HILGARDIA

A JOURNAL OF AGRICULTURAL SCIENCE PUBLISHED BY THE CALIFORNIA AGRICULTURAL EXPERIMENT STATION

Volume 42, Number 11 · May, 1974

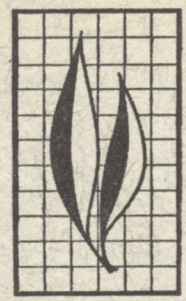
1,3-Dichloropropene and 1,2-Dibromoethane Compounds:

I. Movement and Fate as Affected by Various Conditions in Several Soils

II. Organism-dosage-response Studies in the Laboratory with Several Nematode Species

M. V. McKenry and I. J. Thomason

UNIVERSITY OF CALIFORNIA DIVISION OF AGRICULTURAL SCIENCES



Part I. Movement and Fate as Affected by Various Conditions in Several Soils

The approximate movement and fate of 1,3-dichloropropene (Telone,® D-D,® or Vidden-D®) and 1-2-dibromoethane (EDB) in two soils were predicted using extrapolations from laboratory experiments and soil-vapor phase concentrations obtained from simulated field experiments. The most far-reaching diffusion patterns in mineral soils are those obtained in soils whose moisture content is nearest the wilting point of plants (15 bars moisture tension). As the moisture content of the soil is increased, the diffusion pattern gradually becomes more limited. This effect is most striking when fine-textured soils have moisture contents in excess of ½ bar moisture tension (at the 30.5 cm depth).

Fumigation of warm soils (25°C) results in a faster rate and greater distance of nematicide diffusion. In colder soils (5°C), the rate of diffusion is slower, and the persistence of the chemical is longer, but the total distance of diffusion of an effective dosage is decreased. Increased soil temperatures result in increased rates of hydrolysis of cis- and trans-1,3-D. The same is not true of EDB.

The soil texture and type determine to a large extent the amount of soil moisture present and the size of the connecting air spaces. Soil-air space and size of pores are important, because these chemicals move primarily in the vapor phase and the smaller pores are most easily blocked when water is present. It is important that fumigant applicators are successful in sealing the soil surface and chisel shank hole after an application. Failure to do this results in significant losses to the atmosphere especially, if the subsoil is in a moist to wet condition.

(continued inside back cover)

THE AUTHORS:

I. J. Thomason is Professor of Nematology and Nematologist in the Experiment Station, University of California, Riverside.
M. V. McKenry is Assistant Nematologist in the Experiment Station, University of California, Riverside, and is presently stationed at the San Joaquin Valley Research and Extension Center, Parlier.

Part II. Organism-dosage-response Studies in the Laboratory with Several Nematode Species[1]

INTRODUCTION

CONCENTRATIONS OF ALKYL HALIDE NEMATICIDES in soils can be predicted with some certainty at any given point in the soil profile at any given time. (See Part I in this series.) For the most efficient use of these materials, it is then necessary to determine the relationship of dosage to organism response (dosage-response) and to establish curves based on these data to coincide with the diffusion gradients. As this organism-dosage-response data accumulates, eventually we may be able to answer questions pertaining to (1) the increased growth response of plants; (2) the mode of nematicidal action; and (3) pesticide specificity (Bollen, 1961; Goring, 1962; Mankau and Imbriani, 1971; Martin, Baines, and Erwin, 1957).

In this report, the authors sought to establish the above-mentioned curves for several nematodes under laboratory conditions.

Previous work

Techniques for previous dosage-response studies of the nematicides in question have involved (1) known amounts of toxicant added to sealed jars containing soil and associated organisms (Baines, *et al.* 1966; Moje, Martin, and Baines, 1957); (2) toxicants dissolved in the water phase and test organisms immersed in the solution (Evans and Thomason, 1971); and (3) cumulative data of various field experiments used to predict the relative toxicity of various chemicals.

However, several problems are inherent with these techniques: For instance, hydrolysis and sorption effects were not sufficiently known—so that the exact concentration to which the organism was exposed could not be determined. Also, in the experiments where nematodes were placed in a solution, chemical may have been lost due to its sorption onto the various materials used to construct the exposure flasks, or from escape through seals. (This last would be especially true with EDB.) Hydrolysis of some compounds can also occur under these conditions.

This study

For the experiments described here, dosages were accurately determined by monitoring the vapor phase concentrations throughout the exposure period (Kolbezen, personal communication). Knowledge of the vapor-phase concentrations and the value for Henry's constant permitted us to calculate the water phase concentrations.

[1] Submitted for publication April 12, 1973

MATERIALS AND METHODS

Gas chromatography techniques

For details of these, the reader is referred to Part I in this series.

Technique for exposure of organisms

Fumigation chambers were large (20 to 27 liter) glass bottles. The liquid phase of the toxicant (0.1 ml to 1 ml) was added to the chamber along with large amounts of mineral oil (0.5 to 5 liters). Water (20–40 ml) was also added in order to maintain a relative humidity of 99 to 100 per cent within the chamber. The chamber was sealed with a rubber stopper fitted with a stainless steel tube for removal of vapor-phase samples as indicated in fig. 1. The toxicant was allowed to stabilize within the chamber for several days at a constant temperature. By alternately adding more toxicant or mineral oil, the vapor phase within the chamber could be adjusted to any desired concentration. The fumigation chamber provides a constant concentration of toxicant in the vapor phase. Some toxicant is lost when the lid is removed, but concentrations re-stabilize in approximately one hour.

Dialysis tubing of 1.60 cm in diameter with an average pore size of 48 Angstrom units was filled with approximately ten grams sand (#18 silica sand) and then sealed off on both ends. One ml of tap water containing a known number of nematodes was then added to the air-dry sand by means of a syringe. This technique was similar for all nematode exposures except where cysts of *Heterodera schachtii* Schmidt, 1871, or field soils were involved. White cysts were exposed while attached to freshly harvested host roots. Brown cysts were added simultaneously with the silica sand and 1 ml tap water, or they were already present in the case of the field soil.

Five dialysis bags containing nematodes were suspended from each string from the top of the fumigation chamber. The vapor-phase concentration in the fumigation chamber (hence the actual concentration to which the nematodes were exposed) was sampled periodically.

Test organisms were exposed to the toxicant from 24 hours to seven days. In every case, checks were run simultaneously by placing organisms in chambers containing mineral oil and water but no toxicant.

The usual experiment consisted of placing four strings of five bags each into the chamber, then removing single strings on the first, second, fourth and sixth days. The concentration in the fumigation chamber was lowered, and the experiment was repeated until sufficient data were available to establish the LD_{50}, LD_{95} and the dosage required to kill all of the organisms.

The vapor-phase concentration and the exposure time gave us a dosage value (concentration × time) which was reported as moles/liter · day or ppm · day in the soil-vapor phase.

Temperature of the fumigation chambers was controlled by placing them in constant temperature rooms. Nematodes were preconditioned to the experimental temperature for 24 hours before being exposed to the various toxicants.

This static-type of exposure system was used in all treatments discussed in this section. The dosage-response data obtained from experiments involving the dialysis bags were comparable to similar experiments where we used cheesecloth to hold soil and nematodes.

