THE UNIVERSITY OF MANITOBA

ECOLOGY OF SLUGS IN MANITOBA, AND ACCUMULATION, STORAGE AND EXCRETION OF DDT IN THEIR BODIES

by

Gordon James Howe

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

DEPARTMENT OF ENTOMOLOGY

WINNIPEG, MANITOBA

October 1972

ABSTRACT

The purpose of this study was to observe different aspects of slug ecology in Manitoba, and examine the accumulation, storage and excretion of DDT by slugs. Reference was made to the culture of slugs for use in long-term laboratory experimentation.

A field survey was done in August 1972 with samples of slugs being collected from 19 rural communities and urban areas in the southern part of the province. Three species of slugs are found in Manitoba at present. Agriolimax laeve occurred in nearly all areas sampled, whereas Lehmannia valentiana was found only in 2 greenhouses. Agriolimax reticulatus was found in all but 9 of the sampled areas. This species is responsible for much of economic and asthetic damage done by slugs in Manitoba.

Severe climatic conditions during the summer and winter prevent slugs such as Lehmannia valentiana from becoming established in the field. Severe winter conditions likewise limit Agriolimax reticulatus to winter survival in the egg stage.

Particular reference was made with respect to accumulation, storage and excretion of DDT. ^{14}C-pp-DDT was fed to slugs at 4, 16, 40 and 80 ppm. dry weight in the diet. A variety of food sources were tested as vehicles for adminis-

tering the DDT to the slugs and were exposed to the slugs for up to 104 days.

For both Agriolimax reticulatus and Lehmannia valentiana, rate of accumulation was proportional to time of exposure and level of intake. Agriolimax reticulatus showed a greater rate of accumulation of DDT from both leaf litter and oatmeal pellets than did Lehmannia valentiana. Both species concentrated the majority of the DDT in the hepatopancreas. The rate of excretion was fairly slow when the slugs were feeding on DDT-free food. Of the 20% retained after feeding on DDT for 20 days, 12% was still present in the slug tissue after 79 days. Mucous secretion and production of eggs resulted in little loss of DDT from the tissue.

The possibility of these ubiquitous invertebrates acting as indicators of pesticides in the soil environment was also considered. It is hoped that this study will act as an introduction to the study of terrestrial molluscs in Manitoba.

ACKNOWLEDGEMENTS

The writer wishes to express his sincere appreciation to Dr. G. M. Findlay, Department of Entomology, The University of Manitoba, for his guidance and counsel. I am grateful to Professor A. J. Thorsteinson, Head, Department of Entomology, University of Manitoba, and Dr. A. L. Hamilton, Fisheries Research Board of Canada for their constructive criticism and advise.

I wish to thank Mrs. K. Subrahmaniam for her help with statistical analysis and Mr. L. Perreault for his forbearance throughout the field work.

I wish to sincerely thank Dr. M. G. Dudley for her help and encouragement during the past years.

TABLE OF CONTENTS

Chapter | Page

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Terrestrial slugs are an important component of the ecosystem. In many parts of Europe slugs form a large part of the biomass of a given biotic community. Most species of slugs are herbivorous and detritis feeders, but a few are carnivorous. The amount of food consumed by slugs is considerable, thus in many areas the herbivorous slugs are important primary consumers. Although slugs are apparently utilized as food by predatory organisms, their importance in food chains is undetermined. Climatic conditions and pathogenic organisms are apparently the key factors determining the distribution and abundance of slugs in a given area.

DDT usage increased greatly from 1947 to 1960. Much of the DDT now in the environment was introduced by spraying for agricultural pests, forest pests and mosquito-fly control. Much of the DDT reached the top soil layers directly by spray application, or indirectly by being washed off of plants by precipitation or by being adsorbed to plant material which eventually dies and falls to the ground. The DDT has become bound to the humus and may remain there for several years.

Slugs may be exposed to pesticides by direct contact with the spray or indirectly through the food or from contact with a contaminated substrate. As the highest

densities of slugs tend to be found on the types of cultivated land where much of the DDT application has taken place, DDT and other pesticides are now a integral part of the slug's environment.

Because slugs utilize a wide variety of habitats, are ubiquitous and sedentary, it is quite conceivable that slugs could be used for monitoring the biological availability of pesticides in the lower trophic levels.

DDT was chosen as the study chemical since its accumulation in the higher trophic levels has been well documented and thereby stirred up considerable controversy. The use of DDT labelled with the ^{14}C isotope eliminated contamination errors due to polychlorinated biphenyls and facilitated the analytical measurement of DDT residues.

The objectives of this thesis were:

1) To initiate the study of slug ecology in Manitoba and determine what species are present;
2) To investigate the accumulation, storage and excretion of DDT by slugs.

Chapter 1

LITERATURE REVIEW

Ecology of Slugs

There has been little research published on the ecology and distribution of slugs in Canada. Thus this review will be primarily of research done in the United States and Europe.

Before the settlement of North America there were few native species of slugs present. However, in the last 200 years there has been a rapid introduction of slugs from Europe (Chichester and Getz, 1968). The early introduction of slugs from Europe was reported by Binney (1842). *Agriolimax reticulatus* is found in many of the cultivated areas of North America and *Lehmannia valentiana* is found in greenhouses and similar protected places (Chichester and Getz, 1968).

The ecology of *Agriolimax sp.* has been well documented in Europe (Carrick, 1942; Gould, 1961; South, 1965; and Hunter, 1967, 1968a, b, c). *Agriolimax sp.* breeds throughout the year except in very dry or cold weather, and under laboratory conditions can lay 500 eggs in a season (Carrick, 1942). Many of the slug eggs laid in the field in the latter part of the fall fail to hatch in the spring

(Carrick, 1938). Many species of slugs, including Agriolimax, complete their life cycles in one year. Hunter (1971) reports that Agriolimax reticulatus has two generations a year in England, and that those slugs hatched in the fall will overwinter to produce eggs the following year.

The incubation period of Agriolimax reticulatus eggs varies from 15 days at 20°C to 105 days at 5°C (Carrick, 1942). The eggs of Agriolimax appear to have no structural provisions for resistance to desiccation (Bayne, 1968). Carrick (1942) and Arias and Crowell (1963) determined that turgidity of eggs depends on contact with a moist surface. During development the embryoes become increasingly resistant to extremes of temperature (Carrick, 1942) and weight loss due to desiccation (Bayne, 1969). Bayne (1969) reported that the embryos of Agriolimax reticulatus were able to survive a dehydration weight loss of 60-80%. Fully embryonated eggs that had developed at 15°C and were frozen for 2 hours at -2°C hatched within 3 hours when returned to 15°C (Stephenson, 1968).

Dainton (1954) mentions that adult Agriolimax can survive a weight loss due to desiccation and rapidly regain this moisture when in contact with damp surroundings. Mellanby (1961) found that Agriolimax reticulatus is not completely immobilized even at 0°C and at 0.8°C moves normally. Pinder (1969) found that there were no Agriolimax mortalities at -2.5°C, at -3.5°C they could survive for 35 hours but were killed in 28 hours at -4.5°C.

Kalmus (1942) and Dainton (1954) found that air currents will initiate orthokinetic and klinotactic responses in slugs. Crozier and Pilz (1923) showed that the speed of locomotion in Limax varied directly with temperature, movement being greatest at high temperature. Dainton (1953) concluded that the diurnal activity of slugs was controlled by fluctuations in temperature rather than humidity, which was previously assumed to be the controlling factor. Furthermore, Dainton (1953) concluded that light initially stimulates activity in dark-adapted animals, but the response is short lived.

Most animals are not randomly distributed, but tend to aggregate (Andrewartha, 1961). Agriolimax was found to be aggregated in grassland habitats, but the evidence for aggregation on arable land was not conclusive (South, 1967). Thomas (1948) determined that slug populations could range from 26,000 to 275,000 per acre on cultivated land. Stringer and Pickard (1964) estimated that the population of Agriolimax reticulatus in orchards could exceed one million per acre. However, it is difficult to get an accurate estimate of a slug population, as many of the slugs may be underground (South, 1965).

Bruel and Moens (1958) and Gould (1961) found that slugs were most common in heavy soils where moisture is retained at fairly high levels and that cover crops providing shelter favor high population. Runham and Hunter (1970) reported that with most slug populations there is a vast

surplus of food, and the number of animals feeding can have little affect on the food supply. However, speed of development and initiation of egg production tend to be dependant on the quality of food.

Collinge (1921); Blezard (1967) and Holyoak (1968) reported that several species of birds such as rooks and gulls feed on slugs. Stephenson (1964) found that carabid beetles preyed on Agriolimax. Knutson, Stephenson and Berg (1965) found that the fly Tetanocera plepeia, which is widespread in Canada and the United States, parasitizes Agriolimax sp. Hunter (1967) found that several species of slugs were parasitized by a nematode of the genus Cosmocercoides. Arias and Crowell (1963) found nematodes of the genera (Rhabiditus, Panogrolamus and Diplogaster in Agriolimax. Arias and Crowell (1963) and Brooks (1967) reported that ciliates of the genus Tetrahymena may be responsible for high mortality in slug populations.

Slug Culture

Most research on slugs has been conducted with field collected specimens. Reynolds (1936), Kozcloff (1956) and Arias and Crowell (1963) reported that they had difficulty in maintaining slugs under laboratory conditions. Mortality was blamed on fungus infections, nematodes and protozoa. The standard procedure for slug culture in the past has been to maintain slugs in a container with soil, leafmold or other bulk substance as a substrate (Sivik, 1954; Stephenson,

1961; and Arias and Crowell, 1963). Various foods have been tried with carrot and lettuce considered the best (Sivik, 1954; Stephenson, 1961).

Arias and Crowell (1963) attempted to eliminate fungus and parasites from their cultures by maintaining slugs on a special nutrient agar media impregnated with antibiotics. They were successful in eliminating nematodes but mortality still continued due to the protozoan parasites.

Review of DDT

The organochlorine chemical DDT has been widely used as an agricultural insecticide since 1946. This pesticide has proven invaluable in controlling insect pests and disease-carrying arthropods. During the 1950's and early 1960's, there were reports of large residues of DDT in the soil (Fleming and Maines, 1953; Ginsburg and Reed, 1954; Lichtenstein, 1958; and Woodwell and Martin, 1964). Orchard soils had the highest DDT residue levels, often over 100 ppm. dry weight (Lichtenstein, 1958; Harris et al, 1966). Many fields used for cereals and vegetables had DDT residue levels greater than 5 ppm. (Harris et al, 1966, Duffy and Wong, 1967).

DDT movement in the soil is limited by a tendency to form bonds with inorganic and organic components of soil and a very low water solubility (Ballard, 1971). Therefore, most DDT in the soil is relatively immobile (Lichtenstein, 1958; Woodwell, 1961; Peterson et al, 1971).

The persistence of DDT in the soil is variable with a half-life of 3 to 10 years (Menzie, 1972), depending in part on soil type and climatic conditions (Edwards, 1966).

Davis and Harrison (1966); Wheatly and Hardman (1968) and Davis (1966-68) reported DDT residues in various soil invertebrates, including slugs. Gish (1970) found that snails also contain DDT residues. Gish (1970) reported that terrestrial gastropods are able to accumulate DDT with no noticeable toxic effects.

Little work has been done concerning the metabolic dynamics of DDT and similar compounds in molluscs and other invertebrates. Nimmo et al (1970) found that shrimp were able to concentrate polychlorinated biphenyls from their surroundings. PCB's have similar properties to DDT (Riseborough et al, 1968; Gustafson, 1970). The main site of concentration in the shrimp was the hepatopancreas. Similar results were reported by Dindal and Wurzinger (1971) who found that the terrestrial snail, Cepaea hortensis had higher DDT concentrations in the hepatopancreas than in any other body tissue.

DDT and other pesticides are readily available to snails and slugs, not only in the soil organic matter in which they live, but also on the food which they eat (Waites and van Middelm, 1958). The DDT that slugs and other invertebrates concentrate in their tissues may be readily available to higher trophic levels, by transfer up the food chain (Stickel et al, 1965; Jeffries and Davis, 1966; and Vernon,

1970).

Most organochlorine pesticides, including DDT, are lipophilic and therefore stored in adipose tissue. The lipid content of Agriolimax columbianus is 1.12% of the wet body weight (Thompson and Hanan, 1963).

Chapter 2

MATERIALS AND METHODS

Taxonomy

The slugs described in this thesis were classified using the keys described by Barnes and Weil (1943) and Pilsbury (1948). To verify the classification of two species of slugs collected, samples were sent to Dr. L. Chichester, University of Connecticut, who likewise identified them as Agriolimax reticulatus (Fig. 1) and Lehmannia valentiana (Fig. 2). A third species, Agriolimax laeve (Fig. 3) was identified using the key described by Pilsbury (1948) and a key devised by Dr. Chichester.

Location of Sampling and Collecting Sites

A laboratory culture of Lehmannia valentiana was established from slugs collected in a campus greenhouse. Specimens of Agriolimax reticulatus were collected at the edge of the Faculty of Agriculture experimental plots, University of Manitoba, Winnipeg, in the fall of 1970 and the spring of 1971.

Test Chambers

Four different containers were used for maintaining the slugs:

Fig. 1 Agriolimax reticulatus

Fig. 2 Lehmannia valentiana

Fig. 3 Agriolimax laeve

(a) Large plastic containers 25.5 cm. in diameter by 8.75 cm. high

(b) Small plastic containers 15.24 cm. in diameter by 2.5 cm. high

(c) Large glass jars - 16 oz.

(d) Small glass jars - 8 oz.

Because of the danger of the plastic retaining some of the pesticide, the plastic dishes were disposed of after use. The glass containers were reused after being cleaned in a solution of potassium dichromate and sulfuric acid.

The large plastic containers were used primarily for maintaining slugs prior to experimentation and for culture purposes.

Small plastic containers were used for incubating eggs, maintaining immature slugs and for pesticide experiments. These containers were ideal because of ease of handling and they were large enough to accommodate 10 adult slugs.

The large glass jars were used in DDT accumulation experiments involving leaf litter. Nylon net was used to cover the top of jars. This tended to keep the slugs in the leaf litter instead of on the sides of the container, by allowing for water evaporation from the surface of the substrate.

The small glass jars were used for maintaining slugs individually for a short period of time.

Experimental Conditions

Unless otherwise stated, all slug culturing and experiments were carried out in incubators at 15°C and 100% relative humidity. The slug containers were kept in total darkness except for a short period of daily maintenance, when the containers were opened to ensure adequate oxygen. The humidity was maintained by the addition of distilled water whenever necessary.

All slugs used in a given experiment were of the same age and were normally the immature stages, so that egg laying was not occurring during the experiment. For the 6 days prior to initiation of an experiment the slugs were conditioned to the climatic conditions and food supply to be used in the experiments. Slugs that were collected at the end of the various experiments were maintained on a lettuce diet for a period of time to purge the gastro-intestinal tract. The slugs were weighed and then frozen at -20°C to kill them. The slugs were stored at this temperature until extracted for analytical purposes.

DDT Stock Solution

The DDT used in all experiments was the pp-isomer. Randomly ring-labelled pp-^{14}C-DDT (Specific activity 50 µc./ 2.8 mg.) was obtained from New England Nuclear. Unlabelled pp-DDT was obtained from the Aldrich Chemical Company. Several solvents were tested as DDT carriers for uniformly adding the DDT to leaf litter and oatmeal. Acetone, benzene

and ethanol (95%) were tested. Only ethanol was found to be suitable as it did not solubilize organic components of the leaf litter to as great an extent as did the other solvents.

Unlabelled pp-DDT and pp-^{14}C-DDT were individually dissolved in 95% ethanol. 0.025 millicuries of the ^{14}C-DDT solution was then added to the unlabelled solution. The resulting DDT-ethanol stock solution contained 532,000 dpm. per mg. DDT. When 10 mls. of the stock solution was added to 25 gms. of dry substrate, a DDT level of 80 ppm. dry weight and a ^{14}C specific activity of 42,000 dpm. per gram of dry matter was obtained.

Media and Foods

The feeding habits of slugs vary with the seasonal availability of different foods. To simulate the feeding conditions encountered by slugs in the spring and late fall, samples from the top 1/2 inch of a litter layer were collected in the spring and late fall of 1971. These samples were taken at the Glenlea Research Station from a mixed hardwood biome surrounded by fields on three sides and bordered on the fourth by the Red River. Mature oak, maple, elm and ash trees formed the canopy layer. The ground vegetation consisted primarily of grass, nettles and burdock.

