0.08087	-	2.14	0.86201	7.28	0.6980-1.0325	4.99-10.78			
	X	-	-	-	2.42	-	-	-	-			
Penta-chlorophenol	L.terrestris	8.881(3)*	0.25296	0.43523	0.87	1.81853	65.85	1.3364-2.4560	21.70-285.76			
Penta-chlorophenol	L.terrestris	1.812(5)NS	0.13626	-	2.37	2.16471	146.12	1.9179-2.5534	82.77-357.62	16.803(11)NS	9.264(2)**	5.728(2)-
Penta-chlorophenol	L.terrestris	6.110(3)NS	0.18657	-	1.40	2.04021	109.70	1.6590-2.5018	45.60-317.55			
Penta-chlorophenol	A.longa	0.071(3)NS	0.14726	-	2.12	1.66745	46.50	1.3222-2.0122	21.00-102.85			

		Individual LC50 estimates								Comparison of repeated LC50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LC50 (log scale)	Adjusted S.E. of LC50 (log scale)	Gradient (log scale)	LC50 (log scale)	LC50 (antilog scale)	Fiducial limits of LC50 (log scale)	Fiducial limits of LC50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Penta-chlorophenol	A.longa	3.043(5)NS	0.12146	-	2.13	1.79980	63.07	1.5766-2.1282	37.72-134.33	3.652(11)NS	0.181(2)NS	11.278(2)**
Penta-chlorophenol	A.longa	0.537(3)NS	0.15747	-	1.85	1.17941	15.21	0.8068-1.5331	6.41-34.13			
X	X	-	-	-	2.03	-	-	-	-			
Penta-chlorophenol	A.caliginosa	4.277(3)NS	0.19581	-	1.23	0.70366	5.05	0.2943-1.1412	1.97-13.84			
Penta-chlorophenol	A.caliginosa	0.815(5)NS	0.09062	-	3.93	0.57609	3.77	0.2584-0.7286	1.81-5.35	5.093(11)NS	12.485(2)**	1.160(2)-
Penta-chlorophenol	A.caliginosa	0.001(3)NS	0.13295	-	2.77	0.50000	3.16	0.1951-0.8049	1.57-6.38			
Penta-chlorophenol	E.fetida	11.784(3)**	0.13887	0.27523	1.47	1.09450	12.43	0.8168-1.3998	6.56-25.10			
Penta-chlorophenol	E.fetida	6.906(5)NS	0.07788	-	2.20	1.22430	16.76	1.0733-1.3985	11.84-25.03	26.235(11)** (-)	2.704(2)NS (NS)	1.600(2)NS (NS)
Penta-chlorophenol	E.fetida	7.545(3)NS	0.13102	-	1.65	1.10099	12.62	0.8371-1.3915	6.87-24.63			

		Individual LC50 estimates							Comparison of repeated LC50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LC50 (log scale)	Adjusted S.E. of LC50 (log scale)	Gradient (log scale)	LC50 (log scale)	LC50 (antilog scale)	Fiducial limits of LC50 (log scale)	Fiducial limits of LC50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Chloro-acetamide	L.terrestris	0.737(6)NS	0.05709	-	5.56	1.59294	39.17	1.4726-1.7178	29.69-52.22			
Chloro-acetamide	L.terrestris	1.276(6)NS	0.05831	-	4.35	1.48567	30.60	1.3702-1.6195	23.45-41.64	3.019(17)NS	0.926(2)NS	4.6623(2)NS
Chloro-acetamide	L.terrestris	1.006(5)NS	0.05935	-	5.51	1.66078	45.79	1.5124-1.7753	32.54-59.61			
X	X	8.609(21)NS	0.03435	-	4.67	1.56862	37.04	1.4998-1.6381	31.61-43.47			
Chloro-acetamide	A.longa	1.777(6)NS	0.06037	-	4.02	1.56622	36.83	1.4433-1.6982	27.75-49.91			
Chloro-acetamide	A.longa	3.222(6)NS	0.06013	-	4.15	1.53007	33.89	1.4114-1.6662	25.79-46.36	7.301(17)NS	0.074(2)NS	1.597(2)NS
Chloro-acetamide	A.longa	2.301(5)NS	0.06352	-	4.36	1.64472	44.13	1.4896-1.7699	30.87-58.87			
X	X	8.972(21)NS	0.03585	-	4.02	1.57606	37.68	1.5044-1.6485	31.95-44.52			

		Individual LC50 estimates							Comparison of repeated LC50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LC50 (log scale)	Adjusted S.E. of LC50 (log scale)	Gradient (log scale)	LC50 (log scale)	LC50 (antilog scale)	Fiducial limits of LC50 (log scale)	Fiducial limits of LC50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Chloro-acetamide	A.caliginosa	2.038(6)NS	0.05992	-	5.71	1.50600	32.06	1.3990-1.6746	25.06-47.27			
Chloro-acetamide	A.caliginosa	2.038(6)NS	0.05992	-	5.71	1.50600	32.06	1.3990-1.6746	25.06-47.27	6.244(17)NS	1.013(2)NS	1.816(2)NS
Chloro-acetamide	A.caliginosa	2.169(5)NS	0.06739	-	4.25	1.42447	26.57	1.2805-1.5794	19.08-37.97			
X	X	9.073(21)NS	0.03485	-	5.15	1.48605	30.62	1.4210-1.5633	26.36-36.59			
Chloro-acetamide	E.fetida	2.753(6)NS	0.07215	-	3.31	0.98980	9.77	0.7198-1.1003	5.25-12.60			
Chloro-acetamide	E.fetida	6.811(6)NS	0.08764	-	2.32	0.80220	6.34	0.5807-0.9540	3.81-9.00	9.566(17)NS	4.239(2)NS	2.597(2)NS
Chloro-acetamide	E.fetida	0.002(5)NS	0.05311	-	4.78	1.05313	11.30	0.9002-1.1569	7.95-14.35			
X	X	16.402(21)NS	0.04663	-	2.81	0.92836	8.48	0.8189-1.0101	6.59-10.23			

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Table A7.32.1 Inter- and intra-specific variation in susceptibility of earthworms to test chemicals. Filter paper contact test. Chlordane LT50: hours ($1\mu g.cm^{-2}$ test chemical: filter paper surface)

		Individual LT50 estimates								Comparison of repeated LT50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LT50 (log scale)	Adjusted S.E. of LT50 (log scale)	Gradient (log scale)	LT50 (log scale)	LT50 (antilog scale)	Fiducial limits of LT50 (log scale)	Fiducial limits of LT50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Chlor-dane	A.caliginosa	2.817(8)NS	0.06639	-	4.13	-0.10784	0.78	-0.2837-0.0228	0.52-1.05	10.543(16)NS	13.619(1)***	56.916(1)-
Chlor-dane	E.fetida andrei	7.275(8)NS	0.12833	-	1.06	0.75823	5.73	0.5030-1.0931	3.18-12.39	10.971(16)NS	11.969(1)***	7.473(1)-
Chlor-dane	E.fetida fetida	3.246(8)NS	0.06387	-	2.99	0.95359	8.99	0.8374-1.1088	6.88-12.85			

Table A7.32.2. Inter- and intra-specific variation in susceptibility of earthworms to test chemicals.
Filter paper contact test. Pentachlorophenol LT50: hours ($150\mu g.cm^{-2}$ test chemical: filter paper surface)

		Individual LT50 estimates								Comparison of repeated LT50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LT50 (log scale)	Adjusted S.E. of LT50 (log scale)	Gradient (log scale)	LT50 (log scale)	LT50 (antilog scale)	Fiducial limits of LT50 (log scale)	Fiducial limits of LT50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Penta-chloro-phenol	A.caliginosa	1.101(8)NS	0.02807	-	11.05	0.43379	2.72	0.3635-0.4932	2.31-3.11	1.352(16)NS	0.503(1)NS	7.288(1)**
Penta-chloro-phenol	E.fetida andrei	0.252(8)NS	0.02165	-	14.64	0.53781	3.45	0.4809-0.5847	3.03-3.84	1.936(16)NS	0.000(1)NS	2.209(1)NS
Penta-chloro-phenol	E.fetida fetida	1.685(8)NS	0.02052	-	14.73	0.58232	3.82	0.5326-0.6272	3.41-4.24			

Table A7.32.3. Inter- and intra-specific variation in susceptibility of earthworms to test chemicals.
Filter paper contact test. Chloroacetamide LT50: hours (10 µg.cm^{-2} test chemical: filter paper surface)

		Individual LT50 estimates								Comparison of repeated LT50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LT50 (log scale)	Adjusted S.E. of LT50 (log scale)	Gradient (log scale)	LT50 (log scale)	LT50 (antilog scale)	Fiducial limits of LT50 (log scale)	Fiducial limits of LT50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Chloro-acetamide	A.caliginosa	0.002(8)NS	0.08928	-	7.93	1.31477	20.64	+	+	1.520(16)NS	0.201(1)NS	3.640(1)NS
Chloro-acetamide	E.fetida andrei	1.518(8)NS	0.06002	-	5.83	1.12209	13.25	1.0006-1.2556	10.01 18.01	6.703(15)NS	0.672(1)NS	1.370(1)NS
Chloro-acetamide	E.fetida fetida	5.186(7)NS	0.05976	-	4.63	1.22323	16.72	1.0962-1.3517	12.48-22.48			

Table A7.32.4 Inter- and intra-specific variation in susceptibility of earthworms to test chemicals.
Filter paper contact test. Iodoacetamide LT50: hours (10 µg.cm^{-2} test chemical: filter paper surface)

		Individual LT50 estimates								Comparison of repeated LT50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LT50 (log scale)	Adjusted S.E. of LT50 (log scale)	Gradient (log scale)	LT50 (log scale)	LT50 (antilog scale)	Fiducial limits of LT50 (log scale)	Fiducial limits of LT50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Iodo-acetamide	A.caliginosa	1.887(8)NS	0.02804	-	8.73	0.79245	6.20	0.7411-0.8874	5.51-7.72	3.705(16)NS	0.060(1)NS	0.732(1)NS
Iodo-acetamide	E.fetida andrei	1.818(8)NS	0.02881	-	9.80	0.81841	6.58	0.7702-0.9436	5.89-8.78	14.568(16)NS	7.607(1)**	6.941(1)-
Iodo-acetamide	E.fetida fetida	12.750(8)NS	0.07094	-	3.03	1.14423	13.94	1.0166-1.3190	10.39-20.84			

Table A7.33.1 Intra-specific variation in susceptibility of earthworms to test chemicals.
Filter paper contact test. Chlordane LT50: hours (5 µg.cm^{-2} test chemical: filter paper surface)

		Individual LT50 estimates								Comparison of repeated LT50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LT50 (log scale)	Adjusted S.E. of LT50 (log scale)	Gradient (log scale)	LT50 (log scale)	LT50 (antilog scale)	Fiducial limits of LT50 (log scale)	Fiducial limits of LT50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Chlordane	E.fetida andrei	0.465(9)NS	0.01538	-	18.03	1.40269	25.27	1.3692 1.4377	23.40 27.40	12.833(18)NS	34.185(1)***	0.192(1)-
Chlordane	E.fetida fetida	12.368(9)NS	0.06893	-	2.35	1.41875	26.23	1.2970- 1.5961	19.82- 39.45			

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Table A7.33.2 Intra-specific variation in susceptibility of earthworms to test chemicals.
Filter paper contact test. Iodoacetamide LT50: hours (10 µg.cm^{-2} test chemical: filter paper surface)

		Individual LT50 estimates								Comparison of repeated LT50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LT50 (log scale)	Adjusted S.E. of LT50 (log scale)	Gradient (log scale)	LT50 (log scale)	LT50 (antilog scale)	Fiducial limits of LT50 (log scale)	Fiducial limits of LT50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Iodo-acetamide	E.fetida andrei	0.920(6)NS	0.03836	-	5.85	0.94771	8.87	0.8651-1.0283	7.33-10.67	3.098(12)NS	0.211(1)NS	21.767(1)***
Iodo-acetamide	E.fetida fetida	2.179(6)NS	0.04444	-	5.11	0.66166	4.59	0.5550-0.7492	3.59-5.61			