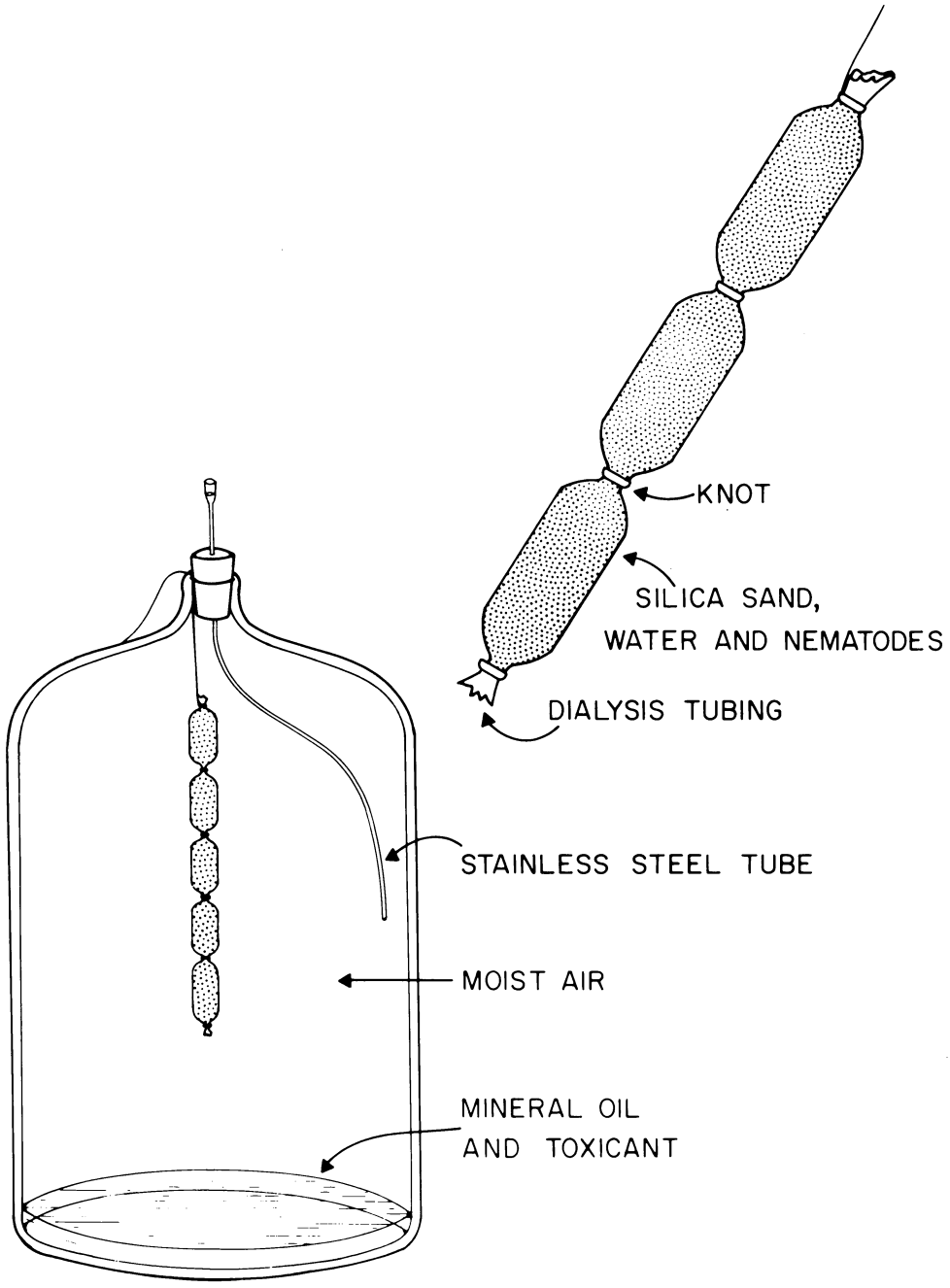


Fig. 1. Fumigation chambers used to expose nematodes to various toxicants.

Bioassay techniques for exposed organisms

Motility and infectivity tests were used to determine the dosage-response curves. A motility test is the quickest and most useful for studying the effect of the toxicant on bacterial and fungal feeding nematodes. This method was used also with plant parasitic nematodes for comparison. The most realistic bioassay for plant parasitic nematodes is to check the number which are able to survive the toxicant and then successfully infect a host plant (Thomason, Van Gundy, and Kirkpatrick, 1964).

Motility tests were made with two genera of free-living nematodes. *Aphelenchus avenae* Bastian, was reared on *Rhizoctonia solani* Kuhn, using the method described by Evans, 1970.[2] The *Eucephalobus* sp. Steiner, was cultured on potato dextrose agar plates containing *Rhizobium* spp. All stages of nematodes were treated simultaneously, and a motility test was used with the results reported as percentage nonmotile. *Aphelenchus avenae* is a good test organism for the components of Telone because the nematodes which are dead assume a straightened position, whereas live nematodes have a slight bend or may be coiled. The motility test was made 24 hours after the end of the exposure in every case. Sometimes, *A. avenae* are only inactivated by the pesticide, and they do recover. At other times, as when narcotized by EDB, the nematodes may take a while to die. No tests were run on the ability of *A. avenae* to reproduce after an exposure period (Evans and Thomason, 1971). Immediately following their removal from fumigation chambers the dialysis bags containing nematodes were individually slit open and the contents—sand, nematodes, and water phase of the toxicant—were placed in a graduated cylinder with approximately 35 ml water. The nematodes plus water were then decanted onto a petri plate, allowed to sit for 24 hours, then visually counted for motility or lack of it. Five hundred nematodes were counted from each dialysis bag.

Infectivity tests involved the placement of exposed nematodes and sand directly into a small plastic pot. A host plant was transplanted to the pot and allowed to grow the proper length of time for symptom expression or appearance of nematodes. For *Meloidogyne* spp., 10-day-old tomato seedlings were used as assay plants, and the root galls were counted 21 to 25 days after placing the nemas upon the roots. The host plants were grown in the greenhouse where air temperature ranged from 22 to 32°C. Potting soil was a loamy sand.

For the *Heterodera schachtii* treatments, 7 to 10-day-old Copenhagen cabbage was used as a host plant and the number of white cysts that had formed on the roots were counted five weeks after placing nematodes with the plant. Again, results were expressed as a per cent of control in comparison to the check plants. Pots containing the cabbage plants were placed in Wisconsin-type temperature tanks and the temperature maintained at 24°C ± 1°. For both the *Heterodera* and *Meloidogyne* spp. approximately 1,500 to 1,700 second-stage juveniles were placed in each dialysis bag, and this resulted in approximately 100 successful infection sites upon the roots of a suitable host plant growing in nontreated soil.

The *Meloidogyne javanica* (Treub) Chitwood, second-stage juveniles were harvested from egg masses on the roots of lima beans. The *M. incognita* Chitwood, 1949, second-stage juveniles were obtained from egg masses on the

[2] Cultures of *Aphelenchus avenae* supplied by H. McKinney.

TABLE 1
THE HENRY'S CONSTANT VALUES FOR THE VARIOUS TOXICANTS STUDIED

Toxicant	Henry's Constant Value (K_h) at following temperature:		
	25°C	15°C	5°C
1,2-D	9	12	24
cis-1,3-D	17	23	43
trans-1,3-D	25	37	76
EDB	35	45	—

SOURCE: Data compiled from the work of Leistra (1970), Goring (1962) and our own laboratory.

roots of tomato. The *Heterodera* brown cysts were obtained by washing the cysts from the soil of naturally infested sugar beet fields in Oxnard or El Centro, California. In some experiments, the soil was not washed, and 10 grams of infested soil, including juveniles and cysts, were exposed to toxicants directly.

