SOME MORPHOLOGICAL AND PHYSIOLOGICAL STUDIES ON THE EFFECTS OF CARBARYL ON THE EARTHWORM, LUMBRICUS TERRESTRIS

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ABSTRACT

SOME MORPHOLOGICAL AND PHYSIOLOGICAL STUDIES
ON THE EFFECTS OF CARBARYL ON THE
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By

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The earthworm, *Lumbricus terrestris* was treated topically with various concentrations of carbaryl in distilled water suspension. At 21 days an LD_{50} of approximately 20 μgs of carbaryl per gm of wet buss-bedding was established for the earthworm under controlled laboratory conditions.

Many non-lethal effects were seen at concentrations of 0.3 to 6.0 μgs of carbaryl per gm of wet bedding. Some of the behavioral responses to the treatment were: (1) reactions to light and chemical stimuli, resulting in a "withdrawal response"; (2) extensive coiling and constricting of the body; (3) swellings and redness in the anterior region of the body of the worm; and (4) formation of blisters.

Oxygen consumption in worms treated with non-lethal concentrations of carbaryl ranged from 115 to 155 μl O_2 per gm per hour and the rates in the controls ranged

from 82-101 μl O_2 per gm per hour. Increases in oxygen consumption were seen in treated animals even though there were no external effects observed.

Histological studies have shown even with non-lethal concentrations of carbaryl that the body wall is damaged. There is extensive proliferation of epidermal cells, and the circular muscle layer shows degenerative effects with epidermal cells replacing the tissues in this area.

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INTRODUCTION

In recent years economic entomologists have become increasingly concerned as to what effect various agricultural chemicals such as insecticides may have on earthworms.

Most of the investigations demonstrating the effects of insecticides on soil invertebrates have been done from a comparative viewpoint: i.e. (1) studies of various invertebrates, involving a specific insecticide, such as carbaryl and (2) studies of various insecticides involving a specific invertebrate, such as the earthworm.

Earthworms occupy a central position in the economy of the soil. Edwards (1968) has estimated that up to 60% of the initial breakdown of plant material is brought about by the earthworm. He has reported many large reductions in populations of various soil invertebrates using various chemicals, including a reduction in populations of earthworms.

LD_{50}: Field and Laboratory Studies of Insecticides, other than Carbaryl on Earthworms

Effects of DDT on Earthworms

There have been many investigations made showing various effects of the insecticide DDT on invertebrates.

However, most of these investigations have been limited to field observations.

Baker (1946) conducted a field study in which he sprayed trees in several plots with DDT. One month later after the foliage had been consumed by earthworms, he observed a sharp decrease in the earthworm population in the sprayed areas, compared to little or no change in the number of earthworms in unsprayed areas.

Fleming and Hadley (1945) applied 50 lb of DDT per acre. Observations made after 26 days showed that partially grown worms were all killed. After repeating this test using full grown worms, no lethal effects were observed, even after more than two months. It was evident that the full grown worms were very resistant to the DDT. In another of their experiments 100 lbs of DDT per acre was used on full grown worms for the same period of time, however, the results were essentially the same.

Hoy (1955) reported a similar study using 40 lbs of DDT per acre and observed no lethal effects. However, Doane (1962) reported a two-year field study in which he showed that earthworms were mildly affected by the application of DDT in concentrations of 25 lb per acre. He also reported that there are some changes that occur in the normal activity and numbers of earthworms following application of the insecticide. The reason for these changes has not been thoroughly examined, especially over large areas.

He suggested that a problem involved in studying the lethal effects of DDT on earthworms is that the earthworms are able to accumulate large amounts of DDT without being killed.

Thompson (1971) found that when DDT concentrations ranging from 1-5 lb. were applied per 10' x 6' plots, that some effects on earthworms were seen, even 5 years after treatment.

Hopkins (1957) reported a field study on English redworms (species undefined) treated with 10% DDT at 15 lb per acre. He found that with this treatment mortality of only 7% was observed after 2 months. He concluded from these data that it would require many years of consistent application of this chlorinated hydrocarbon to endanger the English redworm population.

Martin and Wiggins (1959) determined the tolerance of Hybrid Red Wigglers (species undefined) to various insecticides, including DDT in laboratory studies. They used insecticide concentrations of 0.1, 1.0, 10, 100 and 1,000 ppm (parts per million). The worms were immersed in each concentration for a period of 2 hours. After 24 hours the percent survival was determined. They found that DDT, at concentrations of 0.1, 1.0 and 10 ppm caused no mortality in the worms; 100 ppm caused a 20% mortality and 1,000 ppm caused a 60% mortality.

There are a number of generalizations which may be made from field and laboratory studies on the effects of DDT on earthworms.

DDT seems to have little or no effect on earthworms probably because of their ability to accumulate large quantities of the DDT without any damaging effects. A second generalization is that laboratory studies of related species of worms tend to show no damaging effects of DDT treatment, also suggesting high tolerance levels for the DDT and a third generalization is that length of time of treatment is not critical to the worms, because some experiments were conducted over a period of several years.

Effects of Insecticides Other than DDT on Earthworms

Hoy (1955) reported in addition to the effects of DDT on earthworms, the effects caused by a variety of other insecticides. He found that the chlorinated hydrocarbons, gamma BHC and crude BHC at 2-10 lb active isomer per acre caused significant mortalities of earthworms (species undefined). However, in soil with low organic matter content, crude BHC killed all the earthworms after only 8 weeks of exposure.

Doane (1962) found that when he used chlordane and dieldrin at 10 lb per acre, he had eliminated most of the earthworms, *Lumbricus terrestris* from a field plot one year after treatment.

Edwards (1965) made a field study of various insecticides on various soil invertebrates. In this study he used aldrin, dieldrin, telodrin, BHC, diazinon, and menazon in average dosages of 1-3 lb. per acre and for periods of several weeks to several years. He observed no effects; however, when heptachlor, chlordane, parathion, DNOC and simazine were applied at the same dosages, moderate to large reductions in the numbers of earthworms were noted.

In a more recent field study, Thompson (1971) reported the effects of several insecticides on the numbers of earthworms (Lumbricus sp.). The insecticides used were, dursban, BAYER-37289, endrin, BUX, dasanit, carbofuran and Stauffer N-2596 in various dosages of 1-5 lb. per 10' x 6' plots. The effects of the eight insecticides on the numbers of earthworms were observed after 3 weeks. Dursban and BAYER-37289 had very little effect, but dasanit, carbofuran and Stauffer N-2596 drastically lessened numbers of earthworms. BUX and endrin did not kill more than 60%.

Ruppel et al. (1973) in a study yet unpublished, have investigated some effects on earthworms caused by prophos, aldricarb, Tirpate and carbofuran. They used dosages ranging from several ounces to several pounds. Their results showed that those pesticides proved to be toxic in the laboratory; however, other pesticides such as dyfonate and phyrate caused more than 50% mortality only at the highest dosage, while the other pesticides caused little mortality.

After making these observations, it may be concluded that while DDT seemingly caused little or no damage to the earthworm, other types of pesticides even in small doses may cause more damaging or even lethal effects to the earthworm.

Effects of Carbaryl on Earthworms

Edwards (1965) reported some effects of various chemical residues, including carbaryl on the earthworm. His results showed large reductions in the numbers of earthworms, after 6 months in the soil at average doses of 1.5-2.0 lb of carbaryl per acre.

Thompson (1971) treated earthworms with carbaryl in doses ranging from 1-5 lb per acre for 3 weeks. He noted that the numbers of earthworms were drastically reduced in the treated plots.