Table A7.34 Intra-specific variation in susceptibility of earthworms to test chemicals. Filter paper contact test. 48 hour LC50 ($g.cm^{-2}$ test chemical: filter paper surfa

		Individual LC50 estimates								Comparison of repeated LC50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LC50 (log scale)	Adjusted S.E. of LC50 (log scale)	Gradient (log scale)	LC50 (log scale)	LC50 (antilog scale)	Fiducial limits of LC50 (log scale)	Fiducial limits of LC50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Chlordane	E.fetida andrei	0.000(4)NS	0.04919	-	7.05	-6.11933	7.60×10^{-7}	-6.2431- -5.9890	5.71×10^{-7} - 1.02×10^{-6}	7.952(9)NS	1.987(1)NS	11.877(1)***
Chlordane	E.fetida andrei	7.952(5)NS	0.06439	-	3.72	-5.74528	1.76×10^{-6}	-5.8748- -5.5991	1.33×10^{-6} - 2.51×10^{-6}			
X	X	-	-	-	4.12	-	-	-	-			
Chlordane	E.fetida fetida	5.053(4)NS	0.10523	-	2.49	-5.79503	1.60×10^{-6}	-6.0275- -5.5633	9.39×10^{-7} - 2.73×10^{-6}	5.820(9)NS	7.167(1)**	10.439(1)-
Chlordane	E.fetida fetida	0.767(5)NS	0.04621	-	6.97	-5.40194	3.96×10^{-6}	-5.5213- -5.3051	3.01×10^{-6} - 4.95×10^{-6}			
Iodo-acetamide	E.fetida andrei	0.000(4)NS	0.05580	-	5.99	-6.08741	8.18×10^{-7}	-6.2229- -5.8917	6.00×10^{-7} - 1.28×10^{-6}	2.884(10)NS	0.294(1)NS	1.915(1)NS
Iodo-acetamide	E.fetida andrei	2.884(6)NS	0.05498	-	4.62	-5.96132	1.09×10^{-6}	-6.0705- -5.8205	8.50×10^{-7} - 1.51×10^{-6}			
X	X	5.093(12)NS	0.04367	-	4.63	-6.00530	9.88×10^{-7}	-6.0890- -5.8970	8.15×10^{-7} - 1.27×10^{-6}			

		Individual LC50 estimates								Comparison of repeated LC50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LC50 (log scale)	Adjusted S.E. of LC50 (log scale)	Gradient (log scale)	LC50 (log scale)	LC50 (antilog scale)	Fiducial limits of LC50 (log scale)	Fiducial limits of LC50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Iodo-acetamide	E.fetida fetida	0.268(4)NS	0.09502	-	3.91	-5.65052	2.24×10^{-6}	-5.8618- -5.4392	1.38×10^{-6} - 3.64×10^{-6}	0.455(9)NS	1.782(1)NS	1.203(1)NS
Iodo-acetamide	E.fetida fetida	0.187(5)NS	0.05143	-	6.45	-5.51567	3.05×10^{-6}	-5.6570- -5.4086	2.20×10^{-6} - 3.90×10^{-6}			
X	X	3.439(11)NS	0.05083	-	4.66	-5.56791	2.70×10^{-6}	-5.6802- -5.4684	2.09×10^{-6} - 3.40×10^{-6}			

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Table A7.35.1 Intra-specific variation in susceptibility of earthworms to test chemicals.
Artificial soil test. Chlordane LT50: days (50 mg.kg^{-1} test chemical: dry weight of artificial soil)

		Individual LT50 estimates								Comparison of repeated LT50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LT50 (log scale)	Adjusted S.E. of LT50 (log scale)	Gradient (log scale)	LT50 (log scale)	LT50 (antilog scale)	Fiducial limits of LT50 (log scale)	Fiducial limits of LT50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Chlordane	E.fetida andrei	8.604(7)NS	0.02419	-	5.16	0.72478	5.31	0.6755-0.7742	4.74-5.95	11.838(14)NS	0.198(1)NS	5.594(1)*
Chlordane	E.fetida andrei	3.235(7)NS	0.02345	-	5.64	0.64629	4.43	0.5965-0.6922	3.95-4.92			
X	X	-	-	-	5.40	-	-	-	-			
Chlordane	E.fetida fetida	1.702(7)NS	0.02404	-	6.19	0.56532	3.68	0.5117-0.6103	3.25-4.08	5.940(14)NS	3.285(1)NS	0.926(1)NS
Chlordane	E.fetida fetida	4.239(7)NS	0.03166	-	4.30	0.50578	3.20	0.4344-0.5640	2.72-3.66			
X	X	10.152(16)NS	0.01982	-	5.01	0.53512	3.43	0.4928-0.5722	3.11-3.73			

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Table A7.35.2. Intra-specific variation in susceptibility of earthworms to test chemicals.
Artificial soil test. Iodoacetamide LT50: days (20 mg.kg^{-1} test chemical: dry weight of artificial soil)

		Individual LT50 estimates								Comparison of repeated LT50 estimates		
Chemical	Variable	χ^2 (D.F.) Hetero-geneity	S.E. of LT50 (log scale)	Adjusted S.E. of LT50 (log scale)	Gradient (log scale)	LT50 (log scale)	LT50 (antilog scale)	Fiducial limits of LT50 (log scale)	Fiducial limits of LT50 (anti-log scale)	χ^2 (D.F.) Total Hetero-geneity	χ^2 (D.F.) Parallelism	χ^2 (D.F.) Y intercept
Iodo-acetamide	E.fetida andrei	5.729(7)NS	0.03468	-	3.63	0.51904	3.30	0.4411-0.5831	2.76-3.83	8.409(14)NS	4.457(1)*	1.516(1)-
Iodo-acetamide	E.fetida andrei	2.680(7)NS	0.02714	-	5.54	0.50017	3.16	0.4390-0.5504	2.75-3.55			
Iodo-acetamide	E.fetida fetida	7.641(7)NS	0.02624	-	4.62	0.69906	5.00	0.6454-0.7522	4.42-5.65	15.238(14)NS	2.319(1)NS	6.298(1)*
Iodo-acetamide	E.fetida fetida	7.597(7)NS	0.03339	-	3.41	0.80805	6.43	0.7429-0.8799	5.53-7.58			
X	X	-	-	-	3.94	-	-	-	-			

REFERENCES

Anonymous (1972). Toxicity of metals to rainbow trout, pp. 37-38, Rep. Director Water Pollut. Res. 1971, HMSO, London.

Anonymous (1975). Programme on Man and the Biosphere. Expert consultations on Project 9: Ecological assessment of pest management and fertilizer use on terrestrial and aquatic ecosystems. (Part on pesticides). Final Report. Publ., United Nations Educational, Scientific and Cultural Organisation, Paris, France, 41 pp.

Anonymous (1979). Council Directive 79/831/EEC of 18 September, amending for the 6th time Directive 67/548/EEC. O.J. Eur. Communities L259, pp.10-28.

Anonymous (1981a). Pesticides. 5th edition. Advice and recommendations to be used by national and other authoritories as well as manufacturers concerned with the registration of agricultural and non-agricultural pesticides. Publ., Council of Europe, Strasbourg, France, 108 pp.

Anonymous (1981b). Field Assessment of Soil Texture and Drainage Class. Ministry of Agriculture, Fisheries and Food, Leaflet 796, 4pp.

Anonymous (1981c). ECO 85, UPEC 15: Test guideline for the assessment of toxicity to earthworms (Eisenia foetida Savigny). Proposal for an OECD-guideline laboratory test. Draft BBP AP 3000 bv. OECD Chemicals Testing Programme. Publ., Biologische Bundesanstalt fur Land- und Forstwirtschaft, Braunschweig, West Germany, 8 pp.

Anonymous (1981d). The Analysis of Agricultural Materials. Ministry of Agriculture, Fisheries and Food, Publ., HMSO, London, 226 pp.

Anonymous (1983). Laboratory and field testing of pesticide products for effects on soil macro-organisms. U.K. Pesticides Safety Precautions Scheme. Working Document D6. Publ., Ministry of Agriculture, Fisheries and Food, 3 pp.

Abbott, I. and Parker, C.A. (1981). Interactions between earthworms and their soil environment. Soil. Biol. Biochem. 13, 191-197.

Abbott, W.S. (1925). A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18, 265-267.

Agarwal, H.C., Yadav, D.V. and Pillai, M.K.K. (1978). Metabolism of ^{14}C-DDT in Pheretima posthuma and effect of pretreatment with DDT, Lindane and Dieldrin. Bull. Environ. Contam. Toxicol. 19, 295-299.

An Der Lan, H. and Aspock, H. (1962). Zur Wirkung von Sevin auf Regenwurmer. Anz. Schaedlingskd. 35, 180-182.

Andre, F. (1963). Contribution a l'analyse experimentale de la reproduction des lombriciens. Bull. Biol. Fr. Belg. 97, 3-101.

Ash, C.P.J. and Lee, D.L. (1980). Lead, cadmium, copper and iron in earthworms from roadside sites. Environ. Pollut. Ser. A 22, 59-67.

Aspock, H. and An Der Lan, H. (1963). Okologische Auswirkungen und Physiologische Besonderheiten des Pflanzenschutzmittels Sevin. (1-Naphthyl-N-methylcarbamate). Z. Angew Zool. 50, 343-380.

Atlavinyte, O. (1975). Effect of chemical substances on the activity of Lumbricidae in the process of straw disintegration. In Progress in Soil Zoology (ed. Vanek, J.), pp. 515-519. Publ., Dr. W. Junk, B.V., Amsterdam, Netherlands.

Atlavinyte, O. (1981). The effect of pesticides on the abundance of mites and collembola during the process of decomposition of organic substances. In Noveishie Dostizheniya Sel Skokhozyaistvennoi Entomologii (ed. Semyanov, V.P.). pp.9-13, Publ., Po Materialam Ush Sezda Veo Vilnius, USSR.

Atlavinyte, O., Daciulyte, J. and Lugauskas, A. (1974). The effect of herbicides and insecticides on populations and activities of soil organisms. Dinamika Mikrobiologicheskikh Protsessov v Pochve Obuslovlivayushchie Ee Faktory. Materialy Simpoziuma, Kharkov 1974, pp.137-140, Publ., Akademiya Nauk Estonskoi SSR, Tallin, USSR.

Atlavinyte, O., Daciulyte, J. and Lugauskas, A. (1977). The effect of Lumbricidae on plant humification and soil organism biocenoses under application of pesticides. Ecol. Bull. 25, 222-228.

Atlavinyte, O., Daciulyte, J. and Lugauskas, A. (1978). The influence of TCA on soil organisms and the accumulation of vitamin B12 in soil. Mikrobiologicheskie Protsessov v Pochve I Urozhainost Sel Skokhozyaistvennoi Kultur. Materialy K Respublikanskoi Konferentsii, Vilnius 1973, pp.30-31, Akademiya Nauk Lithuanskoi, Vilnius, USSR.

Atlavinyte, O., Galvelis, A., Daciulyte, J. and Lugauskas, A. (1982). Effects of entobacterin on earthworm activity. Pedobiolog. 23, 372-379.

Atlavinyte, O., Lugauskas, A. and Kilikevicius, G. (1980). Accumulation of organophosphorus insecticides in earthworms and reactions of earthworms and micro-organisms to these substances. In Soil Biology as Related to Land Use Practices. Proceedings of the VII International Soil Zoology Colloquium of the International Society of Soil Science (ed. Dindal, D.L.), pp. 13-24. Pub. Office of Pesticide and Toxic Substances, Environmental Protection Agency, Washington, USA.