The results of the various treatments were reported as the dosage (mole/liter day) required to give a certain degree of control. This dosage-response data was subject to a probit analysis[3] to determine the LD_{50} and LD_{95} within the 5 per cent confidence limits. The lethal dosage values were plotted on linear probability paper, and the $LD_{99.9}$ value was estimated. These results are readily converted to ppm · day (mg/liter · day) dosages using the Henry's constant value of the toxicant for that particular temperature as given in table 1. Natural mortality for *Aphelenchus avenae* and *Eucephalobus* sp. was approximately 4–5 per cent at the end of seven days at 5, 15, or 25°C. The natural mortality of the plant parasites is 5 to 10 per cent, but this is relatively unimportant in the infectivity tests because of the large numbers of nematodes used and the resulting successful degree of galling obtained—even after seven days within the check fumigation chambers (mineral oil and water only) at 5, 15, or 25°C.

RESULTS

Accuracy of dosage-response data

Nematodes were exposed to various concentrations of toxicant for known exposure times. Dosage-response curves were obtained from a minimum of 10 different dosages with five replicates each. Figure 2 depicts the dosage-response curve for *Aphelenchus avenae* when exposed to cis-1,3-D at 25°C. The LD_{50} and LD_{95} for *A. avenae* and cis-1,3-D were 9.1×10^{-6} and 1.7×10^{-5} mole/liter · day, respectively, based on vapor-phase concentrations.

Dosage-response curves for other treatments and organisms were obtained in a similar manner, with fiducial limits within the 5 per cent limit for both the motility and infectivity tests. For the sake of convenience, only the LD_{50} and LD_{95} values are reported in tables 2 and 3, but the slope may be redrawn from those values. As depicted in figure 2, the analytical program, run through the computer, places greatest confidence on those responses in the center of the dosage-response curve.

Infectivity test versus motility test

Infectivity tests were compared to the visual observations made on motility. We tested *Meloidogyne javanica*

[3] This analysis was carried out with the aid of probit analysis program BL-760, supplied by C. K. Huszar, and the use of an IBM-360/$_{50}$ computer.

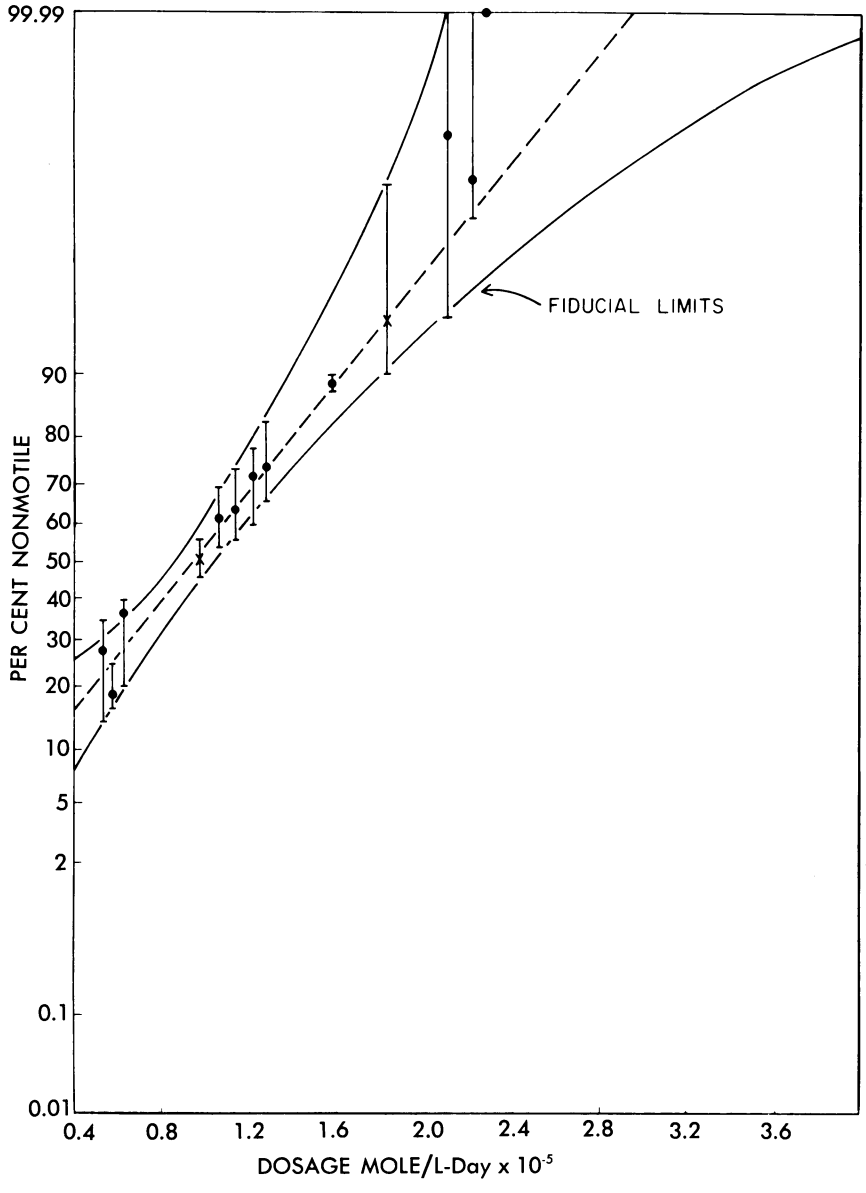


Fig. 2. Dosage response of *Aphelenchus avenae* to cis-1,3-D at 25°C.

using motility and infectivity tests simultaneously. In figure 3 the two tests are compared showing the effect of cis-1,3-D on *M. javanica*. In figure 4 we have shown the response of the same species to EDB. The responses of these two chemicals were dissimilar; however, it does appear that motility tests involving 1,3-D can be made with some confidence. In the case of EDB,

TABLE 2
DOSAGE-RESPONSE DATA FOR VARIOUS ORGANISMS AND TOXICANTS

Temp.	Organism	Chemical	LD_{50}-*		LD_{95}-*	
			Mole/liter • day	Ppm • day	Mole/liter • day	Ppm • day
25°C	*Aphelenchus avenae*					
	(all stages)	cis-1,3-D	1.6×10^{-4}	18.0	3.0×10^{-4}	33.7
		trans-1,3-D	3.7×10^{-4}	40.5	5.6×10^{-4}	62.0
		EDB	2.0×10^{-3}	382.0	3.9×10^{-3}	735.0
	Eucephalobus					
	(all stages)	cis-1,3-D	1.6×10^{-4}	18.0	2.3×10^{-4}	25.0
	Meloidogyne incognita					
	(second stage)	cis-1,3-D	1.2×10^{-4}	13.5	1.9×10^{-4}	21.0
		trans-1,3-D	2.0×10^{-4}	22.5	3.2×10^{-4}	35.0
	M. Javanica					
	(second stage)	cis-1,3-D	1.4×10^{-4}	15.0	1.9×10^{-4}	21.5
		EDB	1.2×10^{-4}	23.0	1.9×10^{-4}	36.0
	Heterodera schactii					
	(second stage)	cis-1,3-D	1.0×10^{-4}	11.2	1.9×10^{-4}	21.0
	(white cyst)	cis-1,3-D	1.6×10^{-4}	17.2	3.2×10^{-4}	36.0
		trans-1,3-D	3.5×10^{-4}	38.6	6.8×10^{-4}	75.0
	(brown cyst)	cis-1,3-D	6.1×10^{-4}	67.5	9.6×10^{-4}	106.0
		trans-1,3-D	8.6×10^{-4}	95.0	1.6×10^{-3}	180.0
15°C	*M. javanica*					
	(second stage)	cis-1,3-D	1.5×10^{-4}	17.0	2.4×10^{-4}	27.0
		trans-1,3-D	2.2×10^{-4}	24.0	3.2×10^{-4}	36.0
		EDB	2.9×10^{-4}	55.0	4.3×10^{-4}	81.0
		1,2-D	6.4×10^{-3}	720.0	———	——
5°C	*A. avenae*					
	(all stages)	cis-1,3-D	7.6×10^{-4}	84.0	1.4×10^{-3}	150.0
		trans-1,3-D	1.3×10^{-3}	145.0	2.6×10^{-3}	285.0
	M. javanica					
	(second stage)	cis-1,3-D	5.2×10^{-4}	57.0	8.3×10^{-4}	92.0
		EDB	1.1×10^{-3}	213.0	1.5×10^{-3}	290.0

* The LD_{50} and LD_{95} values based on concentrations in the soil water phase

the motility test would be of questionable absolute value and only indicative of the actual response obtained.