An Der Lan and Aspock (1962 and 1963) applied 0.3% carbaryl suspensions to the epidermis of the earthworm and noted a number of histopathological effects. They found that there were growths of epithelial cells into other regions of the body wall. Further investigations of histopathological effects of carbaryl showed that there was a breakdown of the cell membrane and the formation of a "syncytium". These earthworms showed the same or similar changes as those treated with carcinogenic hydrocarbons as shown by Gersch *et al*. (1951 and 1957). An Der Lan and

Aspock (1962) concluded that the histopathological effects caused by DDT, carbaryl, chlorinated hydrocarbons or carcinogenic hydrocarbons generally show the same or a similar effect on the epidermis of the oligochaetes.

These data show that carbaryl seems to be a toxic compound for the earthworm and tends to cause various changes in the epidermis of the earthworm, especially when applied directly to the earthworm in low concentrations.

OBJECTIVES OF THE STUDY

There have been a considerable number of field studies studying the effects of various insecticides on various soil invertebrates. Only a few investigations showing the toxic action of carbaryl on earthworms have been reported. Of the investigations which have been conducted, none involves the use of carbaryl in behavioral and metabolic studies and little data have been reported showing histological effects on earthworms.

There are several reasons for using the earthworm, Lumbricus terrestris and carbaryl in this research study: (1) carbaryl is a chemical which is widely used as an agricultural insecticide; (2) according to An Der Lan (1962), carbaryl effects earthworms at very low concentrations; (3) earthworms are easy to maintain; (4) large samples of worms can be studied in a short period of time; (5) earthworms possess a relatively simple organ-system and are therefore easier to study than many other higher invertebrates, from both a gross morphological and histological standpoint.

With these considerations in mind, the major objectives of this investigation were to determine: (1) under controlled condition, what the LD_{50} (lethal dose at which

50% of the animals die) is for the earthworm, treated with various concentrations of carbaryl; (2) whether the normal behavioral patterns (such as reactions to external mechanical stimuli, coiling ability, locomotion and responses to light) are altered significantly, as a result of exposure of the worms to non-lethal doses of carbaryl; (3) what histological changes occur as a result of exposure of worms to lethal and non-lethal dosages of carbaryl over a specified period of time; (4) what metabolic changes, such as changes in respiratory rates, if any, may be observed in the treated animals as a result of exposure of the worms to various sub-lethal concentrations of carbaryl over a period of time.

MATERIALS AND METHODS

Source and Maintenance of Earthworms

The earthworm, Lumbricus terrestris was used in all experiments. The worms were purchased from the Wholesale Bait Company, Hamilton, Ohio. The animals weighed between 2.0-5.0 gms and measured 15-20 cm in length. They were maintained at a constant temperature of 15°C. in buss-bedding in wooden boxes measuring 90 x 45 x 60 cm. Approximately 250 worms were kept in each box.

The maturity of the earthworms was established by the presence of a clitellum and only mature worms were used. Animals were always used within 3 weeks of their purchase.

Buss-Bedding Preparation

Buss-bedding is an artificial soil, prepared by Don Buss Manufacturing Company; Lanark, Illinois. It is a fully nutrient food for the earthworms and only water is needed in the preparation of the bedding for the earthworms.

The buss-bedding mixture was prepared in a ratio of 432 gm dry bedding per liter of tap water. This bedding was thoroughly mixed by hand until evenly moistened.

Pharmacological Procedures

One drawback in the use of carbaryl is the lack of a commercially acceptable emusifiable concentrate (Reeves, 1962). Louloudes et al. (1962) reported the use of a hydroxy-lecithin compound as a solvent for carbaryl and others have suggested acetone or alcohol as solvents for carbaryl (Metcalf, 1961; Dorough and Casida, 1964).

In preliminary tests, acetone and corn oil were tested as solvents for carbaryl, but the results (explained more fully in the "results" section) obtained showed neither of these solvents to be acceptable. There are two reasons why these solvents could not be used: (1) with acetone as the solvent many of the controls died, suggesting that acetone in itself is toxic to the worms; (2) corn oil proved to be a difficult solvent to use in these experiments. It was necessary to use a microliter syringe to make injections and the viscosity of corn oil is such that it cannot be easily forced through the needle of the syringe.

As a result of these preliminary tests the procedures used in the following studies were based primarily on two methods: (1) injections of a carbaryl-distilled water suspension into animals and (2) exposure of worms to buss-bedding which had been wetted with the carbaryl-distilled water suspension.

Source and Preparation of Carbaryl

Carbaryl (1-naphthyl-N-Methylcarbamate) has the trade name Sevin. This synthetic insecticide is a product made by Union Carbide Corporation; Salinas, California and the samples used in these studies were 99% pure and were donated by Dr. Norman Leeling, Pesticide Research Center, Michigan State University, East Lansing, Michigan.

The carbamate, carbaryl is a synthetic derivative of the alkaloid physostigmine or eserine found in the Calabar bean, *Physostigma venonsum*, of French West Africa. Carbaryl is a white crystalline compound, melting point 142°C, relatively insoluble in water (about 0.1%) and somewhat soluble in organic solvents (Metcalf, 1961). However, the 99% pure carbaryl was added to distilled water in various concentrations and suspensions were formed by vigorously agitating the mixture with a magnetic stirrer. This suspension was then added to the prepared buss-bedding mixture or used for injection of animals.

Injection of Carbaryl

The carbaryl for the experiments was prepared using three different solvents: (1) carbaryl dissolved in corn oil; (2) carbaryl dissolved in acetone and (3) carbaryl suspended in distilled water.

The first group of experiments which was performed involved the injection of known amounts of various concentrations of carbaryl into the worms.

There were several experiments conducted using a corn oil-carbaryl mixture. A total of 20 worms per concentration were injected. The total amount injected was: 1×10^{-5}, 5×10^{-5}, 1×10^{-4}, 5×10^{-4}, 1×10^{-3}, 5×10^{-3} and 1×10^{-2} μgs of carbaryl per worm.

There were several experiments conducted using an acetone-carbaryl mixture. A total of 50 worms per concentration were injected. The total amount injected ranged from 1×10^{-2} to 1.6×10^{-1} μgs of carbaryl per worm.

Prior to injections the animals were narcotized for a few minutes in a 0.1% solution of chloretone. A microliter syringe was used for these injections. The controls were injected with 10μl of the solvent. The next group of treated worms were injected with 10μl of the carbaryl-distilled water suspension, in dosages of 0.10, 0.12, 0.14 and 0.16 μgs of carbaryl per worm. The injections were made under the dorsal surface of the animal, just posterior to the clitellum. Careful observations were made for 1-2 minutes to see that the carbaryl was not immediately eliminated. After the injections, the worms were placed in individual milk cartons (9 x 19 x 9 cm.) at 10 worms per box. These milk cartons had been prepared by cutting one side open and sealing the other openings with freezer tape. After the worms and bedding were placed in the boxes, the boxes were covered with porous, clear plastic wrap. During the next 3 weeks, daily observations were made of the treated and control animals.

Topical Application of Carbaryl

To study the effects of topical application of carbaryl to earthworms, the worms were exposed to buss-bedding to which various concentrations of carbaryl had been added.

In these experiments milk cartons prepared as described previously were used. Each box contained 50 gm of buss-bedding. To each of the boxes of buss-bedding 100 ml of tap water was added and was thoroughly mixed with the bedding.

Using a torsion balance 1.5 mgs of the 99% pure carbaryl were weighed and mixed with 10 ml of distilled water. This mixture was added to 150 gm of wet buss-bedding. After this stock of carbaryl and buss-bedding had been thoroughly mixed, several concentrations were obtained from the stock mixture. The concentrations which were obtained from the stock mixture were: 15, 30, 60, 150, 300, 600 and 1,500 μgs of carbaryl per gm wet bedding. Twenty worms were added to each box which contained the various concentrations of bedding mixture. The boxes were then covered with clear, porous plastic wrap, so that:

(1) moisture would be maintained in the bedding and (2) so worms could not escape from the boxes. During the next three weeks, daily observations were made on the number of dead worms, or any obvious symptoms the worms displayed.