Avel, M. (1937). Role joue, dans la regeneration de la tete, par les parties anciennes immediatement voizines de la surface d'amputation. Titres et Travaux Scientifiques de Marcel Avel, pp.46-48. Publ., Bordeaux Imprimerie-Librairie, Delmas, France.

Avery, B.W. (1980). Soil classification for England and Wales (Higher Categories). Soil Survey Technical Monograph No.14, Soil Survey of England and Wales, Harpenden, 67 pp.

Avery, B.W. and Bascomb, C.L. (1974). Soil survey laboratory methods. Soil Survey Technical Monograph No.6, Soil Survey of England and Wales, Harpenden, 83 pp.

Bailey, G.W. and White, J.L. (1964). Review of adsorption and desorption of organic pesticides by soil colloids, with implications concerning pesticide bioactivity. J. Agric. Food Chem. 12, 324-332.

Baker, W.L. (1946). DDT and earthworm populations. J. Econ. Entomol. 39, 404-405.

Barker, G.M. (1982). Short-term effects of methiocarb formulations on pasture earthworms (Oligochaeta:Lumbricidae). N.Z. J. Exp. Agric. 10, 309-311.

Barker, R.J. (1958). Notes on some ecological effects of DDT sprayed on elms. J. Wildl. Man. 22, 269-274.

Barnes, R.D. (1968). Invertebrate Zoology. 2nd edition. Publ., W.B. Saunders and Co., Philadelphia, U.S.A., 743 pp.

Barnett, B.E. (1983). Oligochaetes as indicators of pollution in the Humber estuary, with special reference to Tubificoides benedeni. Environ. Pollut. Ser. A 30, 277-291.

Bascomb, C.L. (1964). A rapid method for the determination of cation exchange capacity of calcareous and non-calcareous soils. J. Sci. Food Agric. 15, 821-823.

Bauer, K. (1964). Studien uber nebenwirkungen von pflanzenchutzmitteln auf die bodenfauna. Mitt. Biol. Bundesanst. Land-u Forstw. 112, 1-42.

Bengtsson, G., Nordstrom, S. and Rundgren, S. (1983). Population density and tissue metal concentration of lumbricids in forest soils near a brass mill. Environ. Pollut. Ser. A 30, 87-108.

Benz, G. and Altwegg, A. (1975). Safety of Bacillus thuringiensis for earthworms. J. Inver. Path. 26, 125-126.

Berankova, J. (1978). Laboratory tests investigating the effect of pesticides and commercial fertilizers on the soil fauna. Ochr. Rosl. 14, 305-312.

Bernstein, I. and Weatherall, M. (1952). Statistics for Medical and Other Biological Students. Publ., E. and S. Livingston Ltd., Edinburgh, 180 pp.

Besch, W.K. (1976a). General principles for biotesting in the field and in the laboratory. Lectures presented at the 4th FAO/SIDA training course on aquatic pollution in relation to the protection of living resources. Bioassays and toxicity testing. Lysekil, Sweden, pp. 16-28, Publ., Food and Agriculture Organisation, Rome, Italy.

Besch, W.K. (1976b). Philosophy behind biotesting methods. Lectures presented at the 4th FAO/SIDA training course on aquatic pollution in relation to the protection of living resources. Bioassays and toxicity testing. Lysekil, Sweden, pp. 29-46, Publ., Food and Agriculture Organisation, Rome, Italy.

Beyer, W.N. (1981). Metals and terrestrial earthworms (Annelida:Oligochaeta). Proceedings of the workshop on the role of earthworms on the stabilisation of organic residues 1 (ed. Appelhof, M.), pp. 137-150. Beech Leaf Press, Kalamazoo, Michigan, U.S.A.

Beyer, W.N., Chaney, R.L. and Mulhern, B.M. (1982). Heavy metal concentrations in earthworms from soil amended with sewage sludge. J. Environ. Qual. 11, 381-385.

Beyer, W.N. and Gish, C.D. (1980). Persistence in earthworms and potential hazards to birds of soil applied DDT, Dieldrin and Heptachlor. J. Appl. Ecol. 17, 295-307.

Bharathi, C. and Subba Rao, B.V.S.S.R. (1984). Toxicity of phosphamidon to the common South Indian earthworm Lampeto mauritii. Bull. Environ. Contam. Toxicol. 32, 295-300.

Bigger, J.H. and Decker, G.C. (1966). Controlling root-feeding insects of corn. A report of a 10 year study. Univ. Ill. Coll. Agric. Exp. Sta. Bull. 716, 24 pp.

Black, W.M. and Neely, D. (1975a). Effect of soil injected benomyl on resident earthworm populations. Pestic. Sci. 6, 543-545.

Black, W.M. and Neely, D. (1975b). Dutch elm disease control with soil injected benomyl; effect on resident earthworm populations. Proc. Am. Phytophathol. Soc. 2, 82.

Blackshaw, R.P. (1980). The effect of benzimidazole fungicides on the ecology of soil fauna in winter wheat. Ph.D. Thesis, University of Newcastle upon Tyne.

Blankwaardt, H.F.H. and Van Der Drift, J. (1961). The influence of soil sterilisation on earthworms in a heated greenhouse. Meded. Dir. Tuinb. 24, 490-496.

Bouche, M.B. (1969) Comparaison critique de methodes d'evaluation des populations de Lombricides. Pedobiolog. 9, 26-34.

Bouche, M.B. (1972) Lombriciens de France. Ecologie et Systematique. Institut National de la Recherche Agronomique, Paris, 671 pp.

Bouche, M.B. (1974). Pesticides et lombriciens: problems methodologiques et economiques. Phytiat. Phytopharm. 23, 107-116.

Bouche, M.B. (1982). Personal communication.

Bouche, M.B. (1984a). Ecotoxicologie des lombriciens. I. Exotoxicite controlee. Acta Oecol. (Oecol. Applic.) 5, 271-287.

Bouche, M.B. (1984b). Ecotoxicologie des lombriciens. II. Surveillance de la contamination des milieux. Acta Oecol. (Oecol. Applic.) 5, 291-301.

Bouche, M.B. (1985). Earthworm toxicological tests, danger assessment and biomonitoring. A methodological approach. Proceedings of the International Conference on Earthworms in Waste and Environmental Management. Cambridge. (in press).

Bouche, M.B. and Beugnot, M. (1978) Action du chlorate de sodium sur le niveau des populations et l'activite biodegradatrice des Lombriciens. Phytiat. Phytopharm. 27, 147-162.

Bouche, M.B. and Gardner, R.H. (1984). Earthworm functions. VIII. Population estimation techniques. Rev. Ecol. Biol. Sol. 21, 37-63.

Bracher, G.A. and Bider, J.R. (1982). Changes in terrestrial animal activity of a forest community after an application of aminocarb. (Matacil). Can. J. Zool. 60, 1981-1997.

Briggs, G.G. (1981). Relationships between chemical structure and the behaviour and fate of pesticides. Proc. 11th Brit. Crop. Prot. Conf. 3, 701-710.

Briggs, G.G. (1982). Personal communication.

Briggs, G.G. and Lord, K.A. (1983). The distribution of Aldicarb and its metabolites between Lumbricus terrestris, water and soil. Pestic. Sci. 14, 412-416.

Broadbent, A.B. and Tomlin, A.D. (1982). Comparison of two methods for assessing the effects of carbofuran on soil animal decomposers in cornfields. Environ. Entomol. 11, 1036-1042.

Brown, G. (1974). The agricultural significance of clays. In Soil Type and Land Capability, Soil Survey Technical Monograph No.4, (ed. Mackney, D), pp. 27-42. Soil Survey of England and Wales, Harpenden.

Buahin, G.K.A. and Edwards, C.A. (1963). The side effects of toxic chemicals in the soil on arthropods and worms. Rep Rothamsted Exp Sta. 1962, 156-157.

Bull, K.R., Roberts, R.D., Inskip, M.J. and Goodman, G.T. (1977). Mercury concentrations on soil, grass, earthworms and small mammals near an industrial emission source. Environ. Pollut. Ser A 12, 135-140.

Burrell, R.E. and Corke, C.T. (1980). Interactions of the solvent acetone with the fungicides benomyl and captan in fungal assays. Bull. Environ. Contam. Toxicol. 25, 554-561.

Busvine, J.R. (1957). A Critical Review of the Techniques for Testing Insecticides. Commonwealth Institute of Entomology, London, 208 pp.

Busvine, J.R. and Nash, R. (1953). Evaluation of new contact insecticides. Bull. ent. Res. 44, 371.

Butler, G.C. (1978). Principles of Ecotoxicology. Scope 12. J. Wiley & Sons., Chichester, 372 pp.

Callahan, C.A. (1985). Earthworm ecotoxicology. Proceedings of the International Conference on Earthworms in Waste and Environmental Management, Cambridge. (in press).

Carley, W.W., Caracciolo, E.A. and Mason, R.T. (1983). Cell and coelomic fluid volume regulation in the earthworm Lumbricus terrestris. Comp. Biochem. Physiol. A Comp. Physiol 74, 569-575.

Carson, R. (1963). Silent Spring. Hamish Hamilton Ltd., 304 pp.

Caseley, J.C. and Eno, C.F. (1966). Survival and reproduction of two species of earthworm and a rotifer following herbicide treatments. Proc. Soil Sci. Soc. Am. 30, 346-350.

Cathey, B. (1982). Comparative toxicities of five insecticides to the earthworm, Lumbricus terrestris. Agric. Environ. 7, 73-81.

Chio, H. and Sanborn, J.R. (1976). The metabolism of ^{14}C-chlordane by the earthworm, Lumbricus terrestris L. Chemosphere 3, 161-166.

Chio, H. and Sanborn, J.R. (1978). The metabolism of atrazine, chloramben and dicamba in earthworms (Lumbricus terrestris) from treated and untreated plots. Weed Sci. 26, 331-335.

Chiou, C.T., Freed, V.H., Schmedding, D.W. and Kohnert, R.L. (1977). Partition coefficient and bioaccumulation of selected organic chemicals. Environ. Sci. Technol. 11, 475-478.

Chisholm, R.D. and Koblitsky, L. (1943). Sorption of methyl bromide by soil in a fumigation chamber. J. Econ. Entomol. 36, 549-551.

Cirelli, D.P. (1978). Patterns of pentachlorophenol usage in the United States of America - An overview. In Pentachlorophenol: Chemistry, Pharmacology and Environmental Toxicology Section I. (Ed. Rao, K.R.), pp.13-18, Plenum Press, New York, U.S.A.

Clements, R.O. and Henderson, I.F. (1977). Some consequences of a prolonged absence of invertebrates from a perennial ryegrass sward. Proc. 13th Int. Grassl. Cong., 1261-1263.

Clements, R.O., Henderson, I.F. and Bentley, B.R. (1982). The effects of pesticide application on upland permanent pasture. Grass Forage Sci. 37, 123-128.

Clutterbuck, C. (1973). Effects of herbicides on soil invertebrates. Ph.D. Thesis, University of London.

Coates, K. and Ellis, D.V. (1980). Enchytraeid oligochaetes as marine pollution indicators. Mar. Pollut. Bull. 11, 171-174.

Cook, A.G., Critchley, B.R., Critchley, U., Perfect, T.J. and Yeadon, R. (1980). Effects of cultivation and DDT on earthworm activity in a forest soil in the sub-humid tropics. J. Appl. Ecol. 17, 21-29.

Cook, M.E. and Swait, A.A.J. (1975). Effects of some fungicide treatments on earthworm populations and leaf removal in apple orchards. J. Hortic. Sci. 50, 495-499.

Cremlyn, R. (1978). Pesticides. Preparation and Mode of Action. John Wiley and Sons, Chichester, 240 pp.

Crossley, Jr., D.A., Reichle, D.E. and Edwards, C.A. (1971). Intake and turnover of radioactive cesium by earthworms (Lumbricidae). Pedobiolog. 11, 71-76.