Upon collection the litter samples were air dried at 80°C for 48 hours to eliminate soil invertebrates and moisture. These dried samples were then stored in sealed plastic bags.

Twenty five gm. samples of the leaf litter were placed in the large glass jars and aliquots of the DDT ethanol solution were added. Control containers were treated with ethanol only. The ethanol was allowed to evaporate from the leaf litter for 72 hours, and then mechanically mixed. Twenty mls. of distilled water were pipetted onto each 25 gm. sample of leaf litter to ensure adequate moisture for the slugs.

As slugs tend to feed on fresh green material during much of the summer, several different foods were tested for use as carriers in the pesticide experiments. Bran, oatmeal, dried lettuce and dried carrot were tested, with oatmeal being the most palatable; thus the slugs consumed this preferred food before the other foods offered. Fresh foods such as lettuce, as used by Dindal and Wurzinger (1971) were found entirely unsatisfactory due to the difficulty in adding a known amount of DDT and the perishable nature of the foodstuff.

Oatmeal proved to be an ideal carrier for DDT, especially when made into pellet form. Both L. valentiana and A. reticulatus could be maintained on a diet of uncontaminated oatmeal for 60 days with no apparent adverse effects.

Oatmeal was ground to a powder in a Sorval blender at 6000 rpm. for 2 minutes. Twenty five gm. samples of this powder were then placed in small glass jars and aliquots of the DDT-ethanol solution were added, or in the case of the

controls, ethanol only. The oatmeal was then allowed to dry for 24 hours, at which time it was again placed in the Sorval blender and mixed at 4000 rpm. for 2 minutes. Fifteen grams of this powder was then placed in an 8 inch x 8 inch flat bottomed glass dish and distilled water was added until all the powder was dampened. The oatmeal was then allowed to dry at room temperature, forming a solid pad. This was broken down to form small pellets which were stored in sealed containers.

These oatmeal pellets became fairly soft when placed in the experimental containers, and the slugs did not aggregate about or on the pellet as much as they did with fresh lettuce. There was no problem with the pellets being scattered about by the slugs as the powdered oatmeal was. Thus the accumulation of DDT could be accurately determined.

Homogeneity of ^{14}C-DDT distribution in the leaf litter and pellets was checked by ^{14}C determination of randomly collected 0.5 gm. samples. The ^{14}C-DDT distribution was found to be very homogeneous in the media.

Analytical Techniques

The extraction procedure used was basically that described by Bligh and Dyer (1959) for the extraction of lipid from tissue. Several modifications had to be made to this procedure due to difficulties in filtration. Frozen slugs (pooled sample) were placed in the blender cup containing chloroform and methanol. This mixture was blended

for 5 minutes at 6000 rpm. in a Sorval blender. The mixture was then filtered through one layer of filter paper (Whatman #2) in a Buchner funnel using a water aspirator. Filtration was rapid provided the water was added directly to the collection flask rather than to the mixture being blended. The filter paper was rinsed with chloroform and then placed in the blender cup with chloroform and homogenized at 4000 rpm. for one minute. This mixture was then suction filtered and rinsed with chloroform before being added to the collection flask. Distilled water was then added to the suction flask and the contents poured into a 250 ml. separatory funnel. The suction flask was then rinsed with methanol which was in turn added to the separatory funnel. This slug extract was allowed to settle for 24 hours before the lower chloroform layer was drained into a 100 ml. volumetric flask. The volume was brought to 100 ml. with chloroform. Subsequently the methanol-water layer was drained into a volumetric flask and the volume brought to 100 mls. by adding methanol.

Scintillation Counting

Ten mls. of the chloroform extract was placed in each of three scintillation vials. The chloroform was evaporated by warming the vials in a Temp-Blok module heater and using a stream of nitrogen gas. When drying was complete, 15 mls. of toluene scintillator solution (see Appendix 1) was added and the samples counted in a liquid

scintillation counter (Nuclear Chicago).

Five ml. samples of the methanol-water layer were likewise counted using a p-dioxane scintillator solution (see Appendix 1). The ^{14}C-counts for both layers were added together to give a total ^{14}C-count for the slug sample. In the scintillation counter two background samples were placed in front of the unknown and single background samples were placed after every 10th unknown sample. All samples including backgrounds were counted for 4000 total counts or 100 minutes. As the machine counting efficiency varied with time, and the efficiency of the scintillation solutions differed, correction factors were used as necessary. The count value for the pooled control samples were subtracted from the test samples to acquire the final DDT levels.

Culture of Agriolimax reticulatus

Initially an attempt was made to maintain slugs at 15°C in 1 gallon jars containing damp leaf litter. Because of the high mortality rate, often reaching 90% in 3 weeks, other alternatives were tested. It was found that mortality was greatly reduced by lowering the temperature from 15°C to 4°C. At this temperature mortality of newly collected slugs was 23% in 3 weeks. As it would have been impossible to run long term experiments at even this mortality, slugs relatively free of disease and parasites were reared in the laboratory.

Eggs were collected from a group of slugs that had a low mortality rate (7.1% in 41 days). These adult slugs had been maintained at 4°C until 500 eggs were obtained. Small plastic containers with tight fitting lids were used for incubating the eggs. Paper towelling (Kimberly-Clark Canada Ltd.) was placed on the bottom of the dishes and 10 mls. of distilled water was added to provide adequate moisture. One hundred eggs were then placed on the paper and the containers incubated at 15°C in the dark. Incubation time varied, the majority of the eggs hatching in 15-25 days.

Upon hatching the slugs were transferred to small plastic containers with a fine brush. Paper toweling was used as a substrate. The containers were stored in unlit incubators at 15°C with a humidity of 100%. Green outer leaves of lettuce were supplied as the primary food with oatmeal and calcium carbonate being given as a supplement once a week. Every 3 days the containers were cleaned and fresh food was added.

Although there was a marked difference in size between individuals, mortality was only 1% at 30 days of age. At this time it was decided that the containers were too crowded and difficult to keep in a sanitary condition. Groups of 50 slugs were put in large plastic containers, with feeding and cleaning being continued at regular intervals. At the end of the second month only 1.3% of the slugs had died, but 23 were discarded as they were too small to be used in experiments. At 126 days of age egg production

started. Twenty-five slugs were randomly chosen for breeding and placed in large plastic containers with paper toweling as a substrate and lettuce for food. The majority of the eggs were deposited in the folds of the paper toweling. Eggs were collected and incubated as before. This second and subsequent generations were maintained without significant mortality.

Culture of Lehmannia valentiana

Lehmannia valentiana was found to be tolerant of laboratory conditions and mortality of field collected slugs was very low (0.1% in 30 days at 15°C). The main stock culture was kept in the dark at 4°C in large plastic containers with damp paper toweling as a substrate. Fifty adult slugs could be maintained under these conditions for 20 days before cleaning was necessary. Lettuce leaves and oatmeal were provided for food.

To obtain high egg production with this species conditions different from those used for A. reticulatus were required. While A. reticulatus would oviposite under damp paper, L. valentiana produced only small numbers of eggs under these conditions. Petri dishes containing moist soil were put in the stock culture containers, and the temperature raised to 15°C. Within 6 days average daily egg production was 5 eggs per slug.

All eggs gathered were stored at 4°C until needed. Small plastic containers with damp paper toweling were used

for incubating the eggs. At 15°C the eggs started to hatch in 17 days, but as with A. reticulatus there was variation in incubation time (17-25 days).

As the young slugs proved highly susceptible to desiccation, they were left in the incubation dishes for 7 days, during which time they were fed lettuce and oatmeal. At 7 days of age the young slugs were placed in small plastic containers with damp toweling as a substrate. The slugs were given lettuce, oatmeal and calcium carbonate. When the slugs were one month old they were transferred to large plastic containers. Four generations were raised without serious mortality.

Chapter 3

EXPERIMENTAL

PART I. - SLUG ECOLOGY

Introduction

The distribution and ecology of slugs in Manitoba has been largely overlooked. Only one species of slug, Agriolimax laeve, is native to Manitoba. In most areas of the province slugs were either unknown or of limited importance prior to 1946. Since then there has been a definite spread of introduced species, with slug damage in market and home gardens becoming more frequent.

Being soft-bodied and lacking a moisture-retaining cover slugs require high moisture levels in their surrounding environment in order to survive. The hot dry summer periods often encountered in Manitoba would prevent colonization of those species lacking behavioral traits enabling them to seek moist conditions. Only those species whose life cycle is adapted to these recurring dry conditions could hope to survive in any but isolated areas. In most soil types, cracks in the soil provide the most readily available source of shelter for slugs. Debris and vegetation provide cover and tend to retain moisture. Slugs have also been observed utilizing holes in the ground made by

rodents, wasps and earthworms. A second limiting factor in Manitoba, severe cold in the winter months, will prevent the colonization of species not tolerant of the severe cold. Only places such as rootcellars, greenhouses and other artificially maintained environments will permit the survival of frost susceptible species.

Slugs can become serious pests of field crops and in greenhouses. Each slug is capable of producing large numbers of eggs, the potential depending on the species. Although individual slugs are capable of consuming large amounts of organic matter, it is what they damage and render unmarketable that causes the greatest economic loss.

Survey of Slug Distribution in Manitoba in 1972

Procedure:

A field survey was conducted in August 1972 of 19 areas in Southern Manitoba. Population densities were determined by counting the number of slugs on the surface of the ground in grid squares (5 ft. by 5 ft.) which were chosen at random in gardens. Four separate gardens were sampled in each of the smaller towns, with 8 sites being sampled in each of Brandon, Portage la Prairie and Swan River. An average of the 4 or 8 sites was used in determining the density classification (Table 1).

Results:

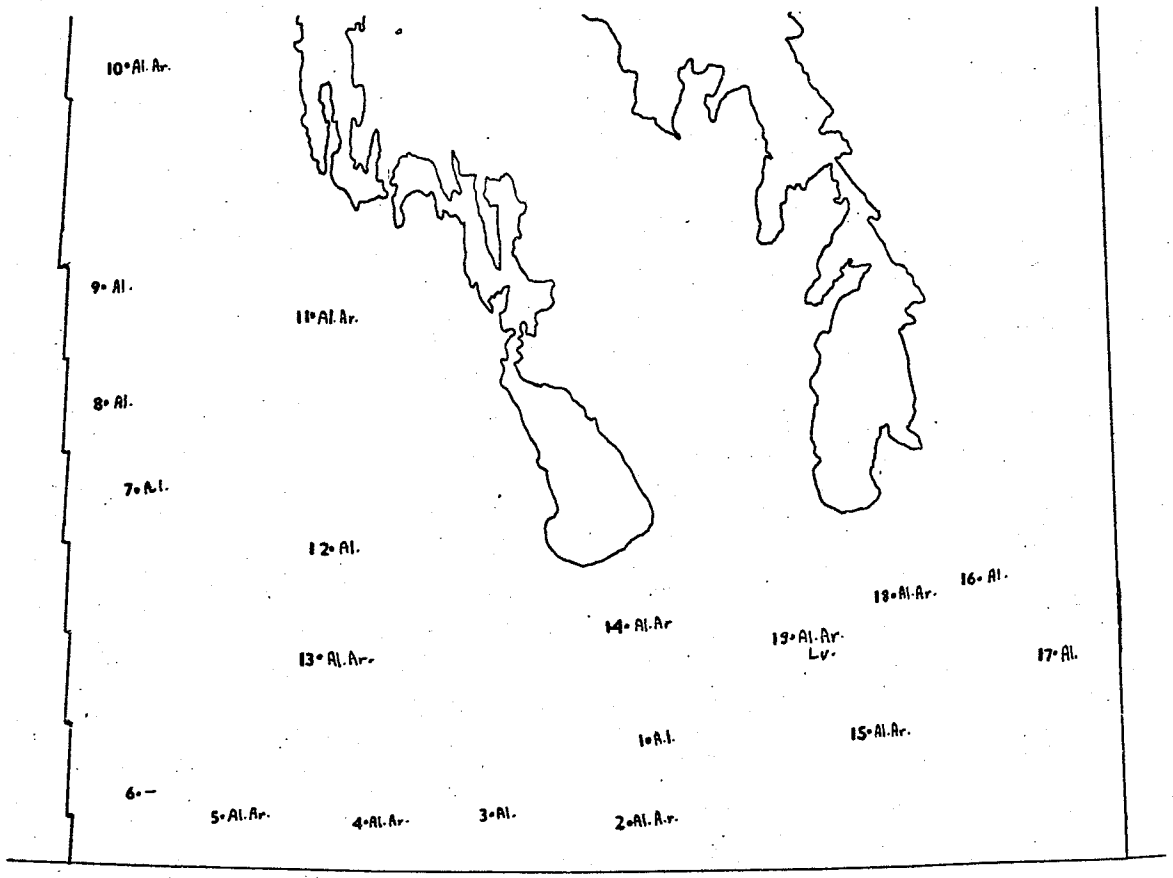
Agriolimax laeve has a wide distribution in Manitoba (Fig. 4). This species was found in 18 of the 19 locations

checked, and it was too dry at these sites for slugs to be found on the surface of the ground. When found in cultivated areas, Agriolimax laeve was most common in the moister parts of gardens and along the edges of cultivated fields. In all localities population densities were low and little or no damage was observed.

Lehmannia valentiana has a very limited distribution in Manitoba, being found only in greenhouses in the Winnipeg area (Fig. 4). It has undoubtably been introduced with plant material. The damage done in these greenhouses is slight despite high slug populations.

Agriolimax reticulatus has a scattered distribution in Manitoba (Fig. 4). Whereas this species has been present in the Winnipeg area for more than 20 years, it has been found in Northwestern Manitoba (Swan River area) for less than 5 years. This implies that the species is still extending its range in Manitoba. Other areas of the province such as Melita and Birtle have reported slight slug damage in the past few years, but in August 1972 Agriolimax reticulatus was not found in these areas. Either Agriolimax laeve was causing this damage or the previous population of Agriolimax reticulatus had decreased or died out. In all areas where this slug was found it was causing aesthetic loss to homegardners and economic loss to market gardens.

Fig. 4 - Distribution of Slugs in Manitoba (1972)



Al - Agriolimax laeve
Ar - Agriolimax reticulatus
Lv - Lehmannia valentiana

1	Carmen	11	Dauphin
2	Morden	12	Minnedosa
3	Pilot Mound	13	Brandon
4	Killarney	14	Portage la Prairie
5	Deloraine	15	Steinbach
6	Melita	16	Pinawa
7	Birtle	17	Rennie
8	Russel	18	Beausejour
9	Roblin	19	Winnipeg
10	Swan River		

TABLE 1

DISTRIBUTION OF SLUGS IN SOUTHERN MANITOBA IN AUGUST 1972

Town	Species Found (1)	Population Densities (2)
Carmen	Al.	low
Morden	Al. Ar.	low, moderate
Pilot Mound	Al.	low
Killarney	Al. Ar.	low, low
Deloraine	Al. Ar.	low, moderate
Melita	-	-
Birtle	Al.	low
Russel	Al.	low
Roblin	Al.	low
Swan River	Al. Ar.	low, high
Dauphin	Al. Ar.	low, low
Minnedosa	Al.	low
Brandon	Al. Ar.	low, low
Portage la Prairie	Al. Ar.	low, low
Steinbach	Al. Ar.	low, low
Pinawa	Al.	low
Rennie	Al.	low
Winnipeg	Al. Ar. Lv.	low, high, low
Beausejour	Al. Ar.	low, low

(1) Species:
Al. = Agriolimax laeve
Ar. = Agriolimax reticulatus
Lv. = Lehmannia valentiana

(2) Population densities:
low = 2 or less/grid square
moderate = 3-4/grid square
high = 4 or more/grid square

Egg Production of Lehmannia valentiana under Laboratory Conditions

Procedure:

Specimens of L. valentiana used were laboratory reared individuals 140 days old. Several of these slugs had started egg production before the initiation of the experiment.

Large plastic containers were used for the experiment. Conditions were the same as those used for slug culture. Ten slugs were placed in each of 4 containers. Eggs were collected daily for a period of 42 days and then weekly for the next 42 days.

Results:

Egg production was low initially but by day 15 had reached a total of 200 eggs. Egg production for the next 27 days fluctuated greatly (Fig. 5), showing rather definite 5 day fluctuations. Periods when egg production was nearly zero were followed by fairly high peaks of production. A total of 6684 eggs were laid in the first 42 days by the 40 slugs. This was an average of 3.98 eggs/slug/day. From day 42 to 84 the weekly egg production fluctuated from 3.39 to 6.50 eggs/slug/day (Table 2). The average egg production per slug for the 84 day period was 4.5 eggs/slug/day.