Respiration Apparatus and Procedures

The respiration rates of earthworms were determined by using a Scholander Microvolumetric Respirometer, Model VR-300 (The Mark Company, Randolph, Mass.).

Some of the preliminary procedures used for determination of the oxygen consumption are noted in the results. The worms were weighed and one worm was placed in each vial with 5 gm of bedding. After 15 minutes equilibration time, the vials were closed and the data recorded every 10 minutes for one hour. A vial containing 5 gm of bedding was used as a control.

Histological Techniques and Light Microscopy

After the three week period of observation of the control and topically treated worms, the worms were removed from the bedding and placed in boxes containing shredded paper for two days so that the worms would clear their digestive tract of materials.

The intact worms were placed in Bouin's fixative for 1-2 days. The worms were then removed and 1-1/2 cm cross sections were cut from each worm at the area just posterior to the clitellum.

The following procedures were used for preparing the tissues for light microscopy.

1. Washing: the tissues were washed for several hours in tap water to remove the excess Bouin's fixative.

2. Dehydration: the tissues were carried through a dehydration series (using various concentrations of alcohol) which involved the removal of water from the tissues. The following series of ethyl alcohol were used in the dehydration: 30, 50, 70, 80, 90, and 100%.

3. Clearing: the tissues were removed from the alcohol and placed in xylene for 30 minutes.

4. Infiltration: the tissues were infiltrated with paraffin for 24 hours at a temperature of 50-52°C.

5. Embedding and Sectioning: the tissues were embedded with paraffin and 12μ thick sections were made.

6. Staining: the tissues were stained with Harris' hematoxylin and double stained with eosin.

7. Mounting: the tissues were mounted using a permanent mounting medium.

The procedures for clearing, infiltrating, sectioning, staining and mounting are modified procedures taken from Humanson's (1972) "Histological Techniques of Animal Tissues".

RESULTS

LD_{50} Studies

Few studies have been conducted showing the effects of carbaryl on earthworms and most of the studies which have been conducted are those which show only lethal dosages of insecticides on earthworms. The only controlled laboratory study was that of An Der Lan and Aspock (1962 and 1963) in which a 0.3% suspension of carbaryl in water was applied to the cuticle. No experiments in which carbaryl was injected directly into worms have been reported.

Injection of Carbaryl: Preliminary Findings

The injected animals were observed daily for a period of 21 days. (See Table I). As can be seen from this table there occurred a comparatively low mortality in the worms at all concentrations. Most deaths occurred within the first two weeks of treatment, and there was no significant increase in the numbers of mortalities from days 13 to 21. These data suggest the following: (1) that the concentrations were too low to cause any large percentage of deaths in 21 days; (2) that since a very small percentage of deaths occurred in the worms after about two

Table I Mortality in earthworms in the 21 days following injections with 10 μl of corn oil-carbaryl mixture.

Concentration (μg/Worm)	No. Worms	% Mortality
Controls	20	20%
1×10^{-5}	20	40%
5×10^{-5}	20	20%
1×10^{-4}	20	40%
5×10^{-4}	20	30%
1×10^{-3}	20	30%
5×10^{-3}	20	30%
1×10^{-2}	20	50%

weeks, the worms were going to survive the effects of the carbaryl and (3) that since 20% of the controls died and 50% is the largest number of mortalities to occur in the treated animals, the solvent used was insufficient for future studies.

As a result of these initial experiments higher concentrations of carbaryl were used in the subsequent experiments. A shorter period of time for treatment and a different solvent were also used.

Several experiments were conducted which involved the injection of a carbaryl-acetone mixture into the earthworms. A group of 20 worms was injected with 10 μl of carbaryl per worm per concentration. The total amount injected at each concentration was 0.01, 0.02, 0.03, 0.04, 0.05 and 0.10 μgs of carbaryl per worm.

The earthworms were observed for a period of 6 days and their mortality rates were recorded. Table II shows that 40% of the controls died within 6 days and that a 100% mortality was achieved at concentrations of 0.04 and 0.10 μgs of carbaryl per worm, after only 6 days of treatment.

To support the previous findings of the carbaryl-acetone experiments, a second group of experiments involving the use of the carbaryl-acetone mixture was conducted with a total of 30 worms per concentration and injected with 10 μl per concentration per worm. In this

Table II Mortality in earthworms in the 6 days following injections with 10 μl of acetone-carbaryl mixture.

Concentration (μg/Worm)	No. Worms	% Mortality
Controls	20	40%
0.01	20	40%
0.02	20	60%
0.03	20	80%
0.04	20	100%
0.05	20	40%
0.10	20	100%

group of experiments, however, the concentrations were increased to 0.10, 0.12, 0.14, 0.16 μgs of carbaryl per worm. After observations for 6 days, the mortality rates were recorded.

As can be seen from Table III, a 30% mortality occurred in the controls, after only 6 days. This table also shows that 0.12 and 0.16 μgs of carbaryl per worm gives a 100% mortality.

There was a significant percentage of deaths in the control worms in this experiment as well as the previous one. This suggests that the acetone may be toxic to the worms, thus it appears that acetone is an unsuitable solvent for these experiments.

In a further attempt to find a more suitable solvent, several experiments were conducted which involved the use of a carbaryl-distilled water suspension. A total of 30 worms per concentration were used. The concentrations of carbaryl used in these tests were 0.10, 0.12, 0.14, and 0.16 μgs per worm, the same as those used in the previous experiments. Observations were made for a period of 5 days.

As can be seen from Table IV no mortality occurred in the controls over a period of 5 days, while in all the carbaryl treated groups there was significant mortality. This suggested that distilled water was a suitable solvent to use in the application of carbaryl to earthworms.

Table III Mortality in earthworms in the 6 days following injections with 10 µl of carbaryl-acetone mixture.

Concentration (µg/Worm)	No. Worms	% Mortality	No. Days 50% Killed	No. Days 100% Killed
Controls	30	30%	--	--
0.10	30	67%	3	--
0.12	30	100%	3	4
0.14	30	67%	4	--
0.16	30	100%	4	5

Table IV Mortality in earthworms in the 5 days following injections with 10 μl of carbaryl-distilled water suspension.

Concentration (μg/Worm)	No. Worms	% Mortality	No. Days 50% Killed	No. Days 100% Killed
Controls	30	0%	--	--
0.10	30	67%	4	--
0.12	30	100%	3	5
0.14	30	67%	3	--
0.16	30	83%	3	--

In a second group of experiments, using the same solvent (distilled water), a total of 6 worms per concentration were used and the concentrations used previously were the same, except that the amount of carbaryl-distilled water injected was decreased from 10µl per worm to 5µl per worm and the worms observed for a period of 30 days. The results of these experiments are recorded in Table V. This table shows that a mortality of 83% was achieved in all concentrations between 8 and 12 days after treatment. No mortalities occurred from day 12 to day 30, suggesting that the animals which were living after day 12, would survive the treatment.

In a third group of experiments, the concentrations, amounts injected and the number of animals used remained the same as in experiment two; however, a test period of 25 days was used. The results are summarized in Table VI. As can be seen from this table, thirteen days were necessary to achieve a 100% mortality in most concentrations.

From these injection experiments several conclusions seem warranted: (1) the type of solvent which seems to be most applicable for the carbaryl is distilled water. No mortalities were seen in the controls and there are few inconsistent patterns in mortality rates at the various concentrations: distilled water was the best solvent to use in these tests; (2) using the carbaryl-distilled water

Table V Mortality in earthworms in the 30 days following injections with 5µl of carbaryl-distilled water suspension.

Concentration (µg/Worm)	No. Worms	% Mortality	No. Days 50% Killed	No. Days 100% Killed
Controls	6	0%	--	--
0.05	6	83%	10	--
0.06	6	83%	12	--
0.07	6	83%	9	--
0.08	6	83%	8	--

Table VI Mortality in earthworms in the 25 days following injections wity 5µl of carbaryl-distilled water suspension.

