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A Harvester Ant Bioassay for Assessing Hazardous Chemical Waste Sites

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May 1985

Prepared for the
U.S. Environmental Protection Agency
under a Related Services Agreement
with the U.S. Department of Energy
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Pacific Northwest Laboratory
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Richland, Washington 99352

SUMMARY

A technique was developed for using harvester ants, *Pogonomyrmex owhyeei*, in terrestrial bioassays. Procedures were developed for maintaining stock populations, handling ants, and exposing ants to toxic materials. Relative toxicities were determined by exposing ants to 10 different materials. These materials included three insecticides, Endrin, Aldrin, and Dieldrin; one herbicide, 2,4-D; three complex industrial waste residuals, wood preservative sludge, drilling fluid, and slop oil; and three heavy metals, copper zinc, and cadium. Ants were exposed in petri dishes containing soil amended with a particular toxicant. Under these test conditions, ants showed no sensitivity to the metals or 2,4-D. Ants were sensitive to the insecticides and oils in repeated tests, and relative toxicity remained consistent throughout. Aldrin was the most toxic material followed by Dieldrin, Endrin, wood preservative sludge, drilling fluid, and slop oil.

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CONTENTS

INTRODUCTION

To address the clean up of hazardous chemical waste sites, a means of prioritizing sites in the order of greatest hazard is needed. Once prioritized, the most hazardous sites can be attended to first. One approach to ranking sites is to identify the relative biological hazard by conducting a series of bioassays based on samples from each site. This approach, despite some imperfections, is far less expensive than a complete characterization for all listed hazardous constituents.

Bioassay techniques have been developed for testing aquatic organisms and terrestrial plants (Porcella 1983), but few have addressed terrestrial animals, perhaps due partly to the difficulty of maintaining large stocks of experimental animals. Terrestrial bioassays that have been used include crickets (Walton 1980; Burkhardt and Fairchild 1967), earthworms, soil respiration, and sclerotia formation by a species of the fungus *Aspergillus* (Thomas et al. 1983). In this study, harvester ants were investigated as an additional terrestrial organism for bioassay.

Harvester ants (genus *Pogonomyrmex*) are common in all arid and semi-arid habitats of the U.S. and could provide a ready supply of test organisms. They construct large, conspicuous nests containing up to 5,000 or more workers. They also represent an organism that lives in intimate contact with the soil and might be expected to come in contact with hazardous wastes in their natural setting. This fact alone eventually may lead to a means of determining the relative toxicity of a site merely by the presence or absence of these ants. Previous studies have shown that harvester ants easily can be maintained as test animals in a laboratory (Gano 1981, Spangler 1973).

The objective of this study was to assess the potential of using a harvester ant bioassay to identify the relative toxicity of various hazardous wastes. Our specific aim was to develop techniques for handling ants, maintaining stock populations, exposing ants to toxicants, and determining the relative toxicities of different toxic materials to ants.

METHODS

Ants and soil used in this study were collected from the Arid Lands Ecology (ALE) Reserve, which is located about 15 miles northwest of Richland, Washington. The harvester ants (Pogonomyrmex owyheei Cole) were excavated by shovel and trowel and brought into the laboratory where they were sorted from the soil and counted out into groups of 120. Each group was maintained in a standard 10 cm diameter polystyrene petri dish. Water was maintained in each dish through a modified 500-lambda micropipette taped to the lid. The large end of the micropipette was plugged with beeswax, which was pierced to make a small air hole. The tip of the micropipette was shortened by about 2 mm, making a larger orifice to allow ants better access to the water. This watering system gave ants access to water without having an open water supply that would promote contamination and fungal growth.

All newly excavated ants were acclimated in an incubator at 21°C for 7 to 10 days to allow time for injured ants to die and be removed before testing began. The only food given was 1 to 2 drops of 10% sugar solution at the beginning of the acclimation period. Thereafter, ants were given distilled water ad libidum. In this study, we found that harvester ants can be maintained under these conditions without food for as long as 4 months without significant losses. However, the ants used in the tests described herein were maintained for less than 6 weeks before being tested.

The 10 materials tested in this study included three insecticides representing chlorinated hydrocarbons used in agriculture, Endrin™(1,2,3,4,10,10-Hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo-endo-5,8-dimethanonaphthalene), Aldrin™(1,2,3,4,10,10-Hexachloro-1,4,4a,5,8,8a-Hexahydro-endo-exo-1,4:5,8-dimethanonaphthalene), and Dieldrin™(1,2,3,4,10,10-Hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-endo,1,4:5,8-dimethanonaphthalene); one herbicide, 2,4-D (2,4-dichlorophenoxy-acetic acid); three complex industrial waste residuals referred to in this paper as wood preservative sludge, drilling fluid, and slop oil; and three heavy metals,

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copper, zinc, and cadmium. The metals were in the chloride salt forms as $CuCl_2 \cdot 2H_2O$, $ZnCl_2$, and $CdCl_2 \cdot 2.5H_2O$.

TEST CONDITIONS

Each compound was amended to soil in individual petri dishes and the ants exposed for a period of 10 or 15 days. Ten grams of Ritzvile silt-loam soil (common to the ALE Reserve) were used per dish for all tests. All concentrations of insecticides, heavy metals, and 2,4-D are expressed in this paper in parts per million (ppm) to soil. Concentrations of the complex waste residuals are expressed as percent waste to soil (wt/wt). The soil in each dish was moistened to provide a 5% soil moisture regime for all tests except test #9 where soil moisture was 10%. We found that if soil moisture was too high, fungi would often grow on dead ants and perhaps influence mortality. To avoid fungal growth, 5% soil moisture was used since it provided enough moisture to prevent desiccation of ants during a 10 to 15 day test. Petri dishes containing amended soil and ants were taped shut during all tests to reduce moisture loss and prevent cross contamination.

Stock solutions of approximately 1000 ppm each were made using the insecticides and 2,4-D. The chemicals were each dissolved in 25 ml of methanol and stored in six 4-ml injectable vials in a freezer. Further dilutions were made for each test by taking the appropriate quantity from the injectable vial and adding it to approximately 4 ml of methanol. This solution was then added to the soil and the methanol allowed to evaporate. Used vials were discarded. The soil in control replicates for these compounds was also treated with methanol, which was allowed to evaporate before water and ants were added.

Dilutions for all tests using the complex waste residuals and the metals were made just prior to exposing the ants. The various concentrations of waste residuals were made by mixing the particular waste with soil to a known concentration (wt/wt) and making further dilutions by adding clean soil. The metals were applied to the soil by dissolving the appropriate quantity in 1 ml of distilled water and adding this solution to the soil in each dish.

ANT SELECTION

Ants used in the initial tests (tests 9, 11, and 12) were selected from a 1-liter beaker containing ants from the stock population. Three replicates of 30 ants each were used for each treatment. To obtain ants for each replicate, the large beaker was held at a slight angle so that the ants could climb up the walls and fall out of the spout one at a time into a receiving beaker. This method provided a quick and easy way to count ants for each replicate; however, it did not provide for the possibility of different sensitivities associated with ants of different vigor. After several tests we realized there were always some ants that were more aggressive than others; i.e., some would climb the walls of the beaker rapidly with mandibles held open, while the last ants to be counted moved slower and were not as aggressive. Consequently, we conducted a test (test #13) with randomly selected subgroups to make up each replicate of 30 ants. One treatment used replicates made up of six randomly selected subgroups of five ants each, and the other treatment used three randomly selected subgroups of 10 ants for each replicate. Three replicates were used for each treatment with the toxicant Endrin at 1.2 ppm.

Subgroups were randomly selected by equally dividing a population of ants and placing each half in a large beaker. Subgroups of five were counted out from one beaker and numbered sequentially as they emerged; then replicates of 30 ants each were obtained by randomly selecting the numbered subgroups. Subgroups of 10 were counted out from the second beaker, and the same procedure was followed.

All successive tests (after tests 9, 11, 12, and 13) were conducted using six randomly chosen subgroups of five ants for each replicate. Once selected, the ants were introduced to petri dishes containing treated soil; the dishes were then sealed with vinyl tape and placed on racks inside a fume hood. The room and the hood were kept dark throughout each test. All dishes were given a number and assigned random positions within the hood to eliminate any possible biases due to position. Randomizing the positions also helped to reduce any bias from knowing the treatment and concentrations of dishes while counting dead ants. Daily mortality counts were taken throughout all tests so that response curves could be plotted for each replicate (see Appendices). These response curves were used for initial evaluation and as a basis for determining concentrations to be used in subsequent tests. The criterion for death was inability to stand. If an ant was lying on its side, it was considered moribund or dead; all moribund and dead ants were tallied together for mortality counts.

MISCELLANEOUS VARIABLES

Along with testing the response of ants to the various toxic materials, additional variables were examined to see what influence they might have on the mortality response. These additional variables were the substrate on which toxins were administered and the age of the ants. These variables were tested only once and used only to generate hypotheses about the influence of different test conditions. The substrate used throughout this study was a silt-loam soil from Richland, Washington. The toxic materials tested were wood preservative sludge at 0.5%, drilling fluid at 1.0%, and slop oil at 7.0%. To examine substrate influence, the mortality response of ants exposed on filter paper was compared with the response of ants exposed on silt-loam soil. A similar test was conducted to explore the influence of different soil types using a clay soil from Ada, Oklahoma, and compared with the silt-loam soil. This test was conducted to explore the potential of using a universal substrate on which to amend toxicants. The compounds for this test were Endrin and Aldrin at 1.0 ppm and Dieldrin at 1.2 ppm.

The age of the ants is one variable that cannot often be controlled. In all tests reported here, the ants were of mixed age. Distinguishing ages of harvester ants is nearly impossible except in mid summer when the young of the year are emerging. During the excavation of one colony in August, however, several hundred newly emerged adult ants were encountered. These callow ants are easily distinguished from the dark red, older ants by their light amber color. All callow ants were sorted from the rest of the population and used in one test to compare mortality responses with those of the older, mixed-age ants. Random groups of callow ants were selected using the procedure discussed above. The toxicant was Dieldrin at 1.0 and 1.2 ppm. The selection of toxicants and concentrations for testing these miscellaneous variables was based on results of previous tests that produced 5D-100% mortality.

STATISTICAL ANALYSES

Assessments of toxicity were based on cumulative survival distributions (CSD) using what is known as a product limit estimate (Kaplan and Meier 1958). This technique differs somewhat from more common methods of analyzing toxicity data. A common method of determining relative toxicity of contaminants is to expose organisms to a series of concentrations of a particular toxicant for a specified time period. At the end of that period, the proportion of organisms that have died at each concentration is determined, and an estimate of an LC_{50} (lethal concentration at which 50% of the organisms die) is then obtained through techniques such as probit analysis. Relative toxicities of various contaminants are then assessed by comparing the LC_{50}s of the contaminants (Finney 1971, Hewlett and Plackett 1979). In the product limit method, the toxicities are compared over an entire time span for a single concentration rather than comparing the toxicities of various toxicants at a single point in time based on several concentrations, as in probit analysis. Relative toxicities are determined by comparing the CSOs of different toxicants at the same concentration.

The equality of the CSDs of different toxicants at the same concentration was tested using two test statistics: a generalized Wilcoxon (Breslow 1970) and a generalized Savage statistic (Mantel 1966). These are tests of equal distribution rather than tests of central tendency, such as a t-test.

In order to determine repeatability of the ant bioassay technique, the CSOs of several toxicants were compared in repeated tests. If the relative toxicity of different toxicants remained the same in each repeated test, it indicated good repeatability of the bioassay technique with respect to determining relative toxicity. This procedure was used for all statistical comparisons of toxicants and concentrations.

The two test statistics also were used to compare CSDs from the different substrates (soil types and filter paper), CSDs from different subgroup sizes (five ants versus ten ants per subgroup), and CSDs of mixed-age ants and callow ants. In addition, estimates of the mean survival time were obtained for each toxicant and concentration in each test. Tests of equality of CSDs and estimates of mean survival times were obtained using BMDP Statistical Software (Dixon 1983). In all cases, the sample size for these tests was 90 ants, obtained from observations of three replicate dishes, each containing 30 ants.

An advantage of the survival distribution approach is that data obtained during the entire exposure time are used rather than a point estimate at an arbitrary termination point. Also, the number of preliminary experiments needed to obtain a series of differing fractional mortalities (as needed for probit analysis) is reduced. In fact, the product limit method is most efficient when all ants die during the experimental period.

RESULTS

Harvester ants were sensitive to the three insecticides and to the three complex waste residuals but not to 2,4-D or copper, zinc, and cadmium. Three range finding tests were conducted using 2,4-D and the metals before terminating the efforts to find a toxic range. One replicate was used in all range finding tests, with the rationale that if significant mortality occurred, the test would be repeated. The final range finding test using 2,4-D was conducted with concentrations of 1,000; 2,000; 4,000; 6,000; 8,000; 9,500; and 16,000 ppm. The highest observed mortality (13%) occurred after 15 days of exposure to 16,000 ppm 2,4-D. However, two control dishes exhibited 3% and 10% mortality at the end of 15 days. We concluded that harvester ants were not sensitive to 2,4-D at concentrations $\leq$ 16,000 ppm and discontinued testing.

Tests using high concentrations of the metals also failed to produce appreciable mortalities after a 14-day exposure at concentrations up to 30,000 ppm for copper, zinc, and cadmium. The second highest concentration was the equivalent amount of material required to produce 16,900 ppm of zinc and 15,000 ppm of copper and cadmium on soil. These equivalents were absorbed onto filter paper instead of soil as a substrate. The greatest mortality after 14 days was only 10% in the treatment using 30,000 ppm copper, while the control for this test had no mortality. Again, we concluded that harvester ants were insensitive to these materials under these test conditions and discontinued testing.

Ants showed more sensitivity to the insecticides. Mortality responses occurred between 0.8 and 1.4 ppm. Table 1 shows mean survival times (MST) in days for all insecticide tests. During test #11 the sensitivity range had not been narrowed for all three insecticides, and relative toxicity could only be tested at one concentration (Aldrin and Dieldrin at 1.8 ppm, Table 1). However, comparing the MSTs gives an indication of the relative toxicity. In later tests (#12, #16, and #17), relative toxicities can be compared more easily where two or more toxicants were repeated two or more times at a specific concentration (Table 1). When the cumulative survival distributions (CSO) were compared for equality, toxicity decreased from

TABLE 1. Mean survival times ($\bar{X}$ ± SE, days) for ants exposed to Aldrin (Ald), Dieldrin (Die), and Endrin (End)

Test No.	Compound	Test Concentrations (ppm) 0.4	0.6	0.8	1.0	1.2	1.4	1.6	1.8	2.2	2.6	3.0	3.4
11	Ald					2.8 ±.12	4.0 ±.15	4.3 ±.19	4.1 ±.09				
11	Die								5.2 ±.12	4.3 ±.10	3.8 ±.09	3.3 ±.07	2.4 ±.06
11	End	12.8 ±.24	12.3 ±.31	11.7 ±.36	8.1 ±.38								
12	Ald			8.6 ±.45	7.7 ±.32	4.8 ±.13							
12	Die			13.2 ±.34	9.4 ±.34	7.1 ±.15	6.4 ±.09	5.1 ±.10					
12	End				12.0 ±.45	11.5 ±.41							
16	Ald			7.8 ±.27	6.6 ±.29	6.2 ±.26							
16	Die			8.4 ±.28	8.2 ±.26	8.2 ±.24							
16	End					8.9 ±.23	8.9 ±.21						
17	Ald				4.0 ±.20	3.3 ±.16							
17	Die				5.4 ±.26	5.0 ±.23							
17	End				7.8 ±.28	8.7 ±.23							

Aldrin to Dieldrin to Endrin. Figure 1 shows this relationship graphically with plots of the step-wise cumulative survival distributions. In all comparisons of the three toxicants, the CSDs were significantly different ($P \leq 0.05$).

The three complex waste residuals, though not as toxic as the insecticides (at the respective concentrations tested), also produced repeatable relative mortality responses. Table 2 provides mean survival times for the concentrations used during each test. The slop oil was much less toxic than wood preservative sludge or drilling fluid. Consequently, comparisons at the same concentrations could not be conducted. Again, comparing the MSTs of similar concentrations indicates the relative toxicity. For example, the MST for drilling fluid at 1.0% in test #9 was 9.2 days, while the MST for slop oil at 1.25% was 14.4 days (Table 2). In all instances where two toxicants were used at the same concentration during a test, the CSDs were statistically compared for equality. In five out of six comparisons, wood preservative sludge was significantly more toxic (i.e., ants died sooner) than drilling fluid ($P \leq 0.01$).

To determine the importance of the substrate on which ants are exposed, CSDs were compared for filter paper and soil (silt-loam). The toxicants and concentrations were Endrin and Aldrin at 1.0 ppm and Dieldrin at 1.2 ppm. The tests using Endrin and Dieldrin both showed significant differences ($P \leq 0.05$); i.e., ants exposed on filter paper died much sooner than ants on soil. The MSTs for Endrin were 1.14 and 11.99 days for filter paper and soil, respectively. The MSTs for Dieldrin were 1.77 and 7.11 days for filter paper and soil, respectively. The test with Aldrin showed no significant differences (alpha = 0.05, $P \geq 0.25$) between filter paper and soil. The MSTs for this test were 7.82 and 7.67 days for filter paper and soil, respectively.

A similar comparison of CSDs was made between a silt-loam soil and a clay soil. The toxicants were slop oil at 7.0%, drilling fluid at 1.0%, and wood preservative sludge at 0.5%. In all three comparisons, ants exposed on silt-loam soil died significantly sooner ($P \leq 0.05$, Table 2).

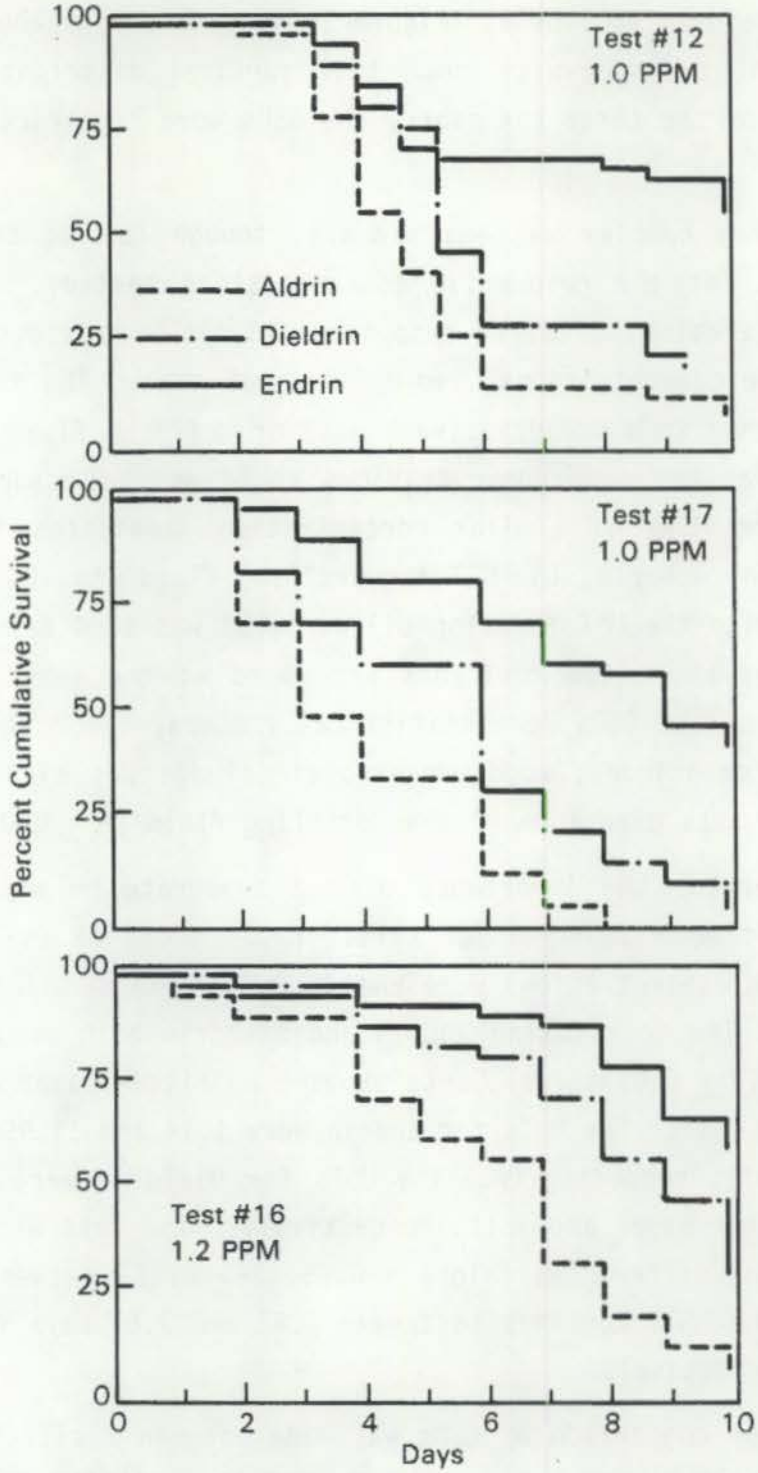

FIGURE 1. Cumulative survival distributions for harvester ants exposed to Aldrin, Dieldrin, and Endrin showing their relative toxicities during three seperate tests.

TABLE 2. Mean survival times ($\bar{X}$ ± SE, days) for ants exposed to wood preservative (WP), drilling fluid (DF), and slop oil (SO) on silt-loam and clay (-Clay) substrates

Test		Test Concentrations (%)										
No.	Compound	0.25	0.30	0.50	0.63	0.75	1.00	1.25	2.5	5.0	7.0	9.0
9	WP	14.4 ±.23		5.3 ±.37		3.3 ±.25	2.4 ±.19					
9	DF	14.9 ±.02		13.2 ±.33		12.0 ±.43	9.2 ±.42					
9	SO				14.8 ±.14			14.4 ±.36	12.2 ±.58	12.3 ±.46		
16	WP		5.3 ±.35	3.8 ±.31		3.4 ±.21						
16	WP-Clay			6.7 ±.38								
16	DF					3.6 ±.27	3.7 ±.31	3.5 ±.30				
16	DF-Clay						5.2 ±.36					
16	SO									4.0 ±.29	4.6 ±.30	5.7 ±.31
16	SO-Clay										7.0 ±.31	
17	WP		1.9 ±.15	1.2 ±.06		1.2 ±.05						
17	DF			2.9 ±.30		1.9 ±.13	1.5 ±.17					
17	SO								2.6 ±.20	2.6 ±.18		2.5 ±.18

The mortality response of ants from mixed-age classes was compared with ants from a young, single-age class. We exposed three replicates of each age class to 1.0 and 1.2 ppm Dieldrin. When the CSDs were compared, the older, mixed-age ants died more rapidly than the young, callow ants (P 0 .001). The mean survival times were 5.40 and 7.90 days for mixed-age and callow ants, respectively, at 1.0 ppm and 5.00 and 6.98 days for mixed and callows, respectively, at 1.2 ppm.

Throughout this study when ants were exposed to marginally toxic concentrations, the mortality response varied considerably among the replicates. At increased concentrations, replicate responses were more similar. In the test comparing mixed-age ants with single-age ants, this variability was not seen at all in the single-age ants. At 1.0 ppm, the MST of the callow ants was not only 2.5 days later, but the responses of the three replicates were nearly identical, while the responses of the mixed-age ants were varied (Figure 2). At 1.2 ppm, the responses of the replicates in the mixed-age treatment became more similar, thus demonstrating the effect of increased concentration (Figure 2).

In another test (test #13), we explored the effect of random selection of ants on test repeatability and possible differences in the mortality responses. Using 30 ants per dish, three subgroups of 10 ants each were randomly allocated per dish for one treatment and six subgroups of five ants per dish randomly allocated for the other treatment. In the 10-ant combinations, there were significant differences ($P \leq 0.005$) in CSDs among the three replicate dishes. These differences suggest that although the same toxicant at the same concentration (Endrin, 1.2 ppm) was used in the three separate dishes, the repeatability was low. However, for the groups composed of subgroups of five ants, there were no significant differences ($P \geq 0.61$) in ant CSDs from the three dishes, which indicates better repeatability using randomly assigned subgroups of five ants per dish.

The results of test #13 should be interpreted cautiously since for both the five and 10 ant subgroup tests there were few mortalities, which may have adversely influenced the test statistics. All subsequent tests were conducted using randomly selected subgroups of five, and a marked improvement was seen within intra-replicate comparisons at the low concentrations.

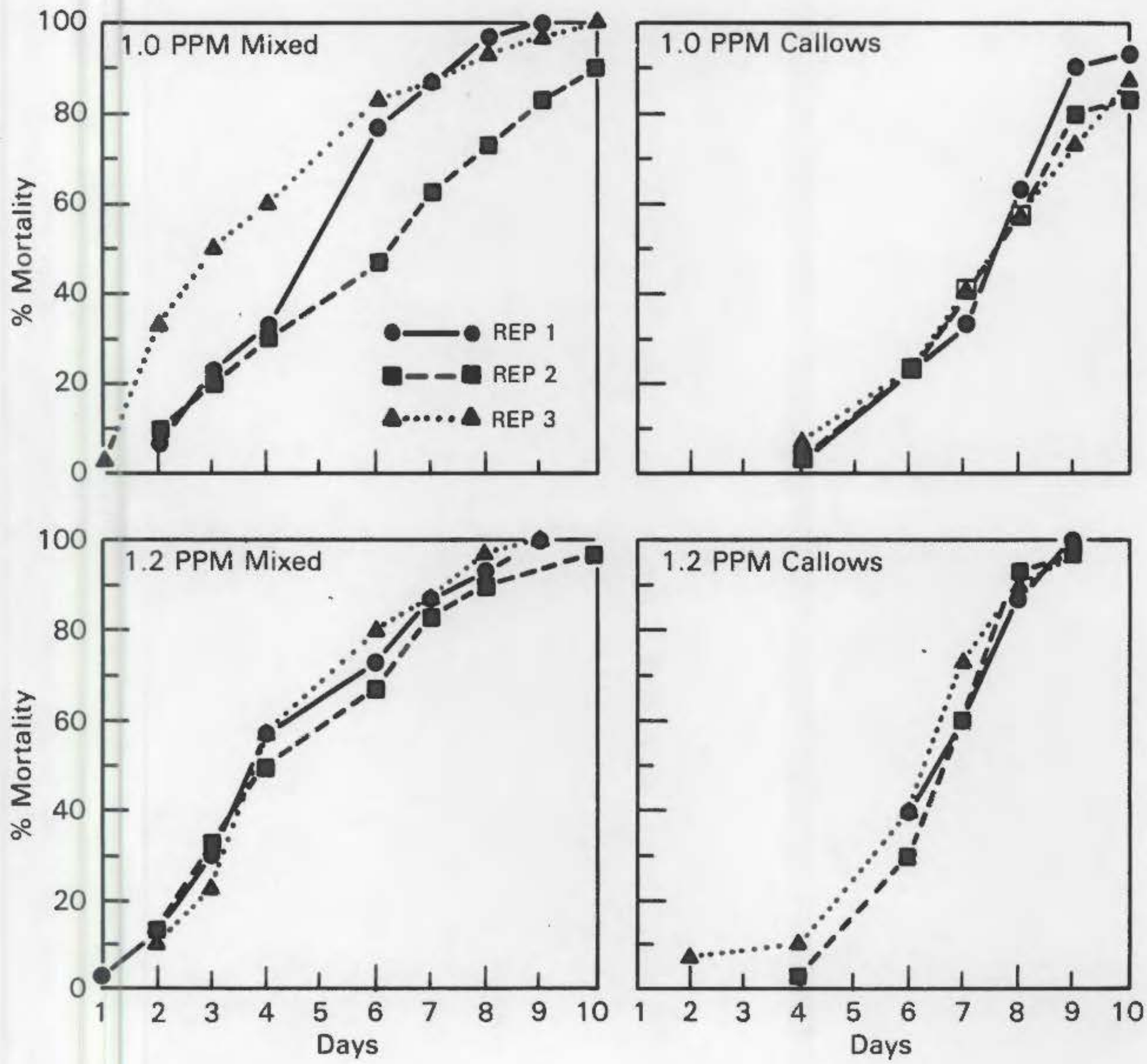

FIGURE 2. Mortality curves for callow ants (callows) and ants of mixed-ages (mixed) exposed to Dieldrin at 1.0 and 1.2 ppm showing the responses of the three replicates (Rep 1, 2, & 3) used in each treatment.