Darwin, C. (1881). The Formation of Vegetable Mould Through the Action of Worms, with Observation of their Habits. Murray, London, 326 pp.

Davis, B.N.K. (1971). Laboratory studies on the uptake of dieldrin and DDT by earthworms. Soil Biol. Biochem. 3, 221-233.

Davis, B.N.K. and French, M.C. (1969). The accumulation and loss of organochlorine insecticide residues by beetles, worms and slugs in sprayed fields. Soil Biol. Biochem. 1, 44-55.

Davey, S.P. (1963). Effects of chemicals on earthworms: A review of the literature. USDI Special Scientific Report. Wildlife No. 74, Washington, USA, 20 pp.

Dawson, R.B., Boyns, B.M. and Shorrock, R.W. (1938). The use of Derris in the control of earthworms. J. Sports Turf Res. Inst. 5, 249-257.

Dawson-Bingley, R.B. (1949). The control of earthworms. Country Life 105, 434 and 437.

Dean-Ross, D (1983). Methods for the assessment of the toxicity of environmental chemicals to earthworms. Regul. Toxicol. Pharmacol. 3, 48-59.

De Medts, A. (1981). Effets de residus de pesticides sur les Lombriciens en terre de culture. Pedobiolog. 21, 439-445.

Devonshire, A.L. (1977). The properties of a carboxylesterase from the Peach-Potato aphid Myzus persicae (Sulz.), and its role in conferring insecticide resistance. Biochem. J. 167, 675-683.

Dikshith, T.S.S. and Gupta, S.K. (1981). Carbaryl induced biochemical changes in earthworm Pheretima posthuma. Ind. J. Biochem. Biophys. 18, 154.

Doane, C.C. (1962). Effects of certain insecticides on earthworms. J. Econ. Entomol. 55, 416-418.

Drandarevski, C.A., Eichler, D. and Domsch, K.H. (1977). Behaviour of Triforine in soil and its influence on microbiological soil processes. Z. Pflanzenkr. Pflanzenschutz. 84, 18-30.

Drewes, C.D. and Callahan, C.A. (1985). Electrophysiological detection of sublethal neurotoxic effects in intact earthworms. Proceedings of the International Conference on Earthworms in Waste and Environmental Management, Cambridge. (in press).

Drewes, C.D., Callahan, C.A. and Fender, W.M. (1983). Species specificity of giant nerve fibre conduction velocity in Oligochaetes. Can. J. Zool. 61, 2688-2694.

Drewes, C.D. and Vining, E.P. (1984). In vivo neurotoxic effects of dieldrin on giant nerve fibres and escape reflex function in the earthworm, Eisenia fetida. Pestic. Biochem. Physiol. 22, 93-103.

Drewes, C.D., Vining, E.P. and Callahan, C.A. (1984). Non-invasive electrophysiological monitoring: a sensitive method for detecting sublethal neurotoxic effects in earthworms. Environ. Toxicol. Chem. 3, 599-607.

DuBois, K.P. and Geiling, E.M.K. (1959). Textbook of Toxicology. Oxford University Press, Oxford, 302 pp.

Duffus, J.H. (1980). Environmental Toxicology. Edward Arnold, London, 164 pp.

Easton, E.G. (1983). A guide to the valid names of the Lumbricidae (Oligochaeta). In Earthworm Ecology. From Darwin to Vermiculture (ed. Satchell, J.E.), pp. 475-485. Chapman and Hall, London.

Ebeling, W. (1963). Analysis of the basic processes involved in the deposition, degradation, persistence and effectiveness of pesticides. Residue Rev. 3, 35-163.

Ebing, K.W. and Haque, A. (1979). Summarising report on previous studies concerning earthworms to test the ecological effects of organic chemicals on soil organisms. 3rd Meeting OECD Expert Group C 'Degradation/Accumulation'. Tokyo, May 21-26, 1979.

Ebing, K.W., Pflugmacher, J. and Haque, A. (1984). Der regenwurm als schlusselorganismus zur messung der bodenbelastung mit organischen fremdchemikalien. Ber. Landwirtsch. 62, 222-255.

Edwards, C.A. (1965). Some side-effects resulting from the use of persistent insecticides. Ann. App. Biol. 55, 329-331.

Edwards, C.A. (1970). Effects of herbicides on the soil fauna. Proc. 10th Brit. Weed Contr. Conf. 3, 1052-1062.

Edwards, C.A. (1974). Soil types and agricultural pest control. Soil Type and Land capability. Soil Survey Technical Monograph No.4 (ed. Mackney, D), pp. 83-90. Publ., Soil Survey of England and Wales, Harpenden.

Edwards, C.A. (1975a). Factors that affect the persistence of pesticides in plants and soils. Pure Appl. Chem. 42, 39-56.

Edwards, C.A. (1975b). Effects of direct drilling on the soil fauna. Outlook Agr. 8, 243-244.

Edwards, C.A. (1976). The uptake of two organophosphorus insecticides by slugs. Bull. Env. Contam. Toxicol. 16, 406-410.

Edwards, C.A. (1977). Investigations into the influence of agricultural practice on soil invertebrates. Ann. App. Biol. 87, 515-520.

Edwards, C.A. (1978). Tests to assess the effects of pesticides on beneficial soil organisms. In Tests for the Ecological Effects of Chemicals, pp. 249-253 (eds. Von Lersner, H., Bourdeau, P.). Publ., Erich Schmidt Verlag, Berlin, West Germany.

Edwards, C.A. (1980). Interactions between agricultural practice and earthworms. Soil Biology as Related to Land Use Practices, Proceedings of the VII International Soil Zoology Colloqium of the International Society of Soil Science (ed. Dindal, D.L.), pp. 3-12. Publ., Office of Pesticides and Toxic Substances, Environmental Protection Agency, Washington, USA.

Edwards, C.A. (1982). Problems caused by the contamination of agricultural land and woodlands by toxic chemicals. Decheniana Beih. 26, 145-150.

Edwards, C.A. (1983a). Earthworm ecology in cultivated soils. Earthworm Ecology. From Darwin to Vermiculture (ed. Satchell, J.E.), pp. 123-137. Chapman and Hall, London.

Edwards, C.A. (1983b). The environmental impact of pesticides: fact and fiction. Med. Fac. Lanabouww. Rijksuniv. Gent 48, 149-155.

Edwards, C.A. and Arnold, M.K. (1963). The side-effects of toxic chemicals in the soil on arthropods and worms. Rep Rothamsted Exp Sta. 1962, p. 156.

Edwards, C.A. and Arnold, M.K. (1964). The side-effects of toxic chemicals in the soil on arthropods and earthworms. Rep Rothamsted Exp Sta. 1963, p. 147.

Edwards, C.A. and Arnold, M.K. (1966). Effects of insecticides on soil fauna. Rep Rothamsted Exp Sta. 1965, p. 186.

Edwards, C.A., Arnold, M.K. and Thompson, A.R. (1966). Effects of insecticides on soil fauna. Rep Rothamsted Exp Sta. 1965, p. 187.

Edwards, C.A., Beck, S.D. and Lichtenstein, E.P. (1957). Bioassay of aldrin and lindane in soil. J. Econ. Entomol. 50, 622-626.

Edwards, C.A. and Dennis, E.B. (1960). Some effects of aldrin and DDT on the soil fauna of arable land. Nature, Lond. 188, 767.

Edwards, C.A., Dennis, E.B. and Empson, D.W. (1967). Pesticides and the soil fauna: effects of aldrin and DDT in an arable field. Ann. Appl. Biol. 60, 11-22.

Edwards, C.A. and Jeffs, K.A. (1965). The persistence of some insecticides in soil and their effects on soil animals. Proc. 12th Int. Congr. Entomol., pp. 559-560.

Edwards, C.A. and Jeffs, K.A. (1973). Uptake of pesticides by earthworms. Rep Rothamsted Exp Sta. 1972, Part 1, pp. 212-213.

Edwards, C.A. and Jeffs, K.A. (1974). Rate of uptake of DDT from soil by earthworms. Nature, Lond. 247, 157-158.

Edwards, C.A. and Lofty, J.R. (1971). Nematicides and the soil fauna. Proc. 6th Brit. Insectic. Fungic. Conf. 1, 158-166.

Edwards, C.A. and Lofty, J.R. (1973). Pesticides and earthworms. Rep Rothamsted Exp Sta. 1972 Part 1, pp. 211-212.

Edwards, C.A. and Lofty, J.R. (1975). The invertebrate fauna of the Park Grass plots. Rep Rothamsted Exp Sta. 1974, Part 2, pp. 133-154.

Edwards, C.A. and Lofty, J.R. (1976). Pesticides and soil fauna. Rep Rothamsted Exp Sta. 1975, Part 1, pp. 128-129.

Edwards, C.A. and Lofty, J.R. (1977). Biology of Earthworms, 2nd edition. Chapman and Hall, London, 333 pp.

Edwards, C.A. and Lofty J.R. (1978). The influence of arthropods and earthworms upon root growth of direct drilled cereals. J. App. Ecol. 15, 789-795.

Edwards, C.A., Lofty, J.R. and Stafford, C.J. (1971). Pesticides and the soil fauna. Rep Rothamsted Exp Sta. 1970 Part 1, p. 194.

Edwards, C.A., Lofty, J.R. and Stafford, C.J. (1972). Pesticides and earthworms. Rep Rothamsted Exp Sta. 1971 Part 1, pp.211-212.

Edwards, C.A., Lofty, J.R. and Stafford, C.J. (1974). Soil fauna: pesticides and earthworms. Rep Rothamsted Exp Sta. 1973, Part 1, p. 204.

Edwards, C.A., Lofty, J.R., Whiting, A.E. and Jeffs, K. (1971). Pesticides and earthworms. Rep Rothamsted Exp Sta. 1970, Part 1, pp. 193-194.

Edwards, C.A., Lofty, J.R. and Whiting, A.E. (1972). Paraquat and slit seeding. Rep Rothamsted Exp Sta. 1971, Part 1, pp. 212-213.

Edwards, C.A. and Neale, R.E. (1983). The use of earthworms in waste disposal and protein production. Basic biology and ecology. Rep Rothamsted Exp Sta. 1982, Part 1, pp. 102-103.

Edwards, C.A., Reichle, D.E. and Crossley, D.A. (1970). The role of soil invertebrates in turnover of organic matter and nutrients. Analysis of temperate forest ecosystems, (ed. Reichle, D.E.), pp. 147-172. Publ., Springer Verlag, Berlin, West Germany.

Edwards, C.A. and Stafford, C.J. (1976). Effects of a herbicide on the soil fauna. Rep Rothamsted Exp Sta. 1975 Part 1, p.129.

Edwards, C.A. and Stafford, C.J. (1979). Interactions between herbicides and the soil fauna. Ann. App. Biol. 91, 132-137.

Edwards, C.A. and Thompson, A.R. (1969). Insecticides and the soil fauna. Rep Rothamsted Exp Sta. 1968, pp. 216-217.

Edwards, C.A. and Thompson, A.R. (1973). Pesticides and the soil fauna. Residue Rev. 45, 1-79.

Edwards, C.A., Thompson, A.R. and Beynon, K.I. (1968). Some effects of chlorfenvinfos, an organophosphorus insecticide, on populations of soil animals. Rev. Ecol. Biol. Sol. 5, 199-224.

Edwards, C.A., Thompson, A.R. and Lofty, J.R. (1968). Changes in soil invertebrate populations caused by some organophosphorus insecticides. Proc. 4th Brit. Insectic. Fungic. Conf., 48-55.

Edwards, P.J. (1982). Personal communication.

Edwards, P.J. (1985). Relative value of laboratory and field toxicity test methods for earthworms. Proceedings of International Conference on Earthworms in Waste and Environmental Management, Cambridge. (in press).

Edwards, P.J. and Brown, S.M. (1982). Use of grassland plots to study the effect of pesticides on earthworms. Pedobiolog. 24, 145-150.