Concentration (µg/Worm)	No. Worms	% Mortality	No. Days 50% Killed	No. Days 100% Killed
Controls	20	0%	--	--
0.05	20	100%	6	13
0.06	20	90%	4	--
0.07	20	100%	4	13
0.08	20	100%	4	8

mixture, in the various concentrations allowed for longer test periods to be used, i.e. longer time to observe the development of symptoms in earthworms.

All of the remaining experiments in this study are based on these preliminary findings.

Topical Application of Carbaryl: LD_{50} Results

In four separate experiments, seven concentrations of carbaryl were used to test the effects of topical application of carbaryl to the earthworm. The worms used in these tests were exposed to the following dosage of carbaryl: 1.5, 3.0, 6.0, 15, 30, 60 and 150 μgs of carbaryl per gm of wet buss-bedding. A total of 81 worms were used for each dosage.

The treated worms were placed in a constant temperature room at 15.5°C, and were observed for symptoms for 3 weeks. The mortality rates were recorded for each group and for each concentration from the first day through the twenty-first day after treatment.

The results of these experiments are presented in Table VII. No mortalities were noted in the controls nor in concentrations of 1.5, 3.0, and 6.0 μgs of carbaryl per gm of wet bedding, therefore, these concentrations were not included in Table VII.

Table VII Mortality of earthworms following topical application of carbaryl: 81 earthworms/concentration.

Days	No. Worms Dead/Concentration (μg/gm) 15μg	30μg	60μg	150μg
1-5	0	0	0	0
6	0	2	3	5
7	1	3	4	11
8	2	3	4	12
9	3	4	11	17
10	5	6	15	19
11	5	8	21	26
12	8	11	25	36
13	9	12	32	46
14	10	15	37	51
15	10	18	40	55
16	10	20	44	58
17	10	26	46	62
18	12	31	49	66
19	12	36	54	70
20	14	41	59	73
21	15	46	60	74

Figure I gives a probit plot of the data in Table VII. From this figure the LD_{50} for the carbaryl on earthworms at 3 weeks is approximately 20 μgs of carbaryl per gm of wet bedding. As can be seen in this table, there is a direct correlation of the lethal effects on the worms to increases in concentration of the carbaryl. The first line at the bottom of the graph represents responses in the worms at 7 days of treatment and each successive line, represents odd days of treatment (9, 11, 13, 15, 17, 19 and 21). This graph also shows that at the dosages tested that more than 3 weeks are necessary to show complete mortality. Since the highest number of days of treatment (21 days) were not sufficient to cause complete mortality, longer time periods are required using these concentrations.

Morphological and Physiological Studies

External Symptoms After Treatment

The symptoms discussed here were noted during the previous LD_{50} studies. Some symptoms were seen in the animals at carbaryl concentrations of 15, 30, 60 and 150 μgs per gm wet bedding after only 1-2 days of treatment. These primary symptoms were: (1) constriction of the body, especially in the area of the clitellum and swellings at the anterior region of the worm; (2) a slight redness throughout the worm's body with a greater intensity in the region of

Figure 1

Figure 1 Percent mortality for earthworms treated with 15, 30, 60 and 150 μgs of carbaryl per gm of buss-bedding. These probit plots show the responses to concentrations beginning on the 7th day (lowest line) and going through odd days to the 21st day of treatment (top line).

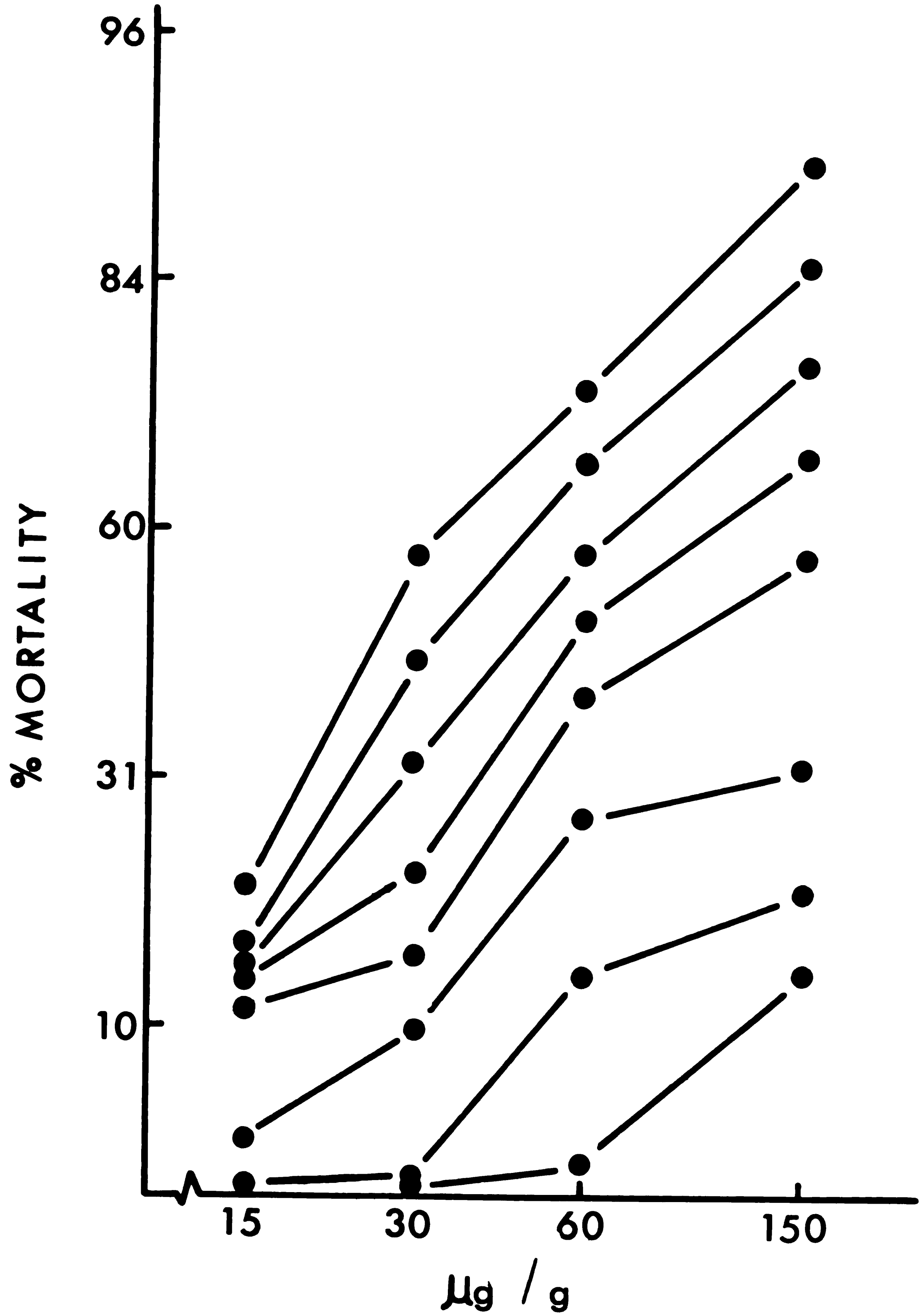


Figure 1

the clitellum; (3) coiling throughout the entire length of the animal; (4) decreased locomotive ability and (5) a decreased "withdrawal ability", i.e. responses to external mechanical stimulation were much less than in the controls. After 10 days of observation, these same symptoms were seen in the animals at the lower concentrations of 1.5, 3.0 and 6.0 μgs carbaryl per gm of wet bedding.