DISCUSSION

One of the main objectives of this study was to determine if harvester ants could be used successfully as a terrestrial bioassay organism. We explored several test conditions and variables that may influence the outcome of tests and evaluated the toxicity of 10 different compounds to harvester ants. Of the compounds tested, the insecticides and the complex waste residuals produced repeatable mortality responses, while the metals and 2,4-D failed to produce a response under the test conditions and concentrations used. Other concentrations or test conditions or a different means of delivering the toxicant to the ants may have produced a different result. However, the technique used here is a facsimile of a natural exposure in the environment and is consistent with our design, an important criterion in developing a bioassay.

Among the tests using insecticides and complex waste residuals, we found that relative toxicities remained consistent throughout. Aldrin was the most toxic followed by Dieldrin, Endrin, wood preservative sludge, drilling fluid, and slop oil. Comparisons of MSTs at particular concentrations were not and should not be made across different tests. In order to make these comparisons, additional knowledge concerning other variables is needed. Some of the variables that might cause test-to-test differences include colony-to-colony variation, time of year ants are collected, and metabolic and nutritional condition of ants. No attempt was made to address these variables. We did address those variables pertaining to possible effects from different substrates and the effect on mean survival times from using a population of mixed ages.

When substrates were tested, the silt-loam soil produced an effect much faster than the clay soil. This is not an unexpected result, since different soil types are likely to have different properties that influence the availability of a compound to biological systems. Filter paper containing Endrin and Dieldrin also produced a response much sooner than soil. If proven to be a suitable substrate, the use of filter paper could be advantageous since it is a standard medium; it provided more sensitivity to the test; and unlike soil, it was not prone to growing fungi on dead ants. However, since we could detect no difference in the CSDs from filter paper and soil when using Aldrin, further testing is needed to explore its potential usefulness. An advantage of soil as a substrate is that contaminated soil from a chemical waste site could be tested directly without having to extract the toxicants for the bioassay.

Age structure is a variable that cannot be controlled when collecting wild populations of ants. When we compared the response of mixed-age, older ants with single-age, younger ants exposed to Dieldrin at 1.0 ppm, more variability was observed among the replicates of older ants. This variability may not be entirely due to a mix of ages but is likely a result of natural heterogeneity or vigor within the population. This comparison illustrates the differences in sensitivity associated with a heterogeneous mix of individuals from a population and the similarity of sensitivity of a homogeneous cast. It also reinforces the need to randomly select ants for use in replicates.

From this study, harvester ants appear promising as a bioassay tool for determining relative toxicities of hazardous wastes. They can be collected by the thousands at any time of the year in nearly every western state and, unlike most other bioassay organisms, can be maintained for extended periods of time with little care. In this study, we showed that harvester ants were sensitive to certain hazardous chemicals, and the bioassays consistently prioritized these chemicals in the order of greatest toxicity to the ants.

REFERENCES

Breslow, N. 1970. A generalized Kruskal-Wallis test for comparing k samples subject to unequal patterns of censorship. Biometrika 57:579-594.

Burkhardt, C. C. and M. L. Fairchild. 1967. Toxicity of insecticides to house crickets and bioassay of treated soils in the laboratory. J. Econ. Entomol. 60:1497-1503.

Dixon, W. J. ed. 1983. BMDP statistical software. Revised printing. Univ. of Calif. Press. 733 p.

Finney, D. J. 1971. Statistical methods in biological assay. Second ed. Griffin Press, London.

Gano, K. A. 1981. Mortality of the harvester ant (Pogonomyrmex owyheei) after exposure to ^{137}Cs gamma radiation. Environ. Entomol. 10:39-44.

Hewlett, P. S. and R. L. Plackett. 1979. The interpretation of quantal responses in biology. Univ. Park Press. Baltimore. 82 p.

Kaplan, E. L. and P. Meier. 1958. Nonparametric estimation from incomplete observations. J. Amer. Statist. Assoc. 53:457-481.

Mantel, N. 1966. Evaluation of survival data and two new rank order statistics arising in its consideration. Cancer Chemotherapy Reports 50:163-170.

Porcella, D. B. 1983. Protocol for bioassement of hazardous waste sites. EPA 600/2:83:054, U.S. Environmental Protection Agency. Corvallis, OR.

Spangler, H. G. 1973. Ant nests for observation and study. Ann. Entomol. Soc. Am. 66:691-692.

Thomas, J. M., J. F. Cline, C. E. Cushing, M. C. McShane, J. E. Rogers, L. E. Rogers, J. C. Simpson, and J. R. Skalski. 1983. Field evaluation of hazardous waste site bioassessment protocols. PNL-4614, Pacific Northwest Laboratory, Richland, Washington.

Walton, B. T. 1980. Differential life-stage susceptibility of Acheta deomesticus to Acridine. Environ. Entomol. 9:18-20.

APPENDIX A

TEST #9

APPENDIX A

TEST #9

Test #9 was the first replicated test using the complex industrial waste residuals and was run for 15 days. The concentrations used were, 1.0%, 0.75%, 0.50%, and 0.25% for both Wood Preservative Sludge and Drilling Fluid. The toxic range for Slop Oil had not been found yet, so only one replicate was used for each concentration. These were 5.0%, 2.5%, 1.25%, and 0.63%. the controls for this test contained water at 10% moisture.

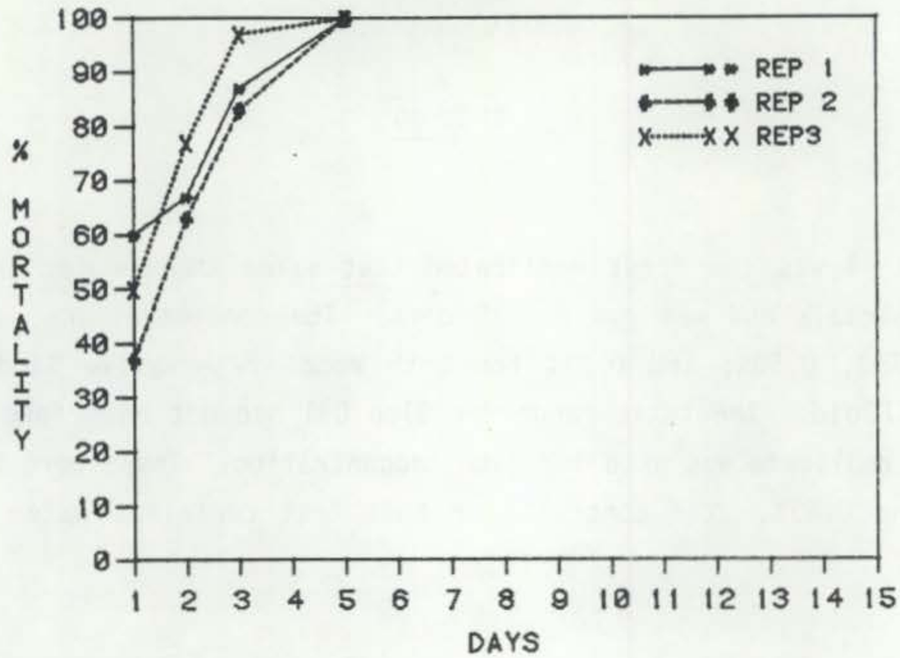

FIGURE A.1. Percent Mortality of Ants Exposed to Wood Preservative Sludge at 1.0%

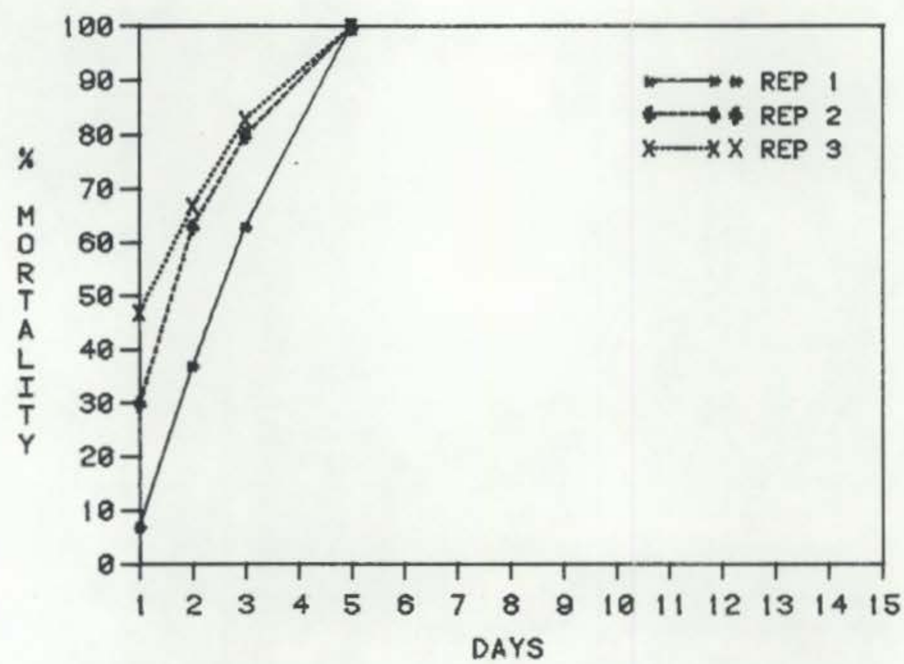

FIGURE A.2. Percent Mortality of Ants Exposed to Wood Preservative Sludge at 0.75%

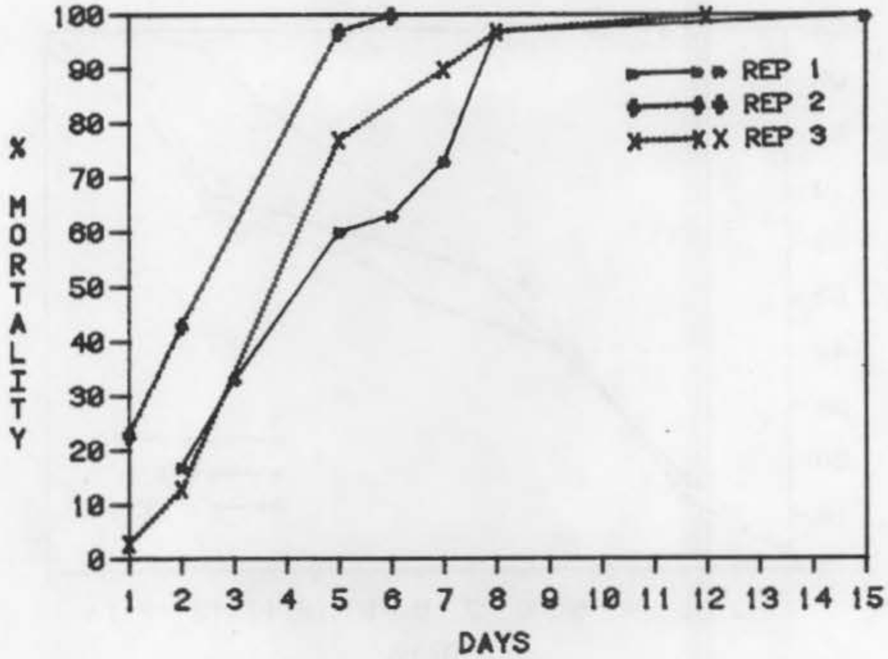

FIGURE A.3. Percent Mortality of Ants Exposed to Wood Preservative Sludge at 0.50%

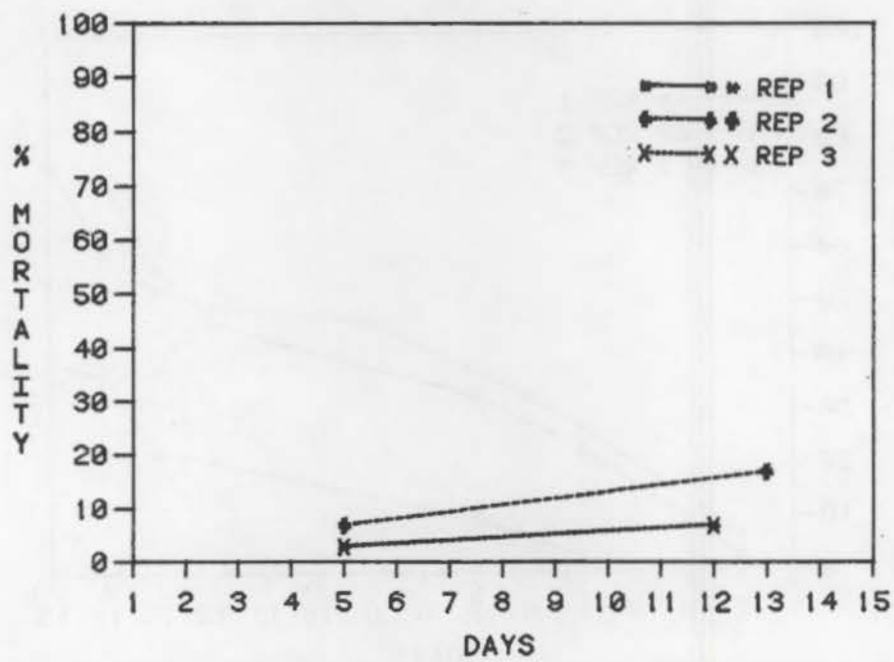

FIGURE A.4. Percent Mortality of Ants Exposed to Wood Preservative Sludge at 0.25%

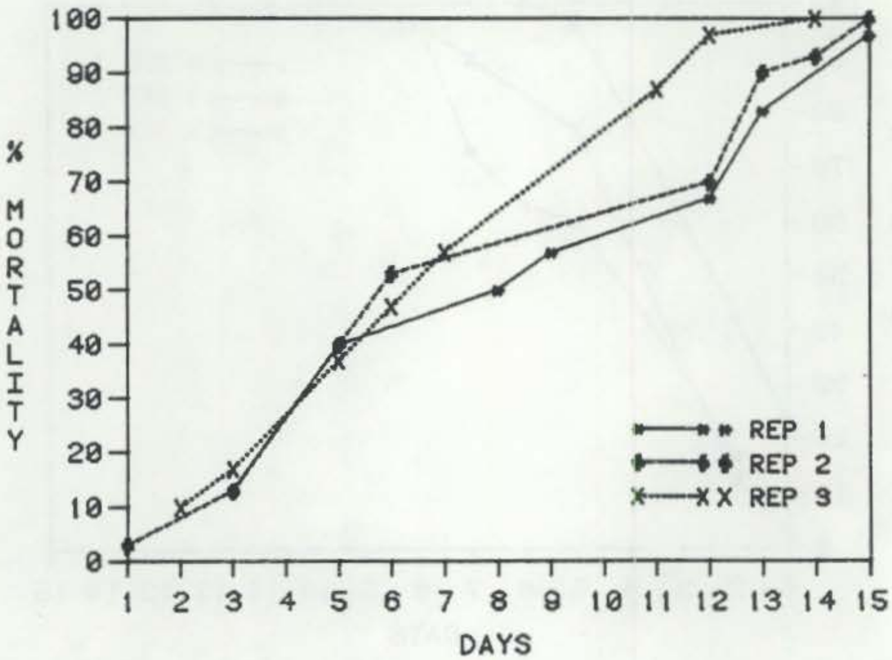

FIGURE A.5. Percent Mortality of Ants Exposed to Drilling Fluid at 1.0%

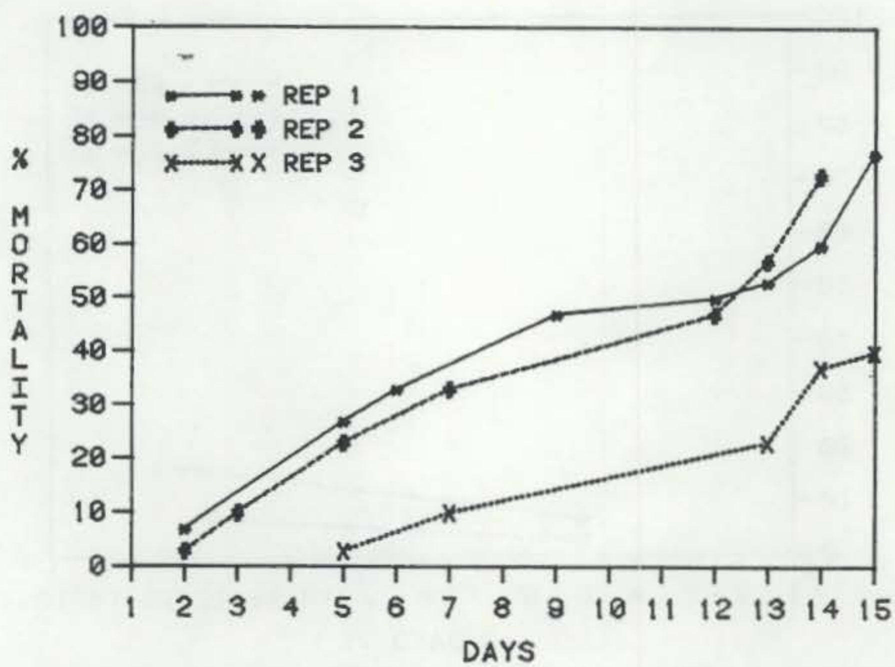

FIGURE A.6. Percent Mortality of Ants Exposed to Drilling Fluid at 0.75%

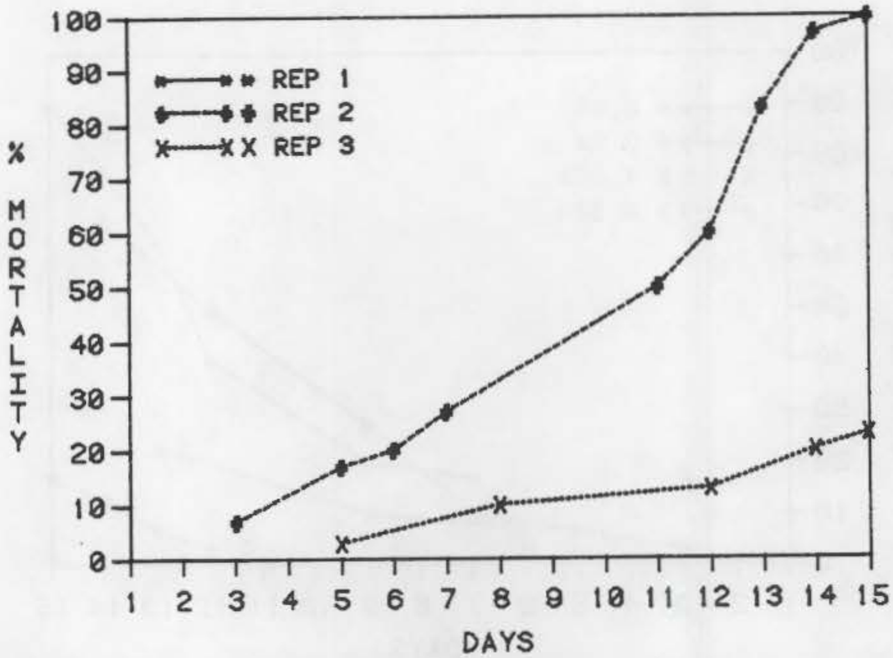

FIGURE A.7. Percent Mortality of Ants Exposed to Drilling Fluid at 0.50%

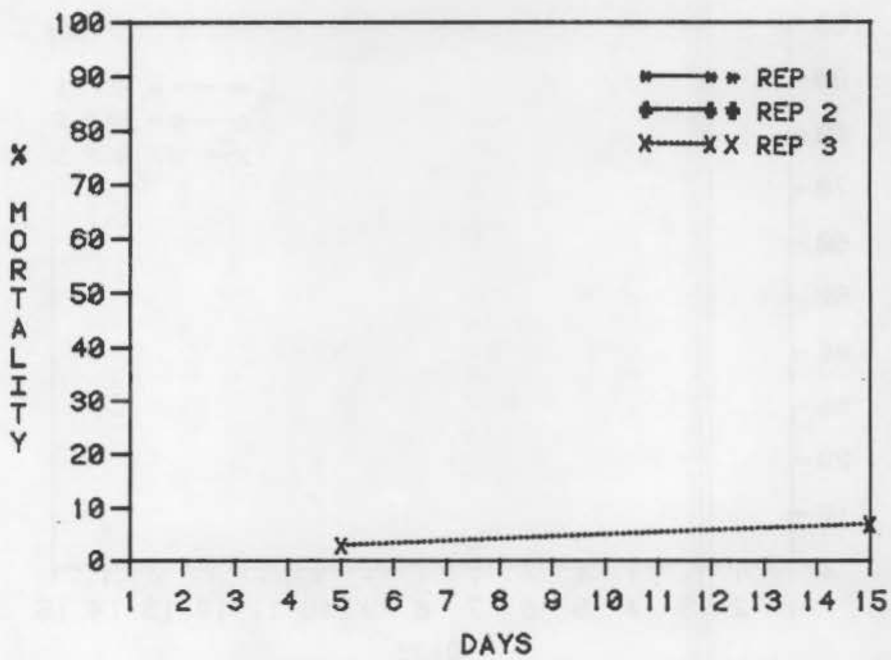

FIGURE A.8. Percent Mortality of Ants Exposed to Drilling Fluid at 0.25%

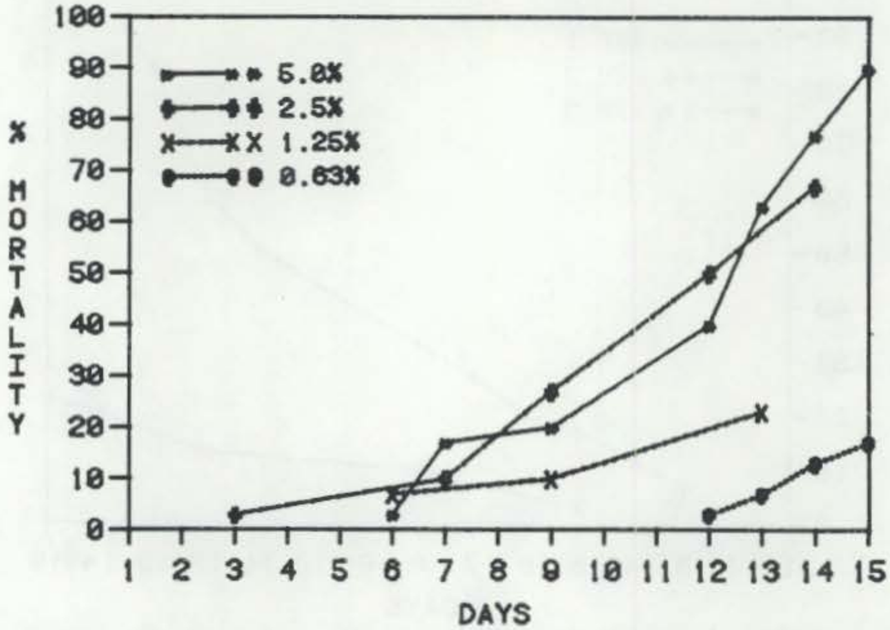

FIGURE A.9. Percent Mortality of Ants Exposed to Slop Oil at 5.0, 2.5, 1.25, and 0.63%

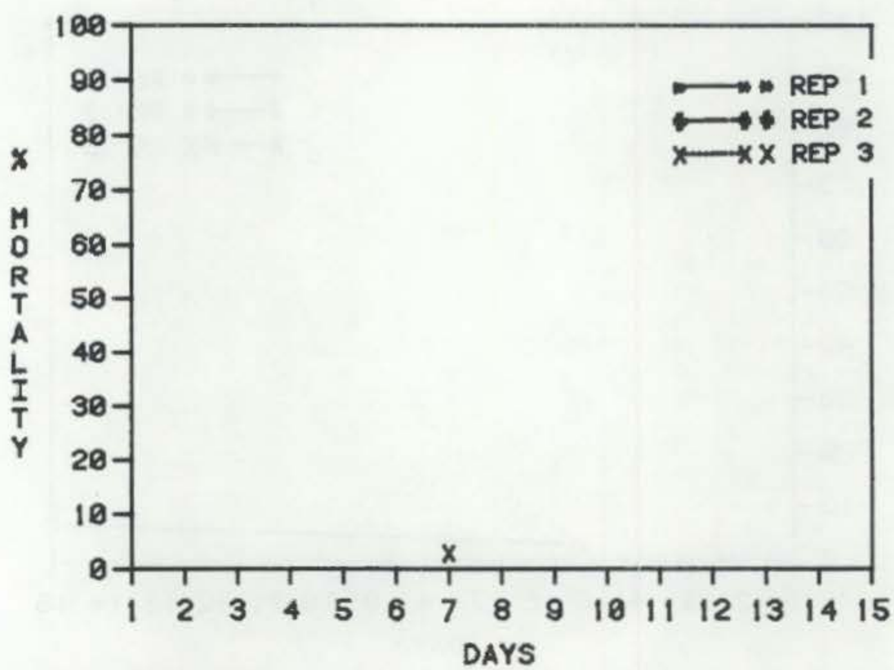

FIGURE A.10. Percent Mortality of Ants Exposed to 10% Moisture as a Control Treatment

APPENDIX B

TEST #11

APPENDIX B

TEST #11

Test #11 was the first replicated test using insecticides. It was a 14 day test using the following concentrations (in ppm):

Endrin	Aldrin	Dieldrin	2,4-D	Controls
0.4	1.2	1.8	400	5 ml Meth Evap
0.6	1.4	2.2	800	H_2O Only
0.8	1.6	2.6		
1.0	1.8	3.0		
		3.4		

The treatments for 2,4-D were not replicated. Two sets of controls were used in this test. The first set was treated as a sham treatment where 5 ml of methanol were added to the 10 g of soil in each dish and then evaporated off and water added for 5% moisture before adding ants. Only one replicate (Rep 2) sustained mortalities, the other two had none. The second set of controls contained soil with only distilled water added at 5% moisture.