Aphelenchus avenae appeared to be more tolerant to EDB than the other organisms tested. The results were not directly comparable, however, since two different types of assay methods were used. When one compares the lethal dosage values of *Meloidogyne javanica, A. avenae,* and *Eucephalobus* sp. when exposed to EDB or cis-1,3-D there is a strong indication that *A. avenae* is, in fact, resistant to EDB (Evans and Thomason, 1971).

Concentration × time studies: their validity

Several toxicological terms have been confused in the literature and should be made clear at this time: dose —the amount of pesticide received by an individual organism; dosage—the amount of pesticide placed in the environment of the organism for a known length of exposure time (concentration × time). Therefore, a nematode exposed to a dosage will pick up a certain dose (Busvine, 1957). Actual dose would depend primarily upon the species of nematode and the kind of toxicant, as shown by Marks, Thomason, and Castro (1968). Soil temperature would determine the amount of toxicant in the soil-water phase.

The authors refer to LD_{50} and LD_{95} as lethal dosages and reserve the term dose for concentrations available at the site of action *within* nematodes or upon their organelles.

TABLE 3

DOSAGE-RESPONSE DATA FOR VARIOUS ORGANISMS AND TOXICANTS AT THE $LD_{99.99}$ PER CENT LEVEL

Organism	Chemical	Temperature	Dosage soil-vapor phase		Dosage soil-water phase	
			Mole/liter • day	Ppm • day	Mole/liter • day	Ppm • day
Aphelenchus avenae	cis-1,3-D	25°C	2.1×10^{-5}	2.3	3.5×10^{-4}	39
	cis-1,3-D	5°C	3.4×10^{-5}	3.8	1.5×10^{-3}	163
	trans-1,3-D	25°C	2.4×10^{-5}	2.7	6.0×10^{-4}	67
	trans-1,3-D	5°C	4.0×10^{-5}	4.4	3.0×10^{-3}	335
	EDB	25°C	1.6×10^{-4}	29.5	5.3×10^{-3}	1000
Meloidogyne javanica	cis-1,3-D	25°C	1.2×10^{-5}	1.3	2.1×10^{-4}	23
	cis-1,3-D	15°C	1.1×10^{-5}	1.2	2.5×10^{-4}	28
	cis-1,3-D	5°C	2.0×10^{-5}	2.2	8.7×10^{-4}	97
	EDB	25°C	5.9×10^{-6}	1.1	2.1×10^{-4}	39
	EDB	15°C	9.6×10^{-6}	1.8	4.7×10^{-4}	87
	EDB	5°C	1.6×10^{-5}	3.0	1.6×10^{-3}	300
Eucephalobus sp.	cis-1,3-D	25°C	1.5×10^{-5}	1.7	2.6×10^{-4}	29
	trans-1,3-D	25°C	1.6×10^{-5}	1.8	4.2×10^{-4}	46
Heterodera schachtii						
(2nd stage)	cis-1,3-D	25°C	1.3×10^{-5}	1.4	2.2×10^{-4}	24
(white cyst)	cis-1,3-D	25°C	2.3×10^{-5}	2.6	4.0×10^{-4}	44
(brown cyst)	cis-1,3-D	25°C	6.0×10^{-5}	6.6	1.0×10^{-3}	112
(brown cyst)	trans-1,3-D	25°C	6.8×10^{-5}	7.5	1.7×10^{-3}	187
(brown cyst)	EDB	15°C	1.2×10^{-4}	23.0	6.1×10^{-3}	1150
*Armillaria mellea**	cis-1,3-D	15°C	2.0×10^{-4}	22.0	4.6×10^{-3}	505

* Unpublished results of Munnecke, McKenry, and Wilbur.

If concentration × time is a valid concept, varying the concentration or time should not appreciably affect the biological response in relation to their product (dosage). To study this, *Aphelenchus avenae* was exposed to various concentrations for various exposure times. The results are shown in figure 5 for cis-1,3-D and EDB. Similar responses were obtained with the other nematodes used in this study. There are limitations to this concept. For instance, when fungi are treated by pesticides there is a threshold point below which kill is not obtained, and the only effect is a cessation of growth during the exposure period. Growth is resumed when the toxicant is removed from the environment, assuming the growth of the competitive organisms has also been stifled. This phenomenon is referred to as fungistasis. It is most noticeable in fungi, since they are more tolerant to these pesticides than are nematodes. Low concentrations of toxicant present in soils for long periods of time may have little direct effect upon nematode control, whereas the indirect effect of competitive and predacious organisms may become more important (Mankau and Imbriani, 1971). The authors worked with exposure times only up to seven days, and for that time period the dosage concept is valid for nematodes; however, nemastasis may well occur at concentrations below a certain threshold level.

Inherent toxicities

The inherent toxicity of 1,2-D, cis-1,3-D, trans-1,3-D and EDB was compared at various temperatures. Table 2 shows a comparison of the LD_{50} and LD_{95} values when several organisms were exposed to these toxicants. For toxicological studies, it is preferable to compare dosages presumably surrounding the nematode, hence, water-phase values were reported.

Various developmental stages of *Heterodera schachtii* were exposed to cis-1,3-D. The response of the infective stage, the eggs contained within the