After 2 weeks many of the animals at the higher concentrations had begun to die. Before death many secondary changes occurred: (1) the worms lost their ability to constrict and coil, and appeared in a totally relaxed state; (2) there was a definite swelling in the area anterior to the clitellum; (3) the discoloration of the body became localized and only the anterior region of the body had a reddish color; the color in the posterior end of the body ranged from yellow to white or colorless; (4) the posterior end of the body became hard and shrunken; (5) almost no movement was seen in the animals, especially in the posterior region of the body; (6) there were little or no responses to external mechanical stimulation; (7) blisters had formed on the surface of the worm and the epidermis appeared to have become very thin and swollen and sometimes these areas of the skin has burst open, resulting in the expulsion of coelomic fluids and waste materials of the gut. When this occurred, there was extensive bleeding in the areas of the ruptures. Most of these lesions occurred in the middle and anterior regions of the animal and (8) large amounts of mucus were secreted from the surface of the body.

During the second and third weeks after beginning of the treatment, the animals treated with the lower concentrations of 1.5, 3.0 and 6.0 μgs carbaryl per gm of bedding, were carefully observed and it was found that most of the primary symptoms, such as coiling, twisting, constricting of the body, especially in the region of the clitellum and redness, had seemingly disappeared and these animals now seemed to have recovered, at least to the extent that it was not possible on external observations to see any physical damage or even distinguish between the treated animals and the control animals.

Some of the other types of behavioral responses of the worms treated with carbaryl were: (1) the treated animals (in all concentrations) after two days, responded to various chemicals, such as 10% KOH, 10% HCl, 1% chloretone and 10% NaCl solutions, which were applied to the epidermis of the worms. In each case, these animals gave a "withdrawal response". When these same chemicals were applied to the treated animals on the tenth day, only the animals in the lower concentrations (of 1.5, 3.0 and 6.0 μgs per gm wet bedding) responded by withdrawing; (2) all treated animals (at all concentrations) on the second day reacted to intense light by showing withdrawal responses. When these animals were stimulated with the light after the tenth day, only the animals at the two highest concentrations of 60 and 150 µgs did not respond to the light; however, on the twenty-first day, only animals at the lowest concentration (1.5µgs) responded to the light.

Based on the data collected here, full evidence of poisoning was not immediately obvious. Deaths continue to occur for a period of time in excess of 3 weeks. The symptoms these treated animals showed seem to follow a definite pattern; first there was an initial stage of degeneration which I have called primary symptoms from which the animals seem to have the ability to recover; secondly there were a set of secondary symptoms from which the animals apparently could not recover.

Respiration Studies: Respiration Rates Before Treatment

In the previous set of experiments the LD_{50} was established for the earthworms in a given population for a period of 3 weeks. However, it was noted that at the lower concentrations of 1.5, 3.0 and 6.0 µgs carbaryl per gm wet bedding, worms though showing some symptoms of poisoning were not killed.

Thus, mortality at these lower concentrations cannot be used as an index of the toxicity of carbaryl for the earthworms. Descriptions of external symptoms are not easily quantifiable, and it seems likely that at concentrations even lower than those used in the previous experiments, deleterious effects may occur with no external manifestations of symptoms.

In an effort to obtain a reliable measure of sublethal effects of carbaryl treatments on earthworms, a series of experiments were undertaken in which oxygen consumption in carbaryl treated worms was measured.

As far as could be determined from the review of the literature, no respiration studies had been conducted showing respiration rates of earthworms after treatment with an insecticide. Johnson et al. (1942) have shown the respiration rates of untreated earthworms to be in the range of 40-50 mm^3 O_2/gm/hr under various laboratory conditions for animals weighing from 2-5 gms and at a temperature of 10°C.

In the initial set of experiments, an attempt was made to establish the pattern of respiration rates in untreated animals was established. In this group of experiments, a total of 32 worms were used. The animals were removed from the bedding and placed into the volumetric respirometer which had a constant temperature of 16.5°C, and the data from the experiments were recorded every ten minutes for one hour.

The following results were obtained. Of the 32 worms used in these experiments, the average oxygen consumption for the worms whose size ranged from 2-5 gm (and whose temperature was 16.5°C) was approximately 93 µl O_2/gm/hr, with the range being from 82-101 µl O_2/gm/hr. These data were collected from four different experiments, using eight animals per experiment. See Table VIII. The rates are higher than those reported by Johnson *et al.* (1942). These experiments I have conducted are at a temperature nearer the optimum for earthworms.

Respiration Studies: Respiration Rates After Treatment

Respiration experiments were conducted in which treated animals were used from previous LD_{50} experiments. Animals of these lower concentrations showed no lethal effects, but did show external symptoms. Non-lethal concentrations of 1.5, 3.0 and 6.0 µgs of carbaryl per gm wet bedding were used. A total of 26 worms per concentration were used for each of the three dosages and 26 untreated control worms were evaluated for each group of treated worms (See Table IX). All animals were tested on the twenty-first day after treatment. The respirometer temperature for all of the experiments was always maintained at 16.5°C. These data were usually collected in the afternoon and early evening. Most of these experiments were conducted in the late spring and summer seasons and only mature worms were used in these tests.

Table VIII Oxygen consumption for untreated animals, µl O_2/gm/hr.

Animal No.	Experiment No. 1	2	3	4
1	86	101	96	101
2	87	87	83	91
3	96	92	84	96
4	101	99	90	99
5	99	98	98	89
6	89	98	98	100
7	94	88	96	83
8	96	97	95	96
Average: (mean)	93	95	92	94

Table IX Oxygen consumption of earthworms

Table IX shows an analysis of animal variability for all earthworms; treated and controls. This table shows 6 groups of oxygen consumption rates of earthworms with one group of controls for each group of treated animals.

Table IX Oxygen consumption of earthworms

Analysis of Animal Variability (µl O_2/gm/hr)

Animal No.	Conc. 0.3	Cont.	Conc. 0.6	Cont.	Conc. 1.2	Cont.	Conc. 1.5	Cont.	Conc. 3.0	Cont.	Conc. 6.0	Cont.
1	105.0	97.8	138.0	79.2	134.4	82.2	121.2	81.6	113.4	84.6	120.6	68.4
2	132.0	139.8	108.6	116.4	127.2	86.4	87.0	88.8	96.0	82.2	163.8	78.0
3	144.6	136.8	144.6	87.0	138.0	116.4	87.0	64.2	112.8	82.2	84.6	69.0
4	135.0	91.2	77.4	78.0	118.2	107.4	131.4	63.0	159.6	76.2	107.4	77.4
5	79.2	79.2	144.6	138.6	63.0	27.6	115.2	96.0	112.8	52.2	142.8	101.4
6	143.4	153.0	173.4	107.4	83.4	17.4	101.4	94.8	84.6	64.8	109.2	84.0
7	143.4	101.4	137.4	156.0	67.2	63.6	130.8	66.0	79.2	84.6	132.6	60.0
8	78.6	92.4	171.0	183.0	72.0	40.8	155.4	114.0	108.0	90.0	166.8	124.2
9	98.4	79.2	105.6	67.2	103.2	60.0	104.4	91.2	85.2	54.0	90.6	87.6
10	86.4	95.4	73.2	83.4	86.4	63.6	112.8	94.2	99.0	43.8	132.6	102.0
11	99.0	75.0	106.2	58.5	105.0	52.2	80.4	105.6	94.2	76.2	115.2	129.6
12	97.8	115.2	61.2	94.2	103.2	82.8	132.6	84.6	93.0	58.2	111.0	94.8
13	82.8	102.0	109.8	68.4	101.4	41.4	105.0	66.6	131.4	76.2	143.4	117.0
14	85.2	73.8	102.0	87.6	114.6	54.0	97.8	84.0	111.0	62.4	141.6	89.4
15	76.8	81.0	102.6	93.6	95.4	66.0	94.2	90.6	70.2	99.6	133.8	136.2
16	86.4	38.8	90.0	81.0	115.2	56.4	111.6	109.2	94.2	96.0	168.6	89.4
17	144.0	84.0	126.0	84.0	132.0	84.0	138.0	84.0	132.0	84.0	210.0	84.0
18	144.0	96.0	144.0	96.0	138.0	96.0	162.0	96.0	192.0	96.0	210.0	96.0
19	132.0	96.0	132.0	96.0	162.0	96.0	192.0	96.0	144.0	96.0	168.0	96.0
20	132.0	108.0	114.0	108.0	132.0	108.0	192.0	108.0	180.0	108.0	198.0	108.0
21	132.0	108.0	132.0	108.0	132.0	108.0	144.0	108.0	162.0	108.0	204.0	108.0
22	138.0	90.0	138.0	90.0	180.0	90.0	126.0	90.0	186.0	90.0	198.0	90.0
23	108.0	102.0	126.0	102.0	132.0	102.0	168.0	102.0	168.0	102.0	168.0	102.0
24	114.0	102.0	132.0	102.0	126.0	102.0	156.0	102.0	162.0	102.0	216.0	102.0
25	138.0	108.0	132.0	108.0	138.0	108.0	174.0	108.0	174.0	108.0	186.0	108.0
26	138.0	102.0	132.0	102.0	174.0	102.0	150.0	102.0	180.0	102.0	204.0	102.0