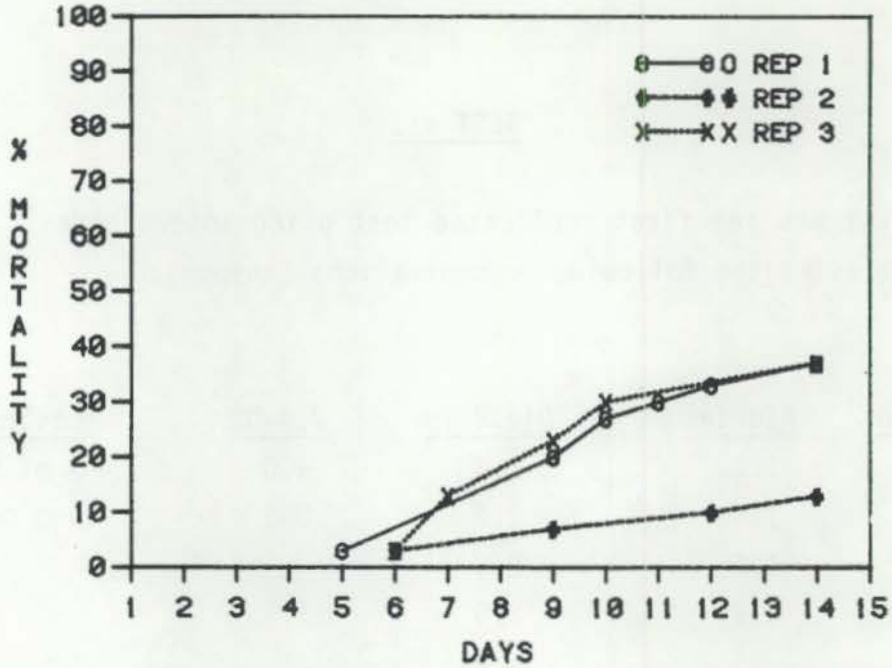

FIGURE B.1. Percent Mortality of Ants Exposed to Endrin at 0.4 ppm

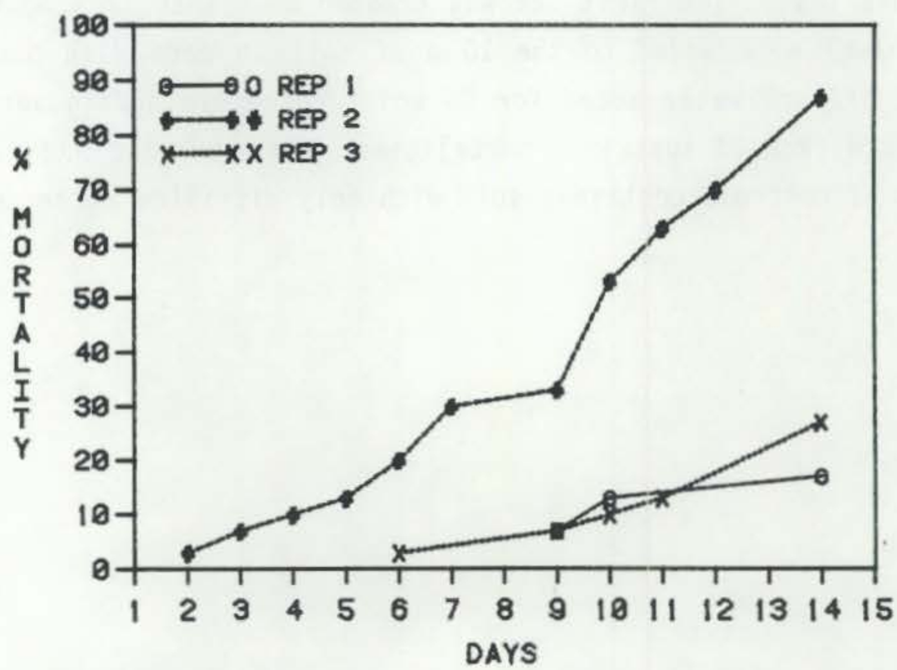

FIGURE B.2. Percent Mortality of Ants Exposed to Endrin at 0.6 ppm

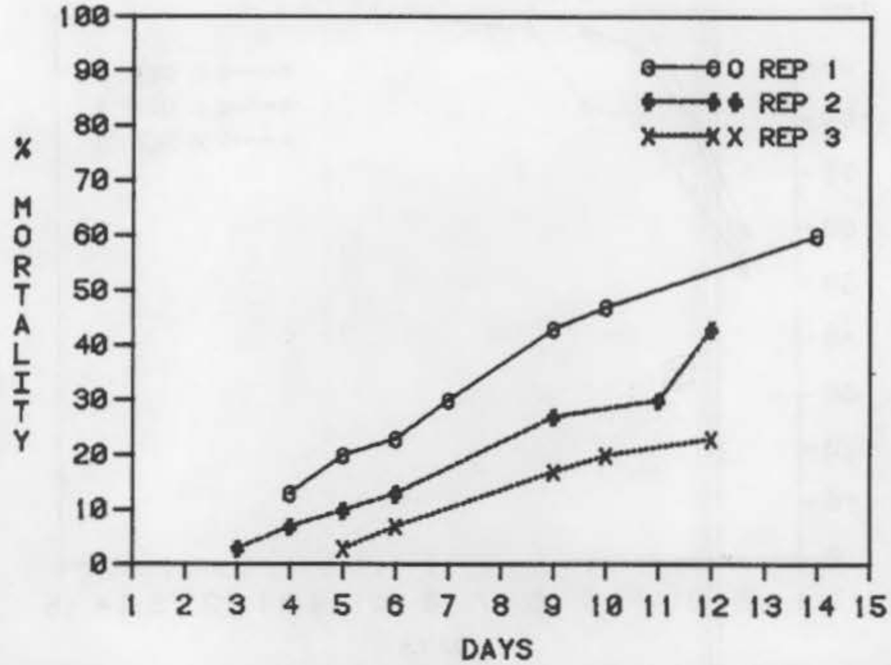

FIGURE B.3. Percent Mortality of Ants Exposed to Endrin at 0.8 ppm

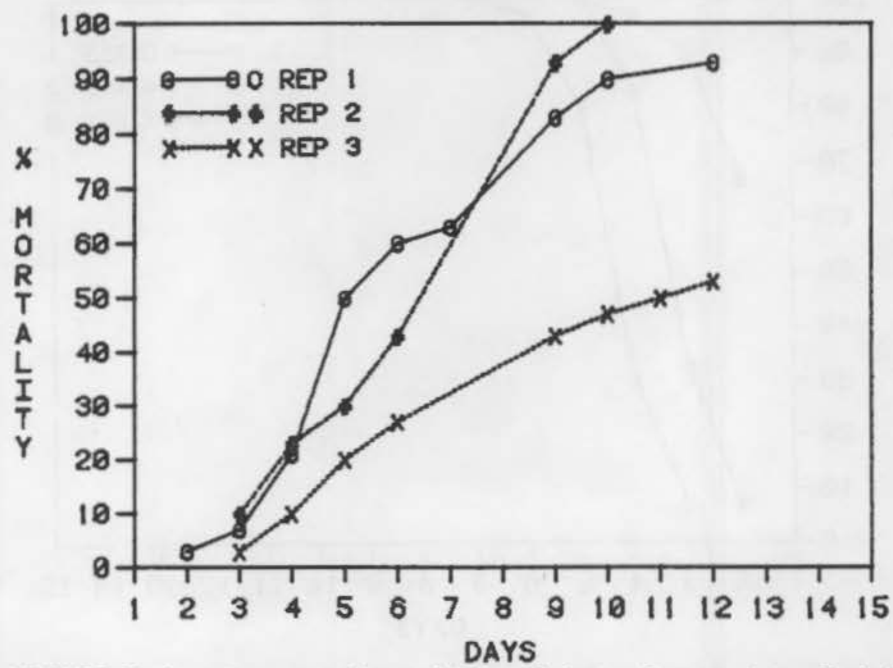

FIGURE B.4. Percent Mortality of Ants Exposed to Endrin at 1.0 ppm

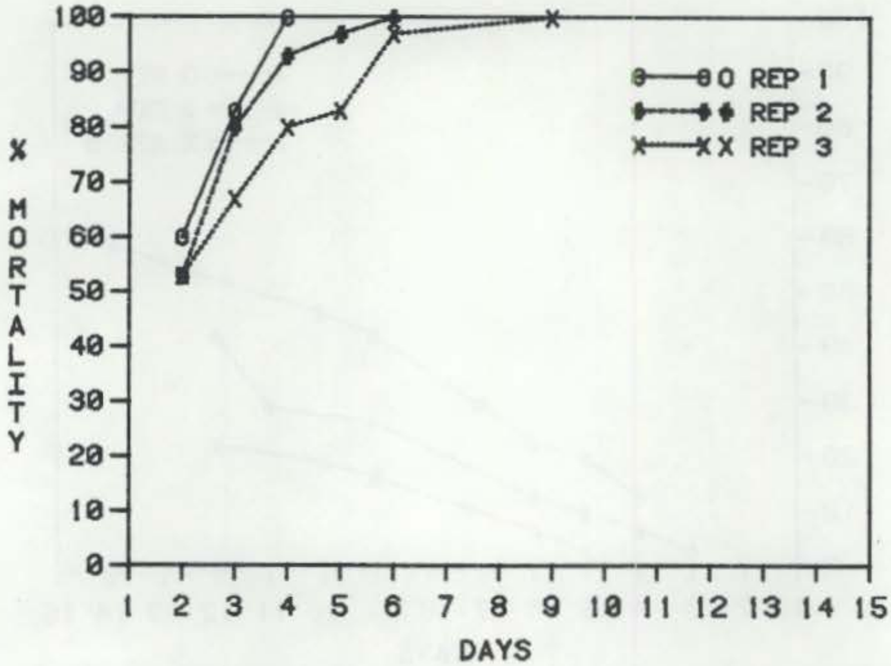

FIGURE B.5. Percent Mortality of Ants Exposed to Aldrin at 1.2 ppm

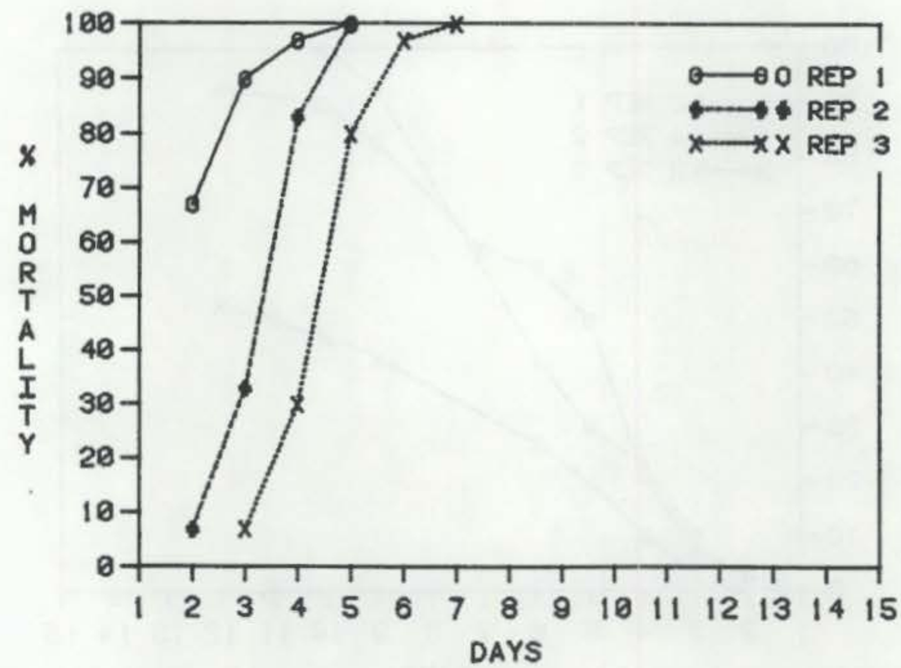

FIGURE B.6. Percent Mortality of Ants Exposed to Aldrin at 1.4 ppm

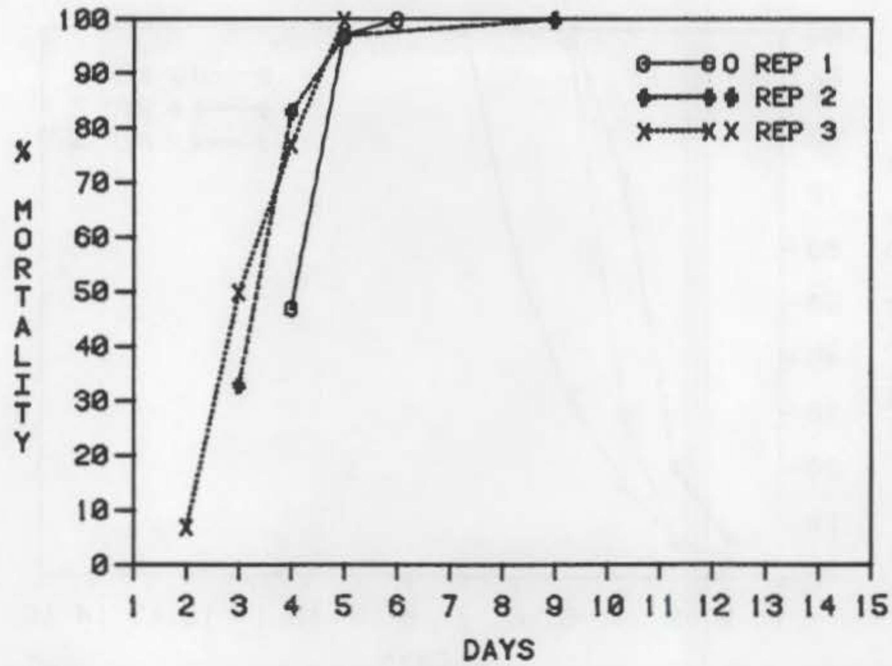

FIGURE B.7. Percent Mortality of Ants Exposed to Aldrin at 1.6 ppm

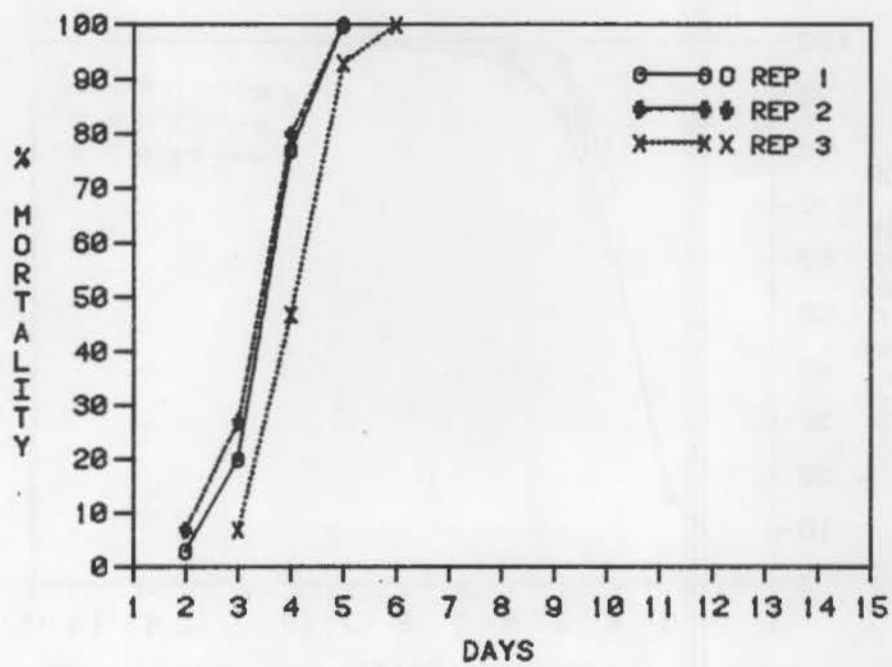

FIGURE B.8. Percent Mortality of Ants Exposed to Aldrin at 1.8 ppm

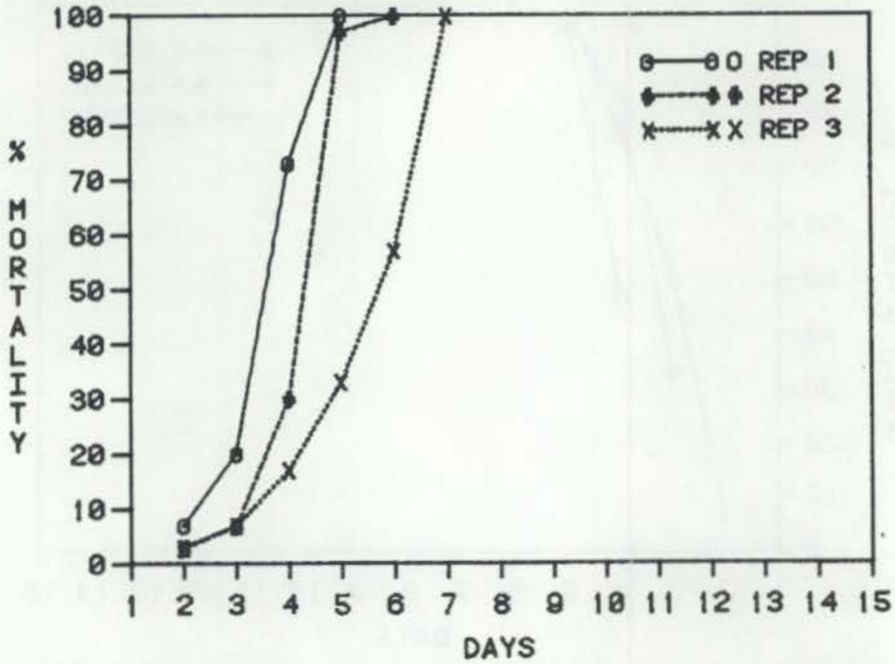

FIGURE B.9. Percent Mortality of Ants Exposed to Dieldrin at 1.8 ppm

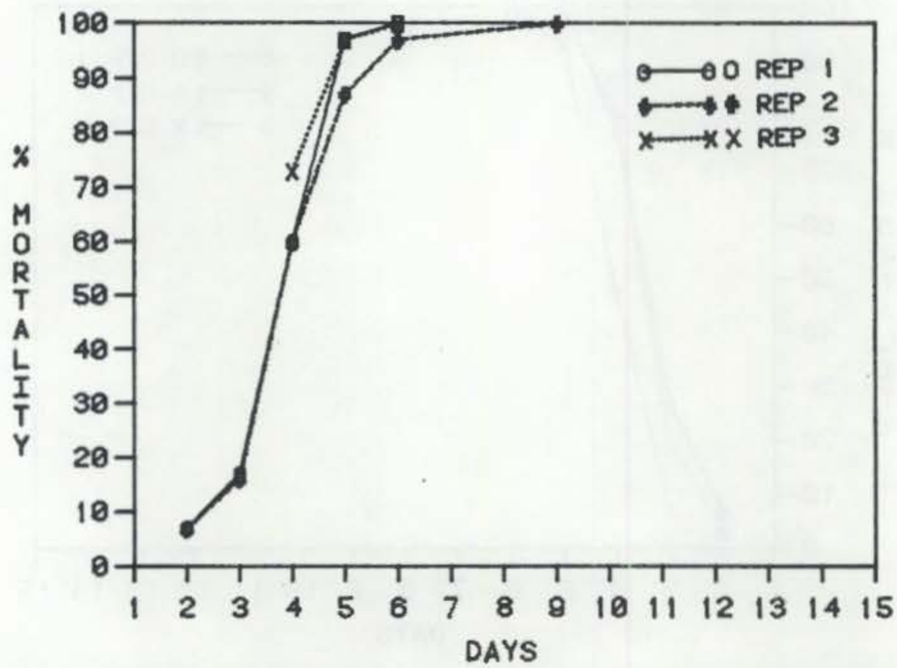

FIGURE B.10. Percent Mortality of Ants Exposed to Dieldrin at 2.2 ppm

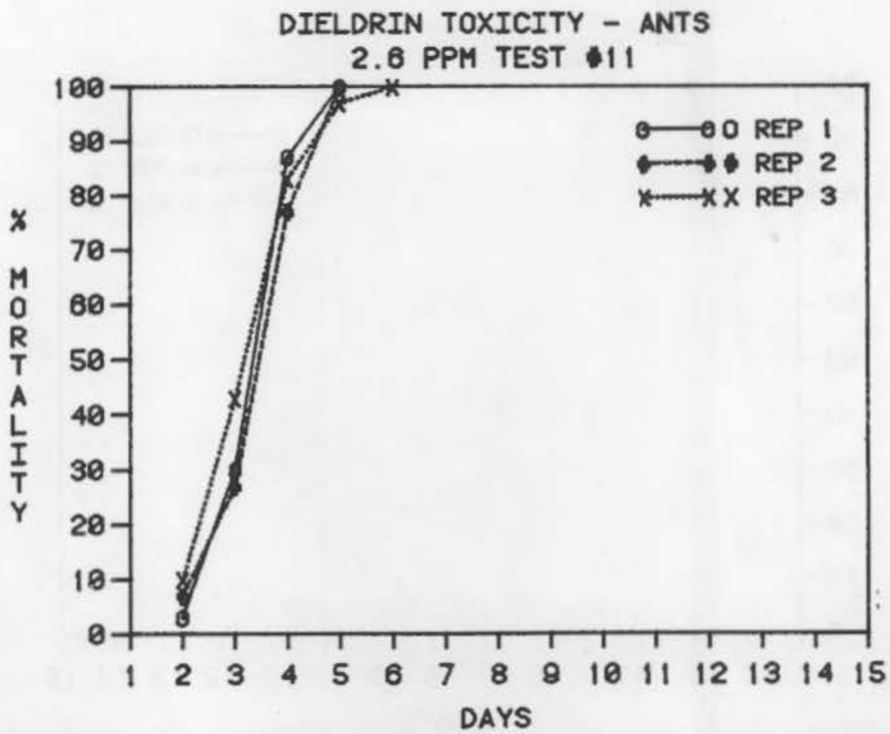

FIGURE B.11. Percent Mortality of Ants Exposed to Dieldrin at 2.6 ppm

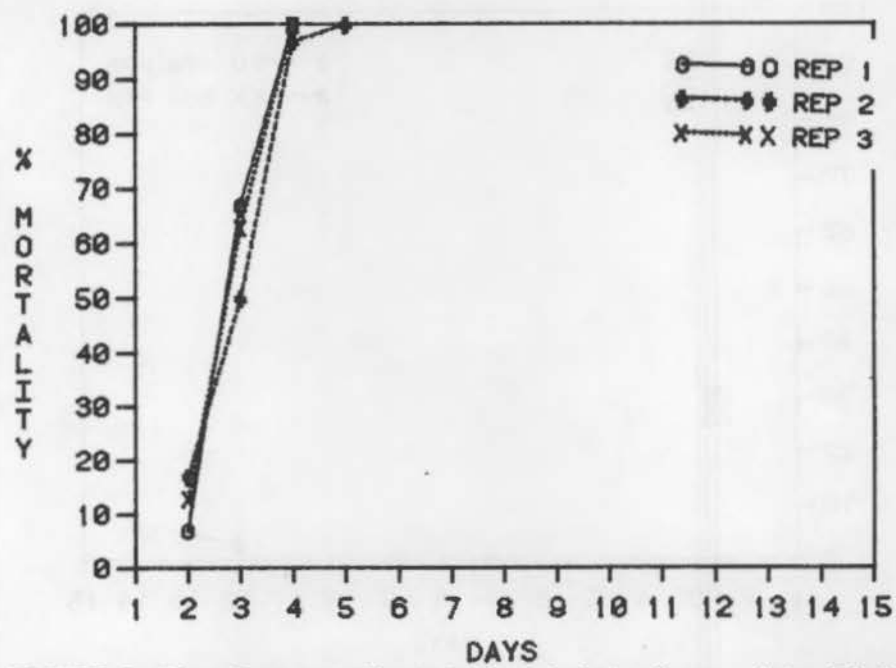

FIGURE B.12. Percent Mortality of Ants Exposed to Dieldrin at 3.0 ppm

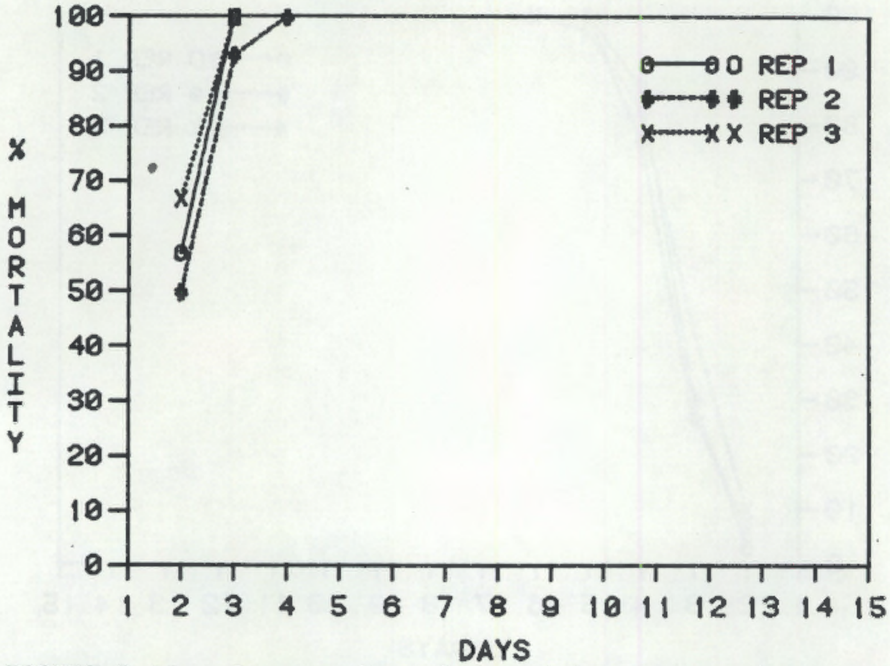

FIGURE B.13. Percent Mortality of Ants Exposed to Dieldrin at 3.4 ppm

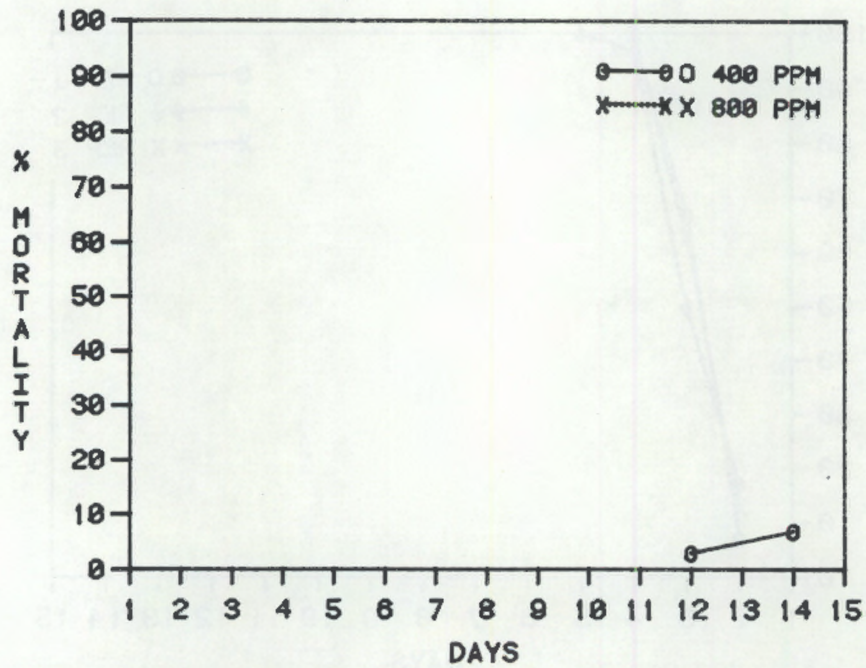

FIGURE B.14. Percent Mortality of Ants Exposed to 2,4-D at 400 and 800 ppm

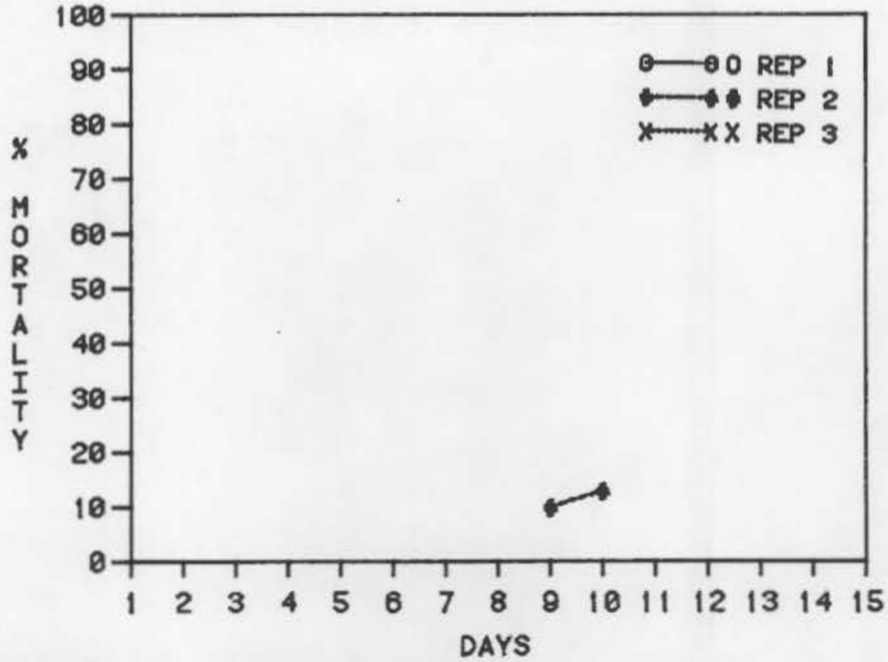

FIGURE B.15. Percent Mortality of Ants Exposed to Soil Treated with Methanol as a Control Treatment