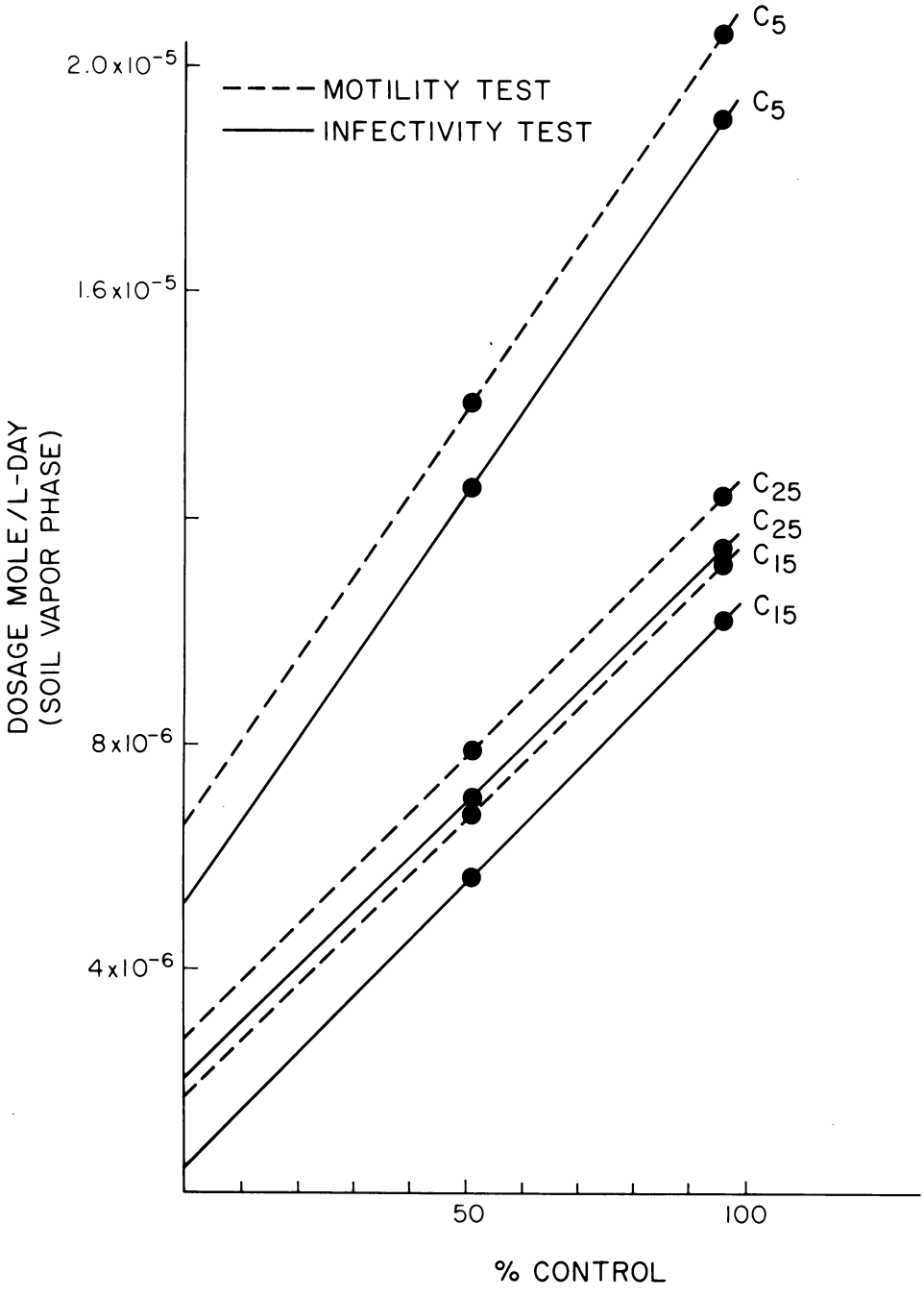


Fig. 3. Dosage response curves for *M. javanica* exposed to cis-1,3-D at 5°C (C_5), 15°C (C_{15}) and 25°C (C_{25}).

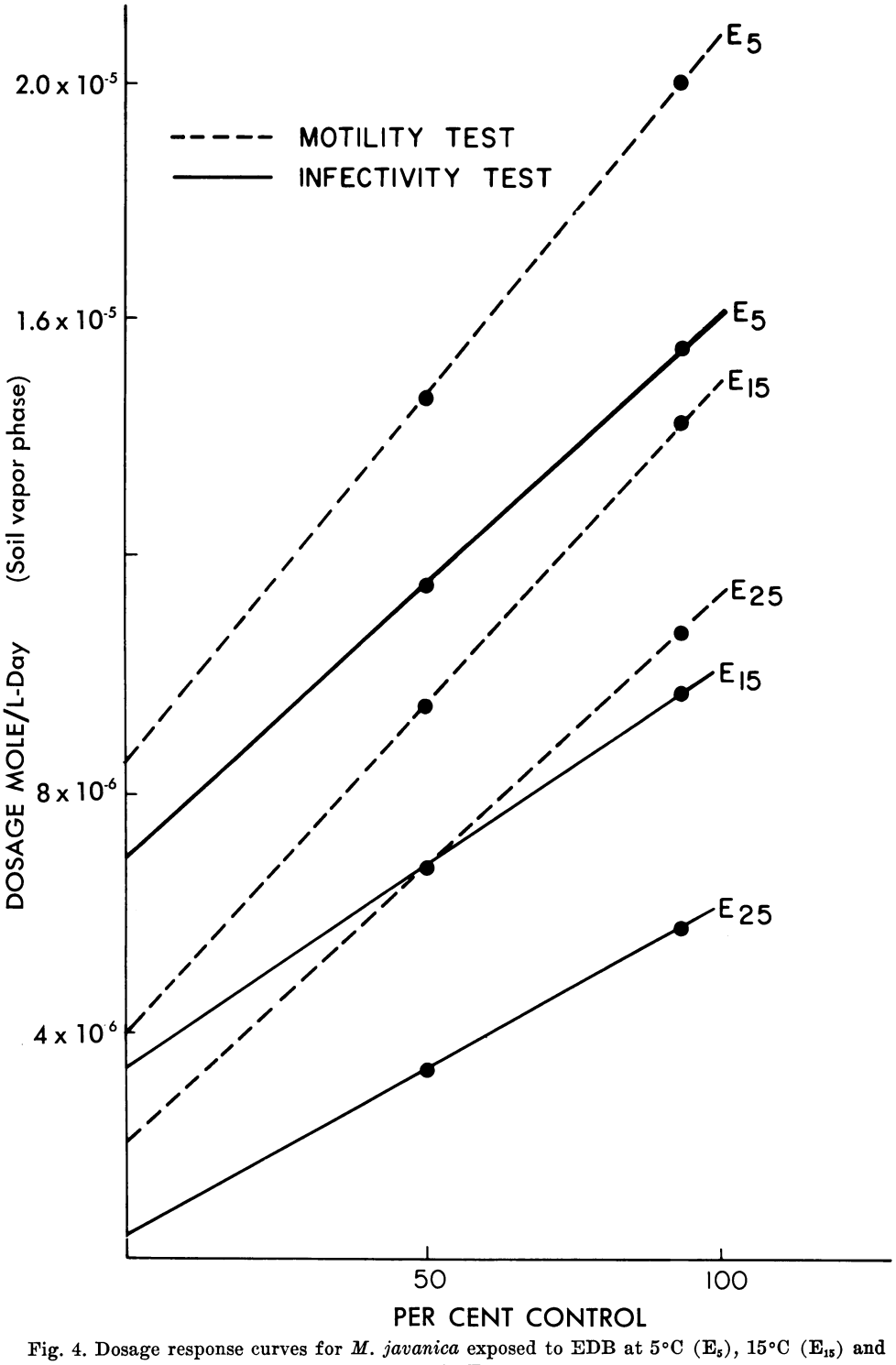


Fig. 4. Dosage response curves for *M. javanica* exposed to EDB at 5°C (E_5), 15°C (E_{15}) and 25°C (E_{25}).