Escritt, J.R. (1955). Calcium arsenate for earthworm control. J. Sports Turf Res. Inst. 9, 28-34.

Escritt, J.R. and Arthur, J.H. (1948). Earthworm control. A resume of methods available. J. Sports Turf Res. Inst. 7, 162-172.

Evans, A.C. (1947). Earthworms. J. Sports Turf Res. Inst. 7, 49-54.

Evans, A.C. and Guild, W.J. McL. (1947). Studies on the relationships between earthworms and soil fertility. I.Biological studies in the field. Ann. App. Biol. 34, 307-330.

Evans, A.C. and Guild, W.J. McL. (1948). Studies on the relationships between earthworms and soil fertility. V. Field populations. Ann. App. Biol. 35, 485-493.

Fanelli, R., Bertoni, M.P., Castelli, M.G., Chiabrando, C., Martelli, G.P., Noseda, A., Garattini, S., Binaghi, C., Marazza, V. and Pezza F. (1980a). 2,3,7,8-Tetrachlorodibenzo-p-dioxin toxic effects and tissue levels in animals from the contaminated area of Seveso, Italy. Arch. Environ. Contam. Toxicol. 9, 569-577.

Fanelli, R., Castelli, M.G., Martelli, G.P., Noseda, A. and Garattini, S. (1980b). Presence of 2,3,7,8-tetrachlorodibenzo-p-dioxin in wildlife living near Seveso, Italy; a preliminary study. Bull. Environ. Contam. Toxicol. 24, 460-462.

Farnham, R.S. (1979). The classification of peat, peatlands and organic soils in the U.S. Proceedings of the 1979 International Symposium on Classification of Peat and Peatlands, Hyytiala, Finland, 194-199.

Fayolle, L. (1979). Consequences of the impact of pollutants on earthworms. III. Laboratory tests. Doc. Pedozool. 1, 34-65.

Ferguson, A. (1980). Biochemical Systematics and Evolution. Blackie, London, 194 pp.

Ferriere, G., Fayolle, L. and Bouche, M.B. (1981). Un nouvel outil, essentiel pour l'ecophysiologie et l'ecotoxicologie: l'elevage des lombriciens en sol artificiel. Pedobiolog. 22, 196-201.

Finlayson, D.G., Campbell, C.J. and Roberts, H.A. (1975). Herbicides and insecticides: their compatibility and effects on weeds, insects and earthworms in the minicauliflower crop. Ann. App. Biol. 79, 95-108.

Finney, D.J. (1971). Probit Analysis. 3rd edition. Cambridge University Press, Cambridge, 333 pp.

Finney, D.J. (1978). Statistical Method in Biological Assay, 3rd edition. Charles Griffin and Co. Ltd., London, 508 pp.

Fisher, S.W. (1984). A comparison of standardised methods for measuring the biological activity of pesticides to the earthworm L. terrestris. Ecotoxicol. Environ. Safety 8, 564-571.

Fleckenstein, J. and Graff, O. (1982). Schwermetallaufnahme aus mullkompost durch der regenwurm Eisenia fetida (Savigny 1826). Landbauforsch Voelkenrode 32, 198-202.

Fleming, W.E. and Hadley, C.H. (1945). DDT ineffective for the control of an exotic earthworm. J. Econ. Entomol. 38, 411.

Flickinger, E.L., King, K.A., Stout, W.F. and Mohn, M.M. (1980). Wildlife hazards from Furadan 3G applications to rice in Texas, U.S.A. J. Wildl. Man. 44, 190-197.

Fox, C.J.S. (1964). The effects of five herbicides on the numbers of certain invertebrate animals in grassland soils. Can. J. Plant Sci. 44, 405-409.

Fox, C.J.S. (1974). Effect of a carbamate and three organophosphorus insecticides on the numbers of wireworms, earthworms, springtails and mites in grassland soil. Phytoprot. 55, 103-105.

Fuhremann, T.W. and Lichtenstein, E.P. (1978). Release of soil-bound methyl [^{14}C]-Parathion residues and their uptake by earthworms and oat plants. J. Agric. Food Chem. 26, 605-610.

Galvyalis, A.G. and Lugauskas, A. (1978). Effect of chlorophos, carbophos, simazine and sodium trichloroacetate on earthworms and microscopic fungi. Liet TSR Mokslu Akad. Darb Ser. C 2, 17-26.

Gantz, R.L. and Slife, F.W. (1960). Persistence and movement of CDAA and CDEC in soil and tolerance of corn seedlings to these herbicides. Weeds 8, 599-606.

Garrec, J.P. and Plebin, R. (1984). Accumulation du fluor dans les vers de terre vivant dans des sol contamines. Environ. Pollut. Ser B 7, 97-105.

Gerard, B.M. (1960). The biology of certain British earthworms in relation to environmental conditions. Ph.D. Thesis, University of London.

Gerard, B.M. (1964). Lumbricidae (Annelida). Synopses of the British Fauna No. 6. The Linnean Society of London, London, 58 pp.

Gerard, B.M. (1967). Factors affecting earthworms in pastures. J. Anim. Ecol. 36, 235-252.

Ghabbour, S.I. and Imam, M. (1967). The effect of five herbicides on three Oligochaete species. Rev. Ecol. Biol. Sol. 4, 119-122.

Gilman, A.P. and Vardanis, A. (1974). Carbofuran. Comparative toxicity and metabolism in the worms L. terrestris and E. foetida. J. Agric. Food Chem. 22, 625-628.

Gish, C.D. and Hughes, D.L. (1982). Residues of DDT, dieldrin and heptachlor in earthworms during two years following application. Special Scientific Report - Wildlife No. 241. Publ., United States Department of the Interior. Fish and Wildlife Service, Washington D.C., USA, 15 pp.

Goats, G.C. (1983). A comparison of field and laboratory methods for testing toxicity to earthworms. p. 713, Proc. 10th Int. Cong. Plant Prot. 2, Brighton.

Goats, G.C. and Edwards, C.A. (1982). Testing the toxicity of industrial chemicals to earthworms. Rep Rothamsted Exp Sta. 1981 Part 1, 105.

Goats, G.C. and Edwards, C.A. (1983). Testing the toxicity of industrial chemicals to earthworms. Rep Rothamsted Exp Sta. 1982 Part 1, 104-105.

Goats, G.C. and Edwards, C.A. (1985). The prediction of field toxicity to earthworms by laboratory methods. Proceedings of the International Conference on Earthworms in Waste and Environmental Management, Cambridge. (in press).

Goring, C.A.I., Laskowski, D.A., Hamaker, J.W. and Meikle, R.W. (1975). Principles of pesticide degradation in soil. In Environmental Dynamics of Pesticides (Eds. Haque, R. and Freed, V.H.), Plenum Press, New York, 387 pp.

Gough, H.C. (1945). A Review of the Literature on Soil Insecticides. Imperial Institute of Entomology, London, 161 pp.

Gould, H.J. (1962). Trials on the control of slugs on arable fields in autumn. Plant Pathol. 11, 125-130.

Graham-Bryce, I.J. (1977). Recent developments in the chemical control of agricultural pests and diseases in relation to ecological effects. Ecological Effects of Pesticides (eds. Perring, F.H. and Mellanby, K.), Linnean Society Symposium Series No. 5, Academic Press, London, pp.47-60.

Grant, W.C. (1955). Studies on moisture relationships in earthworms. Ecology 36, 400-407.

Greenwood, D.E. (1945). Wireworm investigations. Conn. Agric. Exp. Sta. Bull. 488.

Griffiths, D.C., Raw, F. and Lofty, J.R. (1967). The effects on soil fauna of insecticides tested against wireworms (Agriotes spp.) in wheat. Ann. App. Biol. 60, 479-490.

Grigoreva, T.G. (1952). The action of hexachlorane introduced into the soil on soil fauna. Dokl. Usesoyuz. Akad. Sel.-khoz. Nauk Lenina 17, 16-20.

Guthrie, F.E. (1950). Effect of temperature on toxicity of certain organic insecticides. J. Econ. Entomol. 43, 559-560.

Haque, A. and Ebing, W. (1980). Uptake of the herbicide ^{14}C-monolinuron by earthworms and metabolism in soil and earthworm. Jahresber Biolog. Bundesans Land-u Fortswirtshaft, Berlin-u Braunschweig, 1979, p.99.

Haque, A. and Ebing, W. (1983a). Toxicity determination of pesticides to earthworms in the soil substrate. Z. Pflanzenkr. Pflanzenschutz. 90, 395-408.

Haque, A. and Ebing, W. (1983b) Uptake, accumulation and elimination of HCB and 2,4-D by the terrestrial slug, Deroceras reticulatum (Muller). Bull. Environ. Contam. Toxicol. 31, 727-733.

Harris, C.R. (1964). Influence of soil moisture on the toxicity of insecticides in a mineral soil to insects. J. Econ. Entomol. 57, 946-950.

Harris, C.R. (1966). Influence of soil type on the activity of insecticides in soil. J. Econ. Entomol. 59, 1221-1225.

Harris, C.R. (1970). Laboratory evaluation of candidate materials as potential soil insecticides. III. J. Econ. Entomol. 63, 782-787.

Harris, C.R. (1971). Influence of temperature on the biological activity of insecticides in soil. J. Econ. Entomol. 64, 1044-1049.

Harris, C.R. (1972a). Factors influencing the effectiveness of soil insecticides. Ann. Rev. Entomol. 17, 177-198.

Harris, C.R. (1972b). Factors influencing the biological activity of technical chlordane and some related components in soil. J. Econ. Entomol. 65, 341-347.

Harris, C.R. and Hitchon, J.L. (1970). Laboratory evaluation of candidate materials as potential soil insecticides. II. J. Econ. Entomol. 63, 2-7.

Harris, C.R. and Mazurek, J.H. (1966). Laboratory evaluation of candidate materials as potential soil insecticides. J. Econ. Entomol. 59, 1215-1221.

Harris, G.S. (1949). Note on control of earthworms in greenkeeping. N.Z. J. Sci. Technol. Sect. B 31, 40.

Hartenstein, F., Hartenstein, E. and Hartenstein, R. (1981). Gut load and Transit time in the earthworm Eisenia foetida. Pedobiolog. 22, 5-20.

Hartenstein, R. (1982). Metabolic parameters of the earthworm Eisenia foetida in relation to temperature. Biotechnol. Bioeng. 14, 1803-1811.

Hassall, K.A. (1982). The Chemistry of Pesticides. Macmillan, London, 372 pp.

Haverty, M.I. and Robertson, J.L. (1982). Laboratory bioassays for selecting candidate insecticides and application rates for field tests on the Western Spruce Budworm. J. Econ. Entomol. 75, 179-182.

Healy, M.J.R. (1950). The planning of probit assays. Biom. 6, 424-431.

Heimbach, F. (1982) Personal communication.

Heimbach, F. (1984). Correlations between three methods for determining the toxicity of chemicals to earthworms. Pestic. Sci. 15, 605-611.

Heimbach, F. (1985a). Comparison of laboratory methods, using Eisenia foetida and Lumbricus terrestris, for the assessment of the hazard of chemicals to earthworms. Z. Pflanzenkrank Pflanzenschutz. 92, 186-193.

Heimbach, F. (1985b). A comparison of laboratory methods for toxicity testing with earthworms. Proceedings of the International Conference on Earthworms in Waste and Environmental Management. Cambridge. (in press).

Heimbach, F. and Edwards, P.J. (1983). The toxicity of 2-chloroacetamide and benomyl to earthworms under various test conditions in an artificial soil test. Pestic. Sci. 14, 635-636.

Heungens, A. (1968). The influence of DBCP on the soil fauna in azalea culture. Pedobiolog 8, 281-288.

Heungens, A. (1969). L'Influence de la fumure et des pesticides aldrin, carbaryl et DBCP sur la faune du sol dans la culture des Azalees. Rev. Ecol. Biol. Sol. 4, 131-145.