In a second set of experiments, using the same controlled conditions and the same number of animals per concentration, three concentrations, lower than those previously utilized were tested. The new concentrations used were 0.3, 0.6 and 1.2 μgs of carbaryl per gm of wet bedding. No external symptoms were seen in animals treated with these concentrations. All of the data from the previous two groups of experiments are recorded in Table IX, the results of the data analysis are shown in Table X and are graphically shown in Figure 2.

There appears to be a definite relationship between effects of carbaryl and respiration rates. The initial conclusions of these data suggest: (1) that carbaryl treated animals consumed oxygen at a faster rate than did the untreated animals, and (2) that oxygen consumption increased in treated animals even though there may be no external signs of carbaryl poisoning. Thus, oxygen consumption may be used as a measure to show effects of carbaryl on earthworms.

Histological Studies

Data and Observations

An Der Lan and Aspock (1962 and 1963) reported the general biological characteristics of *Lumbricus* sp. after treatment with carbaryl. They noticed that after treating the earthworm with a lethal concentration of 0.3% carbaryl,

Table X Data analysis of oxygen consumption

Concentration	µl O_2/gm/hr Consumed $\pm$ S.D.		Difference
(µg/gm)	By Treated Animals	By Controls	(Treated--Controls)
1. 0.3	115 $\pm$ 25	99 $\pm$ 19	16 µl O_2/gm/hr
2. 0.6	121 $\pm$ 27	99 $\pm$ 26	22 µl O_2/gm/hr
3. 1.2	118 $\pm$ 30	77 $\pm$ 27	41 µl O_2/gm/hr
4. 1.5	129 $\pm$ 32	91 $\pm$ 14	37 µl O_2/gm/hr
5. 3.0	127 $\pm$ 38	83 $\pm$ 18	44 µl O_2/gm/hr
6. 6.0	155 $\pm$ 40	96 $\pm$ 18	59 µl O_2/gm/hr

Figure 2 Graph of respiration rates

Respiration rates of earthworms treated with 0.3, 0.6, 1.2, 1.5, 3.0 and 6.0 μgs of carbaryl per gm of buss-bedding. The dotted line represents the respiration rates of the controls and the solid line represents the respiration rates for the animals treated with the carbaryl.

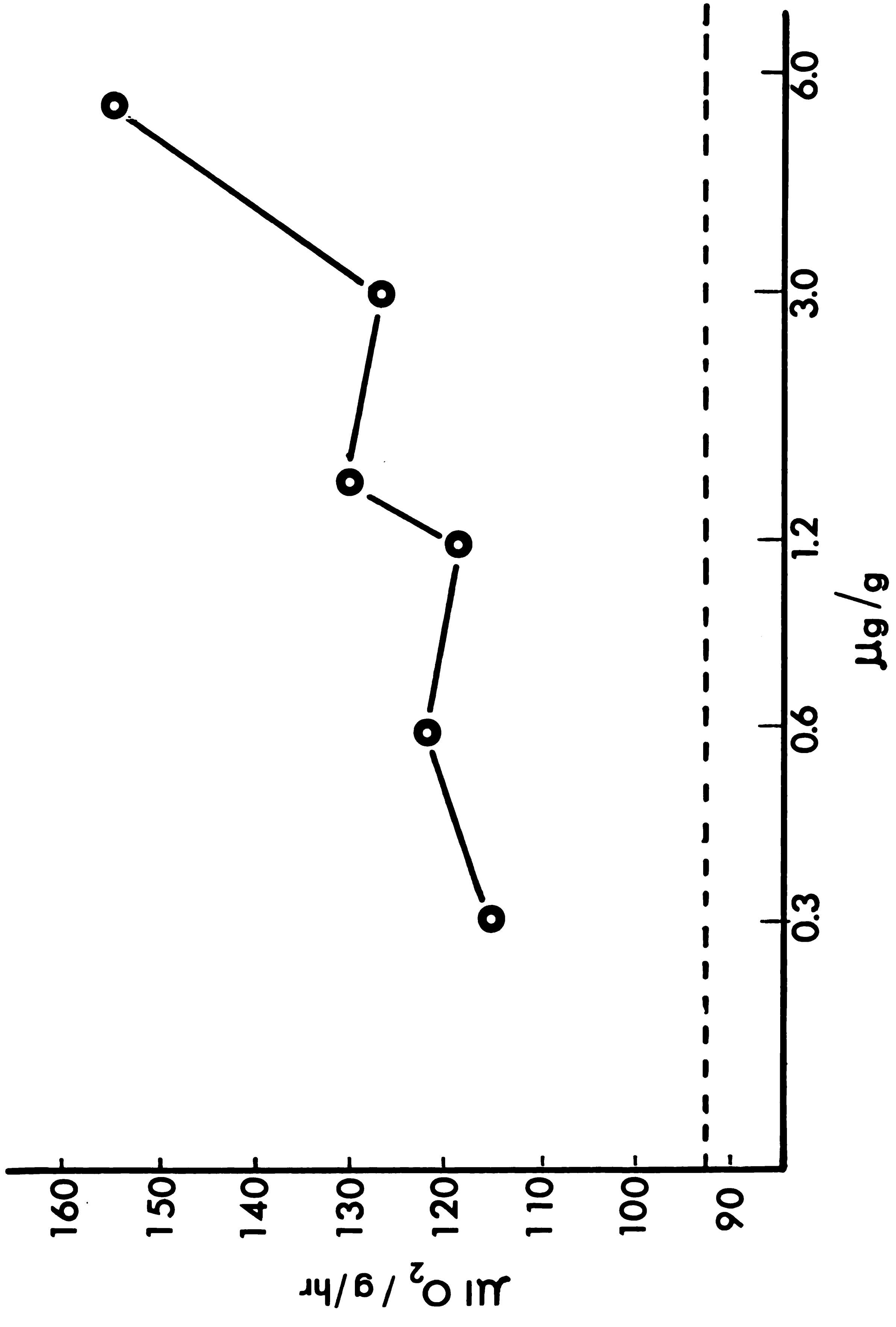


Figure 2

many blisters appeared on the epidermis and ruptures occurred within only a few days after treatment. Based on these observations, they conducted histological studies of the earthworm body wall. The results of these investigations showed that there was a change in the position of the epithelial cells of the epidermis. It was also noted in their investigations that various lesions or "skin cancers" were present. These epidermal cancers were seen only in worms treated with lethal concentrations of carbaryl.

In my experiments with sub-lethal concentrations of carbaryl several non-lethal effects that were caused by carbaryl were noted. Therefore it seemed likely that there would also be some histological effects in earthworms treated with sub-lethal concentrations of carbaryl.