Survival of Introduced Species of Slugs under Dry Conditions

Procedure:

Agriolimax reticulatus and Lehmannia valentiana were

Fig. 5 - Daily Egg Production of Forty L. valentiana
Under Laboratory Conditions

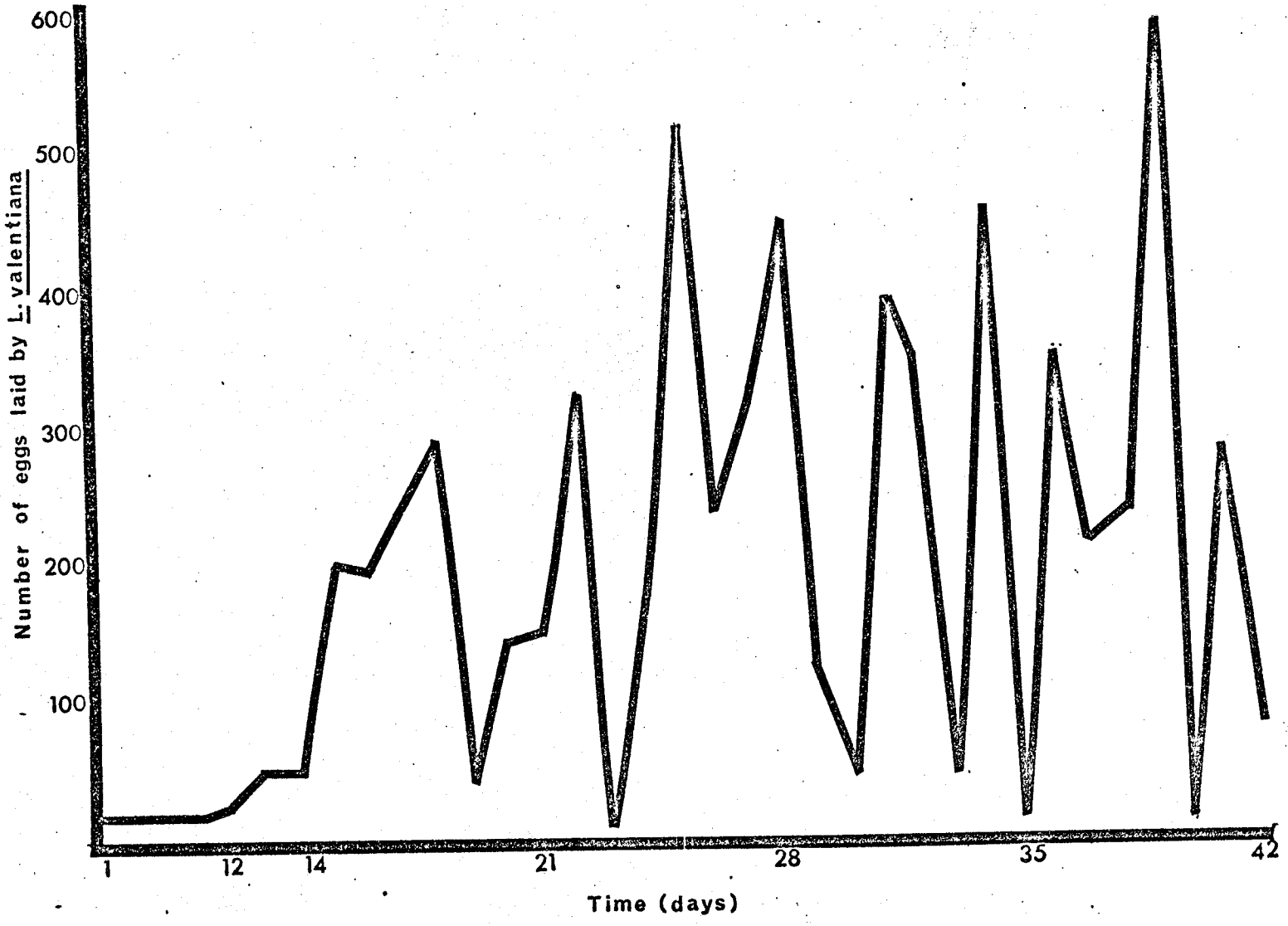

600
500
400
300
200
100
Number of eggs laid by L. valentiana
1
12
14
21
28
35
42
Time (days)

TABLE 2

EGG PRODUCTION OF L. VALENTIANA FOR A PERIOD OF 84 DAYS UNDER LABORATORY CONDITIONS

Day	Number of Eggs	Number of Slugs	Number of Days	Eggs/ Slug/ Day
1 - 42	6684	40	42	3.98
43 - 49	1200	40	7	4.28
50 - 56	1432	40	7	5.11
57 - 63	1820	40	7	6.50
64 - 70	1196	40	7	4.27
71 - 77	928	39	7	3.39
78 - 84	1144	38	7	4.30
Totals	14404		84	

used as the test species. Specimens used were 140 to 168 days old and had been raised at 15°C. Thirty flower pots (8 inch diameter) were filled with 750 gm. of soil (clay loam). Three hundred mls. of distilled water was added to each container. Four hours later the surface of the soil was stirred to give an irregular surface and 3 earthworms (Lumbricus sp.) were added to 15 of the containers. The containers were left in total darkness for 48 hours to allow the earthworms to become established. By this time the earthworms had established several holes to the surface in each of those pots in which they were placed. At this time 3 A. reticulatus and 3 L. valentiana were placed in each of the 30 pots. Nylon net held in place by rubberbands was placed over each container to prevent the slugs escaping and the containers left at room temperature. Within 2 days of introducing the slugs the soil was cracking and both species of slugs in both groups appeared to be congregating in the cracks. Four days subsequent to introduction of the slugs, the soil was dry to a depth of 5 cm. At this time the containers were checked individually for slug survival. By careful removal of soil individual cracks and earthworm holes were excavated.

Results:

Both Agriolimax reticulatus and Lehmannia valentiana were found in the earthworm holes. When cracks in the soil were utilized both species tended to congregate in the

deepest cracks, with the 2 species often being grouped together. When no earthworms were present survival was 80% for L. valentiana and 20% for A. reticulatus (Table 3). Several slugs were found on the surface of the soil, having died of desiccation, but the majority died in the cracks, usually when alone or in small groups. When earthworms were present in the containers, 60% of the slugs were found in the worm holes, several A. reticulatus being found at a depth of 10 cm. Ninety six percent of the L. valentiana and 90% of the A. reticulatus survived in these containers (Table 3). Those slugs found dead in these containers were on the surface of the soil and sides of the containers. Both groups of containers showed considerable amounts of dried mucous on the sides of the containers and on the netting, indicating that considerable locomotion took place prior to selection of a suitable refuge.

Field Survival of Lehmannia valentiana under Manitoba Conditions

Procedure:

The slug Lehmannia valentiana has so far been confined to greenhouses in Manitoba. To determine if field survival is possible, a plot 20 ft. by 60 ft. was used at the University of Manitoba Field Station. This area was plowed in the spring and planted to cabbage and beans, in rows 2 feet apart. One foot squares of fiberboard were placed at the 4 corners and the center of the plot. This

TABLE 3

PERCENT SURVIVAL OF SLUGS UNDER DRY CONDITIONS, IN THE PRESENCE AND ABSENCE OF EARTHWORMS

		L. valentiana	*A. reticulatus*
Earthworms	present	96	90
Earthworms	absent	80	20

plot was surrounded by summerfallow which was cultivated several times during the year. The plot rows were cultivated twice during the summer. Thirty adult L. valentiana were placed at the center of the plot on July 30, 1971. Counts of L. valentiana and Agriolimax sp. (Natural population) under the cabbage plants and wooden boards were made during the summer. The cabbages and boards were left undisturbed in the field from the first snow until the following spring when a check for slug survival was made after the ground was free of snow.

Results:

From Table 4 it can be seen that Lehmannia valentiana was quite capable of surviving under summer conditions in Manitoba. Although all the slugs were not found at the surface at any one time, a certain number may have been buried in the soil or wandered away from the plot. Both species were observed most frequently when the soil was damp. L. valentiana laid eggs which hatched and the young slugs were 6 cm. long by October 25, 1971. In spring (April 10, 1972) a check of the plot showed that all the L. valentiana on the surace of the ground were dead, 8 specimens being found. None of the L. valentiana eggs collected from the plot hatched in the laboratory at 15°C. No evidence of survival appeared on the plot during the following summer.

TABLE 4

NUMBERS OF L. VALENTIANA AND A. RETICULATUS
FOUND FROM AUGUST 1 TO OCTOBER 27 (1971)
ON FIELD PLOT

Species	AUGUST								SEPTEMBER					OCTOBER				
	1	2	4	5	10	15	20	30	4	9	13	24	28	12	15	20	25	27
Agriolimax reticulatus	7	3	9	1	0	2	7	17	12	29	21	14	31	105	71	77	29	69
Lehmannia valentiana	12	2	0	0	0	1	0	0	2	6	2	2	4	18	14	16	8	16

Life Cycle of Agriolimax reticulatus in Manitoba

Procedure:

Field observations were made during 1971 and 1972 in the Winnipeg area.

Results:

Agriolimax reticulatus overwinters in the egg stage except in isolated locations that receive adequate heat during the winter, in which case both adults and juveniles can overwinter. Only 2 adults were found in the springs of 1971 and 1972, both being found in a root cellar.

Eggs hatching in the field were first detected on March 31, 1971, and June 9, 1972. Three hundred eggs taken in April 1972 from 4 locations in the Winnipeg area and incubated in the laboratory at 15°C had a hatchability of 87.5%. Slugs in the field were mature and producing eggs by September 2, 1971, and August 30, 1972. Adult A. reticulatus continued to produce eggs until the first snow in 1971, when observations ceased (October 29).

Discussion

Agriolimax laeve and *Agriolimax reticulatus* are both widely distributed in Southern Manitoba. Because of its low population density and preference for uncultivated areas, it is doubtful if *Agriolimax laeve* could be considered a pest species in this province. *Agriolimax reticulatus* is responsible for nearly all the slug damage in Manitoba, *Lehmannia valentiana* causing only slight damage in greenhouses. Because of the dry conditions that prevail on much of the cultivated land in Manitoba, slug damage is confined mainly to those areas receiving artificial watering (home and market gardens).

Both *Agriolimax reticulatus* and *Lehmannia valentiana* have high egg production. This is a definite advantage to a species colonizing new areas. Although *Lehmannia valentiana* appears more tolerant to dry conditions than *Agriolimax reticulatus*, it is the inability to survive cold conditions that limits it to protected situations (greenhouses). It was previously thought that *Agriolimax reticulatus* overwintered as an adult. *Agriolimax reticulatus* can only overwinter as an adult in protected places. Under natural conditions these slugs lay eggs which overwinter giving rise to the next generation of slugs which mature by late summer. These slugs then produce eggs that hatch the same year to give a subsequent generation and other eggs which overwinter.

PART II.

ACCUMULATION OF DDT

Introduction

After application of a pesticide, the leaf litter and green vegetation of the sprayed area may have pesticides adhering to their surfaces. Precipitation and the seasonal accumulation of dead plant material tend to maintain residue levels in the leaf litter and soil. Since this is the habitat of slugs, they may be subjected to varying levels of pesticide in their diet, depending on the local spray program and the season of the year.

In spring, slugs which have overwintered or hatched become active as soon as their surrounding temperature rises above 0°C. As it may be several weeks before desirable food plants become available, slugs have little choice but to feed on the available organic material, with leaf litter usually being the most readily available.

During the warmer months of the year most species of slugs feed on living plant material. The softer parts of the plants are usually chosen and the slugs may climb several feet to feed on tender shoots, if moist climatic conditions prevail. During dry periods slug movement is limited with the result that leaf litter and subterranean organic material is consumed.

During the last few weeks of the fall, before low temperatures either kill the slugs or force them underground, a change takes place in the quality of food available to the slugs. In the northern latitudes most deciduous plants lose their leaves, thus replenishing the leaf litter. The plants that remain green are usually quite fibrous and unpalatable. As the average daily temperature approaches 0°C, the slugs are forced to remain on or near the soil surface, often taking advantage of the insulation provided by dead leaves and grasses. This dead and dying plant material is the most readily available food for slugs at this time.

Experiments were carried out to determine the rate of uptake of DDT by slugs when maintained on different types of food and substrates.

Accumulation of DDT from aged Leaf Litter

Procedure:

Agriolimax reticulatus and *Lehmannia valentiana* were used as the test species. Specimens used were first generation laboratory reared individuals, 110 days old.

For the experiment 25 gms. of leaf litter (collected spring 1971) was placed in each of 72 large glass jars. Varying aliquots of the ^{14}C-DDT-ethanol stock solution were added to the leaf litter to obtain DDT concentrations of 4, 16, 40 and 80 ppm. dry weight. Sixteen jars were prepared at each concentration. Eight jars were used as controls and only ethanol was added. The ethanol in the jars was allowed

to evaporate at room temperature, and the leaf litter was stirred to help ensure proper mixing. To establish a satisfactory moisture level in the leaf litter, five mls. of distilled water was added to each jar twice a day for 2 days.

One half of the jars at each treatment level and the control jars received 6 Agriolimax reticulatus each and the other half received 6 Lehmannia valentiana each. The tops of the jars were covered with nylon net held in place with rubber bands. The jars were then placed in an unlit incubator at 15°C and left virtually undisturbed for 30 days. Five mls. of distilled water was added every 5 days and samples of the slug population were removed at various intervals.

Slug sampling was done 3, 10, 20 and 30 days after the initiation of the experiment. One slug was randomly picked from each of the 8 jars for each DDT treatment, and 2 slugs were taken from each control jar. This was done at each collection time for the two species. These slugs were maintained on DDT-free food for 10 hours before being killed, to purge the gastro-intestinal tract. The slugs from each treatment were pooled before being analyzed for DDT residues. For statistical analysis see Appendix IIa.

Results:

Agriolimax reticulatus showed a fairly rapid accumulation of DDT from the leaf litter (Fig. 6). At all

different levels of DDT in the leaf litter, the DDT content of the slugs progressively increased over the 30 day period. The body DDT content of slugs kept at 4 and 80 ppm. DDT in the leaf litter increased steadily with time, while those slugs kept at 16 and 40 ppm. DDT showed a relatively slow rate of accumulation until the 20th day, after which the rate of accumulation increased sharply.

The quantity of DDT accumulated in 30 days by the slugs was directly proportional to the DDT content of the leaf litter. For instance, when the DDT content of the leaf litter was increased 5 fold (16 ppm. to 80 ppm.) the slug content of DDT (2.6 µg. on 16 ppm. versus 14.7 µg. on 80 ppm.) increased by slightly more than 5 fold at 30 days.

Lehmannia valentiana showed a more rapid accumulation than did Agriolimax reticulatus during the first 3 days of treatment (Fig. 7). However, with the exception of those slugs maintained at 4 ppm., the rate of accumulation was less than that for Agriolimax reticulatus from day 10 to 30. Otherwise L. valentiana was similar to A. reticulatus in that the DDT content of the slugs was directly related to the DDT content of the leaf litter and the time of exposure to the contaminated media. Food consumption was approximately the same for the 2 species.

Accumulation of DDT from a Preferred Food-Oatmeal

Procedure:

Agriolimax reticulatus and Lehmannia valentiana were

Fig. 6 - Accumulation of DDT from aged leaf litter by A. reticulatus

Each point represents a pooled sample of 8 specimens.