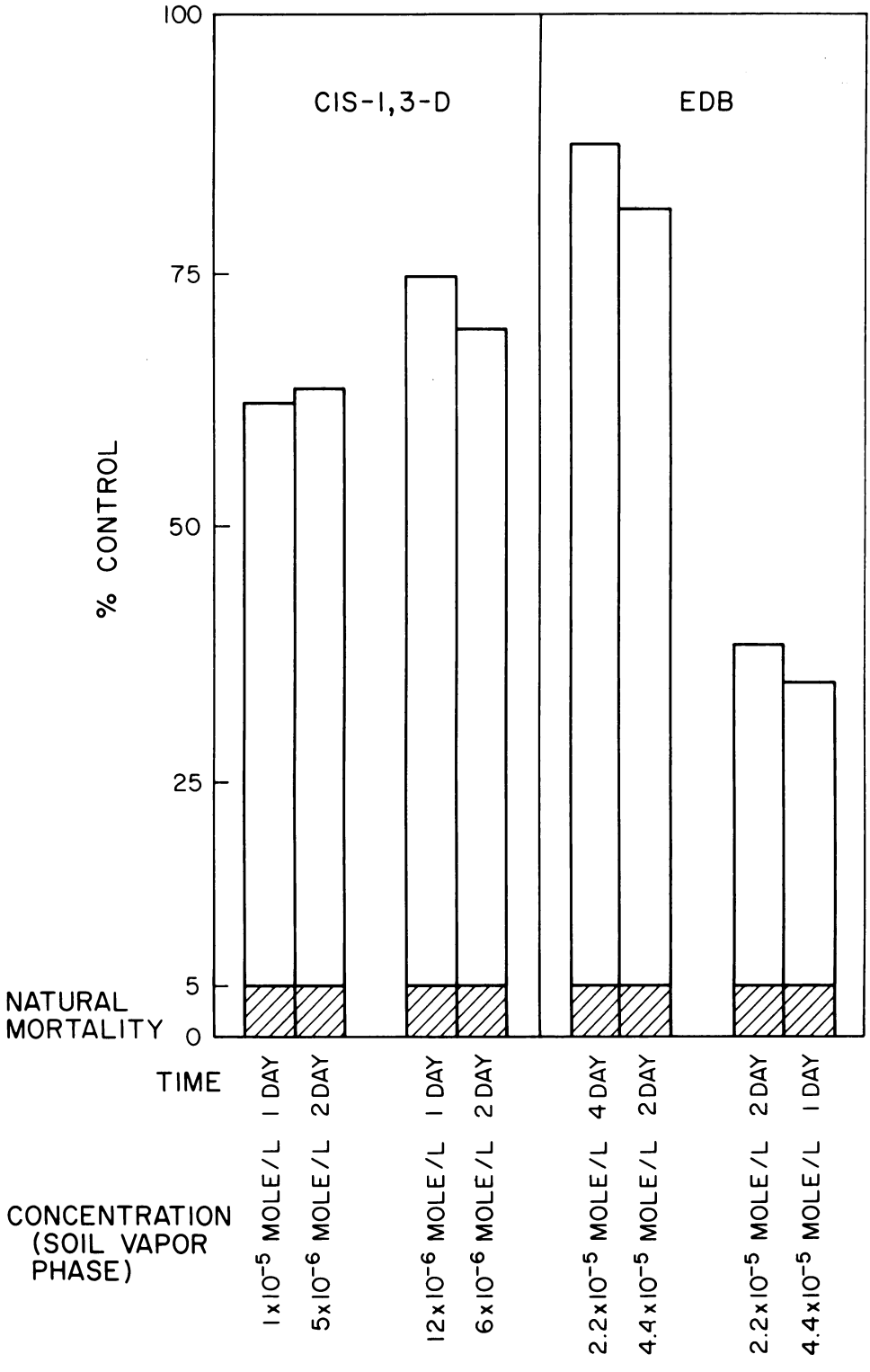


Fig. 5. Effect of varying concentrations and exposure times for *A. avenae* at 25°C using motility tests to measure the response.

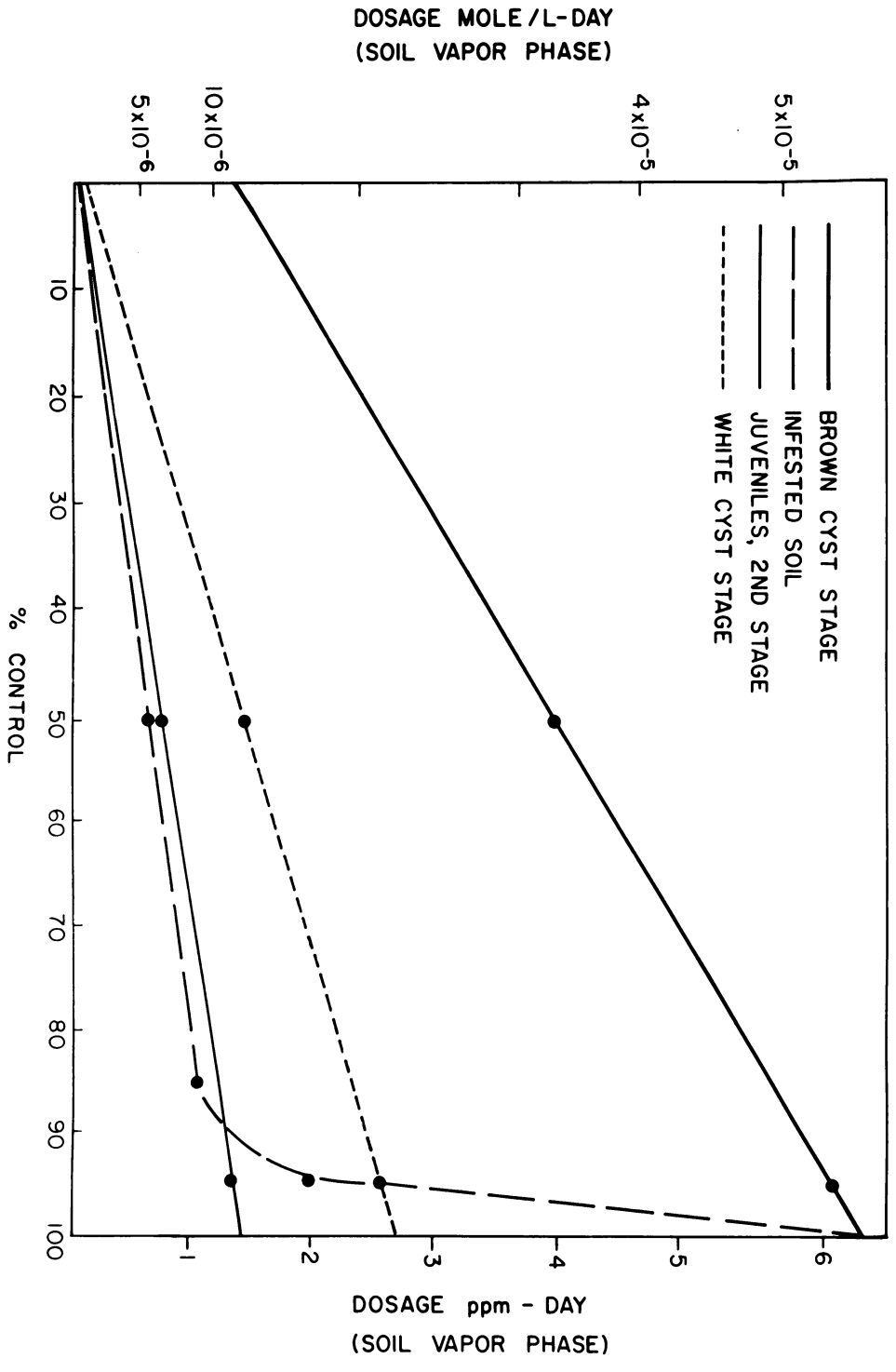


Fig. 6. Dosage response of various stages of *Heterodera schachtii* to cis-1,3-D at 25°C.

white cyst stage, the brown cyst stage, and the response obtained from exposure of a naturally infested soil are shown in figure 6. Brown cysts required five times the dosage as second-stage juveniles of *H. schachtii*. Infested soil may reflect an initial kill of juveniles followed by resistance of brown cysts in the soil.