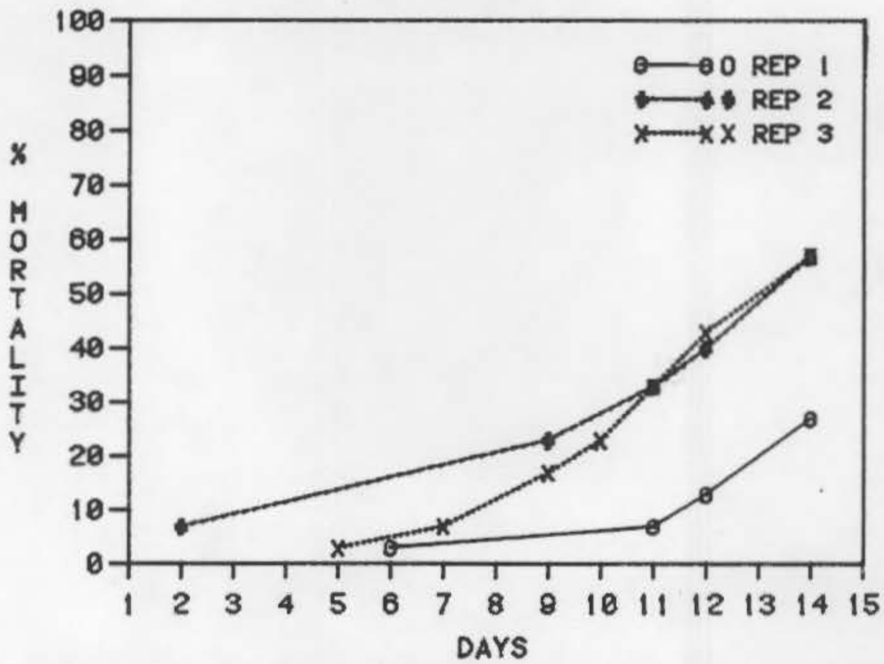

FIGURE B.16. Percent Mortality of Ants Exposed to Soil Treated with 0.5 ml Distilled Water as a Control Treatment

APPENDIX C

TEST #12

APPENDIX C

TEST #12

Test #12 repeated some of the concentrations used in test #11 and included many new concentrations in order to get a better definition of an LC_{50} within a 15 day test period. An additional treatment was included in this test to determine if there was any difference in mortality response based on vigor of the ants in the stock population. Endrin was used at 1.0 ppm for two different treatments. The first treatment included the first 90 ants (30 ants per replicate) to emerge from a beaker containing approximately 1700 ants. The second included the last 90 ants to emerge from the beaker.

Another comparison treatment was included in this test to see if there was a difference in the kind of substrate used. Filter paper was used for a substrate and compared against soil using the same quantity of insecticide for both. Controls for this test were given 5 ml of methanol, evaporated off, and .5 ml water added to give 5% soil moisture before adding ants.

Concentrations of insecticides used in this test include the following (expressed in ppm):

Endrin	Aldrin	Dieldrin	Control
1.0 FIRST Ants	0.8	0.8	Meth Evap.
1.0 LAST Ants	1.D	1.0	
1.2	1.2	1.2	
1.0 F-Paper	1.0 F-Paper	1.4	
		1.8	
		1.2 F-Paper	