DDT content of aged leaf litter (ppm. dry weight)

. . . . 80

— — — 40

------- 16

——— 4

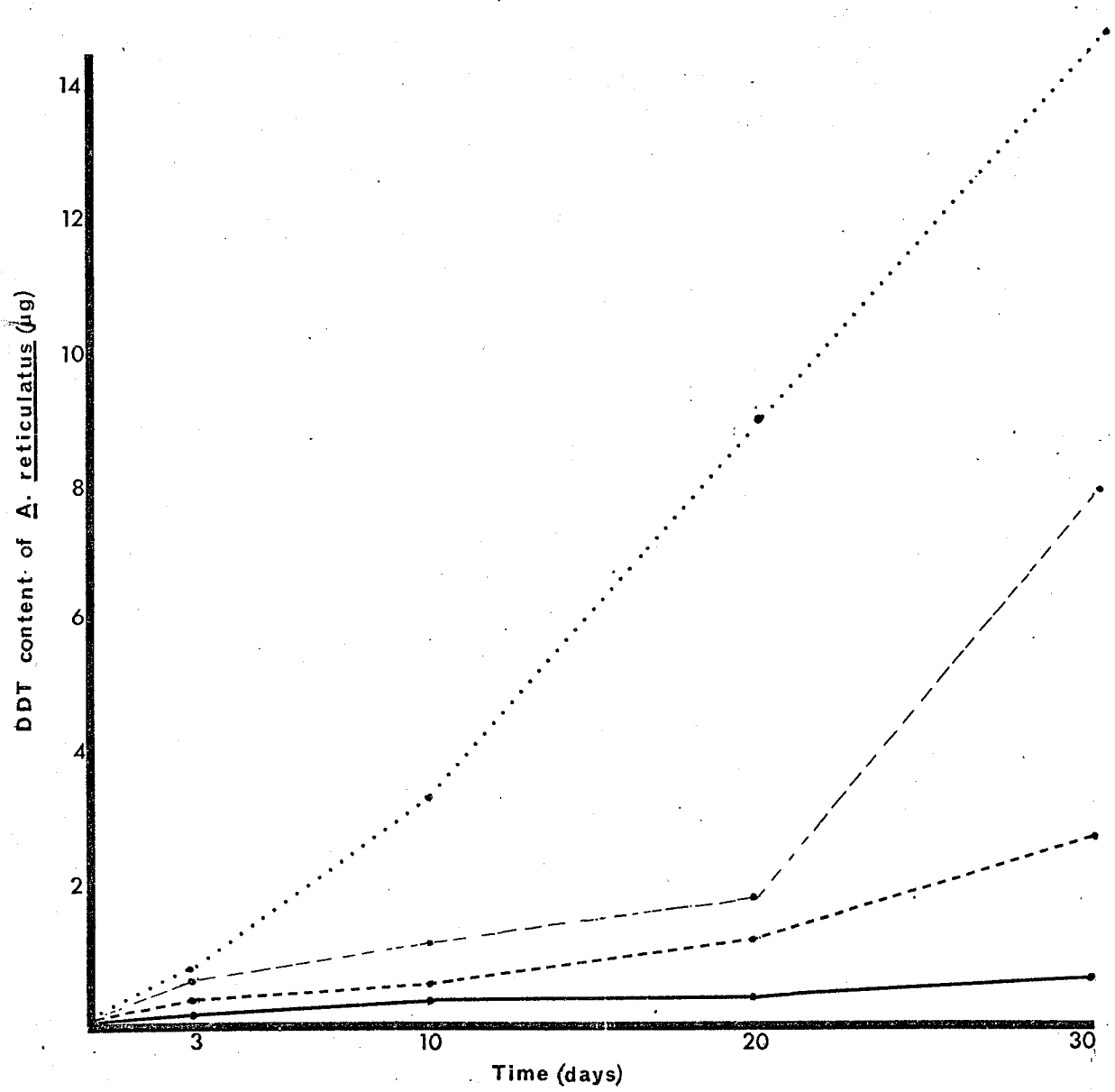
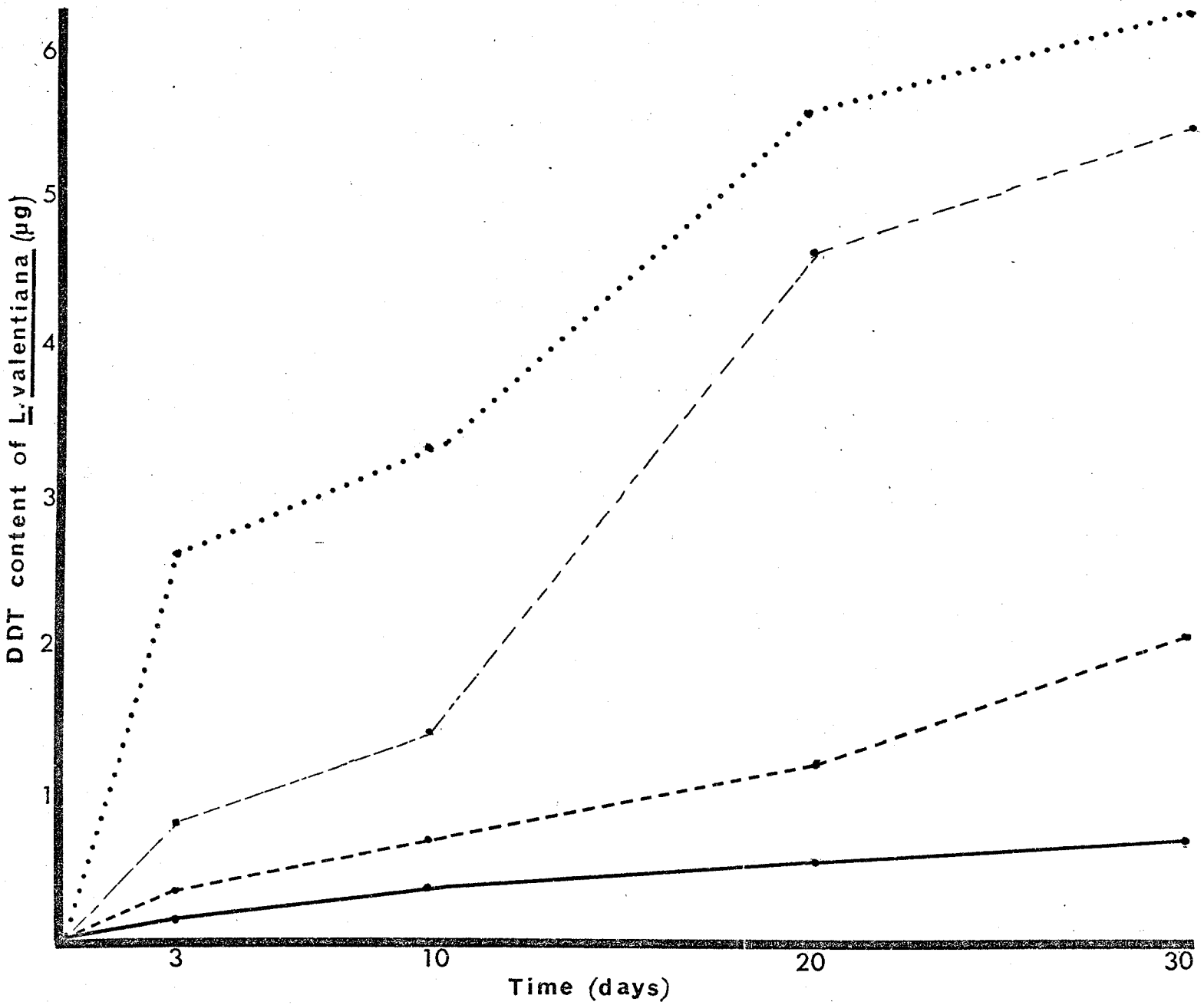

14
12
10
8
6
4
2
DDT content of A. reticulatus (µg)
3
10
20
30
Time (days)

Fig. 7 - Accumulation of DDT from aged leaf litter by L. valentiana

Each point represents a pooled sample of 8 slugs.

DDT content of aged leaf litter (ppm. dry weight)

. . . . 80
— — — 40
------- 16
——————— 4


DDT content of L. valentiana (µg)
6
5
4
3
2
1
3
10
20
30
Time (days)

used as test animals. Specimens used for the experiment were 2nd generation laboratory reared individuals that were 198 days old.

A. reticulatus was fed oatmeal pellets containing 4, 16, 40 and 80 ppm. DDT. L. valentiana were fed DDT at concentrations of 16 and 80 ppm. in the oatmeal pellets. Eight replicates of 6 slugs each were used for each DDT-oatmeal treatment with each species. Four control groups of six slugs each were used for each species. The controls were fed oatmeal pellets to which only ethanol had been added.

Small plastic containers with tight fitting lids were used for test chambers. Two layers of paper toweling (2 inch x 2 inch squares) were placed in the bottom of the containers and 5 mls. of distilled water was added. This kept the relative humidity in the containers at 100%. The containers were stored at 15°C in total darkness except for daily maintenance and sampling of the slug population. The pelleted food was given daily so as to maintain a surplus. The surplus food was removed and dried each day before being reused.

For the A. reticulatus population, sampling was done at 3, 10, 20 and 30 days after the initiation of the experiment. The L. valentiana population was sampled on days 3, 10, 20, 30, 50 and 70. Sampling consisted of picking 1 slug at random from each of the eight jars at each DDT concentration. This was done for each species at each sampling

period. The sampled slugs were maintained on uncontaminated food for 27 hours before being killed. This served to purge the gastro-intestinal tract.

Results:

A. reticulatus showed a rapid accumulation of DDT from the oatmeal pellets (Fig. 8). The body content of DDT was directly related to the DDT content of the ingested material and the length of time the material was ingested.

The rate of accumulation at the 4 and 16 ppm. DDT level tended to be constant with time for the duration of the experiment, as was the rate of accumulation of the slugs at the 40 and 80 ppm. DDT levels of feeding up to day 10. From days 10 to 20 the rate of DDT accumulation was considerably slower, but then from day 20 to day 30 a rapid rate of accumulation took place for the 40 and 80 ppm. DDT levels of feeding.

As depicted in Fig. 9, L. valentiana had a more rapid rate of accumulation the first 3 days than did A. reticulatus. At the 16 ppm. DDT level of feeding the rate of accumulation increased at a relatively constant rate for the 70 days of the experiment. Those slugs maintained on the 80 ppm. DDT level of feeding showed a slow rate of accumulation from day 10 to 20 followed by a rapid accumulation to day 30. From day 30 to 70 there was a steady increase in the accumulated DDT. After 70 days of oatmeal consumption at 80 ppm. DDT the body content (37.2 µg.) was approximately 5 times that

Fig. 8 - Accumulation of DDT from a preferred food (oatmeal) by *A. reticulatus*

Each point represents a pooled sample of 8 slugs.

DDT content of oatmeal pellets (ppm. dry weight)

. . . . 80
— — — 40
------- 16
——— 4

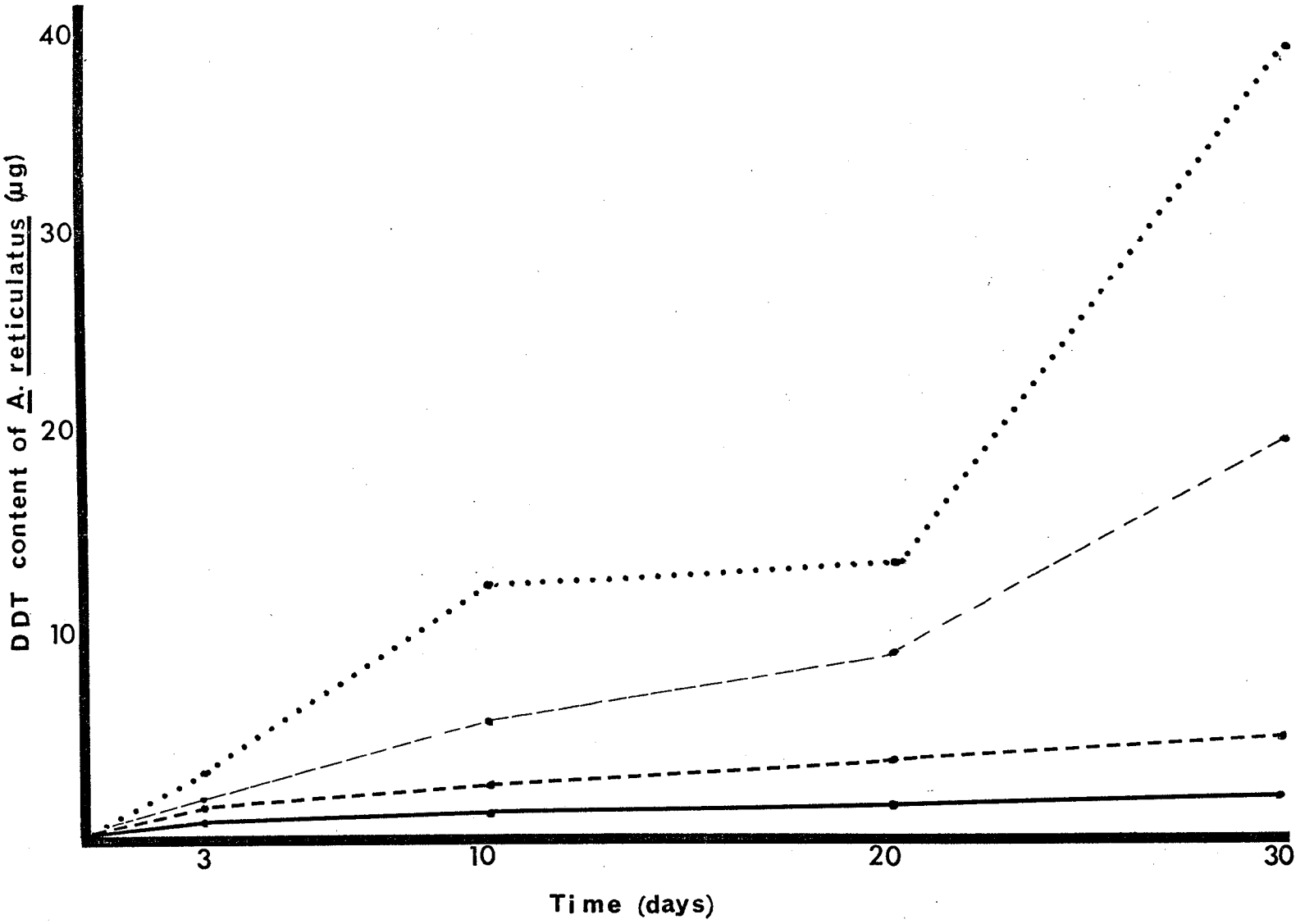

40
30
20
10
DDT content of A. reticulatus (µg)
3
10
20
30
Time (days)

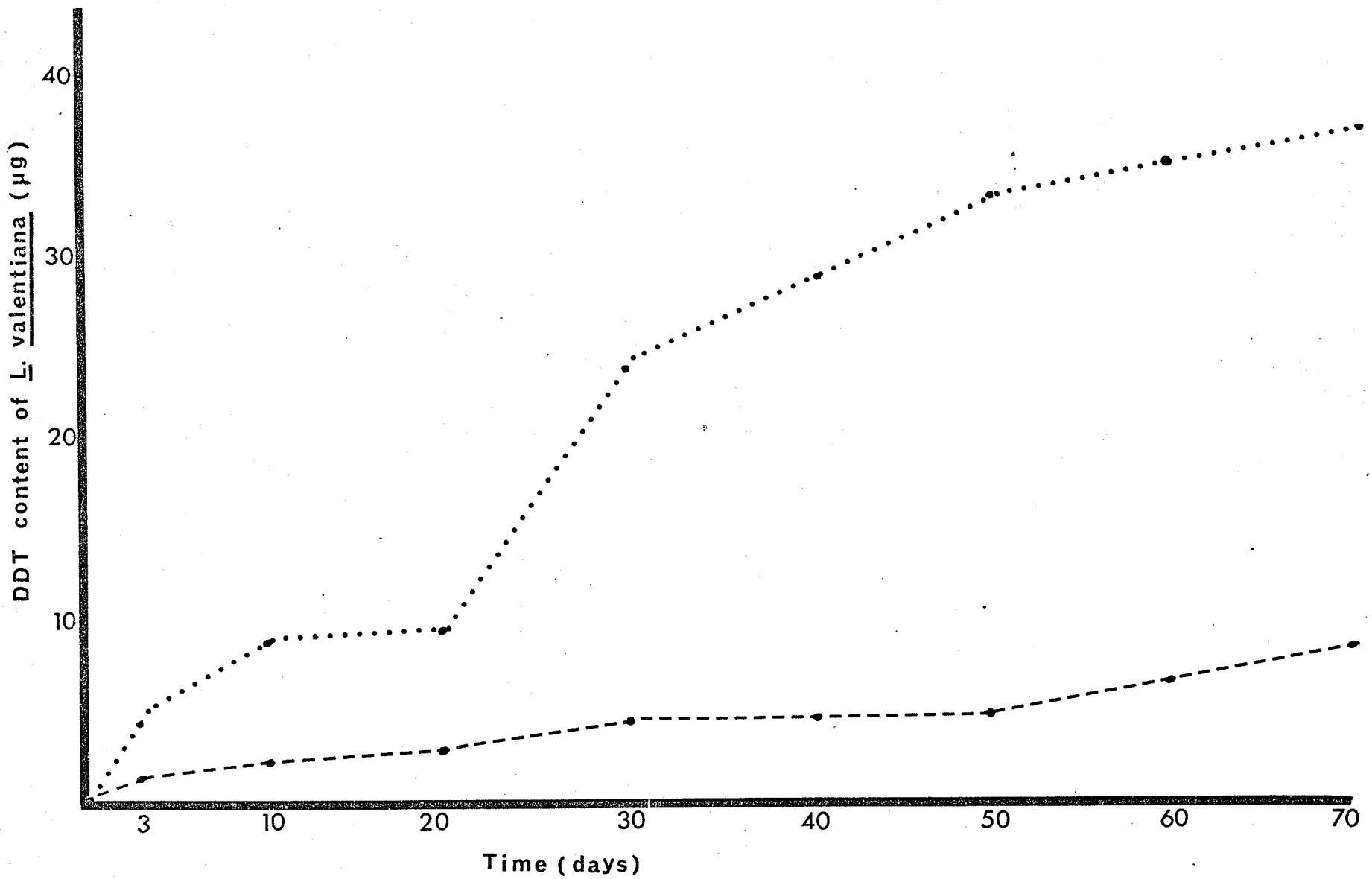
Fig. 9 - Accumulation of DDT from a preferred food (oatmeal) by L. valentiana

Each point represents a pooled sample of 8 slugs.