Temperature

Temperature effects on dosage-response is apparent from figures 3 and 4. The higher the temperature, the lower the dosage required for control particularly with EDB. The temperature effect could conceivably be a result of increased inherent toxicity of the chemical rather than an effect upon the organism. The authors suggest, however, that temperature influences metabolic activity of the nematode, and this is related to toxicant activity. Marks, Thomason, and Castro (1968) have shown that permeation of EDB is not significantly reduced at 5°C; thus the reduced kill is not due to the chemical's not entering the nematode. Nematodes exposed to cis-1,3-D at 25°C are controlled at the LD_{95} level by one-fourth the dosage (soil-water phase) required at 5°C. The effect is even more pronounced with EDB where the difference may be as high as one-eighth.

The compound 1,2-D was tested on the same organisms at the same temperatures. 1,2-D has no role as a control agent unless field application rates of Telone exceed 450 liters/ha. Above this application rate, its value in the control of nematodes is problematical (Youngson and Goring, 1970). In addition, there is no evidence that it acts synergistically with either cis- or trans-1,3-dichloropropene (Moje, 1963). The toxicological data for 1,2-D is not reported, because control was not obtained with the concentrations used.

Dosage required for control of specific organisms

For practical purposes, it is desirable to know the dosage which is required to achieve 100 per cent control of a specific organism. Values for LD_{100} were obtained in our artificial exposure system. It is naive to assume that an LD_{100} is obtainable in a natural soil system, because: (1) blocked pore spaces, especially in moistened finer-textured soils, may protect certain organisms; and (2) the top 3 inches of soil receive a lower dosage due to volatilization.

However, we may assume that when certan dosages above the LD_{95} value are obtained, acceptable control has been achieved. Using linear probability paper, it was possible to obtain values for the $LD_{99.99}$, and this value is used here as an aid to correlating the information on diffusion patterns of the chemical with the amount of toxicant required to achieve control. These values are indicated in table 3. The values are reported as ppm · day in the soil-vapor phase for ease of conversion to the data presented in Part I of this series.

SUMMARY AND DISCUSSION

Before quantifying the biological activity of the various toxicants, an assay method which was adaptable to various groups of soil organisms was needed. This is a difficult task, since no single method is adaptable to all types of organisms. A laboratory toxicological test was needed that, in part, should duplicate the ppm · day dosage of toxicant that one is able to monitor in the field. This would make it possible to estimate the level of control one can anticipate for any organism if he has both the data for soil-vapor phase concentrations in the field (converted to dosage) and the dosage re-

quired to kill various organisms. The predictions could be confirmed by bioassay of soil samples from treated fields.

For *Aphelenchus avenae* and other free-living nematodes exposed to 1,3-D, motility is the most useful criterion for determining the dosage-response curve. When *A. avenae* are exposed to EDB, motility is of less value, presumably because of the effect on reproduction (Evans and Thomason, 1971) and narcotization of the nematodes (Van Gundy *et al.*, 1972). With plant-parasitic nematodes the most useful dosage-response data is that obtained from infectivity tests.

The technique of Munnecke, Wilbur, and Kolbezen (1970) involving exposure of treated *Armillaria mellea* (Vahl) Quel, infected root pieces with nonsterile soils is a good example of a special technique. This technique involves the introduction of competitive organisms and the exposure of infected roots rather than single rhizomorphs of fruiting structures of the fungus. The unpublished results of research by Munnecke, McKenry, and Wilbur are included in table 3 to show the relative toxicity of 1,3-D to the fungus *A. mellea*.

With the organisms studied, the concept of dosage (concentration × time) appeared to be a valid means to measure the responses obtained. The static-type experiment in which nematodes were exposed to toxicants was simple to perform and adaptable to various types of organisms. Perhaps the main advantage of the technique is that concentrations may be periodically monitored (and adjusted if necessary), which results in a high level of accuracy for determining dosages. There were some variations in the dosage-response curves obtained from organisms of the same species. All stages of the free-living nematodes were exposed simultaneously; thus the *Aphelenchus avenae* had varying proportions of the different stages depending upon when they were harvested from the wheat cultures. It was necessary to establish at least ten different dosage values (five replicates each) for a given organism before the LD_{50} and LD_{95} could be obtained within the 5 per cent confidence limits (see fig. 2). This was also true when infectivity tests were the assay method.

Individual toxicants

Dosage-response information for each individual toxicant is necessary, since each toxicant moves through soil at an independent rate. The dosage to which each organism is exposed depends upon the position of the organism in the soil profile. When soil is treated with Telone, the organism is first exposed to 1,2-D and then to cis-1,3-D. If the organism is a significant distance from the line of injection, the trans-1,3-D may never reach its vicinity. Consequently, to apply this toxicological data in the field, one must find the inherent toxicity of each individual component of the Telone or D-D, estimate the dosage of individual components at a particular point in the soil profile, and then add up the dosage for cis- and trans-1,3-D. With EDB the task is simpler, since only one toxic component is involved.

For practical control purposes, 1,2-D is not important as a toxicant to nematodes. In our tests, the trans-1,3-D was approximately 60 per cent as toxic as the cis-1,3-D in the soil-water phase. On a vapor-phase basis, the trans-1,3-D was approximately 90 per cent as toxic as the cis-1,3-D. More important, however, is the distance which the toxic components must travel. The cis-1,3-D moves relatively faster and farther allowing for greater depth of soil penetration. The trans-1,3-D moves more slowly and, most important, is lost to the atmosphere at a slightly slower rate than cis-1,3-D. Since the inherent toxicity of both of these compounds is

relatively high, the trans-1,3-D is probably most significant (in combination with cis-1,3-D) for providing control of organisms in the upper soil surface. The 1,3-dichloropropene containing fumigants have the advantage of the fast movement of cis-1,3-D and the relatively slower movement of trans-1,3-D. EDB moves even slower than trans-1,3-D, but it persists longer and does not move to as great depths as do the 1,3-D components. EDB is equally toxic, but temperature does play an important role here. EDB should not be applied to soils below 15°C, unless they are warming rapidly. Two major factors contributing to this reasoning: (1) the effect of the lowered temperature on the decreased inherent toxicity (Evans and Thomason, 1971; Marks, Thomason, and Castro, 1968); and (2) the effect of lowered temperature on the ability of the toxicant to move through soil (as reported in the first paper of this series) which is even more important.

Temperature

The toxicity of nematicides containing 1,3-D was previously considered to be relatively little affected by temperature. Our results suggest, however, that cis- and trans-1,3-D as well as EDB are less toxic at low temperatures. The effect is most noticeable with EDB, when an increase in temperature from 5 to 25°C results in an eight-fold (soil-water phase) increase in toxicity to *Meloidogyne javanica*. The cis-1,3-D is 4.4 times more effective (soil-water phase) at 25°C than at 5°C, and trans-1,3-D is about 4.7 times as effective.