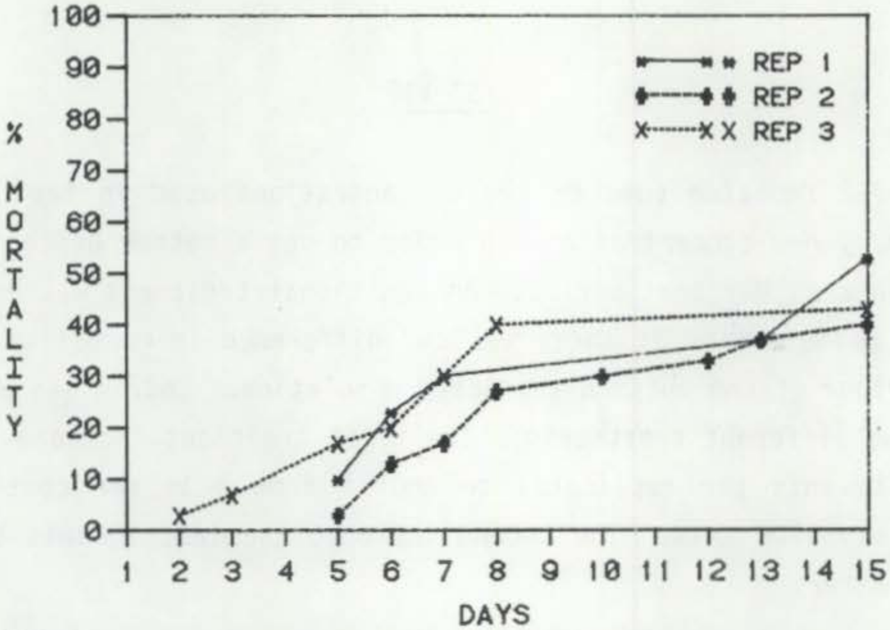

FIGURE C.1. Percent Mortality of "First" Ants Exposed to 1.0 ppm Endrin

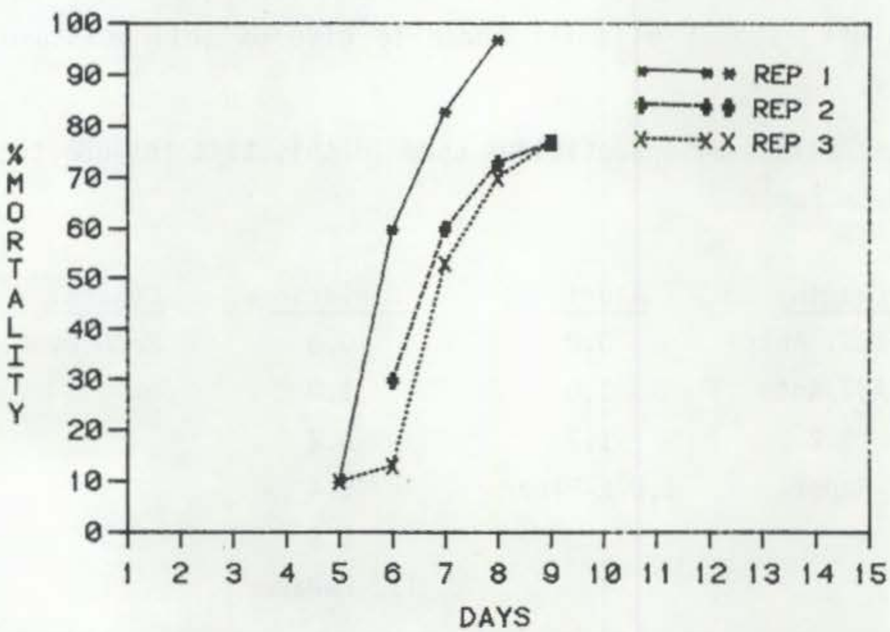

FIGURE C.2. Percent Mortality of "Last" Ants Exposed to 1.0 ppm Endrin

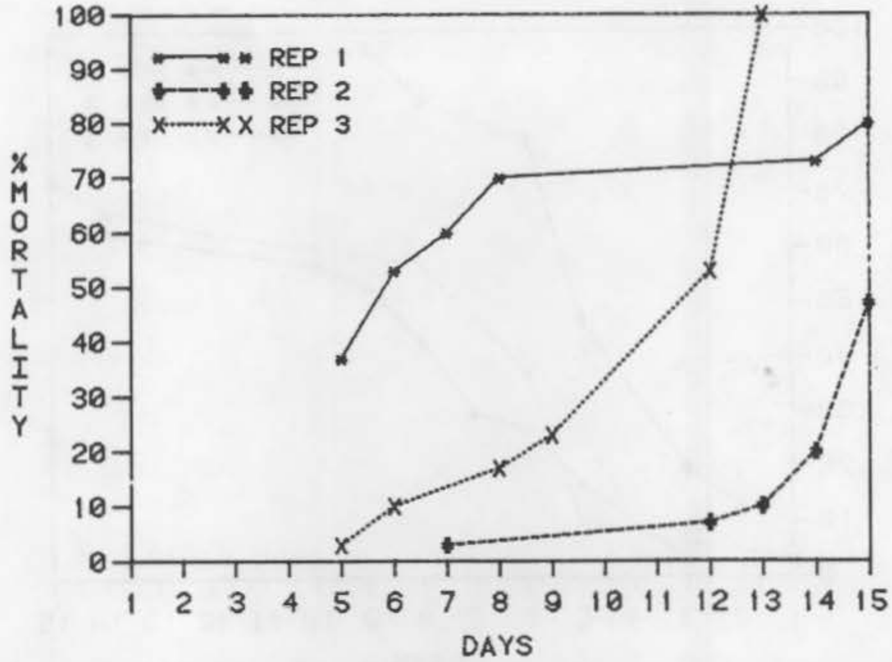

FIGURE C.3. Percent Mortality of Ants Exposed to 1.2 ppm Endrin

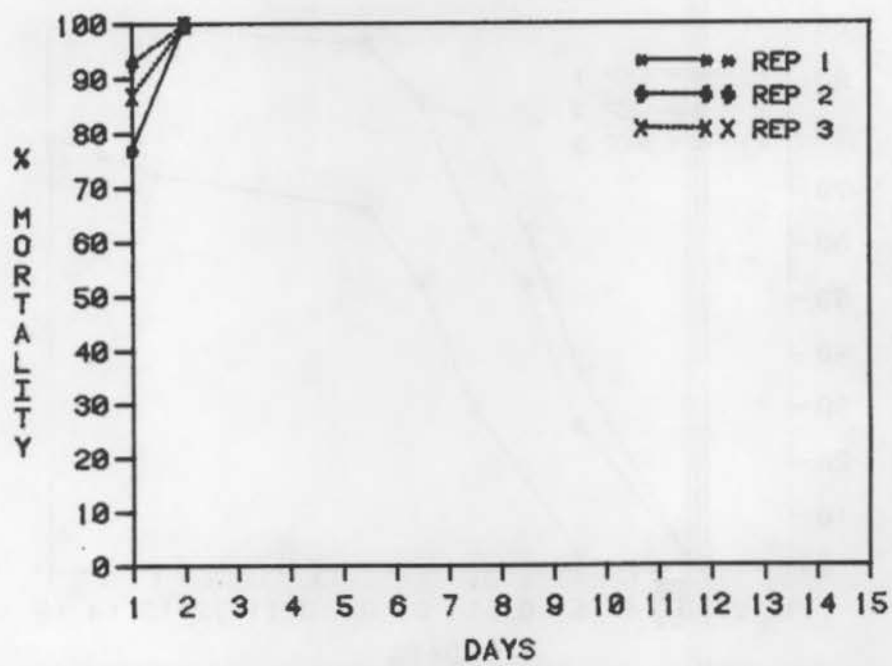

FIGURE C.4. Percent Mortality of Ants Exposed to 1.0 ppm Endrin on Filter Paper

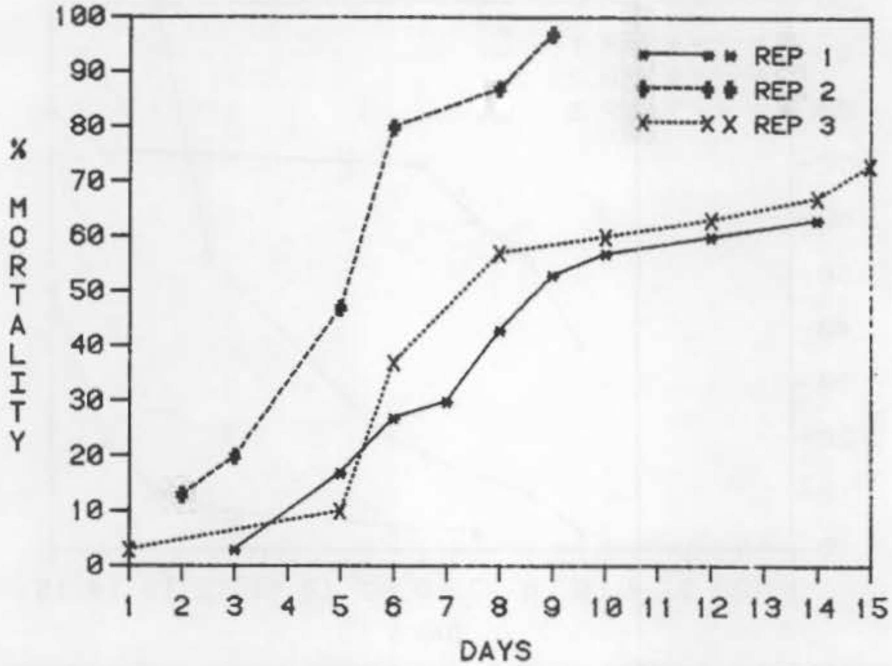

FIGURE C.5. Percent Mortality of Ants Exposed to 0.8 ppm Aldrin

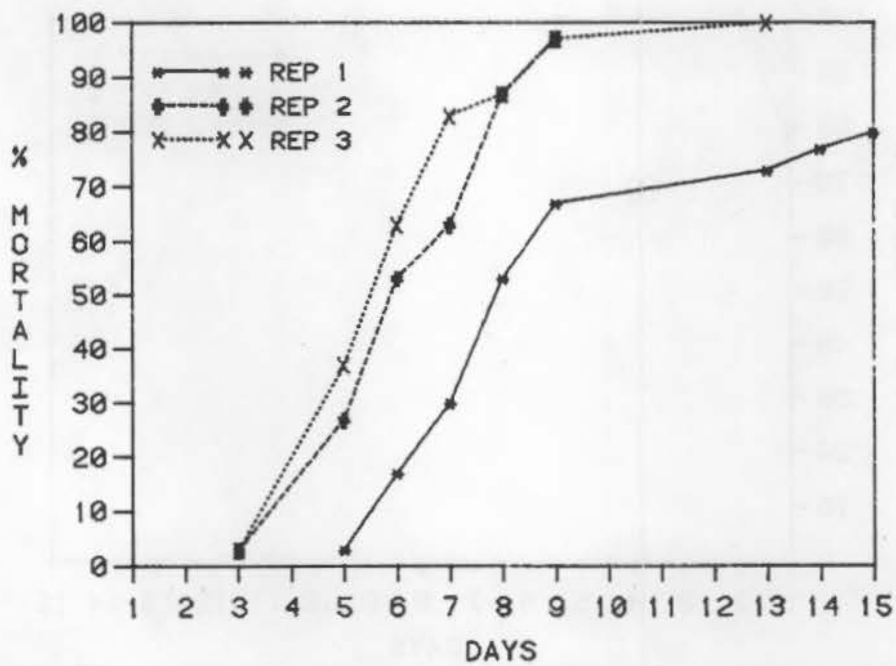

FIGURE C.6. Percent Mortality of Ants Exposed to 1.0 ppm Aldrin

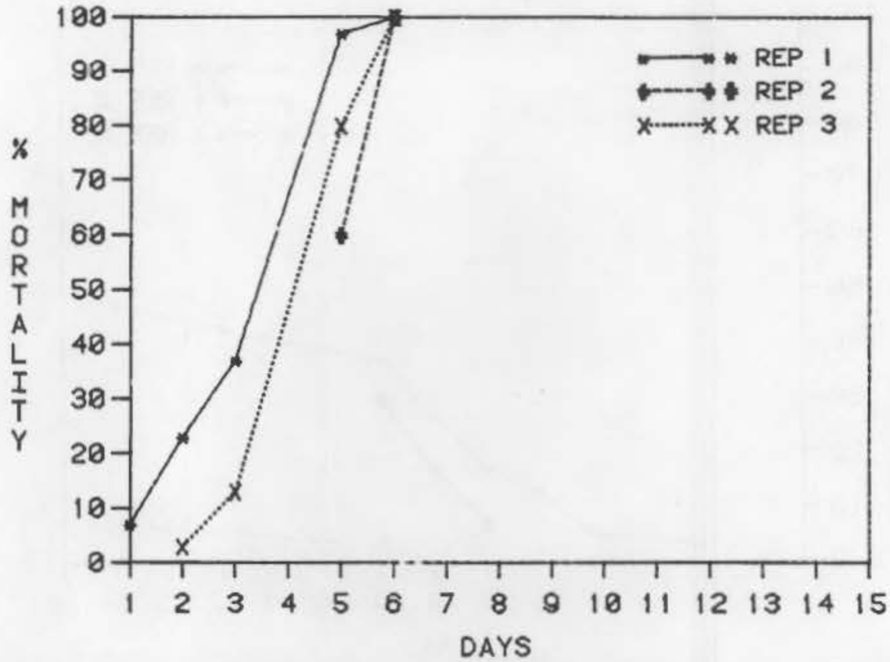

FIGURE C.7. Percent Mortality of Ants Exposed to 1.2 ppm Aldrin

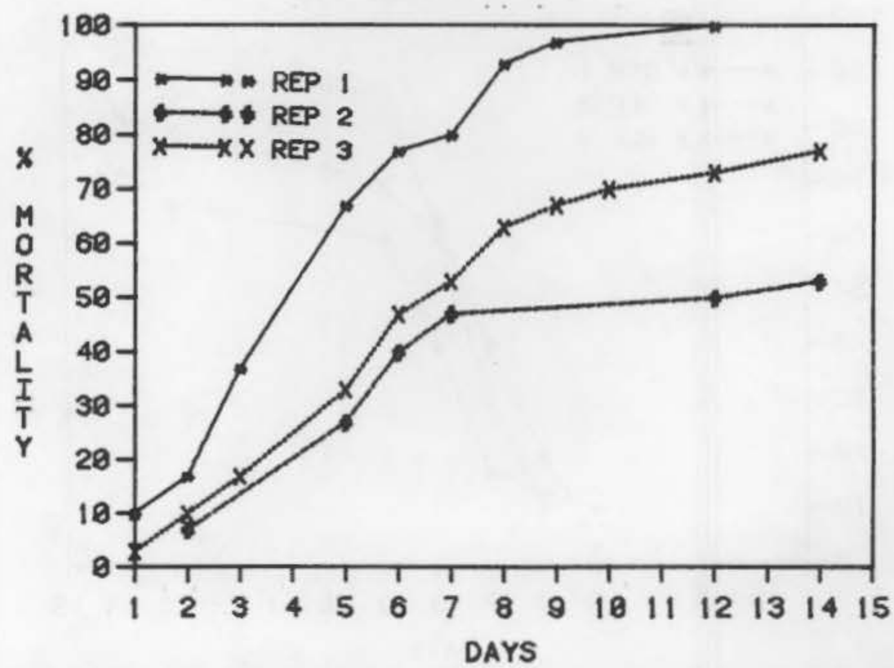

FIGURE C.8. Percent Mortality of Ants Exposed to 1.0 ppm Aldrin on Filter Paper

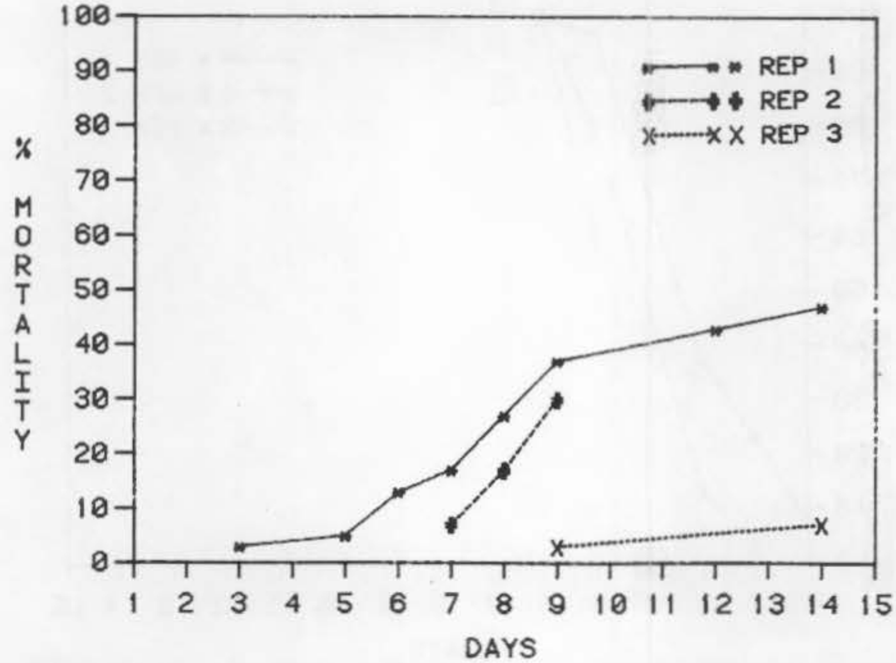

FIGURE C.9. Percent Mortality of Ants Exposed to 0.8 ppm Dieldrin

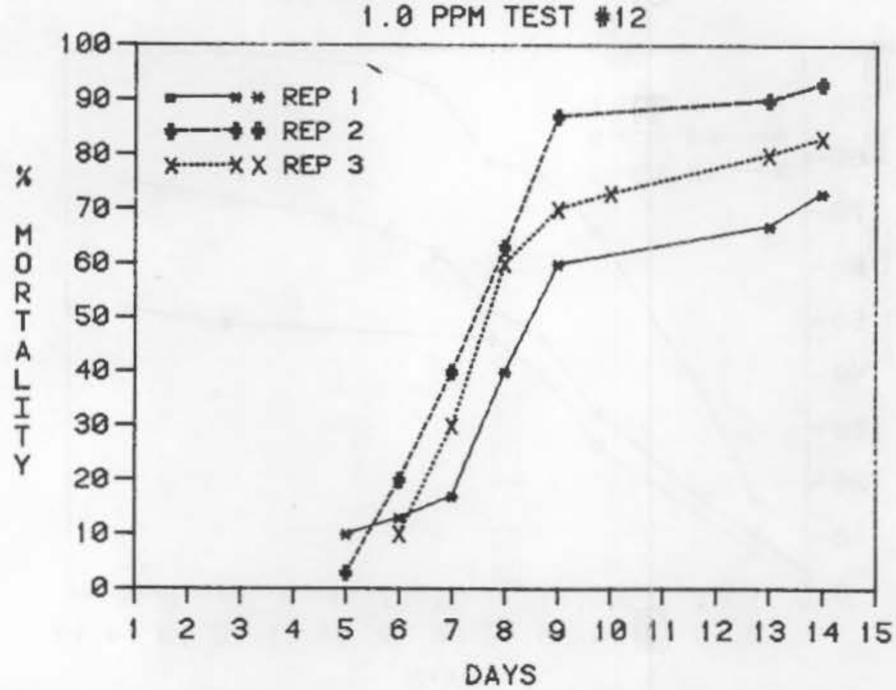

FIGURE C.10. Percent Mortality of Ants Exposed to 1.0 ppm Dieldrin

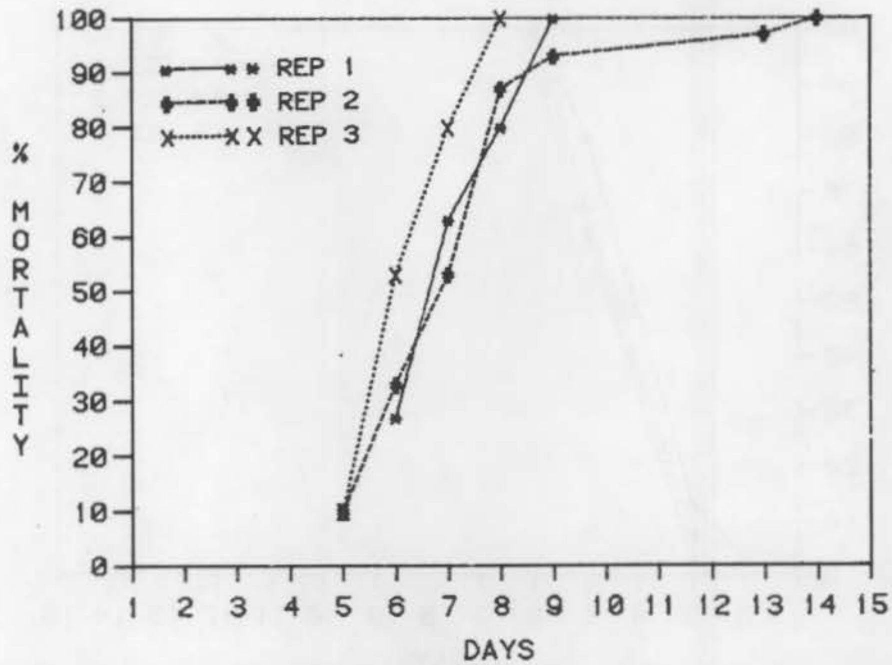

FIGURE C.11. Percent Mortality of Ants Exposed to 1.2 ppm Dieldrin

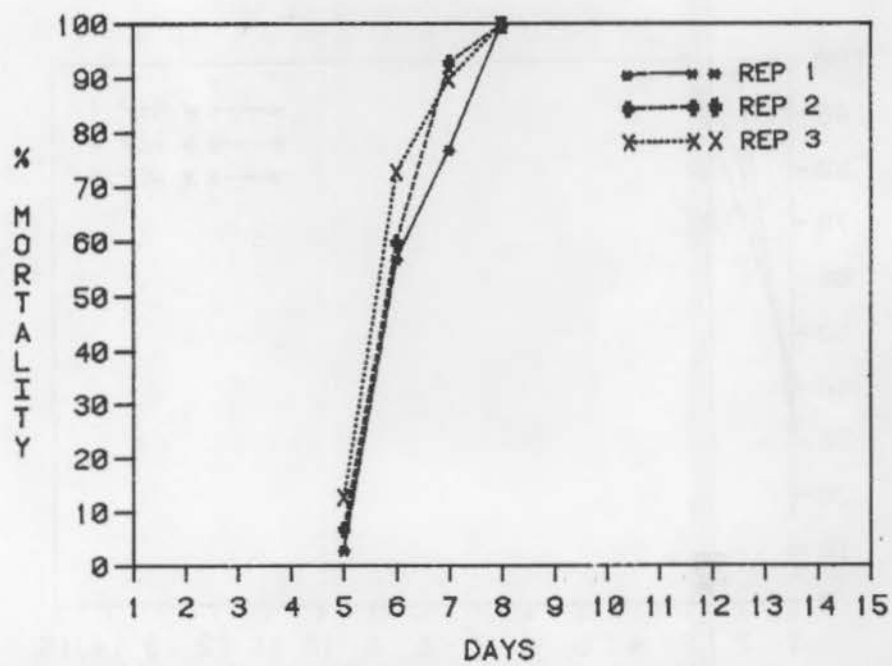

FIGURE C.12. Percent Mortality of Ants Exposed to 1.4 ppm Dieldrin

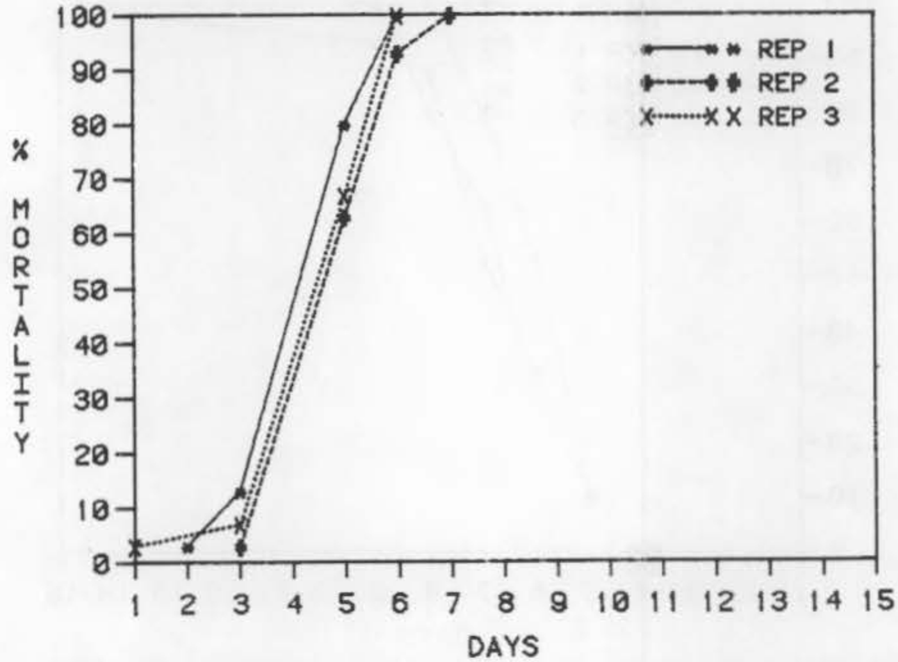

FIGURE C.13. Percent Mortality of Ants Exposed to 1.8 ppm Dieldrin

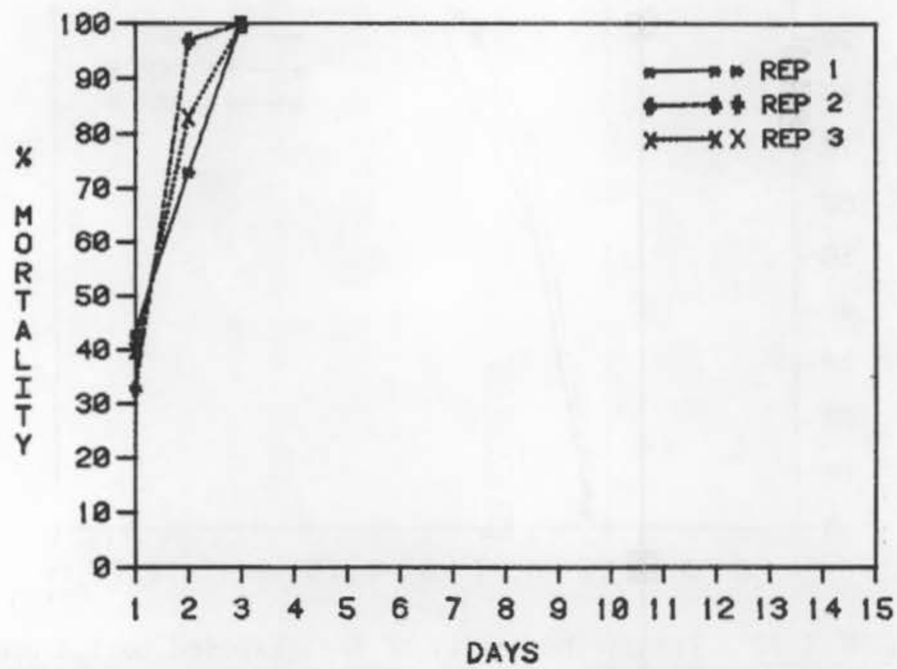

FIGURE C.14. Percent Mortality of Ants Exposed to 1.2 ppm Dieldrin on Filter Paper

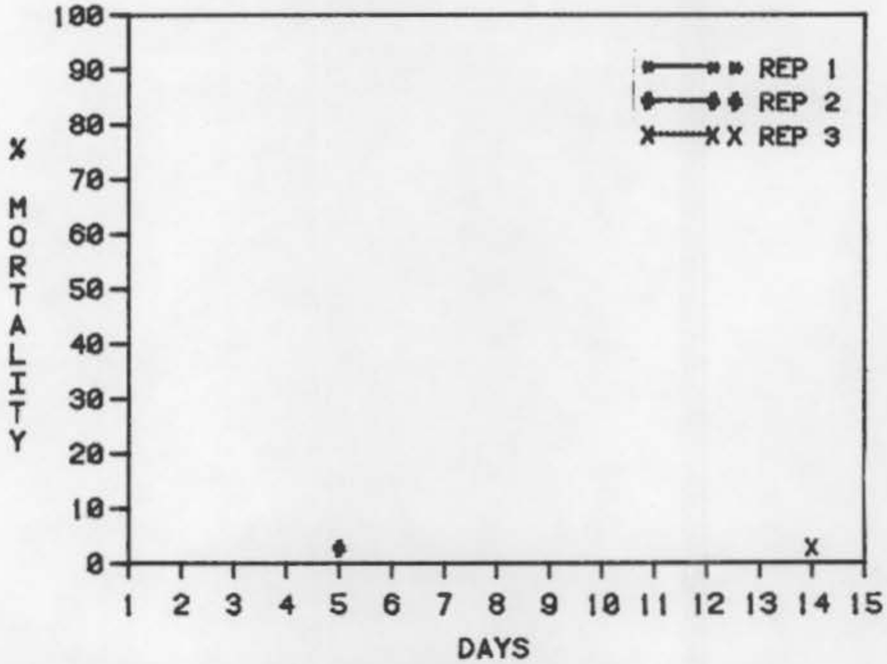

FIGURE C.15. Percent Mortality of Ants Exposed to Soil Treated with Methanol as a Control Treatment

APPENDIX D

TEST #13

APPENDIX D

TEST #13

Test #13 was an attempt to use randomly selected groups of ants to make up the total of 30 ants used for each replicate. The intention was to reduce the variability seen among replicates in past tests. Two differenct group sizes were used (3 groups of 10 and 6 groups of 5) to make up the needed 30 ants per replicate. Both were tested using Endrin at 1.2 ppm. This test was also run for 15 days.

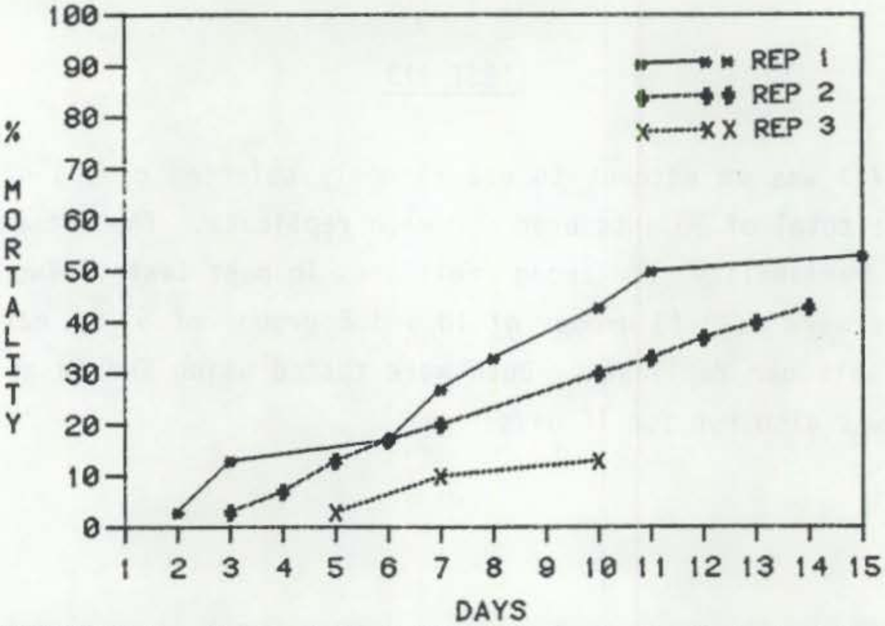

FIGURE D.1. Percent Mortality of Randomly Selected Ants (10 Ants per Group) Exposed to 1.2 ppm Endrin

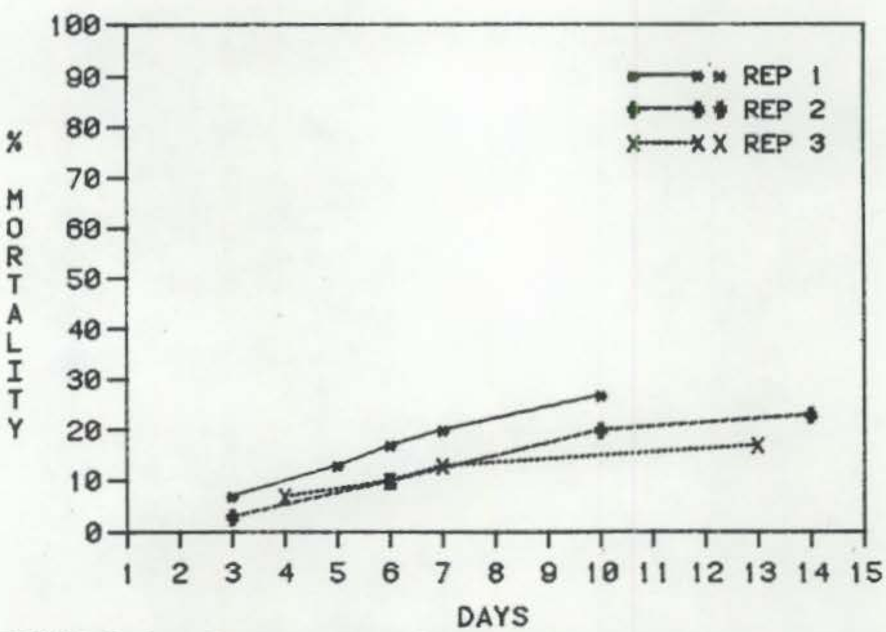

FIGURE D.2. Percent Mortality of Randomly Selected Ants (5 Ants per Group) Exposed to 1.2 ppm Endrin

APPENDIX E

TEST #14

APPENDIX E

TEST #14

Test #14 was the final test using 2,4-D. Ants did not respond in previous tests to concentrations as high as 800 ppm so this test extends that range to 16,000 ppm. One replicate, made up of 6 random groups of 5 ants each for a total of 30 ants, was used for each concentration. The treatments used were as follows: 1000, 2000, 4000, 6000, 8000, 9,500 and 16,000 ppm. Two replicates were used for controls with methanol added to the soil and evaporated off and water added for 5% moisture as in previous tests.

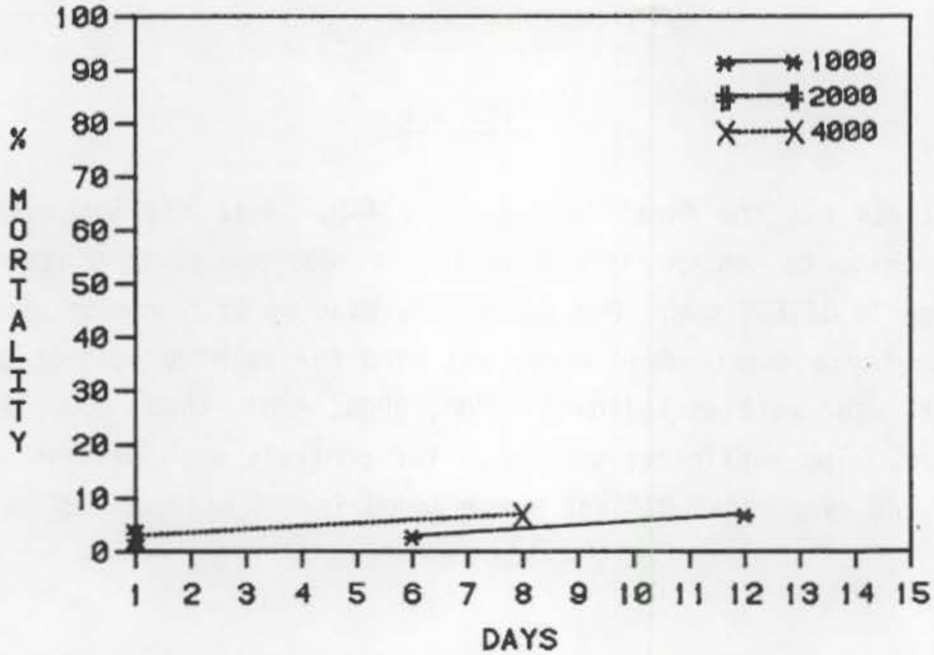

FIGURE E.1. Percent Mortality of Ants Exposed to 1000, 2000, and 4000 ppm 2,4-D

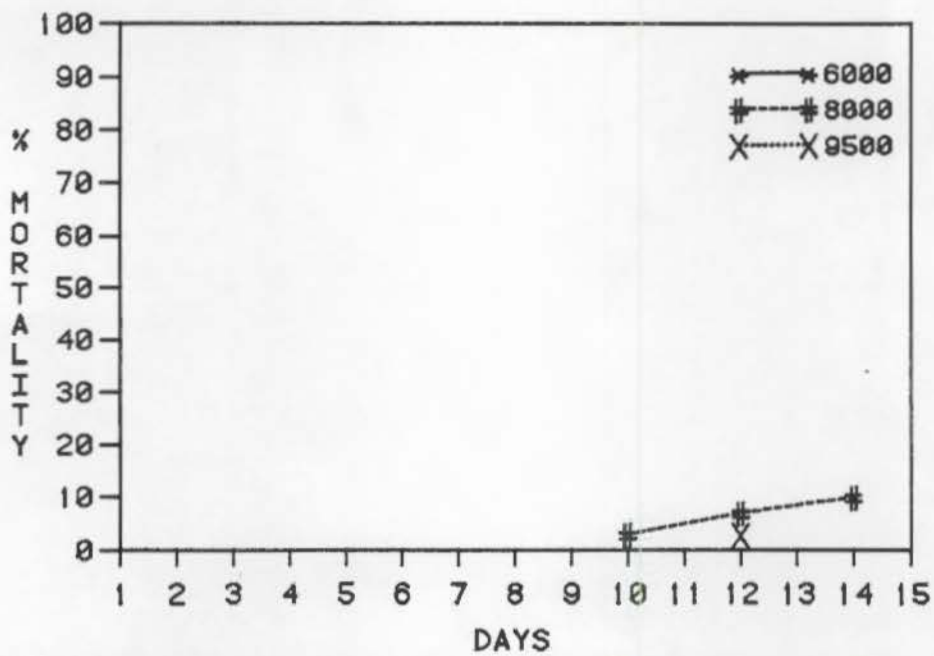

FIGURE E.2. Percent Mortality of Ants Exposed to 6000, 8000, and 9500 ppm 2,4-D

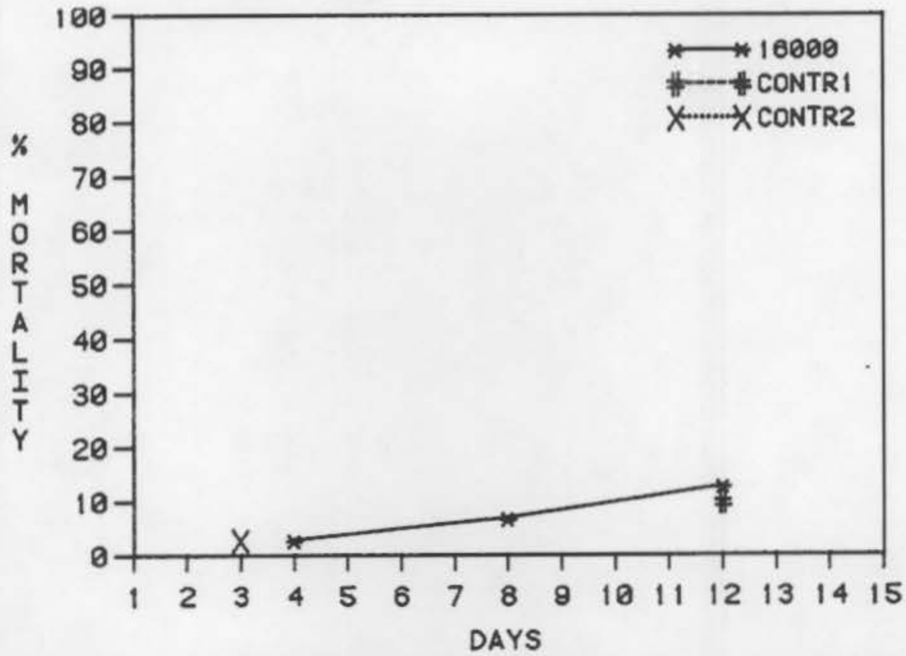

FIGURE E.3. Percent Mortality of Ants Exposed to 16000 ppm 2,4-D and Soil Treated with Methanol Evaporated Off as Controls

APPENDIX F

TEST #15

APPENDIX F

TEST #15

Test #15 was similar to #14 and used high concentrations of the metal chlorides of Cu, Zn, and Cd. Again one replicate of 6 random groups was used for each treatment. The treatments used are as follows:

Copper: 30,000 ppm on 10 g soil.
equivalent of 15,000 ppm on 10 g soil put on filter paper.
equivalent of 7,500 ppm on 10 g soil put on filter paper.

Zinc: 30,200 ppm on 10 g soil.
equivalent of 16,900 ppm on 10 g soil put on filter paper.
equivalent of 7,200 ppm on 10 g soil put on filter paper.

Cadmium: 30,000 ppm on 10 g soil.
equivalent of 15,000 ppm on 10 g soil put on filter paper.

Control: with 1 ml water added.

The metal salts were dissolved in 1 ml of distilled wter and added to 10 g of soil or filter paper. Again the mortality responses were low and not different from the controls after 14 days. The treatments containing zinc had no mortalities during the test. The treatments containing cadmium had only one mortality in the 15,000 ppm concentration.

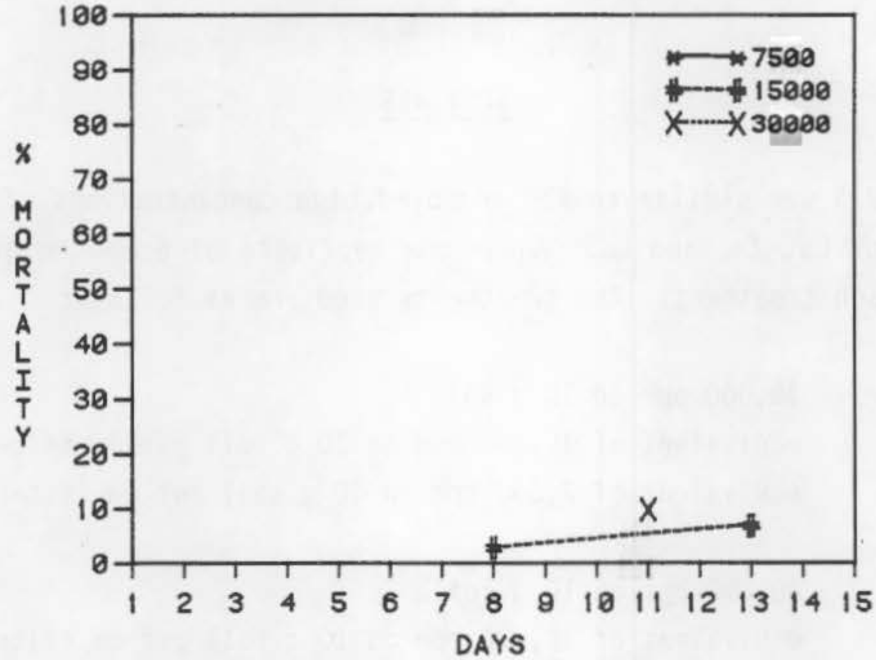

FIGURE F.1. Percent Mortality of Ants Exposed to 7,500, 15,000 and 30,000 ppm Copper

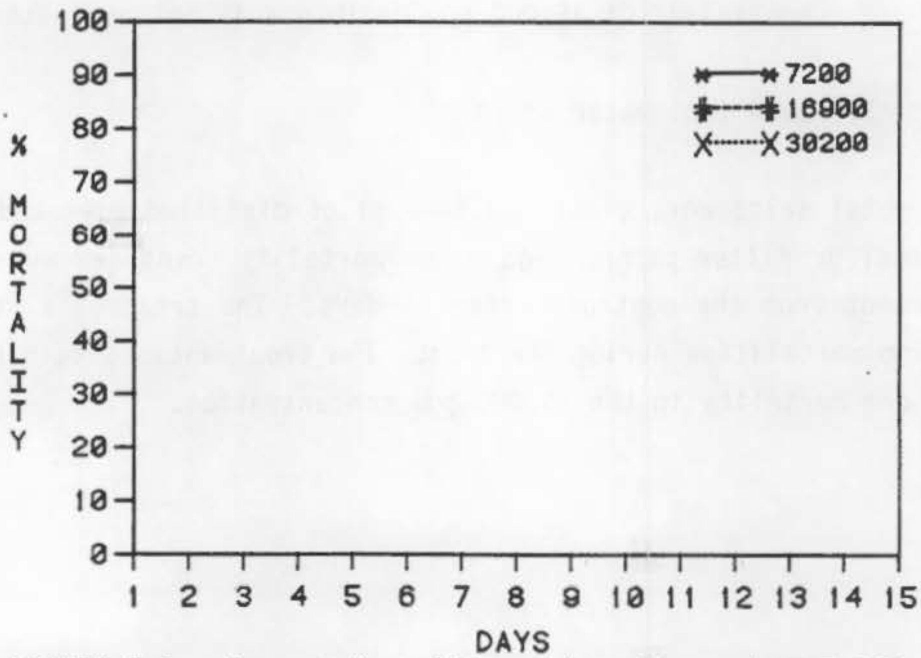

FIGURE F.2. Percent Mortality of Ants Exposed to 7,200, 16,900 and 30,200 ppm Zinc

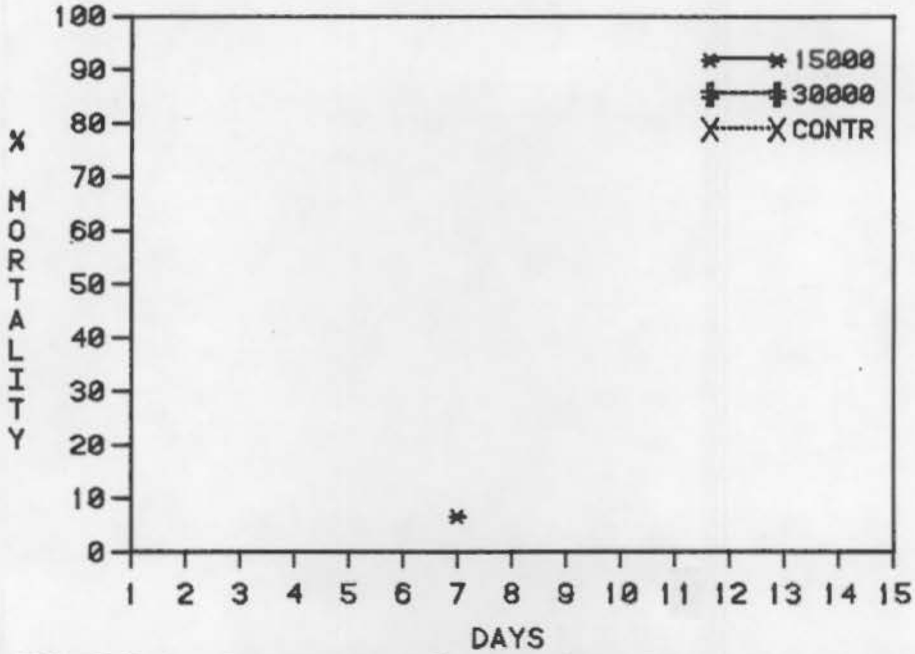

FIGURE F.3. Percent Mortality of Ants Exposed to 15,000 and 30,000 ppm Cadmium and Soil Treated with Water Only as a Control

APPENDIX G

TEST #16

APPENDIX G

TEST #16

Test #16 included many concentrations used in previous tests. Random group sizes of 10 ants and 5 ants per group were again tested using Endrin at 1.2 ppm. Also a comparison was made using Ada soil and Hanford soil as substrates and wood preservative sludge (W.P.) at 0.50%, drilling fluid (D.F.) at 1.0%, and slop oil (S.O.) at 7.0% as toxicants. Daily mortality was recorded for 10 days during this test. The controls for this test were the same as previous tests; treated with methanol, evaporated off, and water added to give 5% moisture. Complex waste residuals and insecticides were tested using the following concentrations:

Endrin (ppm)	Aldrin (ppm)	Dieldrin (ppm)	W.P.
1.2 5 ANTS/GP	0.8	0.8	.75%
1.2 10 ANTS/GP	1.0	1.0	.50%
1.4	1.2	1.2	.50% Ada Soil
			.30%

D.F.	S.O.	Control
1.25%	9.0%	Control-Ants (Hanford Soil 3 Replicates)
1.0%	7.0%	Ada Soil (1 Replicate)
1.0% Ada Soil	7.0% Ada	
.75%	5.0%	