DDT content of oatmeal pellets (ppm. dry weight)

. . . . 80

------- 16


DDT content of L. valentiana (µg)
40
30
20
10
3
10
20
30
40
50
60
70
Time (days)

of slugs feeding on the 16 ppm. DDT contaminated oatmeal (8.6 µg.).

This demonstrates that the accumulation of DDT by the slug is proportional to time for at least 70 days.

Accumulation of DDT from Non-Aged Leaf Litter

Procedure:

Agriolimax reticulatus was used as the test animal. Specimens used were third generation lab-reared slugs, 112 days old. As described previously, 25 gms. of leaf litter (collected October 1971) was added to large glass jars such that 8 jars had DDT levels of 80 ppm. dry weight and 8 had DDT levels of 16 ppm. dry weight. Four controls were used for each DDT concentration, to which only ethanol was added. It was necessary to add water only twice to the leaf litter after initial moistening. Slug sampling was done 3, 10, 20 and 30 days after the initiation of the experiment. One slug was picked at random from each of 8 jars on each treatment.

Results:

A. reticulatus showed a rapid rate of DDT accumulation from the fresh leaf litter (Fig. 10). The DDT residue of A. reticulatus increased at a relatively constant rate over the 30 day experiment. The rate of DDT accumulation was directly proportional to time for the slugs maintained at both DDT concentrations. Again, the DDT accumulated by

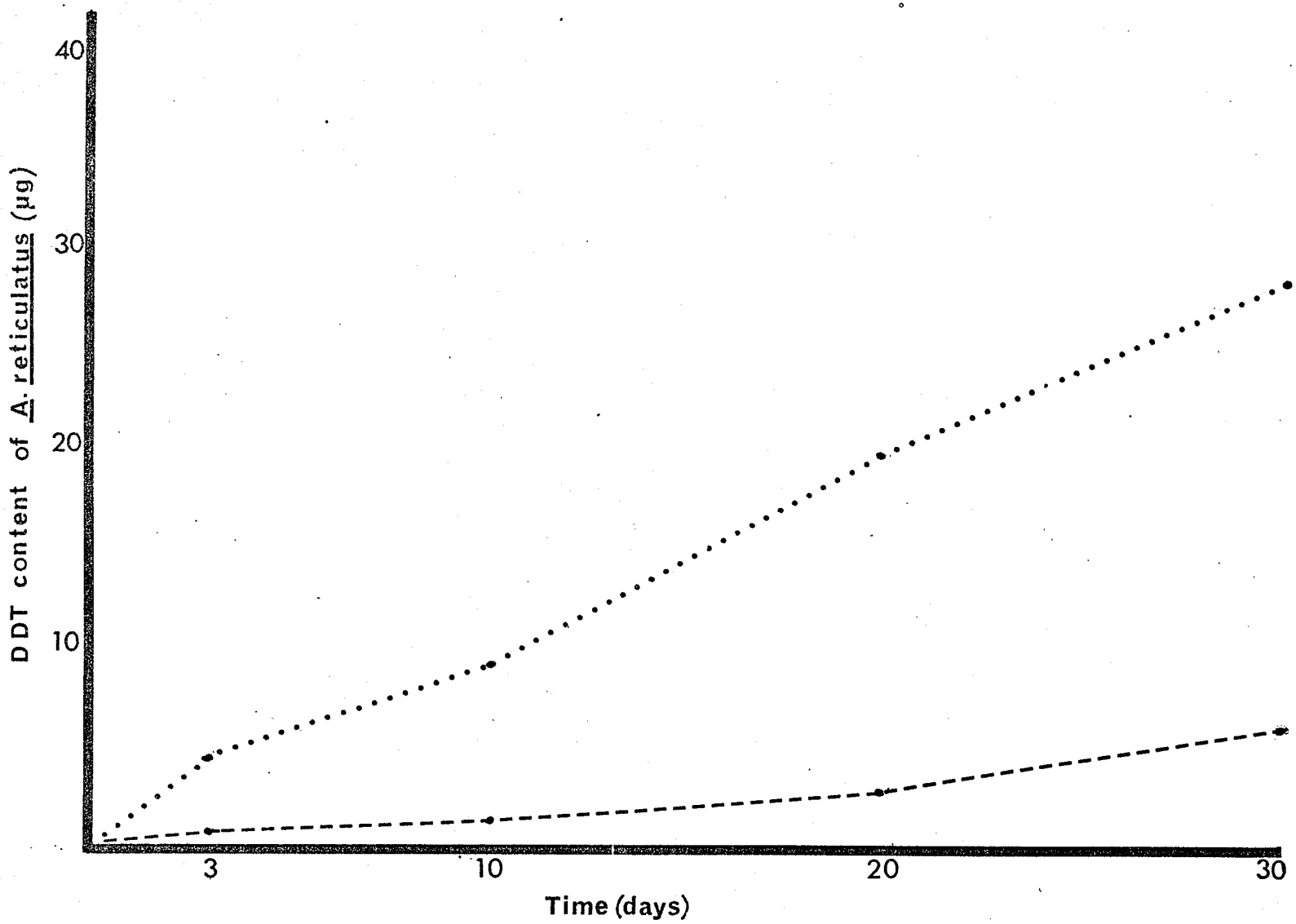
Fig. 10 - Accumulation of DDT from non-aged leaf litter by *A. reticulatus*

Each point represents a pooled sample of 8 slugs.

DDT content of non-aged leaf litter (ppm. dry weight)

. . . . 80

------- 16


40
30
20
10
DDT content of A. reticulatus (µg)
3
10
20
30
Time (days)

the slugs was directly proportional to the DDT content of the leaf litter. At 30 days the slugs on 80 ppm. had a 5 fold greater DDT content than those on 16 ppm.

Accumulation of DDT when Slugs were in a DDT Contaminated Habitat (Leaf Litter) and Receiving a Clean Preferred Food (Oatmeal and Lettuce)

Procedure:

Agriolimax reticulatus was used as the test animal. Specimens used were third generation lab-reared individuals 112 days old.

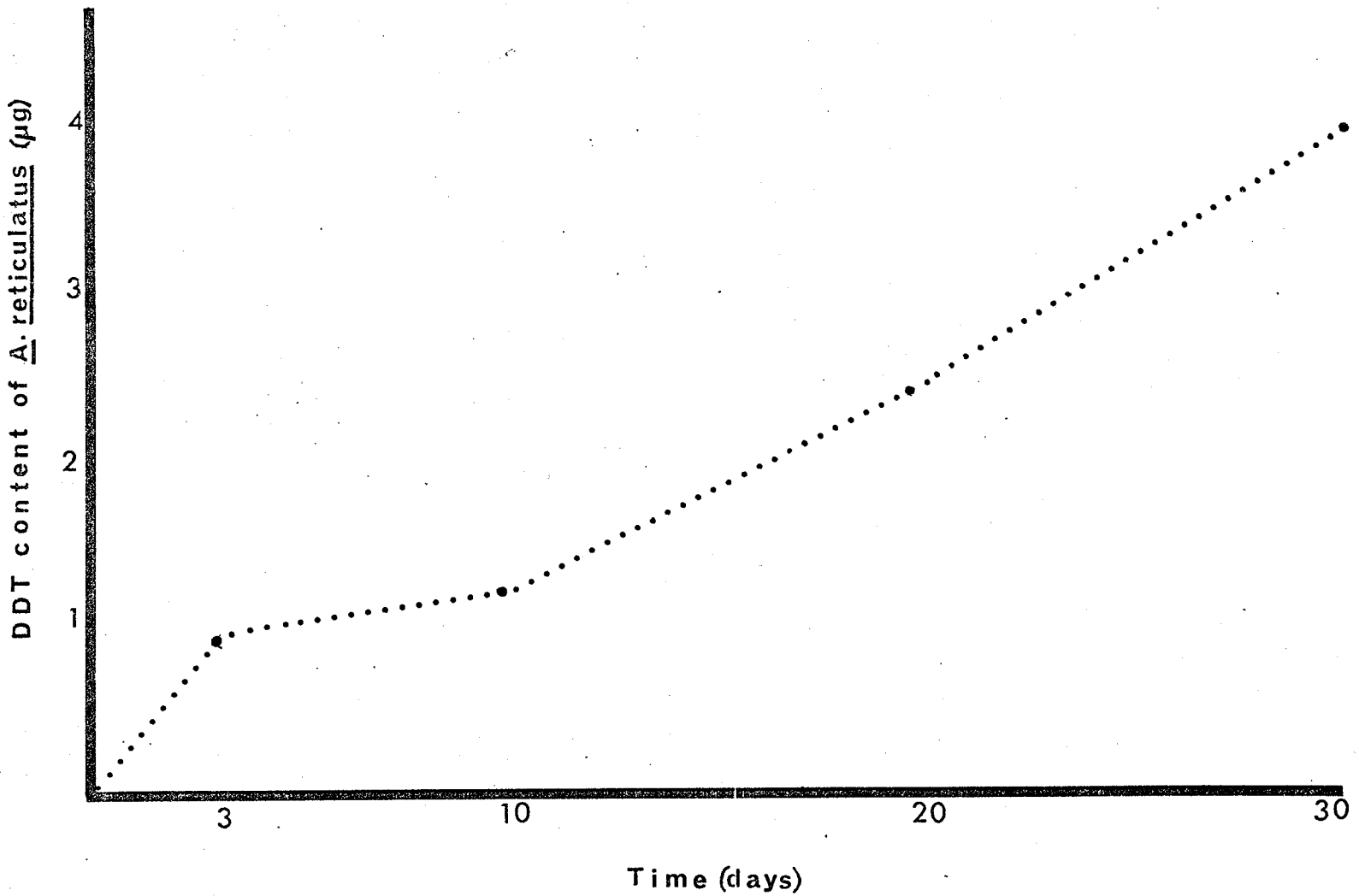
For the experiment 25 gms. of aged leaf litter (collected spring 1971) was placed in each of 10 large plastic containers. DDT was added to 8 containers such that the leaf litter contained 80 ppm. DDT dry weight. Two controls were used to which only ethanol was added. Eight *A. reticulatus* were placed in each of the containers. A plastic petri dish was placed in the center of the container on top of the humus as a food pad. Uncontaminated oatmeal and lettuce were placed in this dish for food. These dishes were necessary otherwise the slugs would ingest some of the leaf litter along with the oatmeal flakes. The containers, with secure lids were placed in an unlit incubator at 15°C for 30 days. After the initial addition of 15 mls. of distilled water, no more was added. The leaf litter substrate was stirred every week as the slugs tended to compact it. Sampling was done 3, 10, 20 and 30 days after the initiation of the experiment. One slug was picked at

Fig. 11 - Accumulation of DDT by A. reticulatus when maintained in a DDT contaminated habitat and fed clean preferred food

Each point represents a pooled sample of 6 slugs.

DDT content of leaf litter habitat (ppm. dry weight)

. . . . 80


DDT content of A. reticulatus (µg)
4
3
2
1
3
10
20
30
Time (days)

random from each of the six containers at each time.

Results:

Under conditions where substrate ingestion should be minimal, A. reticulatus showed a slow increase in DDT residue over the 30 day period (Fig. 11). Rate of accumulation was greatest the first 3 days. From fecal examination it was noticed that leaf litter made up a small part of the diet of slugs maintained under these conditions. This leaf litter was undoubtably the source of DDT reaching the body tissues.

DDT Accumulation When Both DDT Contaminated Food and Substrate was Presented to Immature Slugs

Procedure:

A. reticulatus was used as the test animal. Specimens used were 3rd generation laboratory reared individuals, 60 days old.

For the experiment 50 gms. of leaf litter (collected during spring 1971) was added to 3 large plastic containers. DDT was added to 2 of the containers so that the leaf litter contained 80 ppm. Ethanol was added to the control container. After drying, 15 mls. of distilled water was added to each of the containers, which were then sealed for two days until the leaf litter was sufficiently moist.

Forty A. reticulatus were placed in each of the contaminated containers and 20 in the control container. Oatmeal pellets containing 80 ppm. DDT dry weight were given

as a preferred food. The leaf litter was stirred every two weeks because the slugs were compacting it.

Sampling was done on days 10, 20, 40, 61, 75, 95 and 104. Samples from the controls were taken on days 40 and 104. Eight slugs were picked at random from the test containers, 4 from each container at each sampling period. Eight slugs were taken from the control container.

Results:

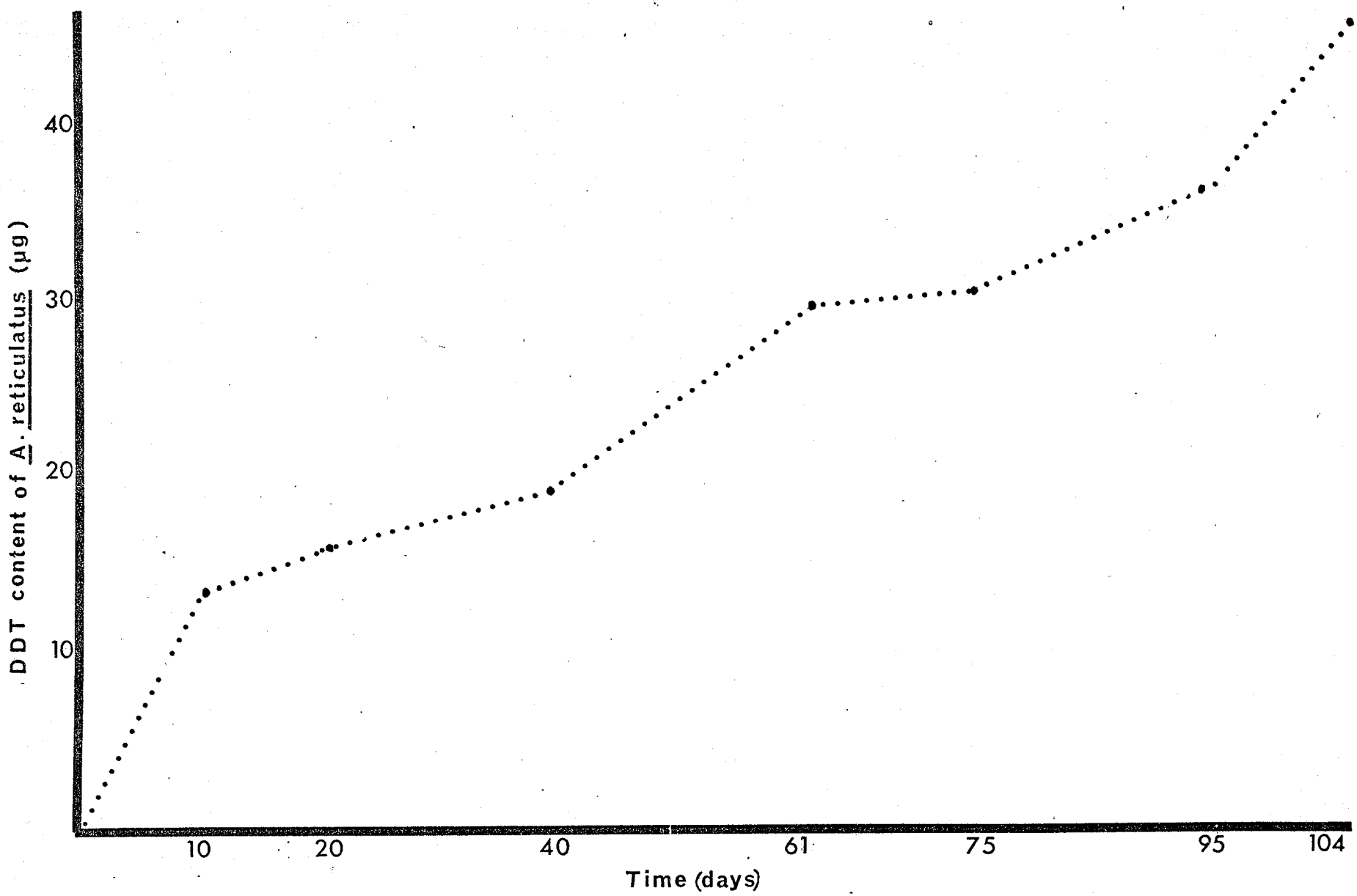
A. reticulatus showed an increase in DDT residues with time (Fig. 12). Initial accumulation of DDT was rapid. The rate of accumulation for the immature slugs for the first 20 days being the same as that for adults maintained on contaminated oatmeal alone (Fig. 8). From day 20 to 104 the rate of accumulation was slow but constant. Food consumption for this period was constant. Fecal examination indicated that very little of the leaf litter was used as food. Egg production started 84 days after the commencement of the experiment when the slugs were 144 days old. Sixty-one percent of these eggs hatched, which was similar to the hatchability of eggs from slugs of the same age maintained under culture conditions and not treated with DDT (63%).