Hirst, J.M., Storey, I.F., Ward, W.C. and Wilcox, H.G. (1955). The origin of apple scab epidemics in the Wisbech area in 1953 and 1954. Plant Pathol. 4, 91.

Hockenyos, G.L. (1939). Laboratory evaluation of soil poisons used in termite control. J. Econ. Entomol. 32, 147-149.

Hoffman, R.A. and Lindquist, A.W. (1949). Effect of temperature on knockdown and mortality of house flies exposed to residues of several chlorinated hydrocarbon insecticides. J. Econ. Entomol. 42, 891-893.

Hoffman, R.A., Roth, A.R. and Lindquist, A.W. (1949). Effect of air temperature on the insecticidal action of some compounds on the sheep tick and on migration of the sheep tick on the animal. J. Econ. Entomol. 42, 893-896.

Hoogerkamp, M., Rogaar, H. and Eijsackers, H.J.P. (1983). The effect of earthworms on grassland on recently reclaimed polder soils in the Netherlands. Earthworm Ecology. From Darwin to Vermiculture (ed. Satchell, J.E.), pp. 85-105. Chapman and Hall, London.

Hopkins, A.R. and Kirk, V.M. (1957). Effects of several insecticides on the English red worm. J. Econ. Entomol. 50, 699-700.

Houpert, G., Jenot, M. and Lardier, P.A. (1982). La sensibilite accrue d'Eisenia fetida (Lumbricidae) aux insecticides carbamates en presence d'atrazine. Bull. Ec. Natl. Super Agron. Ind. Aliment 24, 3-9.

Hoy, J.M. (1955). Toxicity of some hydrocarbon insecticides to earthworms. N.Z. J. Sci. Technol. Sect. A 37, 367-372.

Hyche, I.L. (1956). Control of mites infesting earthworm beds. J. Econ. Entomol. 49, 409-410.

Ilijin, A.M. (1969). The toxic effect of herbicides upon ants and earthworms. Zool. Zh. 48, 141-143.

Inglesfield, C. (1984). Toxicity of the pyrethroid insecticides cypermethrin and WL 85871 to the earthworm, Eisenia foetida Savigny. Bull. Environ. Contam. Toxicol. 33, 568-570.

Ireland, M.P. (1979). Metal accumulation by the earthworms Lumbricus rubellus, Dendrobaena veneta and Eiseniella tetraedra living in heavy metal polluted sites. Environ. Pollut. 19, 201-206.

Ireland, M.P. (1983). Heavy metal uptake and tissue distribution in earthworms. Earthworm Ecology. From Darwin to Vermiculture (ed. Satchell, J.E.), pp. 247-265. Chapman and Hall, London.

Jaenike, J. (1982). 'Eisenia foetida' is two biological species. Megadril 4, 6-8.

Jefferies, D.J. and Davis, B.N.K. (1968). Dynamics of dieldrin in soil, earthworms and song thrushes. J. Wildl. Man. 32, 441-456.

Jefferson, P. (1955). Studies on the earthworms of turf. J. Sports Turf Res. Inst. 9, 6-27.

Johnston, A.E., Poulton, P.R. and McEwen, J. (1981). The soils of Rothamsted Farm. The carbon and nitrogen content of the soils and the effect of changes in crop rotation and manuring on soil pH, P, K and Mg. Rep Rothamsted Exp Sta. 1980 Part 2, pp. 5-20.

Jones, E.W. (1933). The influence of temperature on the toxicity of carbon disulphide to wireworms. J. Econ. Entomol. 26, 887-892.

Kale, R.D. and Krishnamoorthy, R.V. (1979). Pesticidal effects of Sevin (1-napthyl-n-methyl carbamate) on the survivability and abundance of earthworm Pontoscolex corethrurus. Proc. Indian Acad. Sci. Sect. B 88, 391-396.

Kale, R.D. and Krishnamoorthy, R.V. (1982). Residual effect of Sevin on the acetylcholinesterase activity of the nervous system of the earthworm Pontoscolex corethrurus. Curr. Sci. 51, 885-886.

Kalembasa, S.J. and Jenkinson, D.S. (1973). A comparative study of titrimetric and gravimetric methods for the determination of organic carbon in soil. J. Sci. Food Agric. 24, 1085-1090.

Kaplan, D.L., Hartenstein, R., Neuhauser, E.F. and Malecki, M.R. (1980). Physicochemical requirements in the environment of the earthworm Eisenia fetida. Soil Biol. Biochem. 12, 347-352.

Karnak, R.E. and Hamelink, J.L. (1982). A standardized method for determining the acute toxicity of chemicals to earthworms. Ecotoxicol Environ. Safety 6, 216-222.

Kaufman, D.D. (1978). Degradation of pentachlorophenol in soil, and by soil microorganisms. In Pentachlorophenol: Chemistry, Pharmacology and Environmental Toxicology Section I, pp.27-39 (ed. Rao K. R.), Plenum Press, New York, USA.

Kelsey, J.M. and Arlidge, E.Z. (1968). Effects of isobenzan on soil fauna and soil structure. N.Z. J. Agric. Res. 11, 245-260.

Kenaga, E.E. (1972) Guidelines for environmental study of pesticides: determination of bioconcentration potential. Residue Rev. 44, 73-113.

Kenaga, E.E. (1975a). Partitioning and uptake of pesticides in biological systems. Environmental Dynamics of Pesticides (eds. Haque, R. and Freed, V.H.), pp. 217-273. Plenum Press, New York, USA.

Kenaga, E.E. (1975b). Use of biological tests for evaluation of pesticides. Pure Appl. Chem. 42, 285-299.

Kenaga, E.E. (1982). Predictability of chronic toxicity from acute toxicity of chemicals in fish and aquatic invertebrates. Environ. Toxicol. Chem. 1, 347-358.

Keogh, R.G. and Whitehead, P.H. (1975). Observations on some effects of pasture spraying with benomyl and carbendazim on earthworm activity and litter removal from pasture. N.Z. J. Exp. Agric. 3, 103-104.

King, J.W. and Dale, J.L. (1977). Reduction of earthworm activity by fungicides applied to putting green turf. Ark. Farm Res. 26, 12.

Koeman, J.H. (1982). Ecotoxicological evaluation. The Eco- side of the problem. Ecotoxicol. Environ. Safety 6, 358-362.

Kring, J.B. (1969). Mortality of the earthworm Lumbricus terrestris L. following soil applications of insecticides in a tobacco field. J. Econ. Entomol. 62, 963.

Krivoluckij, D.A., Tichomirova, A.L. and Turcaninova, V.A. (1972). Strukturanderungen des tierbesatzes (Land-und bodenwirbellose) unter dem Einfluss der kontamination des bodens mit Sr^{90}. Pedobiolog. 12, 374-380.

Krivolutsky, D., Turcaninova, V. and Mikhaltsova, Z. (1982). Earthworms as bioindicators of radioactive soil pollution. Pedobiolog. 23, 263-265.

Kuenen, D.J. (1957). Time-mortality curves and Abbott's correction in experiments with insecticides. Acta Physiol. Pharmacol. Neerl. 6, 179-196.

Kuhle, J.C. (1983). Ecotoxociological model studies to test the suitability of earthworms as bioindicators. Verh. Dtsch. Zool. Ges. 1983, 147-151.

Laird, J.M. and Kroger, M. (1981). Earthworms. Crit. Rev. Environ. Control. 11, 189-218.

Laverack, M.S. (1963). The Physiology of Earthworms. Pergamon Press, Oxford, 206 pp.

Lebrun, P., De Medts, A. and Wauthy, G. (1981). Comparative ecotoxicology and bioactivity of three carbamate insecticides on an experimental population of the earthworm Lumbricus herculeus. Pedobiolog 21, 225-235.

Leger, R.G. and Millette, G.J.F. (1977). The resistance of earthworms Lumbricus terrestris and Allolobophora turgida to Captan 50 w.p. Rev. Can. Biol. 36, 351-353.

Legg, D.C. (1968). Comparison of various worm-killing chemicals. J. Sports Turf Res. Inst. 44, 47-48.

Lhoste, J. (1975). Preliminary investigations into the action of beet herbicides on the environment. 3e Reunion Internationale sur le Desherbage selectif en cultures de Betteraves, Paris, 1975, pp. 483-493.

Lidgate, H.J. (1966). Earthworm control with chlordane. J. Sports Turf Res. Inst. 42, 5-8.

Liimatainen, A. and Hanninen, O. (1982). Occurrence of cytochrome P-450 in the earthworm L. terrestris. In Cytochrome P-450. Biochemistry, Biophysics and Environmental Implications (eds. Hietanen, E., Laitinen, M. and Hanninen, O.), pp. 255-258. Elsevier, Amsterdam, Netherlands.

Lipa, J.J. (1958). Effect on earthworm and diptera populations of BHC dust applied to soil. Nature, Lond. 181, 863.

Litchfield, J.T. and Wilcoxon, F. (1949). A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther. 96, 99-113.

Lofs-Holmin, A. (1979). A pot method for field experiments with earthworms. Research Report No. 6, Department of Ecology and Environmental Research. Swedish University of Agricultural Sciences, Uppsala, Sweden, 41 pp.

Lofs-Holmin, A. (1980). Measuring growth of earthworms as a method of testing sublethal toxicity of pesticides. Swed. J. Agric. Res. 10, 25-33.

Lofs-Holmin, A. (1981). Influence in field experiments of benomyl and carbendazim on earthworms (Lumbricidae) in relation to soil texture. Swed. J. Agric. Res. 11, 141-147.

Lofs-Holmin, A. (1982a). Influence of routine pesticide spraying on earthworms (Lumbricidae) in field experiments with winter wheat. Swed. J. Agric. Res. 12, 121-123.

Lofs-Holmin, A. (1982b). Measuring cocoon production of the earthworm Allolobophora caliginosa (Sav.) as a method of testing sublethal toxicity of pesticides. An experiment with benomyl. Swed. J. Agric. Res. 12, 117-119.

Lofs-Holmin, A. (1982c). Reproduction and growth of common arable land and pasture species of earthworms (Lumbricidae) in laboratory cultures. Swed. J. Agric. Res. 13, 31-37.

Lofs-Holmin, A. and Bostrom, U. (1985). The use of earthworms and other soil animals in pesticide testing. Conclusions drawn from experiments presented in the literature. Proceedings of the International Conference on Earthworms in Waste and Environmental Management, Cambridge. (in press)

Lord, K.A., Briggs, G.G., Neale, M.C. and Manlove, R. (1980). Uptake of pesticides from water and soil by earthworms. Pestic. Sci. 11, 401-408.

Loxdale, H.D., Castanera, P. and Brookes, C.P. (1983). Electrophoretic study of enzymes from cereal aphid populations. I. Electrophoretic techniques and staining systems for characterising isoenzymes from six species of cereal aphids (Hemiptera: Aphididae). Bull. Entomol. Res. 73, 645-657.

Luckmann, W.H. and Decker, G.C. (1960). A 5-year report of observations in the Japanese beetle control area of Shelden, Illinois. J. Econ. Entomol. 53, 821-827.

Lyons, C., Milsom, N., Morgan, N.G. and Stringer, A. (1972). The effects of repeated applications of the grass suppressant maleic hydrazide on an orchard sward and on the soil fauna. Proc. 11th Brit. Weed Contr. Conf. 1972, 356-359.

Ma, W. (1982). The influence of soil properties and worm-related factors on the concentration of heavy metals in earthworms. Pedobiolog. 24, 109-119.

Ma, W. (1984). Sublethal toxic effects of copper on growth, reproduction and litter breakdown activity in the earthworm Lumbricus rubellus, with observations on the influence of temperature and soil pH. Environ. Pollut. Ser. A 33, 207-219.