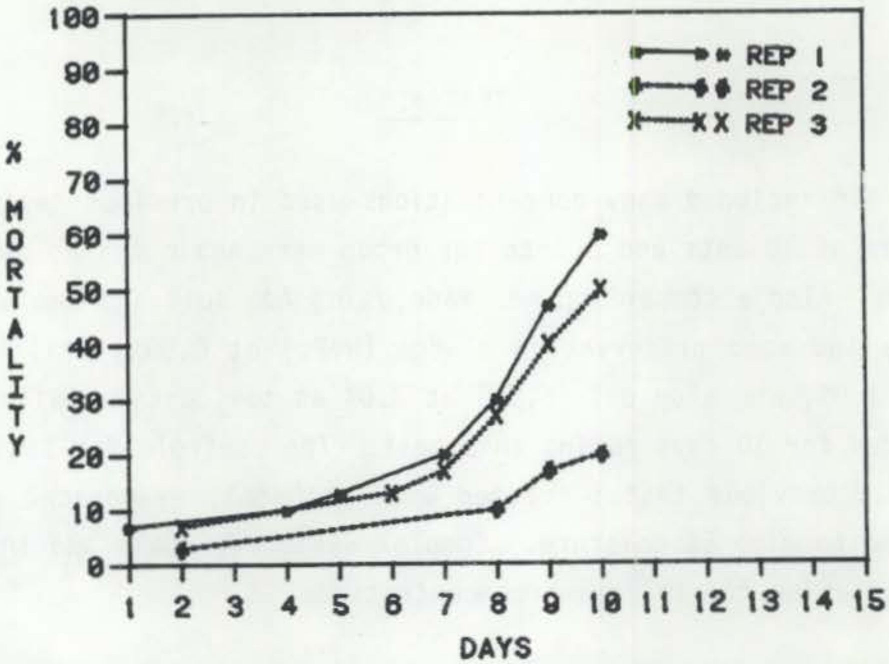

FIGURE G.1. Percent Mortality of Randomly Selected Ants (5 Ants per Group) Exposed to 1.2 ppm Endrin

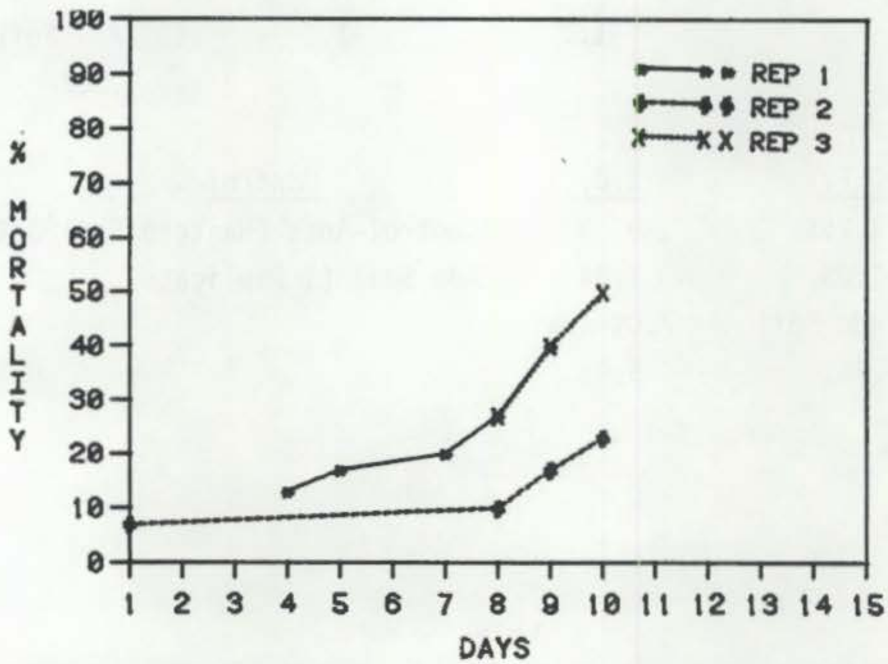

FIGURE G.2. Percent Mortality of Randomly Selected Ants (10 Ants per Group) Exposed to 1.2 ppm Endrin

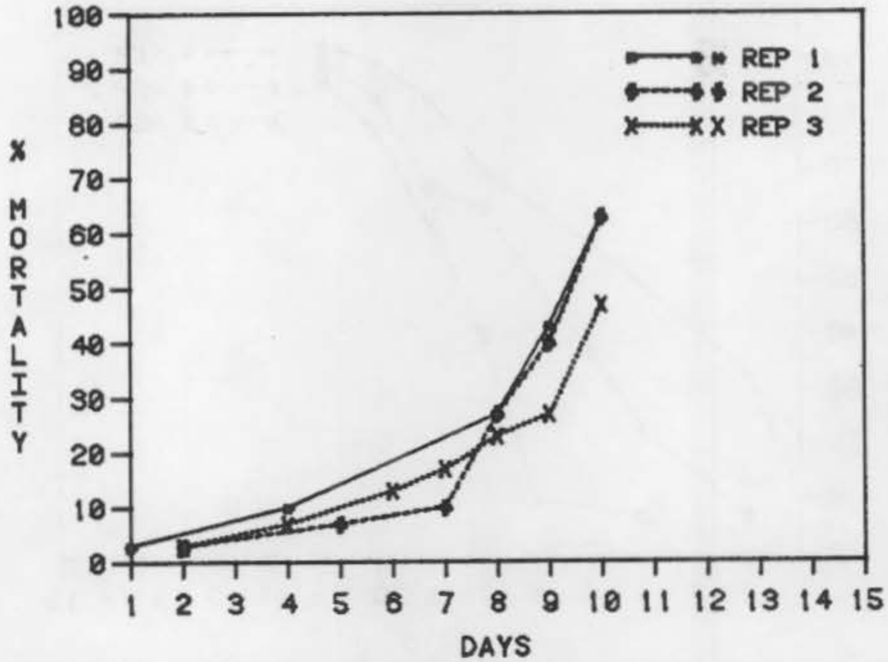

FIGURE G.3. Percent Mortality of Ants Exposed to 1.4 ppm Endrin

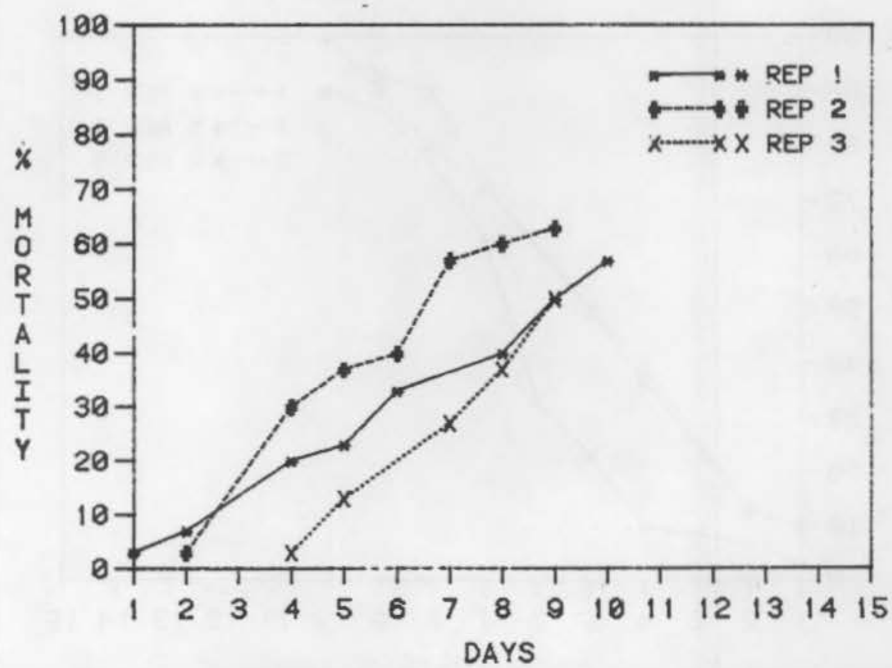

FIGURE G.4. Percent Mortality of Ants Exposed to 0.8 ppm Aldrin

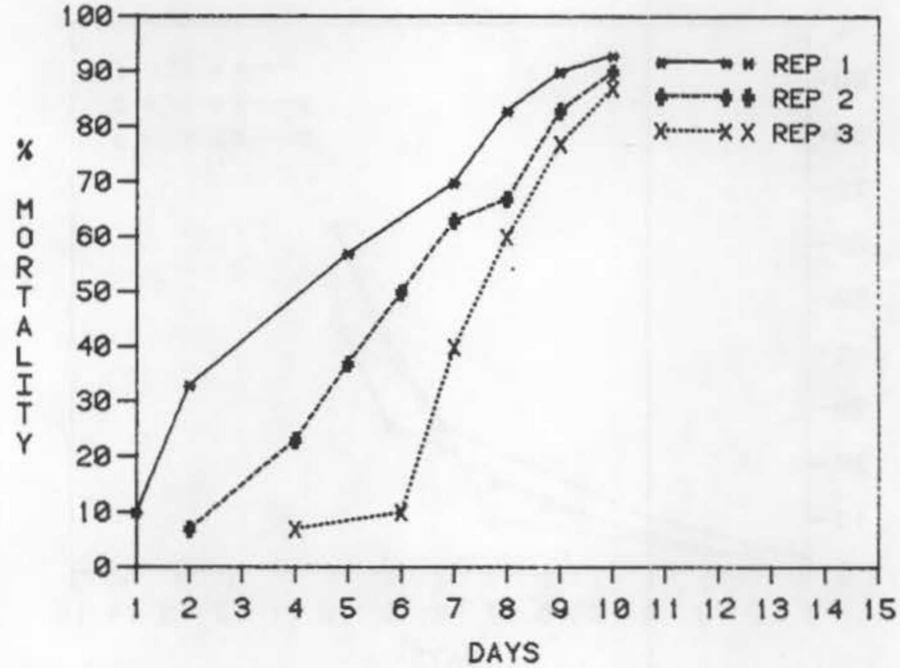

FIGURE G.5. Percent Mortality of Ants Exposed to 1.0 ppm Aldrin

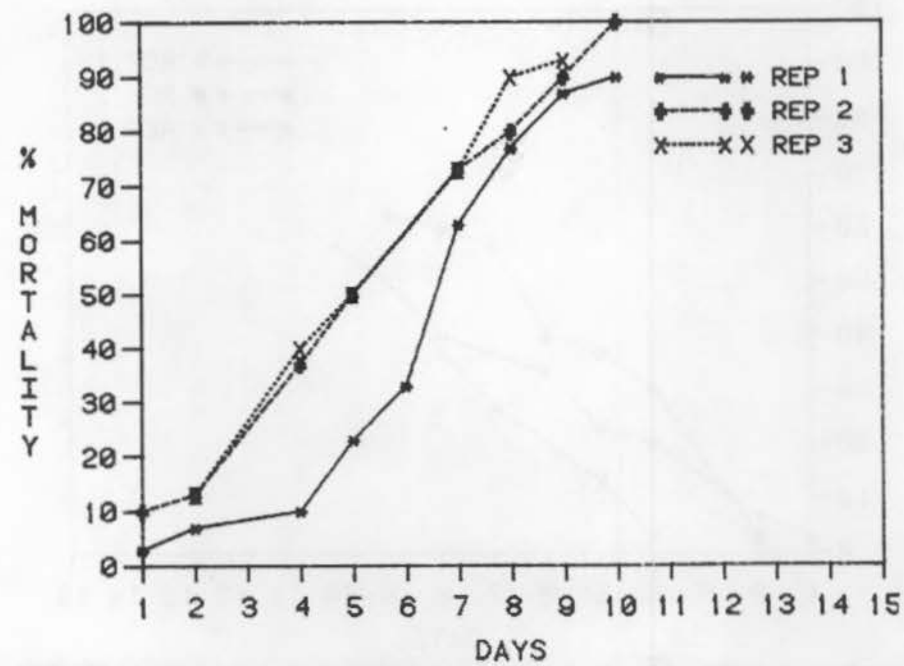

FIGURE G.6. Percent Mortality of Ants Exposed to 1.2 ppm Aldrin

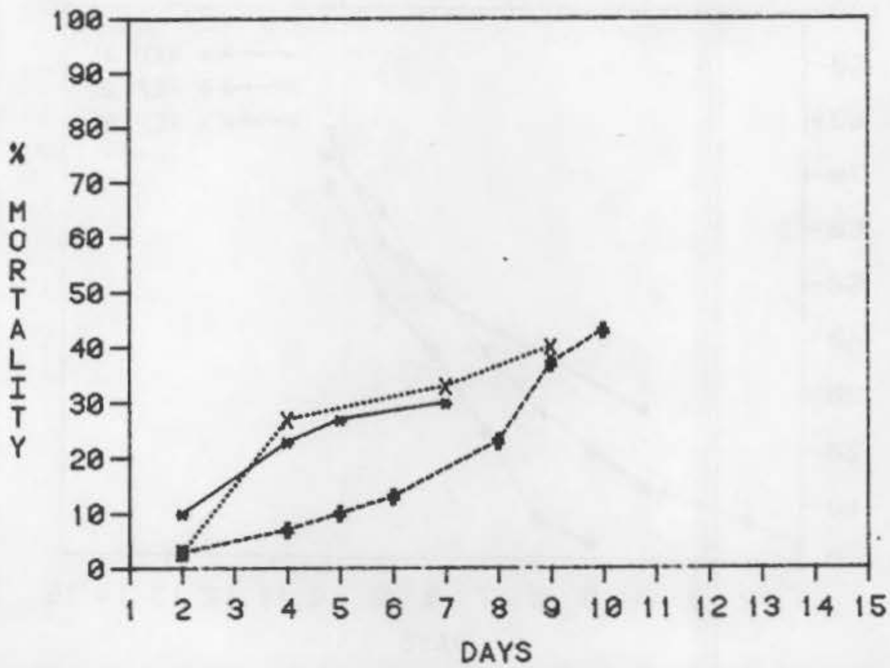

FIGURE G.7. Percent Mortality of Ants Exposed to 0.8 ppm Dieldrin

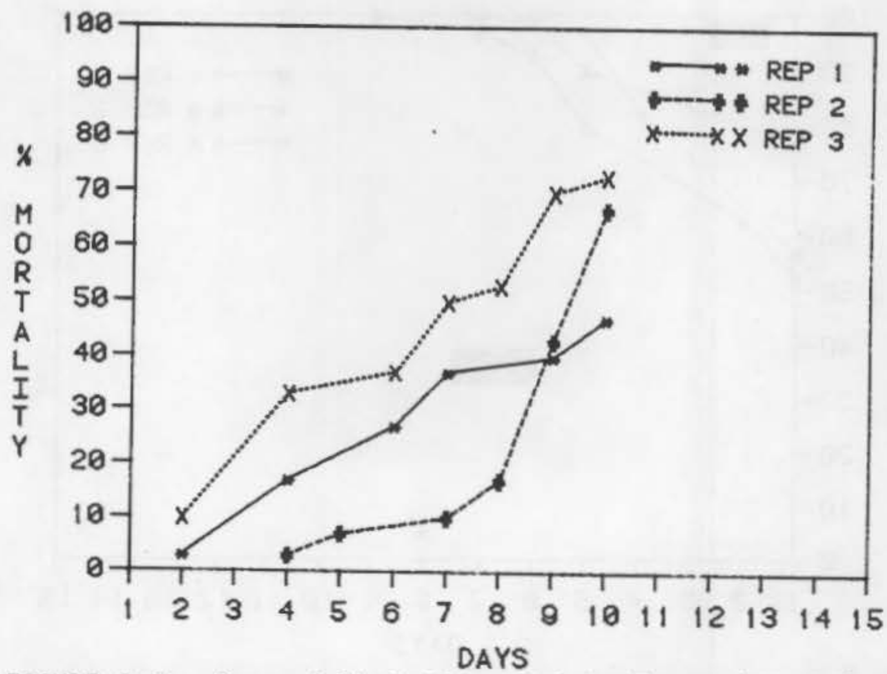

FIGURE G.8. Percent Mortality of Ants Exposed to 1.0 ppm Dieldrin

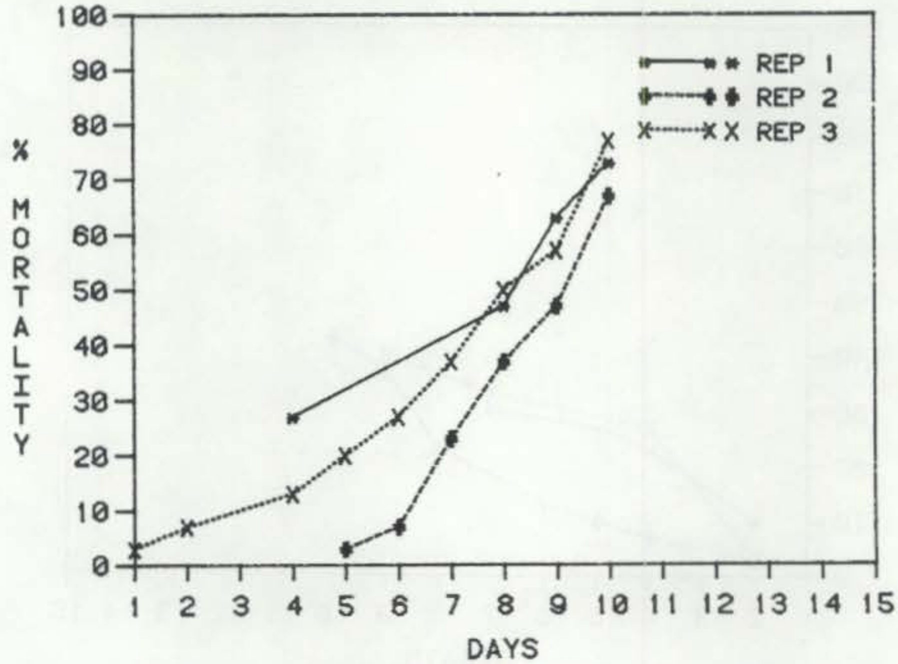

FIGURE G.9. Percent Mortality of Ants Exposed to 1.2 ppm Dieldrin

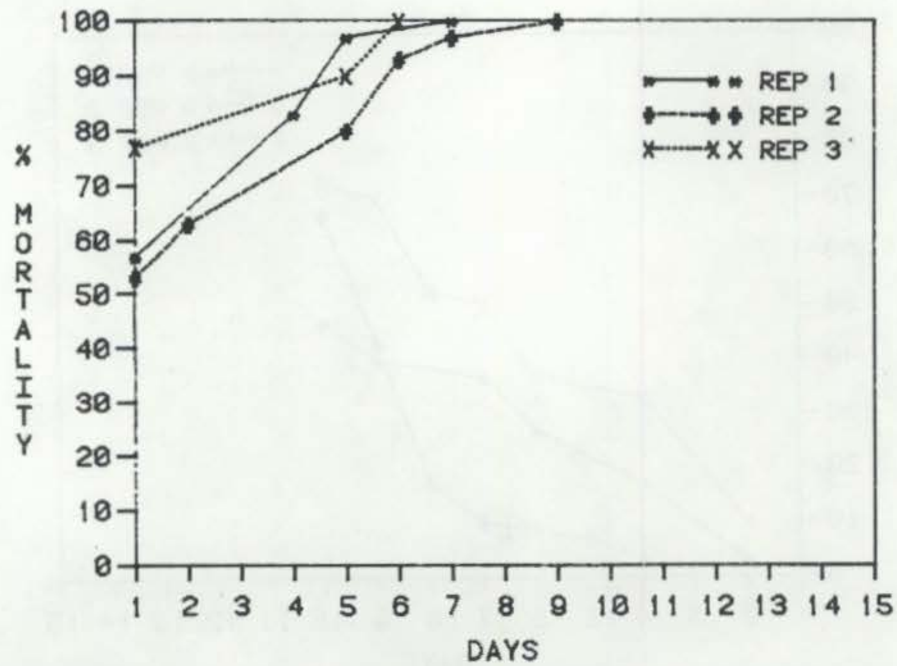

FIGURE G.10. Percent Mortality of Ants Exposed to Wood Preservative Sludge at 0.75%

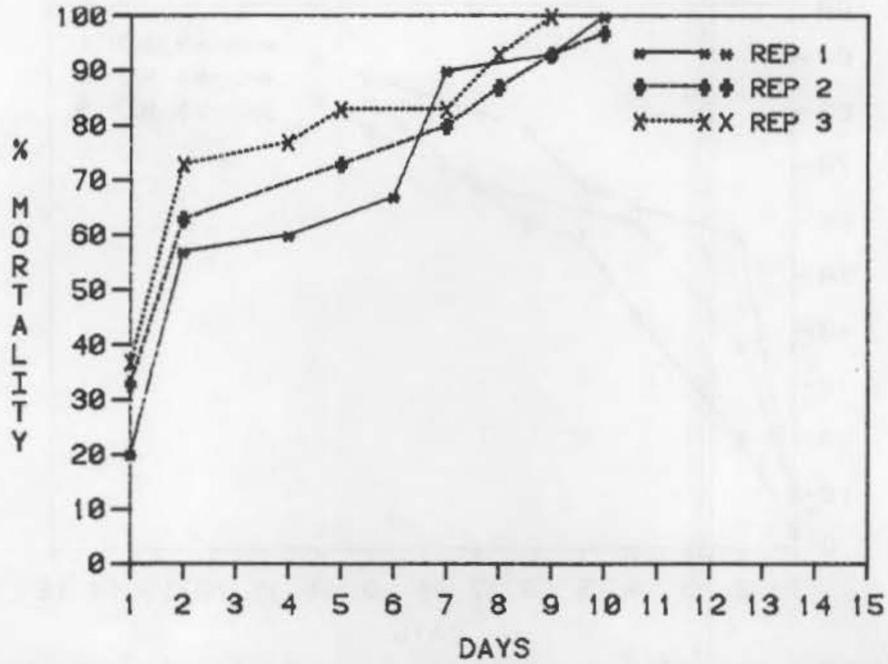

FIGURE G.11. Percent Mortality of Ants Exposed to Wood Preservative Sludge at 0.50%

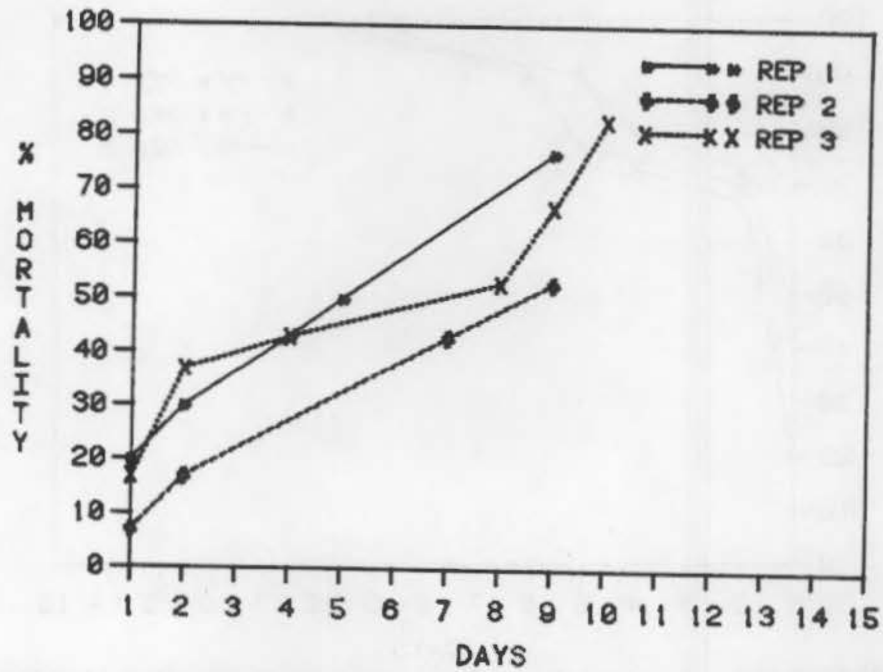

FIGURE G.12. Percent Mortality of Ants Exposed to Wood Preservative Sludge at 0.50% on Ada Soil

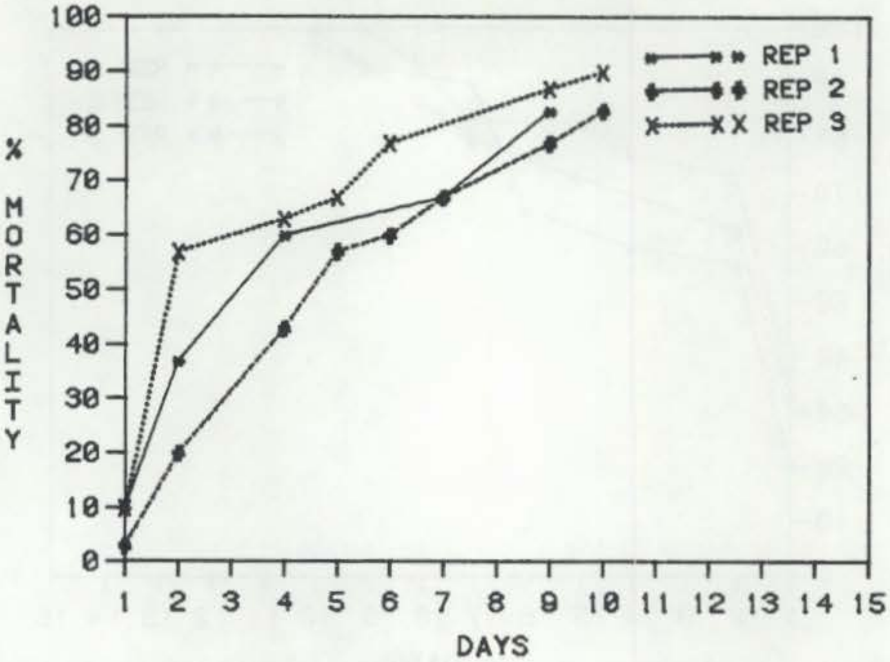

FIGURE G.13. Percent Mortality of Ants Exposed to Wood Preservative Sludge at 0.30%