Fig. 12 - Accumulation of DDT from a contaminated food and habitat by maturing A. reticulatus

Each point represents a pooled sample of 8 slugs

DDT content of oatmeal pellets and habitat (ppm. dry weight)

. . . . 80


DDT content of A. reticulatus (µg)
40
30
20
10
10
20
40
61
75
95
104
Time (days)

Discussion

Under all feeding conditions, both L. valentiana and A. reticulatus were able to accumulate DDT in their body tissues. The DDT accumulated by the slugs in each experiment was directly proportional to the DDT content in the food or substrate and the length of time it was fed.

A. reticulatus showed a slower rate of accumulation the first 3 days than did L. valentiana when they were maintained under the same conditions. From day 3 to the completion of a given experiment A. reticulatus achieved higher residue levels than L. valentiana. Both A. reticulatus and L. valentiana exhibited a slower rate of accumulation at the 40 and 80 ppm. DDT levels from days 10 to 20. Whether this was due to a change in the excretory mechanism or changes in food consumption has not been ascertained.

The 3 diets used for DDT dosing can be ranked in order of increasing palatability, that is, aged leaf litter, non-aged leaf litter and oatmeal. When one compares the DDT content of slug tissues given these 3 foods for 30 days (Table 5) we find that the diets of higher palatability also have resulted in higher DDT accumulation. Thus the more food eaten the more DDT accumulated per unit of time.

Even when uncontaminated preferred foods were available, the slugs accumulated DDT quite readily from contaminated leaf litter used as a substrate. Young slugs maintained until maturity on oatmeal and leaf litter that

contained 80 ppm. DDT showed a rapid accumulation the first 20 days, similar to that for adult slugs feeding on contaminated oatmeal. From day 20 to 30 the rate of accumulation was lower than that for adult slugs. A lower rate of intake may have been responsible for much of the difference between the two groups.

The different experiements conclusively demonstrate that no matter what the food supply is, if the surrounding media contains DDT the slugs will accumulate residues in their tissues. The mature slugs showed a progressive increase in body residues when given DDT continously for 70 days and the immature slugs demonstrated this for 104 days.

No matter what the dosing level, length of dosing period or dosing procedures used, there was never any evidence of a DDT effect upon the slug behavior. Furthermore, egg production seemed unaffected by the treatments.

TABLE 5

COMPARISON OF DDT ACCUMULATION FROM DIFFERENT DIETS IN 30 DAYS BY L. VALENTIANA AND A. RETICULATUS (SAMPLES OF 8 SLUGS)

Species	FOOD SOURCE			
	Aged leaf litter containing 80 ppm. DDT	Preferred Food containing 80 ppm. DDT	Fresh leaf litter containing 80 ppm. DDT	Preferred Food No DDT, Decayed Leaf Litter containing 80 ppm. DDT
Agriolimax reticulatus	14.72 µg.	38.4 µg.	27.6 µg.	3.9 µg.
Lehmannia valentiana	6.18 µg.	25.12 µg.		

PART III - EXCRETION OF DDT

Introduction

The level of DDT in the diet of slugs will vary with climatic conditions and programs for DDT use. For this reason the length of time it takes a slug to excrete DDT is important with respect to magnification of DDT by the slug. The value of slugs as monitors of pesticides would also be determined in part by the length of time residues could be stored in slug tissue. Excretion of DDT can take place by elimination in the feces or through body products such as eggs and the secretion of mucous. Experiments were set up to measure elimination via these various routes.

Excretion of DDT in feces

Procedure:

Lehmannia valentiana was used as the test animal. The specimens used were mature laboratory reared individuals, 285 days old. These slugs were previously used for culture purposes but egg production had ceased.

The slugs were maintained in small plastic dishes with damp paper toweling as substrate. Twelve groups of 8 slugs each were used in the experiment. Ten groups were fed oatmeal pellets containing 80 ppm. DDT as food for 20 days. Two control groups were fed oatmeal to which only ethanol

was added. The containers were kept in the dark at 15°C except for daily maintenance. A known amount of DDT was fed to each group over the 20 day period. This was the basis for calculating the proportion of ingested DDT that was retained in the body tissues.

After the 20 day exposure to DDT in the diet, the slugs were placed in new containers and DDT-free oatmeal and lettuce were given as food. Sampling was subsequently done on days 3, 5, 9, 36, 54 and 79. Two replicates were sampled on day 3 and day 9. On the other sampling days a single container of 8 slugs was sampled. Two containers had to be discarded as one slug died in each of the containers.

Results:

After 20 days of feeding DDT and 3 days of feeding on a DDT free diet, 20.5% of the ingested DDT was retained in the slug tissue (Table 6). The rest of the ingested DDT had been eliminated. Analysis of ^{14}C material in fecal samples showed that there was a definite excretion of DDT taking place for 15 days following the cessation of DDT feeding. From days 15 to 79 no measurable levels of ^{14}C-DDT were recovered in the feces.

Excretion of DDT in Mucous

Procedure:

Lehmannia valentiana was used as the test animal. Specimens used were mature adults collected from a greenhouse.

TABLE 6

DDT RETAINED WHEN FEEDING L. VALENTIANA A DDT-FREE DIET SUBSEQUENT TO A DDT-DOSE PERIOD

Days on DDT-free diet	DDT ingested (µg)	DDT retained (µg)	% of Dose retained
3	80.40	16.140	20.07
3	110.1	23.15	21.02
5	80.01	15.0	18.75
9	112.0	15.01	13.40
9	96.01	13.4	13.96
36	94.2	9.73	10.33
54	63.18	7.80	12.34
79	79.512	10.14	12.76

The slugs were maintained in small plastic dishes, with damp toweling as a substrate. Five groups of 6 slugs each were used. Four of the replicates were fed oatmeal pellets containing 80 ppm. DDT for the duration of the experiment. The control group was fed DDT free oatmeal (no ethanol added).

Sampling was done on days 3, 10, 20 and 30. Mucous was collected by stroking the backs of individual slugs with a steel probe. This method of collection would tend to eliminate contamination due to DDT adhering to the foot area of the slugs. When mucous had been collected from each individual the samples were dried and weighed before being solubilized in Hyamine hydroxide and the DDT residue level determined by ^{14}C determination using the p-dioxane scintillation cocktail.

Results:

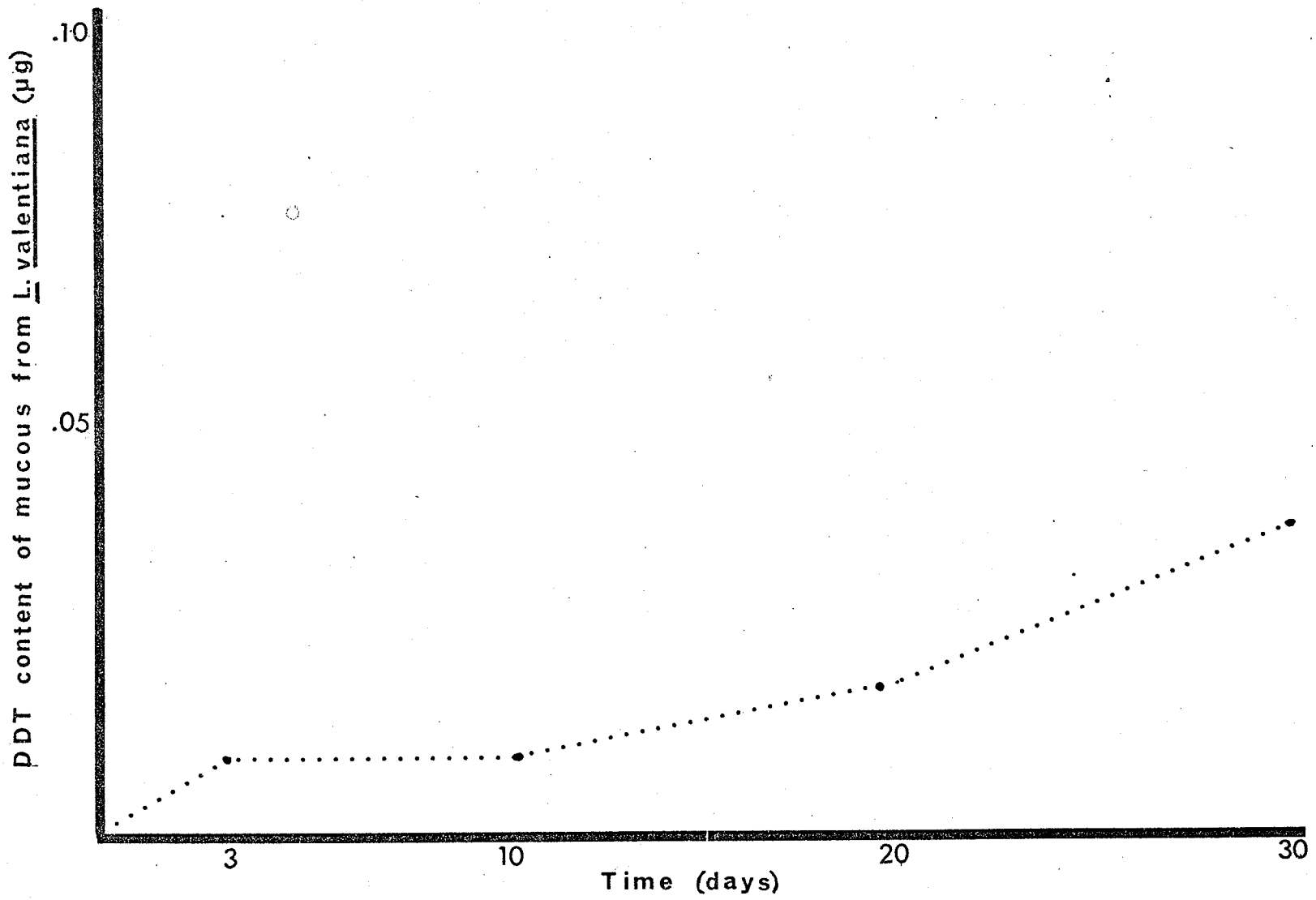
There was a slow but steady increase in DDT residues with time (Fig. 13). There is obviously a direct relationship between the mucous DDT content and the body burden of DDT. Previous experiments have shown that the body burden increases when DDT is fed continuously and this experiment shows that there is a concomitant increase in mucous residue. After 30 days of maintenance on 80 ppm. DDT, the residue level in the mucous was 1.34 ppm., but the values were not high enough to be significantly different from the controls (Appendix IIa).

Fig. 13 - DDT content of mucous from L. valentiana fed oatmeal pellets containing DDT during the experimental period

Each point represents a pooled sample from 24 slugs.

DDT content of oatmeal pellets (ppm. dry weight)

. . . . 80


.10
.05
DDT content of mucous from L. valentiana (µg)
3
10
20
30
Time (days)

Excretion of DDT in Eggs

Procedure:

Lehmannia valentiana was used as the test species. Specimens used were laboratory reared individuals 210 days old that had been used previously for culture purposes.

Large plastic containers were used for the experiment. Conditions were the same as those used for slug culture except that oatmeal pellets containing 80 ppm. DDT were used as food. Ten slugs were placed in each of four containers. Three groups were fed 80 ppm. DDT and the fourth was used as a control, being fed DDT-free food (no ethanol added).

Eggs were removed from the containers daily and discarded except on sampling days. As egg production fluctuated from day to day, it was necessary to combine the eggs from 2 successive days to have a consistently large sample. Sampling was done on days 3-4, 9-10, 19-20 and 29-30. The eggs were dried and weighed before being solubilized in hyamine hydroxide. As the eggs were quite difficult to dissolve, it was necessary to heat the eggs in the solubilizer for 5 days at 75°C before the samples could be analyzed for DDT residues by ^{14}C determination using the p-dioxane scintillation cocktail.

Results:

DDT residues in the eggs were low (Fig. 14). There

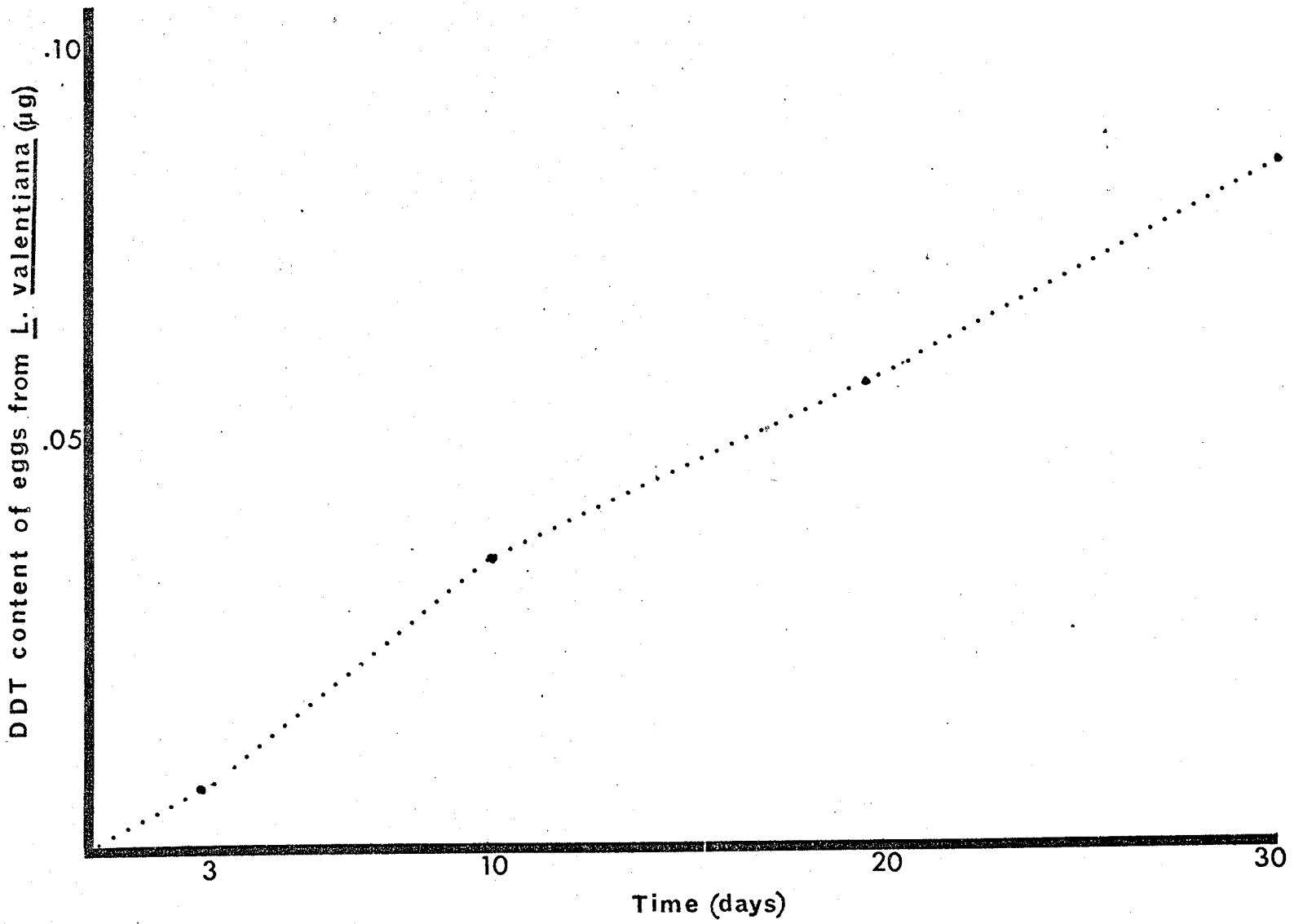
was an apparent increase in DDT egg residues with time, indicating a relationship between egg residues and DDT body burden. However, the values were not high enough to be significantly different from the controls (Appendix IIa).