Lowering the temperature from 25°C to 5°C has the following effects on these toxicants: (1) the concentration present in the water phase of soil is increased approximately three-fold; (2) concurrently, the concentration required for a lethal dosage is increased approximately 4.5-fold for 1,3-D and eight-fold for EDB; and (3) the chemical is hydrolized much more slowly but is reversibly adsorbed to soil colloids to a greater extent, increasing the length of time to which the nematode is exposed. Since Marks, Thomason, and Castro (1968) have shown that nematodes accumulate EDB within their systems at higher concentrations than found in their external water phase, it is conceivable that at lower temperatures this accumulation is increased and that the concentration at the active site within the nematode has increased correspondingly. The overall effect upon those individual nematodes exposed is not changed, but the effect on the toxicant is to decrease the total distance of diffusion and the total number of nematodes exposed.

Developmental stages

The stage of development of the nematode also affects the dosage-response curves. In general, as the metabolic activity of an organism is increased, the amount of chemical required for kill is decreased (Evans and Thomason, 1971; Marks, Thomason, and Castro, 1968). The less active stages of nematodes are less susceptible to the toxicant. The presumed barriers to penetration of nematicides afforded by the presence of the aged cuticular layers (as in the case of *Heterodera schachtii* brown cysts and the eggs within the white cysts of *H. schachtii*) may be of relatively minor importance compared to the metabolic state of the inactive juveniles within eggs. Similarly, nematodes in a state of cryptobiosis (Van Gundy, 1965) could be quite resistant to these toxicants.

What, then, is the effect of moisture content of soil upon metabolic activity of soil organisms? If organisms are much less active at the higher moisture tensions that occur in dry soils (Van Den Brande, 1956), the resultant increase in resistance may be enough to negate the advantages of fumigating

relatively dry soil. This needs further clarification.

Specificity

There is some confusion concerning the inherent toxicity of the various fumigant-type pesticides used for control of soil organisms. The results of laboratory and field research have suggested that methyl bromide is a broad-spectrum pesticide; that the 1,3-D materials have more specificity for nematodes and certain fungi and insects (Wensley, 1953); and that EDB is most effective upon nematodes and insects. To determine these differences, Van Gundy *et al.,* (1972) measured the dosage of methyl bromide required for control of *Meloidogyne* and *Xiphinema* sp. using the dynamic exposure system of Kolbezen. This technique was also used to expose *Armillaria mellea* to obtain lethal dosage values. It is of interest to compare their results with the results we have obtained in a static-type of exposure system.

According to Munnecke, Wilbur, and Kolbezen (1970), 4,800 ppm ($^{V}/V$) at 20°C for one day should give 95 per cent control of *Armillaria mellea* in citrus roots. This value calculated as ppm ($^{W}/V$) for one day equals 18.6 ppm · day. Assuming the K_h for methyl bromide is 3.8, then 70.8 ppm · day in the soil-water phase will give 95 per cent control. According to Van Gundy *et al.* (1972), 660 ppm ($^{V}/V$) for 36 hours will provide 95 per cent control of root knot juveniles using tomato as the bioassay. This calculates to be 900 ppm ($^{V}/V$) for one day or 14.6 ppm · day ($^{W}/V$) in the soil water. The above data for methyl bromide was obtained from a dynamic-type exposure system.

In comparing these results with ours for cis-1,3-D, using a static-type exposure system, we find that methyl bromide has 1.4 times the toxicity to root knot nematode juveniles and 6.7 times the toxicity to *Armillaria mellea* when compared to cis-1,3-D. Methyl bromide is approximately 6.1 times as toxic to the root knot juveniles as is EDB. The reason for methyl bromide's broad spectrum activity is its ability to diffuse through soils at very high concentrations in short periods of time.

Because of the physico-chemical nature of 1,3-D,[4] the concentrations in soils at any one time are much smaller assuming equivalent amounts are applied to the soil. Specificity comes because only fungistatic dosages are available to most of the fungi, whereas, with methyl bromide the organisms are exposed to tremendous amounts of chemical for a short time. Therefore, there is little chance for specificity. EDB is even more specific due to its relatively low concentration for long exposure periods—rendering only fungistatic effects upon the fungi but lethal effects upon the nematodes.

Many soil insects would be expected to reside in the air phase of soils rather than in the water phase. The effect which this would have upon specificity is not certain. However, the inherent toxicity of 1,3-D for insects should be checked, since insects may be relatively easy to control.

LITERATURE CITED

BAINES, R. C., L. J. KLOTZ, T. A. DEWOLFE, R. H. SMALL and G. O. TURNER
1966. Nematocidal and fungicidal properties of some soil fumigants. Phytopathology 56:691–98.

BOLLEN, W. B.
1961. Pesticide, soil microorganism interactions. Ann. Rev. Microbiology 15:69.

[4] The K_h at 20°C is ca. 19.0 for cis-1,3-D and ca. 3.8 for methyl bromide (Goring, 1962).

BUSVINE, J. R.
1957. A critical review of the techniques of testing insecticides. Commonwealth Institute of Entomology, 56, Queen's Gate, S. W. 7, London.
EVANS, A. A. F.
1970. Mass culture of mycophagous nematodes. Journal of Nematology 2(1):99–100.
EVANS, A. A. F., and I. J. THOMASON
1971. Ethylene dibromide toxicity to adults, larvae and moulting stages of *Aphelenchus avenae*. Nematologica 17:243–54.
GORING, C. A. I.
1962. Theory and principles of soil fumigation. Adv. Pest Cont. Res. 5:47–84.
LEISTRA, M.
1970. Distribution of 1,3-dichloropropene over the phases in soil. Jour. Agr. Food Chem. 18:1124–26.
MANKAU, R. and J. IMBRIANI
1971. Tolerance and uptake of 1,2-dibromoethane by nematode-trapping fungi. Phytopathology 61:1492–97.
MARKS, C. F., I. J. THOMASON, and C. E. CASTRO
1968. Dynamics of the permeation of nematodes by water, nematicides and other substances. Exper. Parasitol. 22:321–37.
MARTIN, J. P., R. C. BAINES and D. ERWIN
1957. Influence of soil fumigation for citrus replants on the fungus population of the soil. Proc. Soil Sci. 21:163–66.
MOJE, W.
1963. Toxicity of some halogenated hydrocarbon mixtures to larvae of the citrus nematode *Tylenchulus semipenetrans*. Phytopathology 53:423–27.
MOJE, W., J. P. MARTIN and R. C. BAINES
1957. Structural effect of some organic compounds on soil organisms and citrus seedlings grown in old citrus soil. Jour. Agr. Food Chem. 5:32–36.
MUNNECKE, D. E., W. D. WILBUR and M. J. KOLBEZEN
1970. Dosage response of *Armillaria mellea* to methyl bromide. Phytopathology 60:992–93.
THOMASON, I. J., S. D. VAN GUNDY and J. D. KIRKPATRICK
1964. Motility and infectivity of *Meloidogyne javanica* as affected by storage time and temperature in water. Phytopathology 54:192–95.
VAN DEN BRANDE, J.
1956. Survey of the results of four years experiments on the chemical control of potato-root eelworm. Nematologica 1:81–87.
VAN GUNDY, S. D., D. MUNNECKE, J. BRICKER, and R. MINTEER
1972. Response of *Meloidogyne incognita, Xiphinema index,* and *Dorylaimus* sp. to methyl bromide fumigation. Phytopathology 62:191–92.
WENSLEY, R. N.
1953. Microbiological studies of the action of some selected soil fumigants. Can. Jour. Bot. 31:277–308.
YOUNGSON, C. R. and C. A. I. GORING
1970. Nematicidal activity of 1,3-dichloropropene and 1,2-dichloropropane to three types of plant parasitic nematodes. Pl. Dis. Reporter 54(3):196–99.

4m–4,74(R6435L)P.A.D.