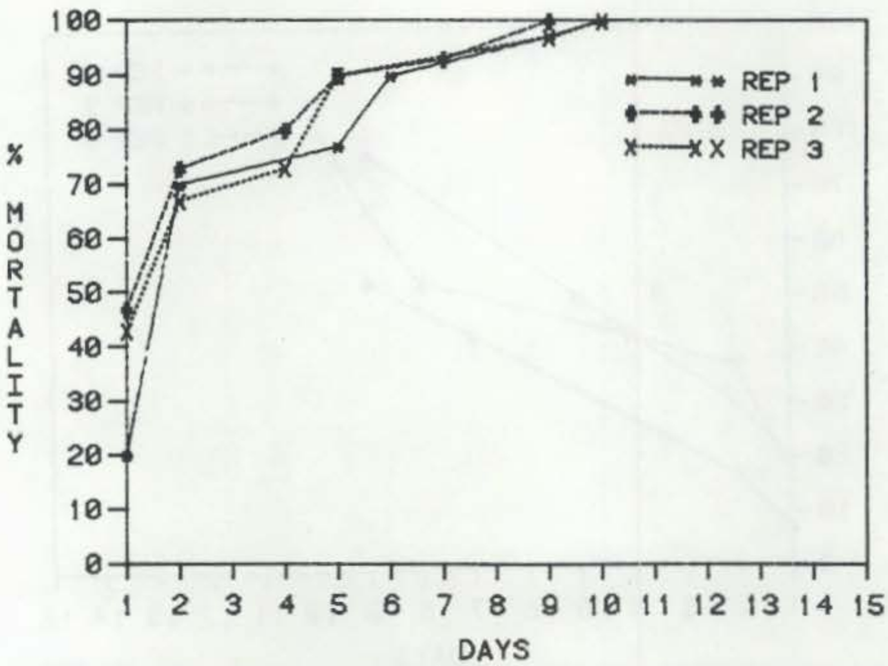

FIGURE G.14. Percent Mortality of Ants Exposed to Drilling Fluid at 1.25%

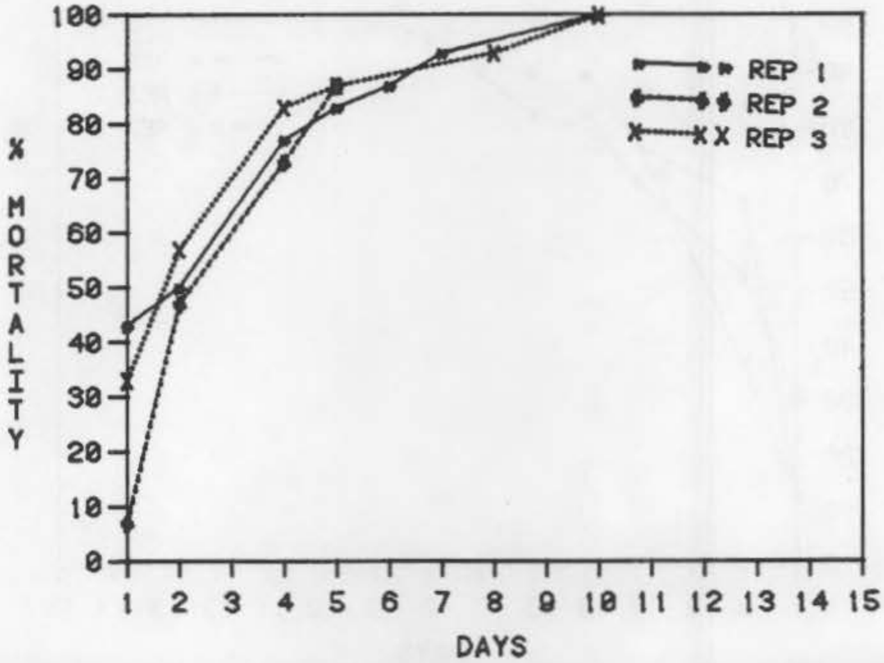

FIGURE G.15. Percent Mortality of Ants Exposed to Drilling Fluid at 1.0%

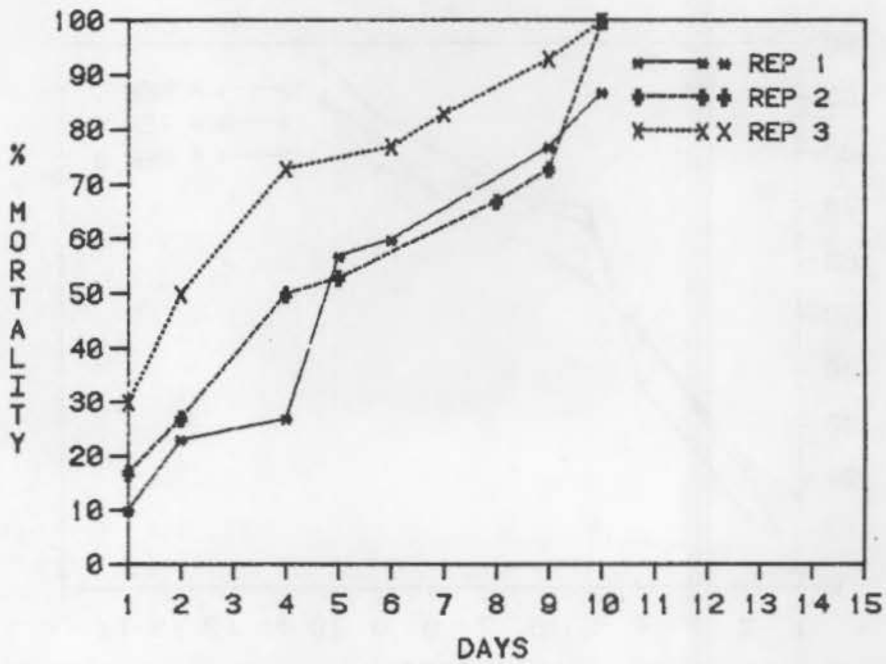

FIGURE G.16. Percent Mortality of Ants Exposed to Drilling Fluid at 1.0% on Ada Soil

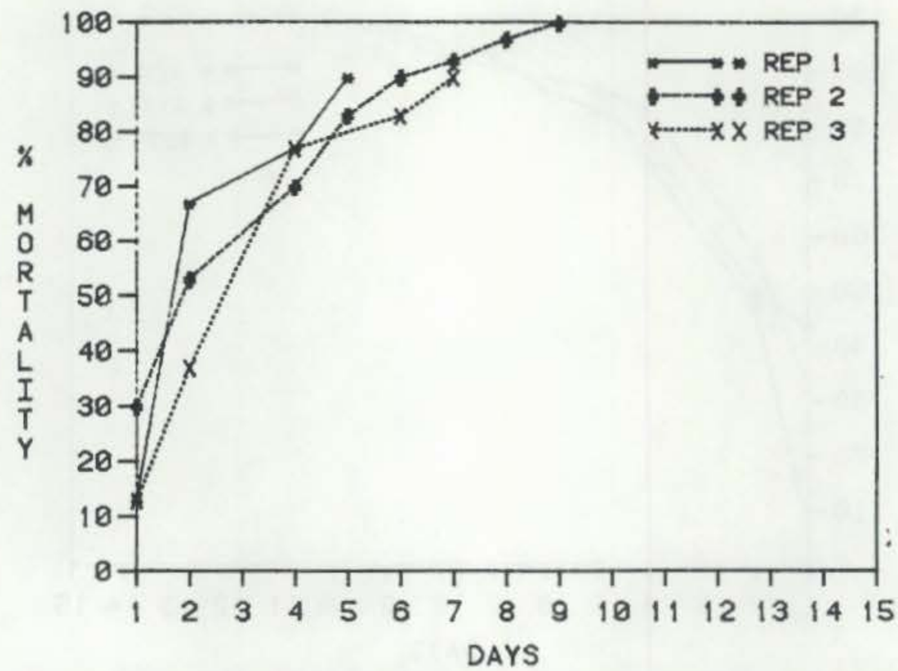

FIGURE G.17. Percent Mortality of Ants Exposed to Drilling Fluid at 0.75%

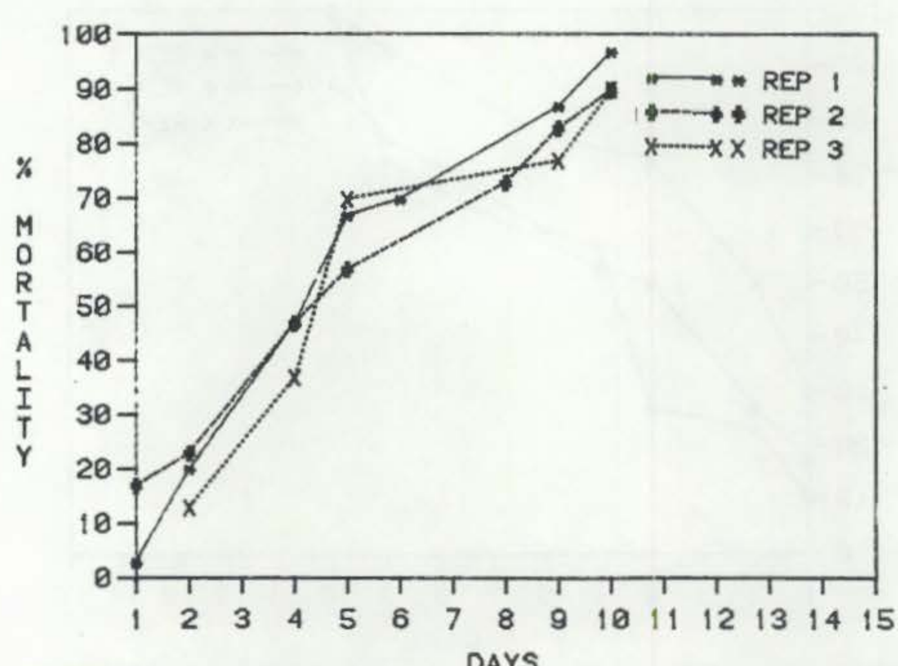

FIGURE G.18. Percent Mortality of Ants Exposed to Slop Oil at 9.0%

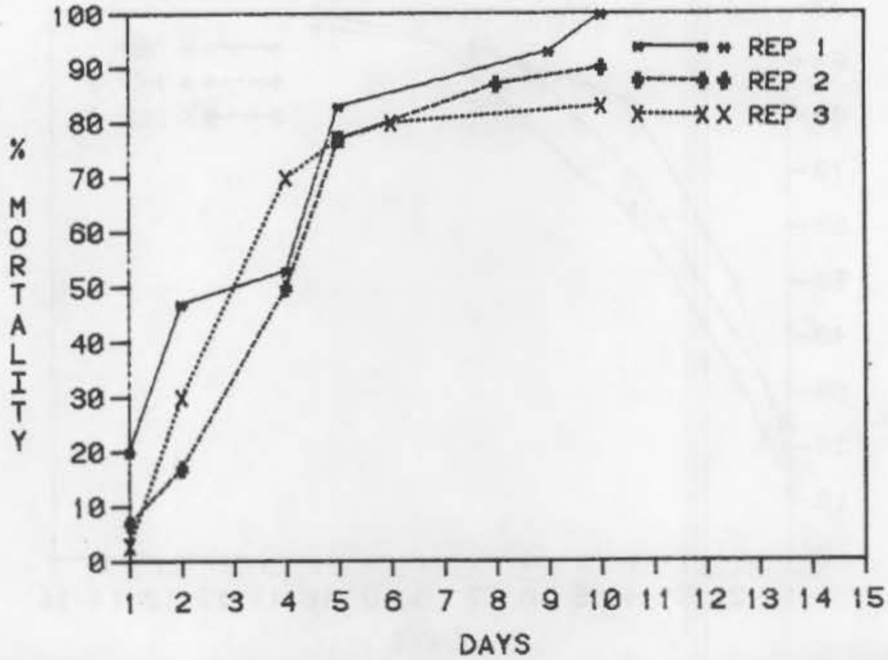

FIGURE G.19. Percent Mortality of Ants Exposed to Slop Oil at 7.0%

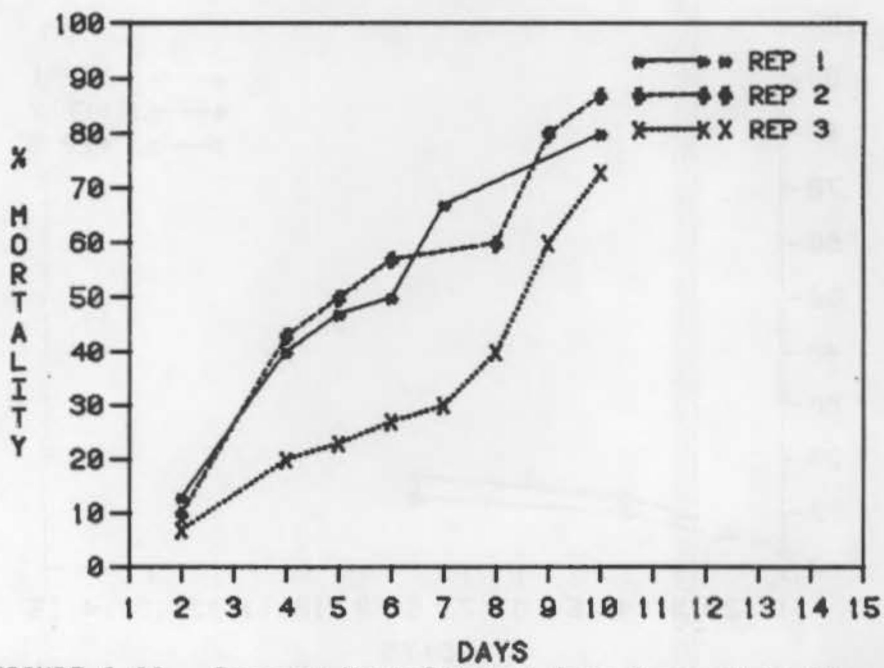

FIGURE G.20. Percent Mortality of Ants Exposed to Slop Oil at 7.0% on Ada Soil

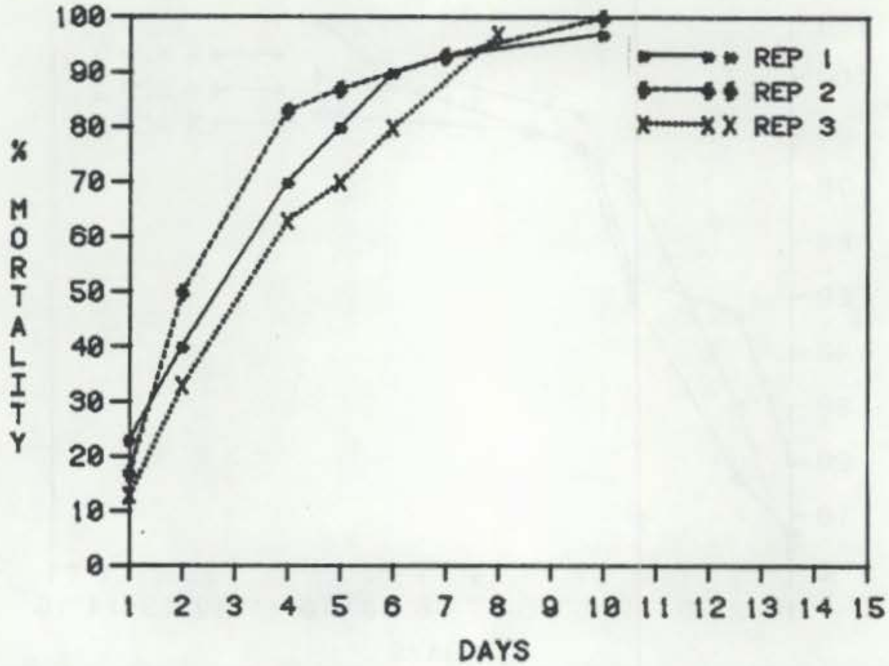

FIGURE G.21. Percent Mortality of Ants Exposed to Slop Oil at 5.0%

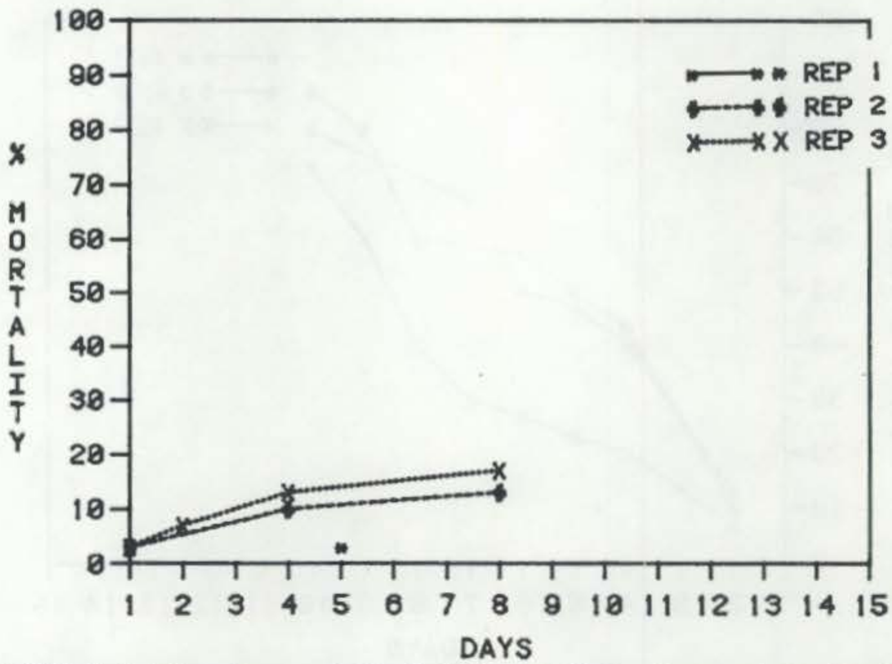

FIGURE G.22. Percent Mortality of Ants Exposed to Hanford Soil Treated with Methanol and Evaporated Off to Serve as Controls

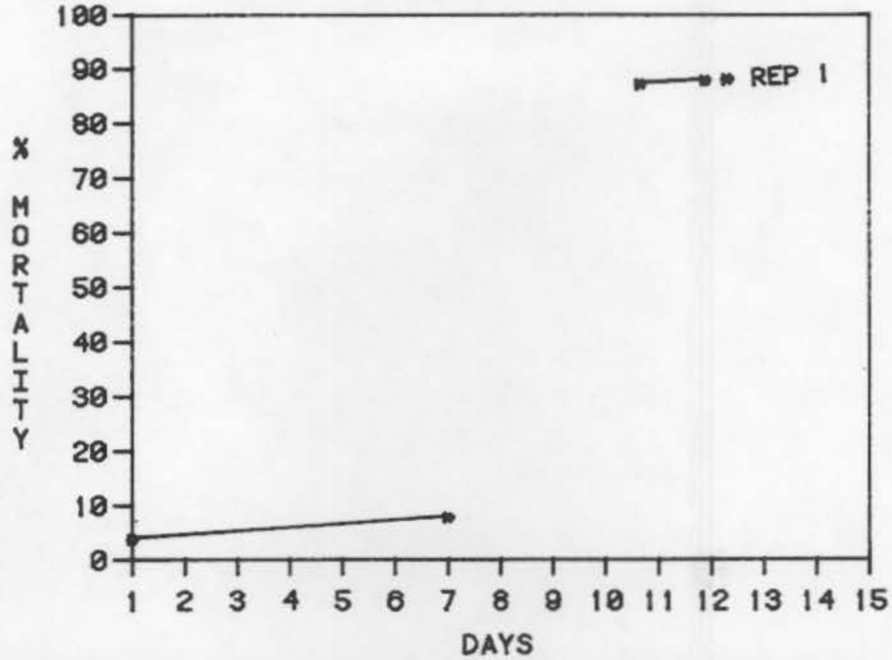

FIGURE G.23. Percent Mortality of Ants Exposed to Ada Soil Treated with Methanol and Evaporated Off to Serve as Controls

APPENDIX H

TEST #17

APPENDIX H

TEST #17

Test #17 used concentrations that had all been tested previously. The concentrations selected were mainly those which had been tested in 2 previous tests. This provided 3 separate tests for which to compare repeatability of the ant bioassay. Four concentrations tested only once previously were selected to help bracket the mortality response. These were wood preservative sludge (W.P.) at .30%, drilling fluid (D.F.) at .50%, and slop oil (S.O.) at 2.5 and 9.0%. All ants were randomly selected in groups of 5. The moisture content in all dishes was 5% water and the duration of this test was 10 days.

The ants used in all previous tests have been adult workers of mixed age. While excavating the colony used in test #17, many newly hatched adult workers were encountered. These young adult harvester ants appear much lighter in color than the dark red adults and are called callows. These ants were sorted out of the population to be tested. They were tested as separate treatments in Test #17 and compared to older mixed age ants using Dieldrin at 1.0 and 1.2 ppm. The control dishes in this test were treated the same as in previous tests with methanol added, evaporated off, and water added back to the soil before introducing ants. The treatments used in this test were identified as follows:

Endrin	Aldrin	Dieldrin	W.P.	D.F.	S.O.	Control
1.0	1.0	1.0	.75%	1.00%	9.0%	
1.2	1.2	1.0 Callow Ants	.50%	.75%	5.0%	
		1.2 Callow Ants	.30%	.50%	2.5%	
		1.2				