In these histological studies earthworms were treated and observed for three weeks after using four concentrations of: 1.5, 3.0, 6.0 and 15 μgs of carbaryl per gm of bedding. The first three concentrations were non-lethal after three weeks of treatment, while 15 μgs of carbaryl per gm of bedding resulted in some mortality. A control group of animals was also observed for three weeks, but no external changes were observed in this group.

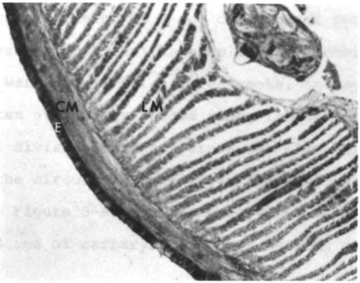
The transverse section as seen in Figure 3-A shows the body wall of a normal untreated worm, it shows longitudinal and circular muscle fibers, and the epidermis.

Figure 3

A. Photomicrograph of the normal earthworm body wall (12μ thick in transverse section). In the normal or control worm the longitudinal muscle layer (LM) consists of pennate bundles, and several layers of circular muscle fibers (CM) which lie adjacent to the longitudinal layer on one side and the epidermis (E) on the other side. X40

B. Photomicrograph of earthworm body wall (12μ thick in transverse section), after treatment with carbaryl at 3 μgs of carbaryl per gm of bedding for 3 weeks. The longitudinal muscle layer (LM) shows little or no change, however, the circular muscle layer (CM) has been affected by showing what appears to be degenerating tissues and lesions in the epidermal layer (arrow). X40

A



B

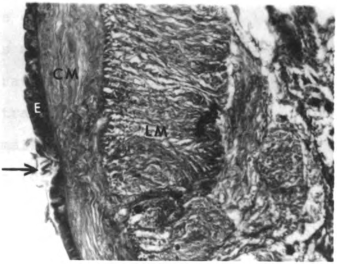


Figure 3

At a concentration of 1.5 μgs of carbaryl per gm of bedding, no gross histological changes were seen, the treated animals appearing identical to the control animals.

At a concentration of 3 μgs of carbaryl per gm of bedding, however, significant changes in the histology of the body wall were seen. After three weeks, the epidermal layer was broken at various places along its surface. Increased cell division in these areas was also observed. In addition, the circular muscle layer appeared to be degenerating. Figure 3-B gives an example of an animal treated with 3 μgs of carbaryl per gm of bedding for 3 weeks.

Animals were also treated with 6 μgs of carbaryl per gm of bedding for three weeks. An example of the typical results of this treatment is shown in Figure 4-A. This section shows some very definite lesions and breaks in the epidermal layer of tissues. There also appears to be a degeneration of the circular muscle layer and a proliferation or extensive growth of epidermal cells into the areas where the lesions and breaks have occurred. This degeneration and proliferation of tissues is more extensive than at the lower concentrations.

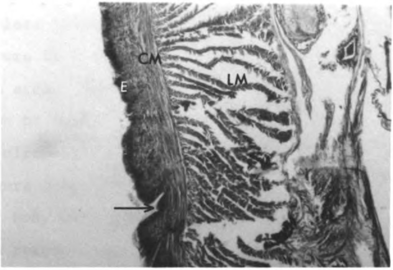
When animals were treated with 15 μgs of carbaryl per gm of bedding for three weeks, a small percentage (5-10%) of the total population of the earthworms were killed. Even though most of the worms survived during this

Figure 4

A. Photomicrograph of earthworm body wall (12 μ thick in transverse section) after treatment with carbaryl at 6 μgs of carbaryl per gm of bedding for 3 weeks. This section shows that the longitudinal muscle layer (LM) appears to have minor changes from the control. There are lesions in the epidermis (E) and circular muscle layer (CM). There is a proliferation of epidermal cells where the lesions have occurred (arrows). X40

B. Photomicrograph of earthworm body wall (12 μ thick in transverse section) after treatment with carbaryl at 15 μgs of carbaryl per gm of bedding for 3 weeks. The longitudinal muscle layer (LM) does not exist in bundles as in the controls, but appears to be very irregular in shape. The circular muscle layers (CM) have degenerated and there is a proliferation of epidermal cells in these affected areas. X40

A



B

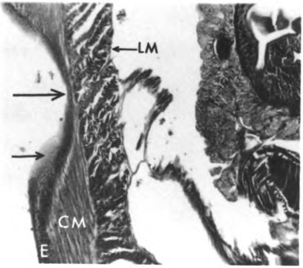


Figure 4

period of time, very extensive damage was seen in the histological sections of almost all of the worms (Figure 4-B). In particular areas there is extensive degeneration and almost complete loss of the circular muscle layer. In addition there is a great proliferation of epidermal cells in the same area. This extensive growth of cells and degeneration of muscle fibers occurs at random throughout the entire circumference of this anterior region of the worm.

Figure 5-A, 5-B, 6-A and 6-B shows similar cross-sections of body wall tissues as seen in Figures 3-A, 3-B, 4-A and 4-B respectively at a much higher magnification (X430).

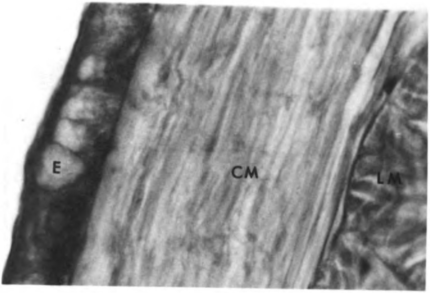
From these histological observations it is evident that even at non-lethal concentrations of carbaryl, extensive histopathological changes occur. These changes may be due to increased mitotic cell divisions. Thus, from these histological studies as well as the respiration studies, it would appear that simple utilization of mortality data by itself to assess the impact of carbaryl treatment on worms does not give an accurate account of the potential changes carbaryl may cause in earthworm populations. Because the earthworms were exposed to the carbaryl via the bedding and the pathological changes which occurred affected the epidermal and circular muscle layers first it appears likely that the earthworms are killed or affected via topical application of the insecticide rather than by ingestion of the insecticide in their food.

Figure 5

A. Photomicrograph of a normal (control) earthworm body wall (12 μ thick in transverse section) showing the epidermis (E), circular muscle layer (CM) and a portion of the longitudinal muscle layer (LM). X430

B. Photomicrograph of an earthworm body wall (12 μ thick in transverse section), which has been treated with 3 μgs of carbaryl per gm of bedding. This section shows several changes in the epidermis (E) and circular muscle layer (CM). Here minor changes can definitely be observed in the epidermal layer (arrow) which differ remarkably from the epidermis of the controls. X430

A



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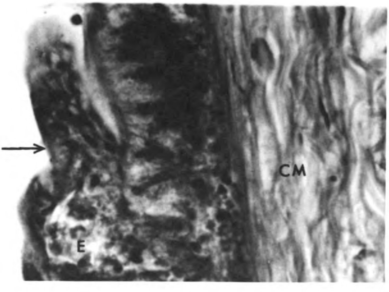


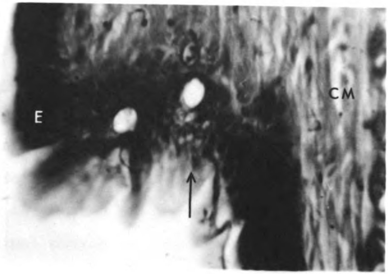
Figure 5

Figure 6

6-A is a photomicrograph of an earthworm body wall (12 μ thick in transverse section) which has been treated with 6 μgs of carbaryl per gm of bedding. This section (arrow) shows the beginning degeneration of the circular muscle layer (CM) and growth of the epidermal layer of cells (E). X430

6-B is a photomicrograph of an earthworm body wall (12 μ thick in transverse section), which has been treated with 15 μgs of carbaryl per gm of bedding. This section (arrow) shows an extensive proliferation of the epidermal cell (E) in the regions of the circular muscle layer (CM) and the epidermis (E). X430

A



B

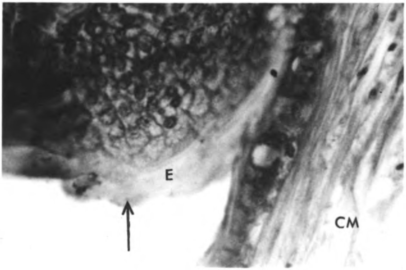


Figure 6

DISCUSSION

The results obtained in this study show that the earthworm, Lumbricus terrestris, is affected both morphologically and physiologically by carbaryl. Both injection and topical applications cause effects. Histological evidence indicates that one major site of action of carbaryl on earthworm is the body wall (cuticle, epidermis, circular and longitudinal muscle layer).