Ma, W., Edelman, Th., Van Beersum, I. and Jans, Th. (1983). Uptake of cadmium, zinc, lead and copper by earthworms near a zinc-smelting complex: influence of soil pH and organic matter. Bull. Environ. Contam. Toxicol. 30, 424-427.

Malone, C.R. and Reichle, D.E. (1973). Chemical manipulation of soil biota in a fescue meadow. Soil Biol. Biochem. 5, 629-639.

Marquenie, J.M. and Simmers, J.W. (1985). Wormwatching: a method to assess potential bioavailability of contaminants: PCB's, PCA's and heavy metals. Proceedings of the International Conference on Earthworms in Waste and Environmental Management, Cambridge. (in press).

Martin, N.A. (1976). Effect of four insecticides on the pasture ecosystem. V. Earthworms (Oligochaeta: Lumbricidae) and arthropoda extracted by wet sieving and salt flotation. N.Z. J. Agric. Res. 19, 111-115.

Martin, N.A. (1980). Earthworm (Oligochaeta: Lumbricidae) populations and late summer pasture renovation. N.Z. J. Agric. Res. 23, 417-419.

Martin, N.A. (1982). The effects of herbicides used on asparagus on the growth rate of the earthworm Allolobophora caliginosa. Proc. 35th N.Z. Weed and Pest Control Conf., pp. 328-331.

Martin, N.A. (1983). Personal communication.

Martin, L.W. and Wiggans, S.G. (1959). Tolerance of earthworms to certain insecticides, herbicides and fertilizers. Okla Agric. Exp. Stn. Process Ser. P, pp. 334.

Martinucci, G.B., Crespi, P., Omodeo, P., Osella, G. and Traldi, G. (1983). Earthworms and TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) in Seveso. In Earthworm Ecology. From Darwin to Vermiculture (ed. Satchell, J.E.), pp. 275-283. Chapman and Hall, London.

Milne, D.L. and DuToit, W. (1976). The effect of citrus nematicides on the earthworm population in the soil. Citrus Grow. Sub-Trop. Fruit J., pp. 13 and 15.

Moeed, A. (1975). Effects of isobenzan, fensulfothion and diazinon on invertebrates and microorganisms. N.Z. J. Exp. Agric. 3, 181-185.

Morgan, M. (1984). Personal communication.

Moriarty, F. (1977). Prediction of ecological effects by pesticides. In Ecological Effects of Pesticides (eds. Perring, F.H. and Mellanby, K.). Linnean Society Symposium Series, No. 5, pp.165-173, Academic Press, London.

Morrison, F.O. (1950). The toxicity of hexachlorocyclohexane to certain microorganisms, earthworms and arthropods. Ont. Entomol. Soc. Ann. Rep. 80, 50-57.

Morrod, R.S. (1982). Factors affecting the distribution of pesticide drenches in soil using the fungicide metazoxolon as a model. Pestic. Sci. 13, 49-59.

Murchie, W.R. (1958). Biology of the oligochaete Eisenia rosea (Savigny) in an upland forest soil of southern Michigan. Am. Midl. Nat. 66, 113-131.

McColl, H.P. (1984). Nematicides and field populations of enchytraeids and earthworms. Soil. Biol. Biochem. 16, 139-143.

McIntosh, A.H. (1961). Graphical and other short statistical methods for "All-or-None" bioassay tests. J. Sci. Food Agric. 12, 312-316.

McIntosh, A.H., Bateman, G.L., Chamberlain, K., Dawson, G.W. and Burrell, M.M. (1981). Decreased severity of potato common scab after foliar sprays of 3,5-dichlorophenoxyacetic acid, a possible antipathogenic agent. Ann. App. Biol. 99, 275-281.

McKenry, M.V. and Naylor, P. (1975). Red worms are sensitive to 1,3-dichloropropene nematicides. J. Nematol. 7, 326-327.

McLaren, R.G., Swift, R.S. and Williams, J.G. (1981). The adsorption of copper by soil materials at low equilibrium solution concentrations. J. Soil Sci. 32, 247-256.

Nakatsugawa, T. and Nelson, P.A. (1972). Studies of insecticide detoxication in invertebrates: an enzymological approach to the problem of biological magnification. In Environmental Toxicology of Pesticides (eds. Matsumura, F., Boush, G.M. and Misato, T.), pp. 501-524. Academic Press, New York, USA.

Neale, R.E. (1984). Personal communication.

Neale, R.E. and Edwards, C.A. (1982). Basic biology of earthworms feeding on wastes. Rep Rothamsted Exp Sta. 1981, Part 1, p.103.

Nelson, P.A., Stewart, R.R., Morelli, M.A. and Nakatsugawa, T. (1976). Aldrin epoxidation in the earthworm Lumbricus terrestris L. Pestic. Biochem. Physiol. 6, 243-253.

Neuhauser, E.F. and Edwards, C.A. (1985). The scientific basis for vermiculture. Proceedings of the International Conference on Earthworms in Waste and Environmental Management, Cambridge. (in press).

Neuhauser, E.F., Malecki, M.R. and Loehr, R.C. (1984). Growth and reproduction of the earthworm E. fetida after exposure to sublethal concentrations of metals. Pedobiolog 27, 89-97.

Niklas, J. and Kennel, W. (1978). Lumbricid populations in orchards of W. Germany and the influence of fungicides based on copper compounds and benzimidazole derivatives upon them. Z. Pflanzenkr. Pflanzenschutz. 85, 705-713.

Øien, N. and Stenersen, J. (1984). Esterases of earthworms. III. Electrophoresis reveals that Eisenia fetida (Savigny) is two species. Comp. Biochem. Physiol. C Comp. Pharmacol. 78, 277-282.

Olson, H.W. (1928). The earthworms of Ohio. Ohio Biol. Surveill. Bull. 17, 47-90.

Parkin, E.A. (1951). Biological tests of insecticides for stored-product insects. J. Sci. Food Agric. 2, 136-141.

Pasteur, N. and Sinegre, G. (1975). Esterase polymorphism and sensitivity to Dursban organophosphorus insecticide in Culex pipiens pipiens populations. Biochem. Gen. 13, 789-803.

Patel, H.K. (1960). Earthworms in tobacco nurseries and their control. Indian Tobacco 10, 56.

Piearce, T.G. (1981). Losses of surface fluids from Lumbricid earthworms. Pedobiolog. 21, 417-426.

Polivka, J.B. (1951). Effect of insecticides upon earthworm populations. Ohio J. Sci. 51, 195-196.

Prusch, R.D. and Otter, T. (1977). Annelid transepithelial ion transport. Comp. Biochem. Physiol. A. Comp. Bioc. 57, 87-92.

Puustjarvi, V. (1979). Classification of virgin peat with regard to the requirements of horticultural peat use. Proceedings of the International Symposium on Classification of Peat and Peatlands, Hyytiala, Finland, September 17-21 1979, pp. 239-242.

Raw, F. (1959). Estimating earthworm populations by using formalin. Nature, Lond.. 184, 1661-1662.

Raw, F. (1960a). Observations on the effect of hexoestrol on earthworms and other soil invertebrates. J. Agric. Sci. 55, 189-190.

Raw, F. (1960b). Earthworm population studies: a comparison of sampling methods. Nature, Lond. 187, 257.

Raw, F. (1961). The effect of Bordeaux mixture on the earthworm population of apple orchards. Report of a symposium on Insecticides, Fungicides and the Soil, pp. 37-39. Rothamsted Experimental Station, Harpenden.

Raw, F. (1965). Current work on side effects of soil-applied organophosphorus insecticides. Ann. App. Biol. 55, 342-343.

Raw, F. and Lofty, J.R. (1962). The effect of chemical control on pests and on other arthropods and worms in the soil. Rep Rothamsted Exp Sta. 1961, p. 146.

Raw, F. and Lofty, J.R. (1964). The side-effects of toxic chemicals in the soil on arthropods and earthworms. Rep Rothamsted Exp Sta. 1963, p. 149.

Reinecke, A.J. and Nash, R.G. (1984). Toxicity of 2,3,7,8 TCDD and short term bioaccumulation by earthworms (Oligochaeta). Soil Biol. Biochem. 16, 45-49.

Reinecke, A.J. and Venter, J.M. (1985). Sublethal ecotoxicological studies with the earthworm, Eisenia fetida (Lumbricidae). Proceedings of the International Conference on Earthworms in Waste and Environmental Management, Cambridge. (in press).

Rhett, R.G., Simmers, J.S. and Lee, C.R. (1985). Eisenia fetida used as a biomonitoring tool to predict the potential bioaccumulation of contaminants from contaminated dredged material. Proceedings of the International Conference on Earthworms in Waste and Environmental Management, Cambridge. (in press).

Richards, A.G. and Cutkomp, L.K. (1946). Correlation between the possession of a chitinous cuticle and sensitivity to DDT. Biol. Bull. 90, 97-108.

Richards, S.K. and Arme, C. (1982). Integumentary uptake of dissolved organic materials by earthworms. Pedobiolog 23, 358-366.

Roark, J.H. and Dale, J.L. (1979). The effect of turf fungicides on earthworms. Ark. Acad. Sci. Proc. 33, 71-74.

Roberts, B.L. and Dorough, H.W. (1984). Relative toxicities of chemicals to the earthworm Eisenia foetida. Environ. Toxicol. Chem. 3, 67-78.

Rombke, J. (1984). Personal communication.

Ruppel, R.F. and Laughlin, C.W. (1977). Toxicity of some soil pesticides to earthworms. J. Kans. Entomol. Soc. 50, 113-118.

Ruppel, R.F., Laughlin, C.W. and Fogg, R. (1973). Toxicities of some insecticides to earthworms. Proc. North Cent. Branch Entomol. Soc. Am. 28, 189.

Rushton, S.P. and Luff, M.L. (1984). A new electrical method for sampling earthworm populations. Pedobiolog. 26, 15-19.

Satchell, J.E. (1955a). The effects of BHC, DDT and parathion on soil fauna. Soil Fert. 18, 279-285.

Satchell, J.E. (1955b). Some aspects of earthworm ecology. In Soil Zoology (ed. Kevan, D.K.Mc.E), pp. 180-201. Butterworths, London.

Satchell, J.E. (1955c). An electrical method of sampling earthworm populations. In Soil Zoology (ed. Kevan, D.K.Mc.E.), pp. 356-364, Butterworths, London.

Satchell, J.E. (1963). Nitrogen turnover by a woodland population of Lumbricus terrestris. In Soil Organisms (eds. Doeksen, J. and Van der Drift, J.), pp. 60-66. North-Holland Publishing Co., Amsterdam, Netherlands.

Satchell, J.E. (1967). Lumbricidae. In Soil Biology (eds. Burgess, A. and Raw, F.), pp. 259-322. Academic Press, London.

Satchell, J.E. and Dottie, D.J. (1984). Factors affecting the longevity of earthworms stored in peat. J. App. Ecol. 21, 285-291.

Saunders, D.G. and Forgie, C.D. (1977). Some effects of phorate on earthworm populations. Proc. 30th N.Z. Weed Pest Contr. Conf., 222-226.

Schread, J.C. (1952). Habits and control of the oriental earthworm. Conn. Storrs. Agric. Exp. Stn. Bull. 556, 15 pp.

Silver, A. (1974). The Biology of Cholinesterases. Elsevier, New York, 596 pp.

Smirnoff, W.A. and Heimpel, A.M. (1961). Notes on the pathogenicity of Bacillus thuringiensis var. thuringiensis Berliner for the earthworm, Lumbricus terrestris Linnaeus. J. Insect Pathol. 3, 403-408.

Springett, J.A. (1981). A new method for extracting earthworms from soil cores, with a comparison of four commonly used methods for estimating earthworm populations. Pedobiolog. 21, 217-222.

Springett, J.A. and Syers, J.K. (1984). Effect of pH and calcium content of soil on earthworm cast production in the laboratory. Soil. Biol. Biochem. 16, 185-189.

Stanley, P.I. (1983). The role of field trials in the assessment of the environmental hazards from pesticides. Pestic. Sci. 14, 634.