Fig. 14 - DDT content of eggs from L. valentiana fed oatmeal pellets during the experimental period.

Each point represents a pooled sample of eggs from 30 slugs.

DDT content of oatmeal pellets (ppm. dry weight)

. . . . 80


.10
.05
DDT content of eggs from L. valentiana (µg)
3
10
20
30
Time (days)

Discussion

The proportion of ingested DDT that is retained by slugs on a day to day basis is fairly low. Only one-fifth of the ingested DDT was present in slugs at the end of a 20 day ingestion experiment. DDT was excreted in the feces fairly rapidly from the slugs for a period of 15 days immediately after withdrawal of the DDT contaminated diet. After this initial decrease in DDT body burden a fairly constant level was maintained for 64 days. The initial decrease was presumably due at least in part to the slow excretion of DDT residues retained in the digestive tract. After 79 days the residues retained were similar to those for 9 days. Following the feeding of DDT there was a short period during which DDT was slowly excreted, thus allowing the body burden to reach an equilibrium state. This residue pool has a very slow turnover rate. When excretion is virtually zero during the equilibrium period, it is doubtful if much DDT could be lost through mucous or egg production.

When mucous was collected from slugs while they were feeding on DDT contaminated food, only low residue levels were found. The residues did increase with time during the feeding period. As the mucous was collected from the back of the slugs, the risk of dermal uptake was eliminated. Mucous production may be of minor importance in DDT excretion when the slugs are feeding on DDT contaminated

food.

Egg production appears to be of negligible importance with respected to excretion of DDT. The residue levels in eggs were low. However, considering the chemical nature and greater weight of the egg shell compared to the rest of the egg, the actual DDT level of the egg contents may have been much greater than for the whole egg.

In these three experiments 80 ppm. DDT was fed to each group of slugs ad libitum. After feeding for 20 days, .02 µg. DDT was found in the mucous sample and .06 µg. was in the egg sample. In a similar experiment, 3 days following a 20 day DDT feeding experiment, elimination of ingested DDT via the feces was 64-87 µg.

There is no disputing the conclusion that feces are the major route of DDT elimination by the slug.

PART IV - STORAGE OF DDT

Introduction

Certain body components such as lipids act as a storage reservoir for DDT. As certain body tissues in slugs have higher lipid levels than others, it would be expected that DDT residues would vary between different tissues. Mobilization of DDT and its metabolites in an organism tends to follow the utilization of stored lipid. This utilization of lipid is governed in part by food consumption and energy expenditure.

Slugs like other animals, are subject to periods of low food consumption due to unfavourable environmental conditions. During such periods slugs would be required to utilize their lipid reserves for energy. DDT concomitantly mobilized would be either stored in other tissues or excreted.

DDT Storage in Tissues

Procedure:

Agriolimax reticulatus and Lehmannia valentiana were used as the test species. Specimens of L. valentiana used were mature laboratory reared individuals 125 days old. Specimens of A. reticulatus used were mature field collected individuals approximately 9 months old. Both species were

maintained in the laboratory for 90 days at 4°C prior to experimentation. As it was previously found that mortality for A. reticulatus was high when slugs were moved to a warmer temperature, the experiment was carried out at 4°C.

Thirty-five A. reticulatus were used for the experiment, 5 being used as controls. Twenty-five L. valentiana were used with 5 of these being used as a control.

For the experiment, small glass jars with tight fitting tops were used to house individual slugs. Damp paper toweling was used as a substrate. These containers were kept in total darkness except during daily maintenance. Oatmeal pellets containing 80 ppm. DDT were given as food. Individual records of food consumption were kept. Food was supplied so that there was always a surplus available to each slug.

After the 20 days of feeding on the contaminated pellets the slugs were starved for 24 hours before being frozen. Each slug was dissected into 7 different tissue groups. Individual tissues were placed in separate scintillation vials, dried and then weighed. These individual samples were then solubilized in Hyamine hydroxide prior to DDT determination by ^{14}C analysis using the p-dioxane scintillation cocktail.

Results:

For A. reticulatus the hepatopancreas was found to have the highest DDT content and the highest DDT concentra-

tion per unit dry weight (Table 7). Sixty-nine percent of the DDT body burden (72.5 µg.) was found in this tissue. The digestive tract had the next highest DDT content (21.62 µg.) which amounted to 20.6% of the total residues. The other 5 tissues each had approximately 2% of the total residues. Thirty-eight percent of the DDT consumed had been retained in the body tissues during the 20 day feeding period.

Similar results were found for L. valentiana (Table 8), which also had the highest residue levels in the hepatopancreas (29.7 µg.) and digestive tract (5.42 µg.). These quantities were 73 and 13% of the body residue respectively. The other 5 groups of tissues each had approximately 3% of the total residues. L. valentiana retained 14.6% of the DDT consumed.

Hepatopancreas Residues When Fed High Concentrations of DDT

Procedure:

Agriolimax reticulatus was used as the test species. Specimens used were laboratory reared individuals 120 days old.

The slugs were maintained in small plastic containers with damp paper toweling as a substrate. Eight slugs were placed in each of 10 containers. Four groups were fed 240 ppm. DDT in oatmeal pellets and 4 groups were fed 480 ppm. DDT in the pellets. Two groups were used as controls

TABLE 7

DDT CONCENTRATIONS OF INDIVIDUAL TISSUES FROM *A. RETICULATUS* MAINTAINED 20 DAYS ON OATMEAL CONTAINING 80 PPM. DDT

Tissue	Tissue Weight (gms.)	µg. DDT in Tissue[4]	ppm. DDT dry weight
Dorsal Carcass	1.4603	3.686	2.52
Foot	.7652	2.004	2.62
Female Reproductive Tract	.9162	1.652	1.80
Albumen Gland	1.2604	2.680	2.13
Male Reproductive Tract	.3443	.836	2.43
Digestive Tract	.6369	21.620	33.95
Hepatopancreas	.8583	72.554	84.53
Totals	6.2446[1]	105.032[2][3]	

(1) Total for 30 slugs
(2) Total for 30 slugs
(3) 276.4 µg. DDT was consumed by the 30 slugs
(4) Mean and standard deviation Appendix IIc.

TABLE 8

DDT CONCENTRATIONS OF INDIVIDUAL TISSUES FROM *L. VALENTIANA* MAINTAINED 20 DAYS ON OATMEAL CONTAINING 80 PPM. DDT

Tissue	Tissue Weight (gms.)	µg. DDT in Tissue	ppm. DDT dry weight
Dorsal Carcass	.804	2.380	2.960
Foot	.3578	.780	2.179
Female Reproductive Tract	.3304	1.540	3.457
Albumen Gland	.4454	.584	1.311
Male Reproductive Tract	.120	.280	2.330
Digestive Tract	.292	5.420	18.561
Hepatopancreas	.270	29.700	110.0
Totals	2.6196 (1)	40.684 (2) (3)	

(1) Total for 20 slugs
(2) Total for 20 slugs
(3) 278.6 µg. DDT was consumed by the 20 slugs

and were fed pellets to which ethanol had been added.

Sampling was done on the 15th and 30th day when two groups at each concentration were sampled. The slugs were starved for 48 hours before freezing. Upon dissection of each slug the hepatopancreas was removed, placed in an individual scintillation vial and dried before weighing. The tissue was then solubilized in Hyamine hydroxide before DDT levels were determined by measuring the ^{14}C content using p-dioxane as a scintillation cocktail.

Results:

Food consumption was normal for 12 days and then decreased with time. Food consumption by the controls was fairly constant with time. No mortalities occurred at either DDT intake level. There was a very rapid concentration of DDT residues in the hepatopancreas (Fig. 15). Although there was an increase with time in tissue DDT concentration at both levels of feeding, the rate of uptake from day 15 to day 30 was very slow in comparison to that for the first 15 days. This period of rapid tissue accumulation of DDT coincided well with the period of most rapid intake. At 30 days the tissue concentration (681 µg.) at the 480 ppm. level of feeding was approximately twice that at the 240 ppm. level of feeding (310 µg.).

Changes in DDT Storage under Varying Feeding Conditions

Procedure:

Lehmannia valentiana was used as the test species.

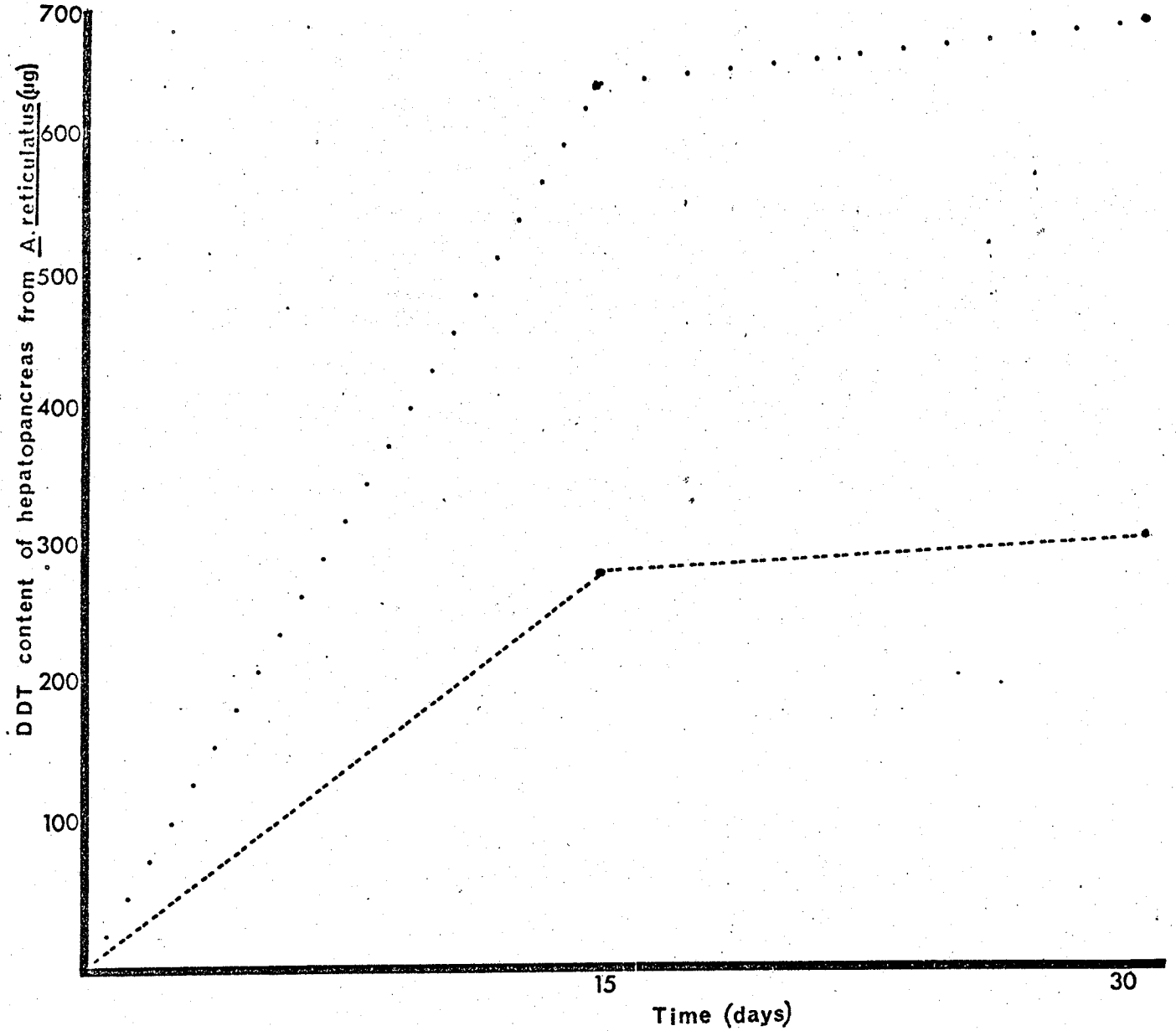
Fig. 15 - Concentration of DDT in hepatopancreas of A. reticulatus under high concentration feeding

Each point represents a pooled sample of 16 slugs.

DDT content of oatmeal pellets (ppm.)

. . . . 480

------- 240


700
600
500
400
300
200
100
DDT content of hepatopancreas from A. reticulatus(µg)
15
30
Time (days)

Specimens used were laboratory reared individuals 112 days old.

Slugs were maintained in small plastic containers with damp paper toweling as a substrate. Six slugs were placed in each of 12 containers. Nine groups were fed 80 ppm. DDT in oatmeal pellets, Three groups were used as controls and were fed oatmeal pellets to which only ethanol was added.

The slugs were maintained on DDT contaminated food for 20 days. After 20 days 3 replicates were put on DDT-free food for 3 days and then terminated by freezing. The remaining 6 groups were placed in clean containers. For the following 30 days 3 of these groups were fed DDT-free food and 3 were starved. After this thirty day treatment the slugs were frozen.

Individual DDT levels were determined for each slug. Tissues were placed in individual scintillation vials, dried, weighed and then solubilized with Hyamine hydroxide before determination of DDT residues by measuring ^{14}C content. The DDT levels for the slugs were totalled so that DDT levels were available for each group of slugs.

Results:

The slugs had retained 12.42% of the ingested DDT at the end of the 20 day feeding period (Table 9). Following 30 days of feeding uncontaminated food the slugs had retained 10.07% of the ingested DDT and after 30 days of starvation

the percent of ingested DDT retained was still 8.75%. This confirms the previous findings that feeding DDT-free food subsequent to DDT feeding can promote a small reduction in DDT body burden. Stavation which promotes lipid utilization does not cause much more of a reduction in body burden than the feeding of DDT-free food (not significantly different - see Appendix IIb).

TABLE 9

DDT RETAINED BY L. VALENTIANA UNDER DIFFERENT FEEDING CONDITIONS

Group	DDT (1) Consumed (µg)	DDT Body Burden at 20 days (µg)	DDT Body Burden (2) at 50 days (µg)	Proportion retained (%)
Fed 80 ppm DDT for 20 days	179.644	8.078 7.344 6.896 Total 22.318	-	12.42
Fed 80 ppm DDT for 20 days - DDT-free food for 30 days	203.448	-	5.469 6.118 8.902 Total 20.49	10.07
Fed 80 ppm DDT for 20 days - starved 30 days	274.55	-	8.83 7.94 7.25 Total 24.02	8.75

(1) Consumed in 20 day feeding period by the 3 replicates (18 slugs).

(2) 50 days consisted of a 20 day feeding period followed by 30 days of feeding a DDT-free food or a 30 day starvation period.

Discussion

Agriolimax reticulatus showed a much higher retention of DDT (38%) from food than did *Lehmannia valentiana* (14.6%). When 80 ppm. DDT was fed to *A. reticulatus* and *L. valentiana* the distribution of DDT between the 7 tissues analysed was very similar for the two species. The hepatopancreas had by far the highest tissue DDT residues in both species, being 69-73% of the body burden. When fed high DDT levels in the food (480 ppm.), hepatopancreas DDT levels of 640 ppm. dry weight were found. This would indicate that this tissue has a very high storage potential for DDT. The digestive tract had the second highest residue levels (13.3-20.6%), while the remaining 5 tissue groups all contained very low residues (2-3%).

The slugs showed a definite ability to retain DDT residues even when starved for 30 days. Although both starvation and feeding DDT-free food indicated a lower slug residue after 30 days, this reduction was not statistically significant.

After 30 days exposure to 240 and 480 ppm. DDT in the diet, the slugs exhibited no abnormal behavior with respect to movement and reproduction. However, high concentration of DDT in the diet decreased food consumption after 12 days.