Toxicity of Carbaryl for Earthworms

There have been few previous studies of the toxicity of carbaryl on earthworms. Edwards (1965) in field studies reported effects of carbaryl residues (at doses of 1.5 to 2 lbs per acre) up to 6 months after treatment. The effect was a large reduction in the number of earthworms. Thompson (1971) also reported data from field studies on the effects of carbaryl on earthworms. He found that when he used various concentrations ranging from 1 to 5 lbs per 10' x 6' plots and 3 weeks for treatment, caused a 60% reduction in the number of earthworms in the treated plots. An Der Lan and Aspock (1962) in controlled laboratory experiments, tested the effects of a 0.3% suspension of carbaryl brushed onto the cuticle of the worms. They found that this treatment was lethal to the earthworms and in addition, caused a variety of histopathological changes in the worms. My studies show an LD_{50} for carbaryl of approximately 20 μgs per gm of wet bedding after 21 days of treatment.

Because the methods of application have been so different in all of the previous studies conducted up to the present time, it is impossible to make direct comparisons between by results and those of other workers; however, as a result of my studies several points should be made about the toxicity of carbaryl for earthworms. First, any determination of the toxicity of carbaryl for earthworms is heavily dependent on the dosage time used, i.e. my results show that more than 3 weeks were needed for complete mortality in the earthworm population tested. Thus, mortality studies involving only a few hours or days of treatment will not give a good measure of the full effects on earthworm populations that long term exposure to carbaryl might have.

A second point is that the use of mortality of worms in laboratory studies may not be an accurate indicator of the impact of carbaryl applications in the field on earthworm populations. My data do indicate that at concentrations of carbaryl considerably below those at which mortalities occur, the worms are so adversely affected, and their survival in the field might be considerably lessened.

The results I have obtained on respiration rates have indicated a consistent increase in rates at doses ranging from 0.3 to 6.0 μgs of carbaryl per gm of wet bedding at 3 weeks. These are concentrations considerably below those at which lethal effects were shown in my laboratory studies. My results show growths of epithelial cells from the epidermis into the area of circular muscles, degeneration of the circular muscle fibers and a change in the normal position of the longitudinal muscles of the body wall at carbaryl concentrations below the lethal level.

A third point which should be made is that extrapolation from my data to expected results in the field must be done with considerable caution. Several factors may change the ways in which worms are affected in field studies: (1) since the breakdown of carbaryl will vary greatly with time and soil composition, it is difficult to know if carbaryl has been changed to its various metabolities before, during or after exposure; (2) the worms in field studies are not labeled, therefore, there is no accurate way to be sure that the worms initially exposed to the carbaryl are the same as those observed days, weeks or even months later; (3) the application of carbaryl is usually in the form of a wettable powder, sprayed or dusted on the soil surface. Using this technique it is difficult to know how many worms are actually being exposed to the carbaryl.

Comparison of Carbaryl Effects on Other Animals

The toxicity of carbaryl as seen in the present study for earthworms is similar to that Sugiyama (1971 and 1972) and his co-workers determined for the silkworm, Bombyx mori and the black-tipped leafhopper, Bothrogonia japonica. The LD_{50} for the silkworm was calculated to be 12.6 μgs carbaryl per gm of body weight for the 2nd day of the 4th instar and 15.2 μg carbaryl per gm body weight for the 4th day of the 5th instar with topical application. LD_{50} value of carbaryl to the black-tipped leafhopper was determined with topical application to be equivalent to 0.99 μg per gm body weight for the nymph and 1.46 μg per gm body weight for the adult. The 20 μg per gm wet bedding LD_{50} I have determined for earthworms is in the same general area. Reeves (1962) reported laboratory studies of acute oral toxicity of carbaryl. He reported an LD_{50} for the rat of 510-560 mg per kg; for the rabbit 710 mg per kg and for the guinea pig 280 mg per kg of body weight.

An Der Lan and Aspock (1962) reported an LD_{50} of 540 μg per gm body weight for the flatworm, Crenobia alpina, using topical application of the carbaryl. My LD_{50} value of approximately 20 μgs carbaryl per gm of wet bedding indicates a much different toxicity to earthworms, suggesting a broad spectrum of effectiveness of carbaryl to invertebrates.

Mode and Site of Action of Carbaryl

My studies, as well as those of An Der Lan and Aspock (1962) suggest that one site of action of carbaryl is the body wall. The results of their investigations as well as mine showed extensive growths of epithelial cells, degenerating circular muscle fibers, a change in the position of the longitudinal muscle bundles and breaks in the cuticle throughout the body surface of the animal.

The symptoms observed in the carbaryl treated earthworms do not appear to be specific to carbaryl.

Gersch (1951 and 1957) reported effects of various dyes and stains on the body wall of the earthworm. His results showed growths of epithelial cells and degenerating tissues of the circular muscle layer of the body wall. He also noted lesions and swellings of the body wall.

In a similar paper, Cooper (1968) reported tumors and growths in earthworm body wall, which were the results of foreign materials being released into the coelomic cavity. Thus, it appears possible that the histopathological changes I have seen in the earthworm in response to carbaryl treatments are generalized responses and not specific responses to carbaryl as such.

O'Brien (1967) suggested that carbaryl, like many other methylcarbamates, is a fairly potent inhibitor of cholinesterase in mammals and suggested that many symptoms which may accompany their action, such as lachrymation, salivation, excretion, convulsions and even death are caused by the anticholinesterase activity. He noted that only a few studies have been reported in insects and mammals showing the mode of action of the carbamate carbaryl.

Casida (1963) suggested that the carbamates act in insects and mammals as autonomic drugs, stimulating structures innervated by cholinergic nerves. This parasympathetic action results from inhibition of acetylcholinesterase to allow local accumulation of acetylcholine.

Even though no studies have been found on the mode of action of carbaryl in earthworms, it is possible that earthworms respond in a very similar manner to the mode of action reported previously for insects and mammals.

Since the importance of acetylcholine and cholinesterase in earthworms has not been clearly established, it will only be through further detailed investigations that it will be possible to determine precisely the site of action of carbaryl in the earthworm.

SUMMARY

1. An LD_{50} of 20 μgs of carbaryl per gm of wet bedding has been established for earthworms. The method of application was topical, the temperature during the treatment period was 15.5°C, the solvent used was distilled water and the period of treatment was 21 days.

2. Using non-lethal doses of carbaryl, several behavioral and morphological effects were seen: lack of "withdrawal responses" to chemical and light stimuli, coiling, twisting and constricting of the body and discoloration of the epidermis of the treated animals.

3. There were increased rates of oxygen consumption for <u>Lumbricus terrestris</u>, after three weeks of treatment, with non-lethal doses of carbaryl, even in animals which did not show any external symptoms of treatment.

4. The histological studies show that treatment of worms with non-lethal concentrations produce a number of structural changes in the body wall. Some of these effects were: proliferation of epithelial cells of the epidermis into the circular muscle layer and lesions throughout the surface of the body wall.

5. These lethal and sub-lethal effects were observed at concentrations of carbaryl as low as 0.3 μgs per gm of bedding and the effects of carbaryl treatment at these low concentrations seems not to be specific for the earthworm, since other compounds may cause the same types of effects in the earthworm.

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