Stenersen, J. (1979a). Action of pesticides on earthworms. Part I. The toxicity of cholinesterase-inhibiting insecticides to earthworms as evaluated by laboratory tests. Pestic. Sci. 10, 66-74.

Stenersen, J. (1979b). Action of pesticides on earthworms. Part II. Elimination of parathion by the earthworm Eisenia foetida (Savigny). Pestic. Sci. 10, 104-112.

Stenersen, J. (1979c). Action of pesticides on earthworms. Part III. Inhibition and reactivation of cholinesterases in Eisenia foetida (Savigny) after treatment with cholinesterase-inhibiting insecticides. Pestic. Sci. 10, 113-122.

Stenersen, J. (1980a). Esterases of earthworms. Part 1. Characterisation of the cholinesterases in Eisenia foetida (Savigny) by substrates and inhibitors. Comp. Biochem. Physiol. C Comp. Pharmacol. 66, 37-44.

Stenersen, J. (1980b). Esterases of earthworms. Part 2. Characterisation of the cholinesterases in the earthworm Eisenia foetida (Savigny) by ion exchange chromatography and electrophoresis. Comp. Biochem. Physiol C Comp. Pharmacol. 66, 45-51.

Stenersen, J. (1981). Personal communication.

Stenersen, J. (1984). Detoxication of xenobiotics by earthworms. Comp. Biochem. Physiol. C Comp. Pharmacol. 78, 249-252.

Stenersen, J., Gilman, A. and Vardanis, A. (1973). Carbofuran: its toxicity to and metabolism by the earthworm (Lumbricus terrestris). J. Agric. Food Chem. 21, 166-171.

Stenersen, J., Guthenberg, C. and Mannervik, B. (1979). Glutathione S-transferases in earthworms (Lumbricidae). Biochem. J. 181, 47-50.

Stenersen, J. and Øien, N. (1980). Action of pesticides on earthworms. IV. Uptake and elimination of oxamyl compared with carbofuran. Pestic. Sci. 11, 396-400.

Stenersen, J. and Øien, N. (1981). Glutathione S-transferases in earthworms (Lumbricidae). Substrate specificity, tissue and species distribution and molecular weight. Comp. Biochem. Physiol. C Comp. Pharmacol. 69, 243-252.

Stephan, C.E. (1977). Methods for calculating an LC_{50}. Aquatic Toxicology and Hazard Evaluation (eds. Mayer, F.L. and Hamelink, J.L.), pp. 65-84. Publ., American Society for Testing and Materials. Washingon, USA.

Stephenson, R.R. (1984). Evaluation of a rapid range-finding test for use in acute lethality studies with fish. Environ. Pollut. Ser. A 35, 75-81.

Stewart, V.I. and Scullion, J. (1985). Earthworms, soil structure and the rehabilitation of former opencast coal mining land. Proceedings of the International Conference on Earthworms in Waste and Environmental Management, Cambridge. (in press)

Stiff, M.J. (1971). The chemical states of copper in polluted freshwater and a scheme of analysis to differentiate them. Water Res. 5, 585-599.

Stringer, A. and Lyons, C.H. (1974). The effect of benomyl and thiophanate-methyl on earthworm populations in apple orchards. Pestic. Sci. 5, 189-196.

Stringer, A. and Lyons, C.H. (1977). The effect on earthworm populations of methods of spraying benomyl in an apple orchard. Pestic. Sci. 8, 647-650.

Stringer, A. and Wright, M.A. (1973). The effect of benomyl and some related compounds on Lumbricus terrestris and other earthworms. Pestic. Sci. 4, 165-170.

Stringer, A. and Wright, M.A. (1976). The toxicity of benomyl and some related 2-substituted benzimidazoles to the earthworm Lumbricus terrestris. Pestic. Sci. 7, 459-464.

Stringer, A. and Wright, M.A. (1980). The toxicity of methiocarb and its breakdown products to earthworms. Rep. Long Ashton Res. Sta. 1979, 120-121.

Stuurman, F.J. and Kamp, H.A. (1971). The control of earthworms in sports grounds. Tijdschr Ned. Heidemaatsch. 82, 148-155.

Sun, Y.P. (1950). Toxicity index - an improved method of comparing the relative toxicity of insecticides. J. Econ. Entomol. 43, 45-53.

Sun, Y.P. (1966). Correlation between laboratory and field data on testing insecticides. J. Econ. Entomol. 59, 1131-1134.

Takahashi, K. and Sakai, Y. (1982). The effect of the surfactants to use with herbicides in the earthworms in citrus orchards. Weed Res. Jap. 27, 10-15.

Taylor, R.N. (1982). Insecticide resistance in houseflies from the Middle East and North Africa with notes on the use of various bioassay techniques. Pestic. Sci. 13, 415-425.

Thompson, A.R. (1971). Effects of nine insecticides on the numbers and biomass of earthworms in pasture. Bull. Environ. Contam. Toxicol. 5, 577-586.

Thompson, A.R. (1973). Pesticide residues in soil invertebrates. Environmental Pollution by Pesticides (ed. Edwards, C.A.), pp. 87-133. Plenum, London.

Thompson, A.R. and Edwards, C.A. (1974). Effects of pesticides on non-target invertebrates in freshwater and soil. Pesticides in Soil and Water, (ed. Guenzi, W.D.), pp. 341-386. Publ., Soil Science Society of America, Wisconsin, USA.

Thompson, A.R. and Sans, W.W. (1974). Effects of soil insecticides in southwestern Ontario on non-target invertebrates: earthworms in pasture. Environ. Entomol. 3, 305-308.

Thompson, L.M. and Troeh, F.R. (1973). Soils and Soil Fertility. MacGraw-Hill, New York, USA, 544 pp.

Tomlin, A.D. (1977). Culture of soil animals for studying the ecological effects of pesticides. Crop Protection Agents: Their Biological Evaluation (ed. McFarlane, N.R.), pp. 541-555. Academic Press, London.

Tomlin, A.D. (1981). Effects on soil fauna of the fungicide, benomyl, used to control earthworm populations around an airport. Prot. Ecol. 2, 325-330.

Tomlin, A.D. and Gore, F.L. (1974). Effects of six insecticides and a fungicide on the numbers and biomass of earthworms in pasture. Bull. Environ. Contam. Toxicol. 12, 487-492.

Tomlin, A.D., Tolman, J.H. and Thorn, G.D. (1981). Suppression of earthworm Lumbricus terrestris populations around an airport by soil application of the fungicide benomyl. Prot. Ecol. 2, 319-323.

Torstensson, N.T.L. (1975). Degradation of 2,4-D and MCPA in soils of low pH. Environ. Qual. Saf. 3, 262-265.

Van Den Brande, J. and Heungens, A. (1969). Influence of repeated applications of nematicides on the soil fauna in begonia culture. Neth. J. Plant Pathol. 75, 40-44.

Van Rhee, J.A. (1967). Development of earthworm populations in orchard soils. In Progress in Soil Zoology (eds. Graff, O. and Satchell, J.E.), pp. 360-371. North Holland Publ. Co., Amsterdam, Netherlands.

Van Rhee, J.A. (1969). Effects of biocides and their residues on earthworms. Meded Rijksfac Landbouwwet Gent 34, 682-689.

Van Rhee, J.A. (1977). Effects of soil pollution on earthworms. Pedobiolog 17, 201-208.

Verloo, M.G. (1980). Peat as a natural complexing agent for trace elements. Acta Hortic. 99, 51-56.

Wadley, F.M. and Sullivan, W.N. (1943). A study of the dosage-mortality curve. J. Econ. Entomol. 36, 367-372.

Waitt, A.W. (1975). Pesticide legislation and industry. Pestic. Sci. 6, 199-208.

Walker, C.R. (1979). Methods of reducing hazard of pesticides to fish, wildlife and habitat. Proc. 9th Int. Cong. Plant Prot. 1, 304-308.

Wallwork, J.A. (1970). Ecology of Soil Animals. McGraw-Hill, London, 283 pp.

Walton, W.R. (1928). Earthworms as pests and otherwise. Farmers Bulletin 1569. United States Department of Agriculture.

Way, M.J. and Scopes, N.E.A. (1965). Side effects of some soil applied systemic insecticides. Ann. App. Biol. 55, 340-341.

Way, M.J. and Scopes, N.E.A. (1968). Studies on the persistence and effects on soil fauna of some soil-applied systemic insecticides. Ann. App. Biol. 62, 199-214.

Weber, J.B. and Weed, S.B. (1974). Effects of soil on the biological activity of pesticides. Pesticides in Soil and Water, (ed. Guenzi, W.D.), pp. 223-256. Publ., Soil Science Society of America Inc., Madison, Wisconsin, USA.

Weber, J.B., Weed, S.B. and Sheets, T.J. (1972). Pesticides: how they move and react in the soil. Crops and Soils Mag. 25, 14-17.

Weinbach, E.C. and Garbus, J. (1969). Mechanism of action of reagents that uncouple oxidative phosphorylation. Nature, Lond. 221, 1016-1018.

Wershaw, R.L., Burcar, P.J. and Goldberg, M.C. (1969). Interaction of pesticides with natural organic material. Environ. Sci. Technol. 3, 271-273.

Wheatley, G.A. and Hardman, J.A. (1964). Insecticides and chlorinated hydrocarbons and organic phosphorus compounds and residues in soil and water, carrots and earthworms. Rep. Natn. Veg. Res. Sta. 15, 63-65.

Wheatley, G.A. and Hardman, J.A. (1968). Organochlorine insecticide residues in earthworms from arable soils. J. Sci. Food Agric. 19, 219-225.

White, G. (1853). Letter 34 (1777) Worms. A Natural History of Selborne. Routledge, London, 428 pp.

White, G.C. (1980). Effects of dinoseb sprays on earthworms. Rep. East Malling Res. Sta. 1979, p.46.

White, J.L. (1976). Clay-pesticide interactions. Bound and Conjugated Pesticide Residues (eds. Kaufman, D.D., Still, G.G., Paulson, G.D. and Bandal, S.K.), pp.208-218, ACS Symposium Series No. 29. Publ., American Chemical Society, Washington, USA.

Wiese, I.H. (1964). Some biological studies on the inactivation of insecticides by various soil types. S. Afr. J. Agric. Sci. 7, 823-836.

Williams, C.B. (1973). Field tests of four insecticides against the Douglas Fir Tussock Moth in Oregon. Perm. Assoc. Comm. Proc., pp.77-83.

Wilson, K.W. (1975). The laboratory estimation of the biological effects of organic pollutants. Proc. R. Soc. Lond. B 189, 459-477.

Wright, M.A. (1977). Effects of benomyl and some other systemic fungicides on earthworms. Ann. App. Biol. 87, 520-524.

Wright, M.A. and Stringer, A. (1973). The toxicity of thiabendazole, benomyl, methyl benzimidazol-2-yl carbamate and thiophanate-methyl to the earthworm, Lumbricus terrestris. Pestic. Sci. 4, 431-432.

Wright, M.A. and Stringer, A. (1980). Lead, zinc and cadmium content of earthworms from pasture in the vicinity of an industrial smelting complex. Environ. Pollut. Ser. A 23, 313-321.

Yadav, D.V., Pillai, M.K.K. and Agarwal, H.C. (1976). Uptake and metabolism of DDT and Lindane by the earthworm, Pheretima posthuma. Bull. Environ. Contam. Toxicol. 16, 541-545.

Yokoyama, V.Y., Pritchard, J. and Dowell, R.V. (1984). Laboratory toxicity of pesticides to Geocoris pallens (Hemiptera: Lygaeidae), a predator in Californian cotton. J. Econ. Entomol. 77, 10-15.

Zachariae, G. and Ebert, K.H. (1970). Does chemical pest control in forests endanger earthworms? Chlorinated hydrocarbon insecticides. Pedobiolog. 10, 407-433.


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