Chapter 4

DISCUSSION

Alien slugs are now a definite part of the fauna of Manitoba. As to where they came from and how they were spread can only be speculated. However, the predominant route of introduction was likely associated with plant material being introduced. In the survey conducted in southern Manitoba in 1972, the native species, Agriolimax laeve, was found in nearly all the gardens that were sampled. Population densities in all areas were low and little economic loss was being caused by this species. Although the introduced species, Agriolimax reticulatus, occurred less frequently in gardens of southern Manitoba than A. laeve, most of the slug damage could be attributed to this species. A. reticulatus has a life cycle that is adapted to the environmental conditions of Manitoba, and thus has been able to extend its range outside of protected places such as greenhouses. Within the last 20 years A. reticulatus has extended its range over much of southern Manitoba. The other introduced species, Lehmannia valentiana was not found in gardens in the survey and occurs at the present time in 2 greenhouses in the Winnipeg area, where it is a pest primarily from the aesthetic point of view. This is attributed to its inability to adapt to the Manitoba environment.

With the increasing importance of vegetable and fruit production in Manitoba, in greenhouses during the winter months and market gardens in summer, serious slug damage by introduced species of slugs is a definite possibility. The lack of safe and suitable control measures for use in these moist localities may accentuate this problem. Agriolimax reticulatus is capable of producing more than 200 eggs/year under Manitoba conditions. For this reason, slug control measures carried out before egg production starts in late August will control the present population and greatly reduce the slug population the next year.

The use of slugs as experimental animals depends in part on the ability to maintain a population long enough to carry out necessary experimentation. Sivak (1954) and Arias and Crowell (1963) reported difficulty in maintaining slugs under laboratory conditions due to high death losses that were attributed to pathogenic organisms. By hatching eggs in the laboratory we were able to obtain relatively disease free populations for use in this study. These disease free populations were maintained on experiments for up to 104 days without significant death losses.

Harris et al (1966) and Duffy and Wong (1967) found some DDT residues in the soil in cultivated areas of Eastern Canada higher than those used in the accumulation experiments, (4, 16, 40 and 80 ppm. dry weight). The top 1/2 inch of soil and 1/2 inch of overlying vegetation from 9 seperate areas in the Winnipeg area contained an average of 26.5 ppm. (dry

weight) DDT and metabolites (Brust, 1970). Both A. reticulatus and L. valentiana exhibited a definite body retention of DDT from various foods contaminated at 4, 16, 40 and 80 ppm. dry weight. The amount of DDT accumulated in the slug tissue was dependent on the level of DDT intake, the length of intake period and the quantity of food consumed. Similar results were reported for earthworms by Edwards (1970).

Elimination of DDT from slugs was rapid during the actual feeding period. In 20 days A. reticulatus eliminated 62% and L. valentiana eliminated 80% of the ingested DDT. It is quite probable that most of the ingested DDT and hence most of the eliminated DDT had never been assimilated by the slugs. That is, it was passed straight through the gastrointestinal tract.

When DDT was removed from the diet of L. valentiana an additional 8% of the ingested DDT was eliminated in 9 days. From day 15 to 79 elimination was negligible, with retention being 12% of the DDT ingested. DDT excretion was not greatly affected by starvation or maintenance on DDT-free food. Three elimination routes to quantitate the relative importance in DDT excretion were investigated. Elimination of DDT through mucous production was found to be low and by egg production was negligible. The fecal route was the major route of DDT elimination.

Dindal and Wurzinger (1971) found that the snail Cepaea hortensis had the highest DDT residues in the hepatopancreas and digestive tract. Our results are very

similar, in that both A. reticulatus and L. valentiana had the highest DDT residues in the hepatopancreas and second highest in the digestive tract. The DDT residue level in the hepatopancreas of A. reticulatus reached 640 ppm. (dry weight) after ingesting a 480 ppm. diet of DDT for 30 days, indicating that this tissue has a very high DDT storage capacity.

When fed high concentrations of DDT in the diet (480 ppm.), A. reticulatus showed a reduction in food consumption after 12 days of feeding. However, movement, reproduction and other behavioral traits continued normally for a 30 day period. Thus implying that high DDT intakes can be tolerated by this species.

Chapter 5

SUMMARY

Two introduced species of slugs, Agriolimax reticulatus and Lehmannia valentiana, are now established in Manitoba. A. reticulatus is found in nearly all southern areas of the province. Severe climatic conditions prevent L. valentiana from becoming established outside of greenhouses.

Both A. reticulatus and L. valentiana are able to accumulate DDT from their environment. The quantity accumulated depends on concentration in the food, the length of consumption period and the amount of food consumed.

Elimination of DDT in the feces is rapid during consumption of contaminated food. When the contaminated food was removed, fecal elimination continued for 15 days, after which the body burden of DDT remained fairly constant for at least 64 days. Elimination of DDT through mucous secretion and egg production was negligible.

The hepatopancreas and digestive tract had the greatest storage capacity of DDT. Residue levels as high as 640 ppm. were found in the hepatopancreas. The body burden of DDT was not significantly affected by starvation or maintenance on DDT-free food.

Apart from changes in food consumption, levels of

DDT as high as 480 ppm. in the diet produced no noticeable affect on behavior or reproduction of slugs.

CONCLUSIONS

Agriolimax reticulatus is ubiquitous in the cultivated areas of North America and Europe, and still extending its range. As this slug has a life cycle well adapted to the climatic conditions of Manitoba, it will undoubtable remain an aesthetic and economic pest. As Agriolimax reticulatus is ubiquitous, relatively sedentary and exhibits a rapid accumulation of DDT from the environment, it is possible that this slug may be useful for monitoring the biological availability of pesticides. The feeding behavior of slugs is such that a wide variety of organic material is utilized. By sampling a slug population in the late fall, determinations of pesticide residues in the primary trophic levels is possible. Thus a species that is most often a pest may have some beneficial use in today's agricultural complex.

BIBLIOGRAPHY

Andrewartha, H. G. 1967. Introduction to the study of animal populations. The University of Chicago Press.

Arias, R. O. and H. L. Crowell. 1963. A contribution to the biology of the grey garden slug. Bull. Calif. Acad. Sci. 62:83.

Ballard, T. M. 1971. Role of humic carrier substances in DDT movement through forest soil. Soil Sci. Soc. Amer. Proc. 35:145.

Barnes, H. F. and J. W. Weil. 1944. Slugs in gardens: their numbers, activities and distribution. Part I, J. Anim. Ecol. 13:145.

Bayne, C. J. 1968. A study of the desiccation of egg capsules of eight gastropod species. J. Zool. Lond. 155:401.

_____. 1969. Survival of the embryos of the grey field slug Agriolimax reticulatus, following desiccation of the egg. Malacologia 9:391.

Binney, A. 1842. Description of some of the species of naked airbreathing mollusca inhabiting the United States. Boston Soc. Nat. Hist. J. 4:163.

Blezard, E. 1967. Non-marine mollusca of lakeland. Trans. Carlisle Nat.Hist. Soc. 11:48.

Bligh, E. and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. Canad. J. Biochem. Physiol. 37:911.

Brooks, W. M. 1966. The role of the holotrichous ciliates Tetrahymena limacina and Tetrahymena rostrata as parasites of the grey field slug, Deroceras reticulatus. H.D. thesis, University of California, Berkeley.

Bruel, W. E. van den, and R. Moens. 1958. Remarques sur les facteurs écologiques influençant l'efficacité de la lutte contre les limaces. Parasitica. 14:135.

Brust, R. A. 1972. DDT residues in soil in the Winnipeg area. Manitoban Entomologist (in press).

Carrick, R. 1938. The life history and development of Agriolimax agrestis L., the grey field slug. Trans. Roy. Soc. Edinb. 59:563.

_____. 1942. The grey field slug Agriolimax agrestis L. and its environment. Ann. Appl. Biol. 29:43.

Chichester, L. F. and L. L. Getz. 1968. Terrestrial slugs. The Biologist. 50:148.

Collinge, W. E. 1921. The starling: Is it injurious to agriculture? J. Minist. Agric., Fish. 27:1114.

Crozier, W. J. and G. F. Pilz. 1923. The locomotion of Limax. 1. Temperature coefficient of pedal activity. J. Gen. Physiol. 6:711.

Dainton, B. H. 1954a. The activity of slugs: I. The induction of activity by changing temperatures. J. Exp. Biol. 31:165.

_____. 1954b. The activity of slugs: II. The effect of light and air currents. J. Exp. Biol. 31:188.

Davis, B. W. and R. B. Harrison. 1966. Organochlorine insecticide residues in soil invertebrates. Nature. 211:1424.

Davis, B. W. K. 1966-68. Organochlorine residues in soils and invertebrates. Report Monks Wood. Experimental Station. 13.

Dindal, D. L. and K. H. Wurzinger. 1971. Accumulation and excretion of DDT by the terrestrial snail, Cepaea hortensis. Bull. Envir. Contam. Toxical. 6:362.

Duffy, J. R. and W. Wong. 1967. Residues of organochlorine insecticides and their metabolites in soils in the Atlantic provinces of Canada. J. Agr. Food Chem. 15:457.

Edwards, C. A. 1970. Persistent pesticides in the environment. Reprinted from Critical Reviews in Environmental Control. 1:8.

Fleming, W. E. and W. W. Maines. 1953. Persistence of DDT in soils in the area infested by the Japanese beetle. J. Econ. Entomol. 46:445.

Ginsburg, J. M. and J. P. Reed. 1954. A survey of DDT accumulation in soils in relation to different crops. J. Econ. Entomol. 47:467.

Gish, C. D. 1970. Organochlorine insecticide residues in soils and soil invertebrates from agricultural lands. Pestic. Monit. J. 3:241.

Gould, H. 1961. Observations on slug damage to winter wheat in East Anglia. Pl. Path. 10:142.

Gustafson, C. G. 1970. PCB's - prevalent and persistent. Envir. Sci. Technol. 4:814.

Harris, C. R.; Sans, W. W. and J. R. Miles. 1966. Exploratory studies on occurrence of organochlorine residues in agricultural soils in S. W. Ontario. J. Agr. Food Chem. 14:398.

Holyoak, D. 1968. A comparative study of the food of some British Corvidae. Bird Study. 15:147.

Hunter, P. J. 1967. The effect of cultivations on slugs of arable ground. Pl. Path. 16:153.

_____. 1968a. Studies on slugs of arable ground. II. Life cycles. Malacologia. 6:379.

_____. 1968b. Studies on slugs of arable ground. I. Sampling methods. Malacologia. 6:369.

_____. 1968c. Studies on slugs of arable ground. III. Feeding habits. Malacologia. 6:391.

_____. 1971. A note on nematodes in the alimentary canal of slugs. Proc. Malac. Soc. Lond. 37:385.

_____. 1971. The leap-frogging slug. Nature. 229: 349.

Jefferies, D. J. and B. N. Davis. 1966. Dynamics of dieldrin in soils, worms and songthrushes. J. Wildlife Manage. 32:441.

Kalmus, H. 1942. Anemotaxis in soft-skinned animals. Nature, London. 150:524.

Knutson, L. V.; Stephenson, J. W. and C. O. Berg. 1965. Biology of a slug killing fly, Tetaflocera elata (Diptera:Sciomyzidae). Proc. Malac. Soc. Lond. 36:213.

Kozloff, E. W. 1956. Experimental infection of the grey garden slug, Deroceras reticulatus by the Holotrichous ciliate Tetrahymena pyriformes. J. Proto-zool. 3:17.

Lichtenstein, E. P. 1958. DDT accumulation in mid-western orchard and crop soils treated since 1945. J. Econ. Entomol. 50:386.

Meenakshi, V. R. and B. T. Scheer. 1968. Studies on the carbohydrates of the slug Agriolimax columbianus with special reference to their distribution in the reproductive system. Comp. Biochem. Physiol. 26:1091.

Menzie, C. M. 1971. Fate of pesticides in the environment. Ann. Rev. Entomol. 17:199.

Mellanby, K. 1961. Slugs at low temperatures. Nature. 189:944.

Nimmo. D. R.; Blackman, R. R.; Wilson, A. J. and J. Forester. 1971. Toxicity and distribution of Aroclor 1254 in the pink shrimp Penaeus duorarum. Mar. Biol. 2:191.

Peterson, J. R.; Adams, R. S. and L. K. Cutkomp. 1971. Soil properties influencing DDT bioactivity. Soil Sci. Soc. Amer. Proc. 35:72.

Pilsbury, H. A. 1948. Land Mollusca of North America (north of Mexico). Acad. Nat. Sci. Philad.

Pinder, L. C. 1969. The biology and behavior of some slugs of economic importance. Ph.D. thesis. University of Newcastle-upon-tyne.

Reynolds, B. P. 1936. Colpoda steini, a facultative parasite of the land slug, Agriolimax agrestis. J. Parasit. 22:48.

Riseborough, R. W.; Rieche, P., Peakall, D. B., Herman, S. G. and M. N. Kirven. 1968. Polychlorinated biphenyls in global ecosystem. Nature. 220:1098.

Runham, N. W. and P. J. Hunter. 1970. Terrestrial Slugs. Hutchinson & Co. Ltd.

Sivak, F. P. 1954. A technique for slug culture. Nautilus. 67:129.

South, A. 1965. Biology and ecology of Agriolimax reticulatus and other slugs: spacial distribution. J. Anim. Ecol. 34:403.

Stephenson, J. W. 1961. A Culture Mehtod for Slugs. Malacological Soc. 43.

_____. 1964. Slug Predators. Rep. Rothamsted Exp. Stn. 188.

_____. 1968. A review of the biology and ecology of slugs of agricultural importance. Proc. Malac. Soc. Lond. 38:169.

_____, and L. V. Knutson. 1968. A resume of recent studies of invertebrates associated with slugs. J. Econ. Ent. 59:356.

Stickel, W. H.; Hayne, D. W. and L. F. Stickel. 1965. Effects of heptachlor contaminated earthworms on woodcocks. J. Wildlife Manage. 29:132.

Stringer, A. and J. A. Pickard. 1963. The DDT content of soil and earthworms in an apple orchard at Long Ashton. Rept. Agr. Hort. Res. Sta. Univ. Bristol, 127.

Thomas, D. C. 1948. The use of metaldehyde against slugs. Ann. Appl. Biol. 35:207.

Thompson, G. A. and D. J. Hanahan. 1963. Identification of α glycerol ether phospholipids as major lipid constituents in two species of terrestrial slugs. J. Biol. Chem. 238:2628.

Vernon, J. D. R. 1970. A preliminary study of the food of the common gull on grassland in the autumn and winter. Bird Study. 17.

Waites, R. E. and C. H. van Middelem. 1958. Residue studies of DDT and malathion on turnip tops, collards, snap beans and lettuce. J. Econ. Entomol. 51:306.

Wheatly, G. A. and J. A. Hardman. 1968. Organochlorine insecticide residues in earthworms from arable soils. J. Sci. Food Agric. 19:219.

Woodwell, G. M. 1961. The persistence of DDT in a forest soil. For. Sci. 7:194.

_____, and F. T. Martin. 1964. Persistence of DDT in soils of heavily sprayed forest stands. Science. 145:481.

APPENDIX I

Scintillator Cocktails

(1) PPO - Toluene

5.0 gm. PPO (2, 5-Diphenyloxazole)
0.3 gm. POPOP (1, 4-bis-2- (5-Phenyloxazolyl)-Benzene)
1.0 l. toluene

(2) PPO - Dioxane

100.0 gm. naphthalene
6.0 gm. PPO (2, 5-Diphenyloxazole)
1.0 l. dioxane (scintillation grade)

APPENDIX II

(a) Linear regression and students-t test with a level of significance of .05 were used. Test values were compared with control values to determine if ^{14}C-DDT levels were significantly higher than background levels in the controls. This method was used for all accumulation and excretion experiments. Values not statistically significant were for excretion of DDT in mucous and eggs.

(b) Difference of means test with a level of significance of .05 to determine if DDT level in each test group differed.

(c)

Tissue	µg. DDT	$\bar{x}$	Standard deviation
Dorsal carcass	3.68	.123	.126
Foot	2.00	.067	.027
Female Reproductive Tissue	1.65	.055	.028
Albumen Gland	2.68	.089	.168
Male Reproductive Tissue	.83	.028	.018
Digestive Tract	21.62	.721	.612
Hepatopancreas	72.55	2.416	1.31