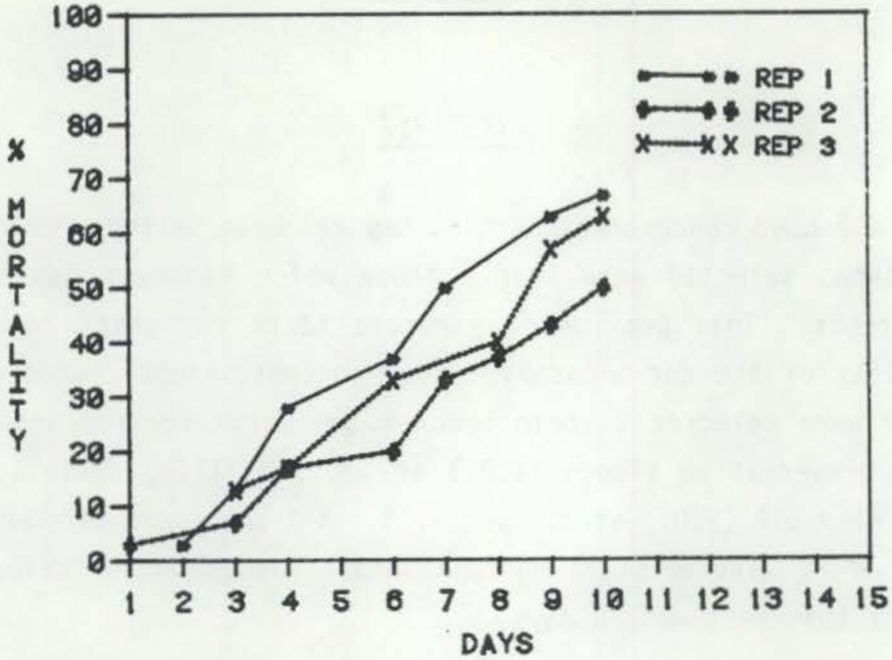

FIGURE H.1. Percent Mortality of Ants Exposed to 1.0 ppm Endrin

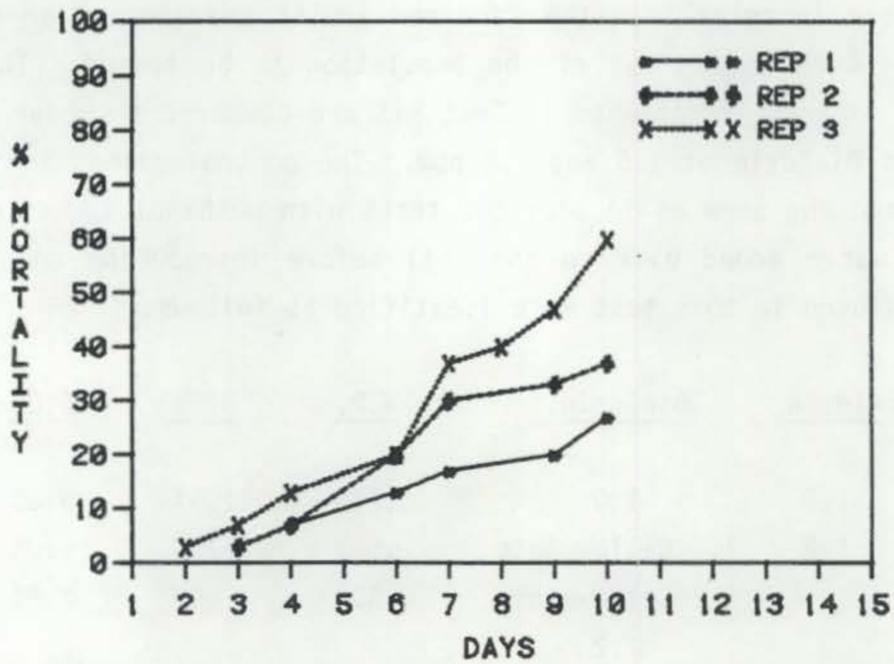

FIGURE H.2. Percent Mortality of Ants Exposed to 1.2 ppm Endrin

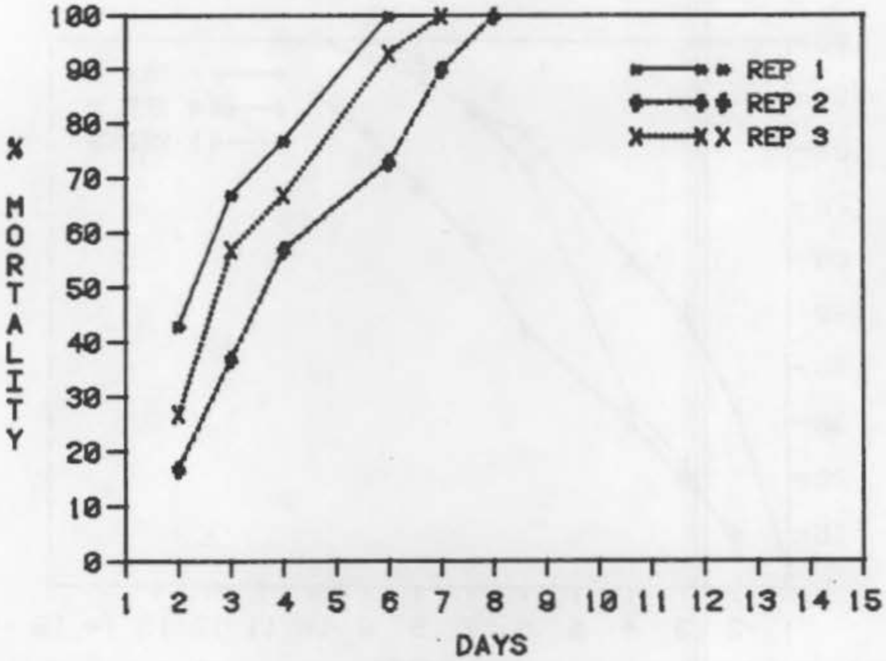

FIGURE H.3. Percent Mortality of Ants Exposed to 1.0 ppm Aldrin

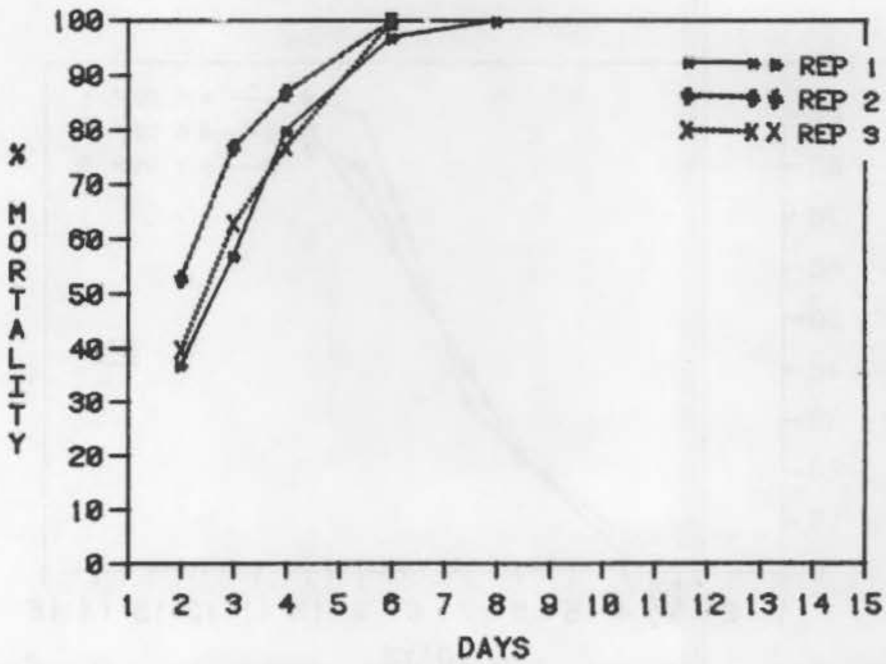

FIGURE H.4. Percent Mortality of Ants Exposed to 1.2 ppm Aldrin

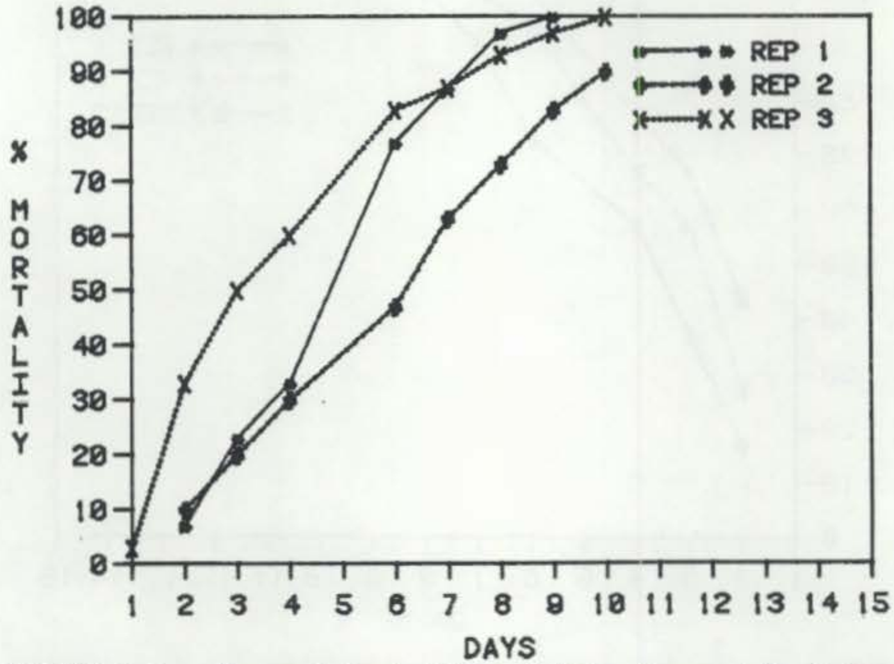

FIGURE H.5. Percent Mortality of Ants Exposed to 1.0 ppm Dieldrin

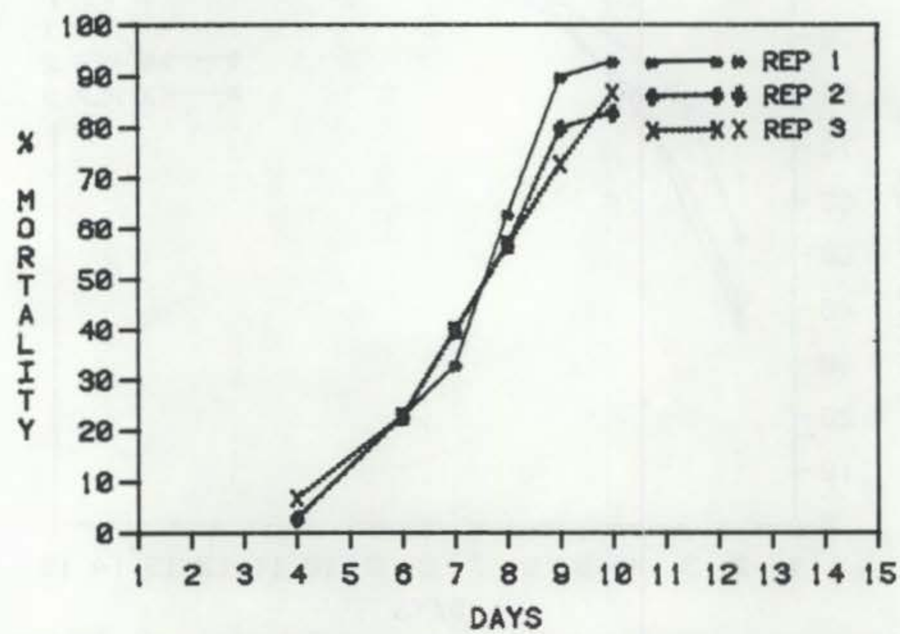

FIGURE H.6. Percent Mortality of Callow Ants Exposed to 1.0 ppm Dieldrin

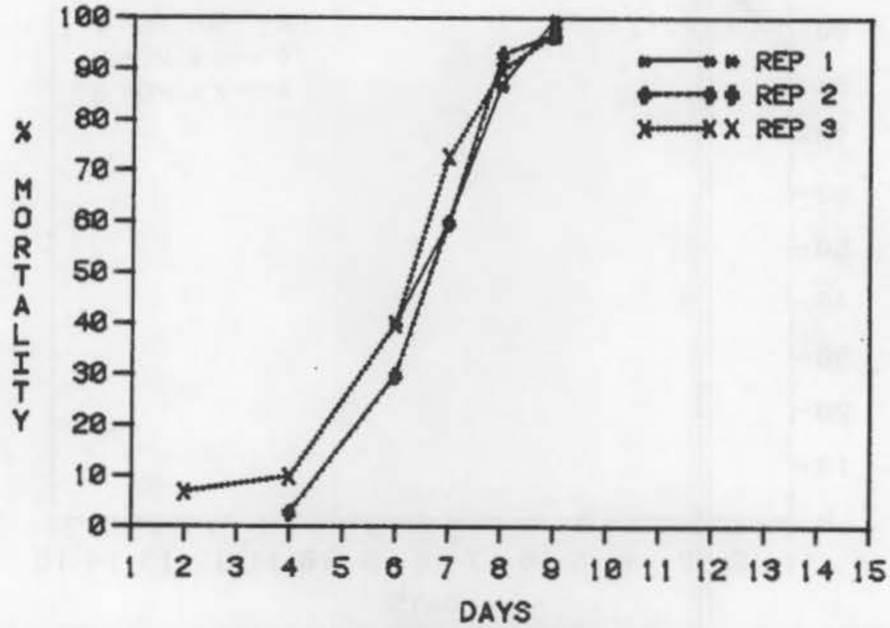

FIGURE H.7. Percent Mortality of Callow Ants Exposed to 1.2 ppm Dieldrin

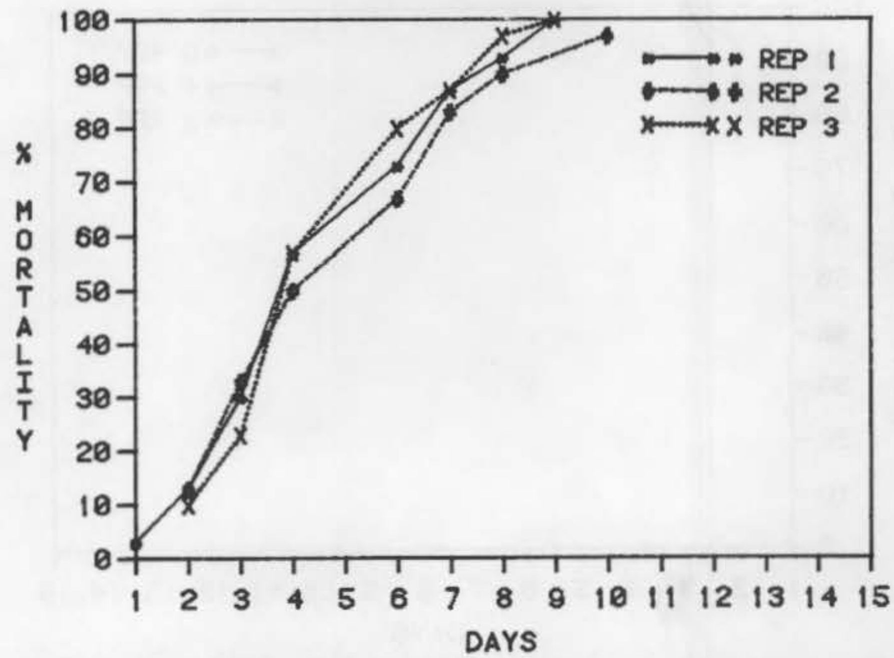

FIGURE H.8. Percent Mortality of Ants Exposed to 1.2 ppm Dieldrin

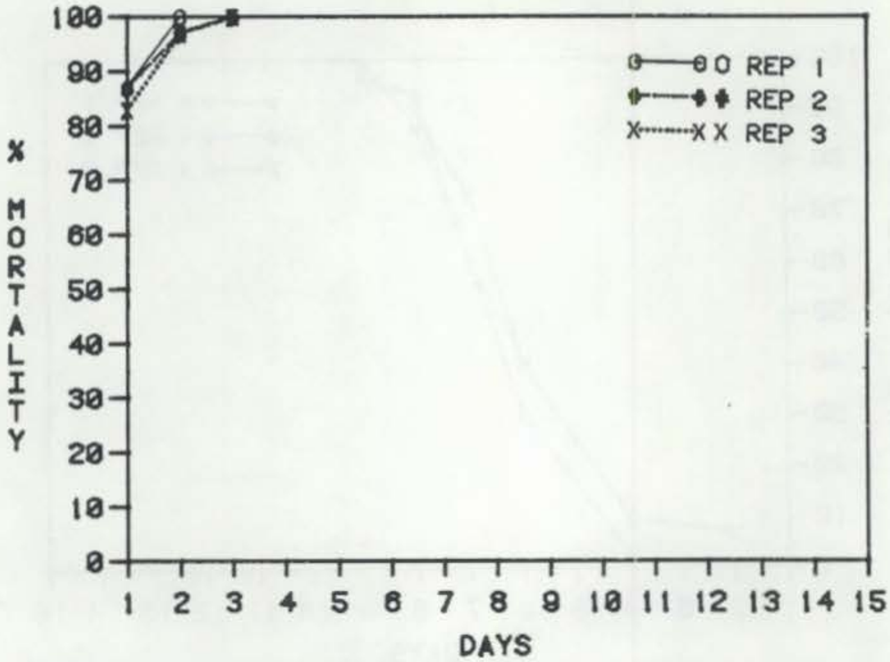

FIGURE H.9. Percent Mortality of Ants Exposed to Wood Preservative Sludge at 0.75%

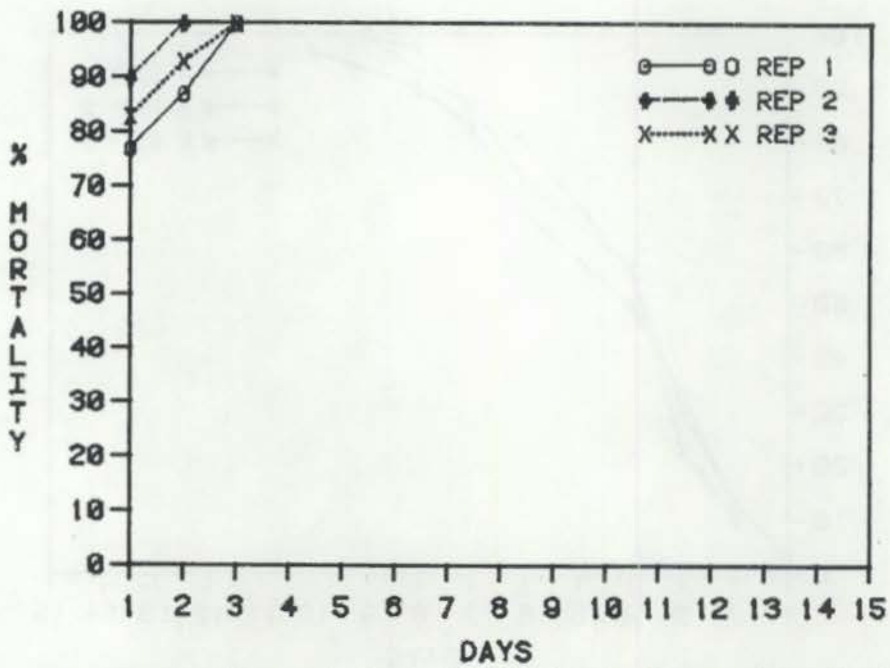

FIGURE H.10. Percent Mortality of Ants Exposed to Wood Preservative Sludge at 0.50%

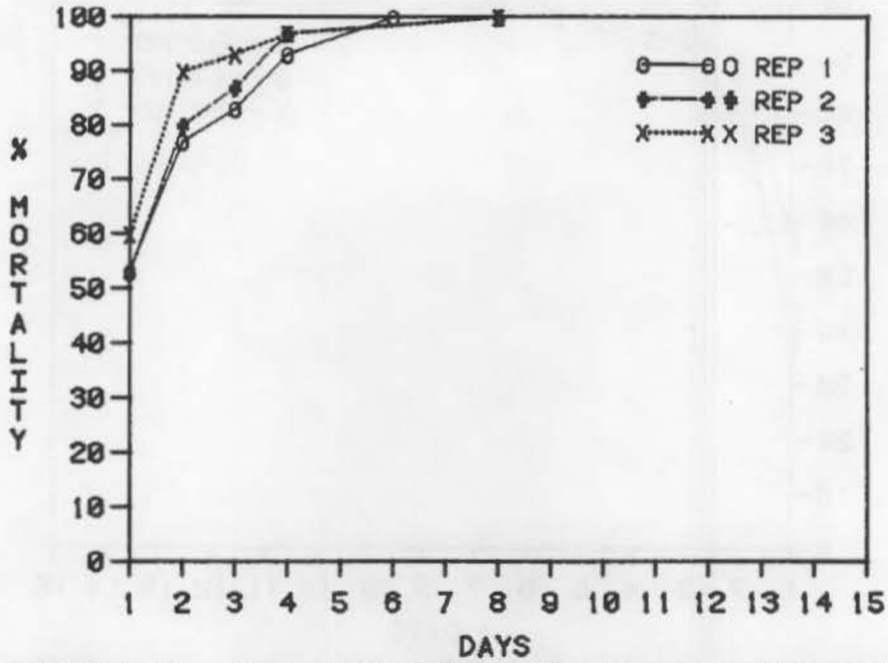

FIGURE H.11. Percent Mortality of Ants Exposed to Wood Preservative Sludge at 0.30%

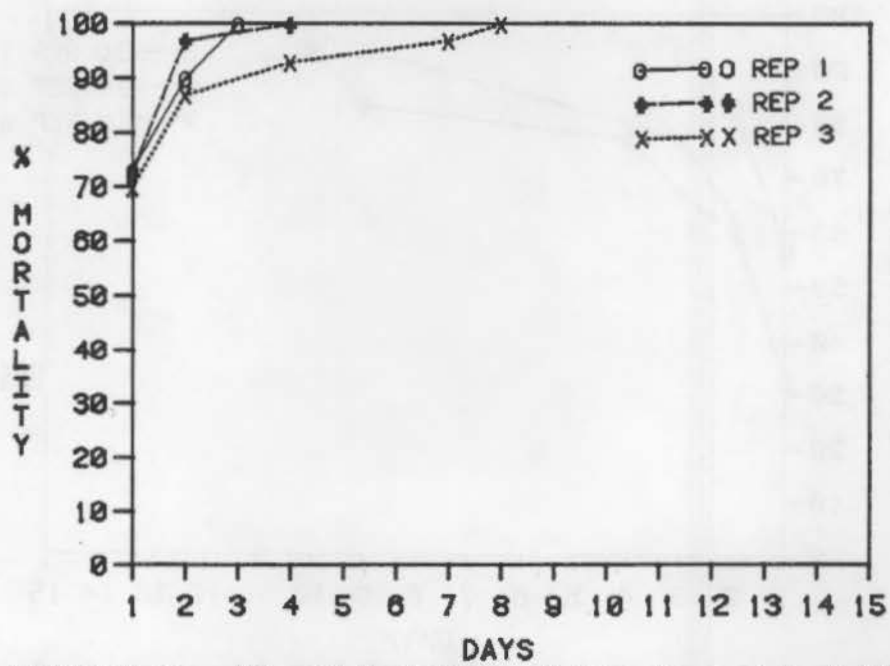

FIGURE H.12. Percent Mortality of Ants Exposed to Drilling Fluid at 1.0%

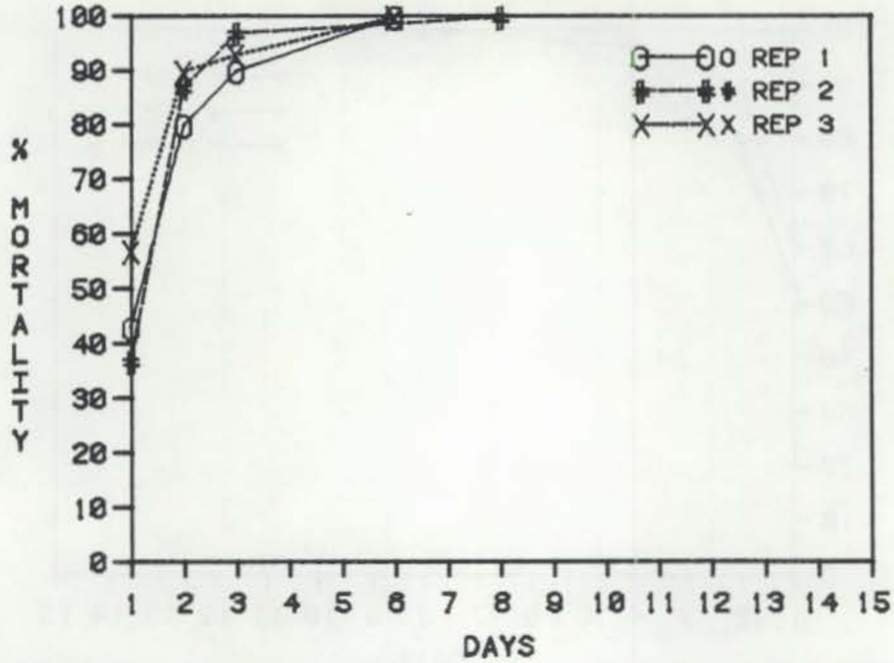

FIGURE H.13. Percent Mortality of Ants Exposed to Drilling Fluid at 0.75%

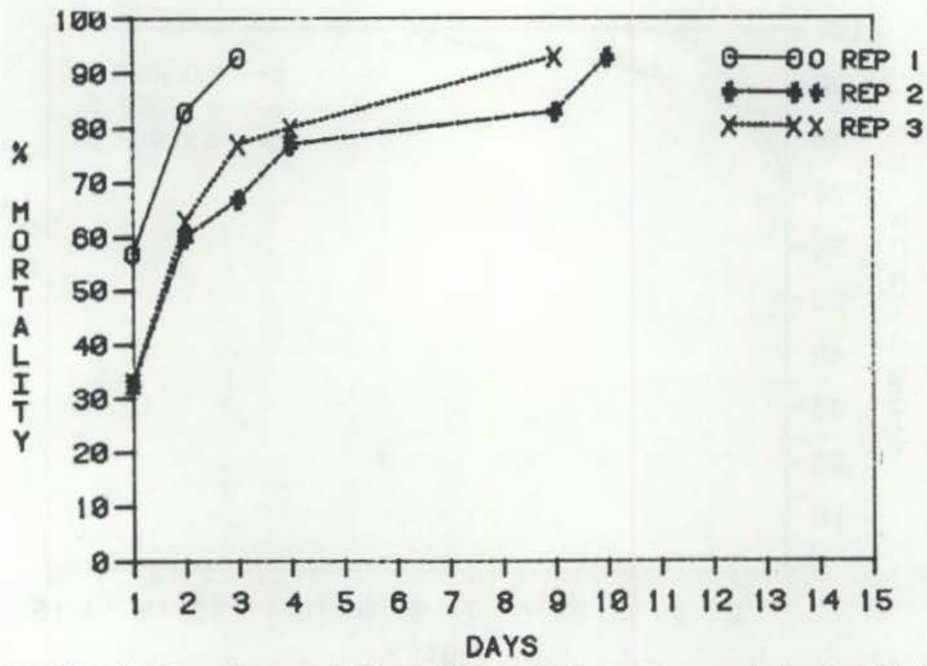

FIGURE H.14. Percent Mortality of Ants Exposed to Drilling Fluid at 0.5%

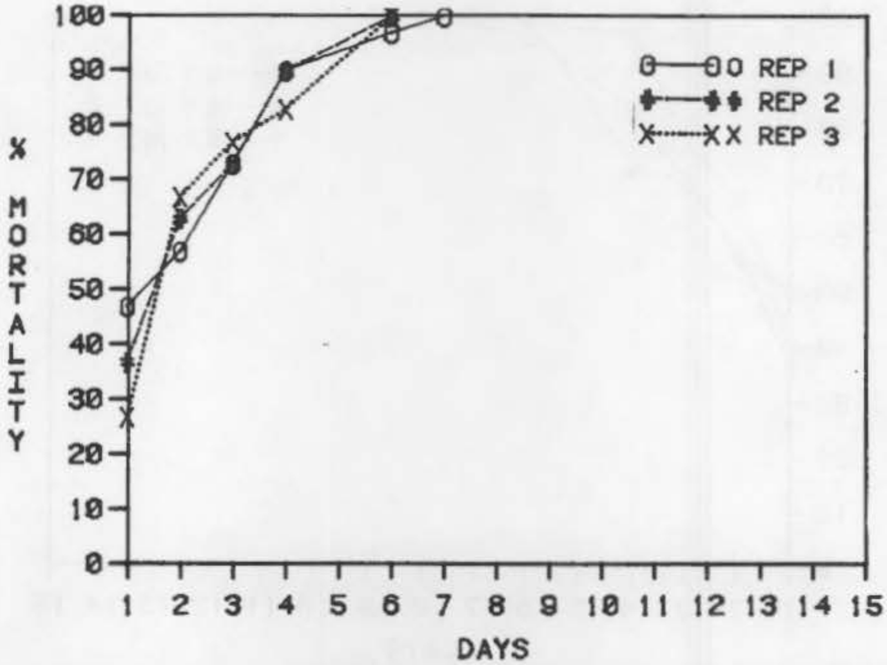

FIGURE H.15. Percent Mortality of Ants Exposed to Slop Oil at 9.0%

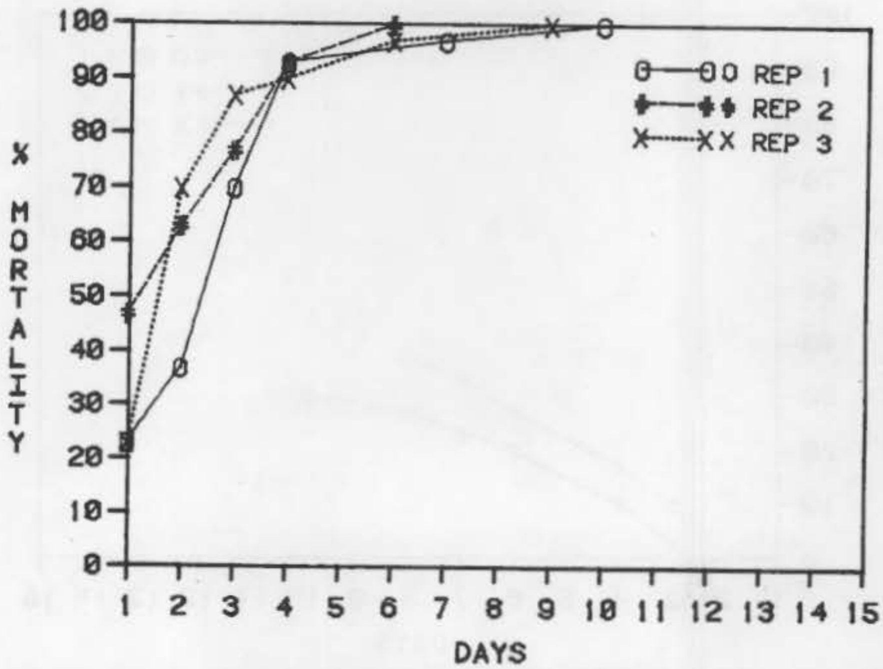

FIGURE H.16. Percent Mortality of Ants Exposed to Slop Oil at 5.0%

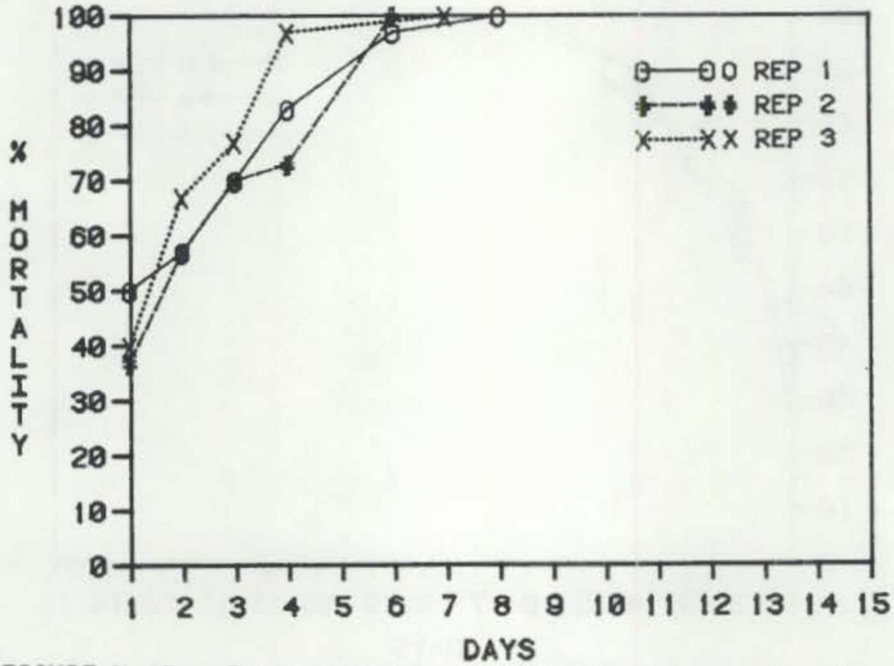

FIGURE H.17. Percent Mortality of Ants Exposed to Slop Oil at 2.5%

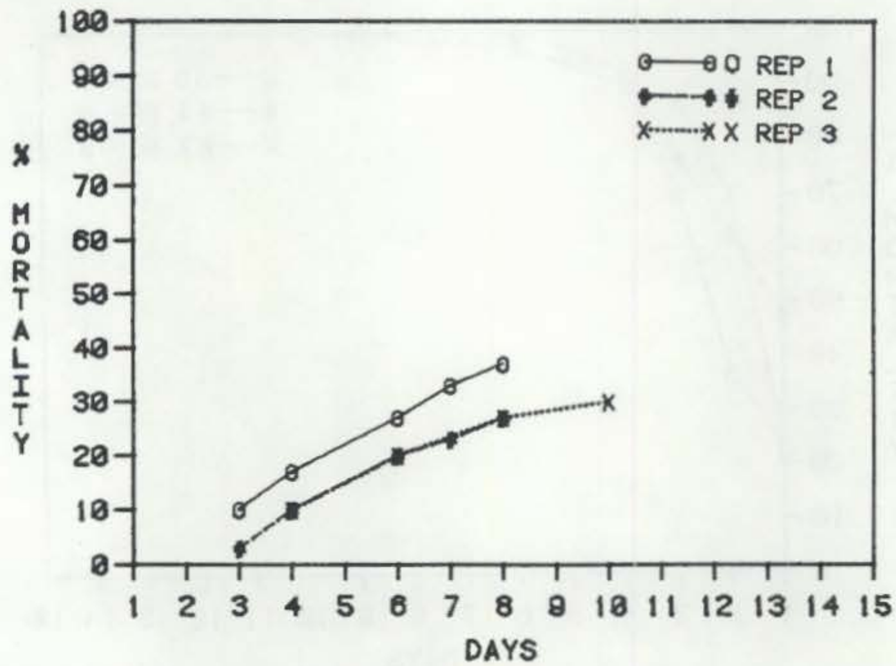

FIGURE H.18. Percent Mortality of Ants Exposed to Soil Treated With Methanol and Evaporated Off to Serve as Controls

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