THE UNIVERSITY OF MANITOBA

# ACCUMULATION AND ELIMINATION OF CESIUM-137 BY FIVE AEDINE SPECIES OF MOSQUITOES AND BY A COMMON PREDATOR, NOTONECTA UNDULATA

by

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ABSTRACT

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Cesium-137 as a radioactive label for entomological investigations in the laboratory is described.

Accumulation and elimination of $^{137}Cs$ by five aedine species of mosquitoes (Aedes aegypti, A. atropalpus, A. dorsalis, A. triseriatus, and A. vexans) are studied. Species differences exist in both accumulation and elimination rates for a variety of reasons. The pupal and adult studies are inconclusive.

Accumulation and elimination of cesium-137 by 4th instar normal and degilled larvae in potassium and potassium-free medium is studied. Most of the radiocesium enters the larvae via the anal papillae. Potassium in the medium decreases the amount of radiocesium accumulated but not the rate of uptake. Carbon particles are used to show that degilled larvae increase their gut uptake. Elimination is not affected by either removal of the anal gills or the presence of potassium.

A study of cesium-137 accumulation and elimination by an aquatic Hemipteran, Notonecta undulata, the common backswimmer, shows a decrease in radiocesium uptake by the nymphs prior to molting. Adult backswimmers have a single

accumulation rate and a two phase elimination. The food consumption of adult backswimmers is calculated.

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# CHAPTER 1

# GENERAL INTRODUCTION

## Contamination of the Aquatic Environment

It has been estimated that by the year 2000 more than 115 GW of electricity will be generated in Canada by nuclear power stations (Crawford and Häussermann, 1975). This increase in nuclear generating capacity together with the use of radioisotopes in industry and medicine, has raised public concern about the impact on the environment of the radioactive waste products of the nuclear industry (Citizen Action Group, 1974). Fallout from nuclear weapons tests conducted in the atmosphere and articles which have appeared in the popular press describing the difficulties associated with the handling of radioactive wastes stress the need for accurate assessment of the capacity of the environment to assimilate radioactive materials without incurring an unacceptable risk of biological damage caused by ionizing radiation.

The biosphere has been exposed to radiation since the beginning of geological time. The various life-forms inhabiting the planet have evolved in a natural radiation background, exposed to an annual dose which varies from 90 mrad to 1500 mrad depending on the geographical location (UNSCEAR, 1972). This background radiation results from naturally occurring radioactive minerals in the rocks and soil of the planet, and from cosmic rays. To this natural

background there has now been added a man-made component: fallout from nuclear weapons tests, tailings from uranium mining and ore processing operations, and radioactive wastes from nuclear power stations and fuel reprocessing plants. The man-made radiation component, however, amounts to less than 3% of the natural background radiation (UNSCEAR, 1972). If the Moscow agreement which bans the testing of nuclear weapons in the atmosphere and in water continues, the major source of man-made radionuclides entering the environment, will be the nuclear fuel cycle.

Canadian nuclear power stations use a natural uranium fueled, heavy water moderated reactor, the CANDU reactor (AECL, 1976). In an accident situation it is the aquatic component of the environment that will be exposed to the greatest potential impact from nuclear power generation in Canada.

## The Aquatic Insects

The most abundant and diverse group of animals on the Earth are to be found in the class Insecta, many of which spend their juvenile stages in an aquatic habitat and the adult stage on land. Hence, because of the large transfer of biomass to the terrestrial environment which occurs when aquatic forms emerge as adults, there exists the potential for translocation of radionuclides from water to land (Peredel'skii and Bogatrov, 1959). The magnitude of such transfer will depend on environmental

temperature and the extent to which aquatic insects, and other forms, ingest, assimilate and egest radioactive wastes released to the aquatic environment. The aquatic dipterans together with their hemipteran predators constitute a large fraction of the aquatic invertebrates.

Cesium-137

The fission reaction which occurs in the core of a nuclear reactor generates more than 200 radioactive nuclides, the fission products (Katcoff, 1960). However, the majority of these fission products have half-lives of only a few hours, days, or months. It is the few fission products that have half-lives measured in years, and are isotopes of elements which may take part in metabolic processes, such as iodine, or chemically similar to them, such as strontium is to calcium, that are the major concern. These are the biologically important fission products. Such a fission product is cesium-137. It has a high fission yield (6%) and a 30 year half-life (Katcoff, 1960). Radiocesium also mimics the metabolically important element potassium in many physiological processes (Davis, 1963), therefore, it is distributed throughout the animal body following ingestion and assimilation. The amount of radiocesium ingested and the residence time or biological half-life in the animal, determine the radiation dose received and subsequent biological damage (Guthrie, 1962; Davis and Foster, 1958).

The use of radiocesium and other radionuclides to study biogeochemical cycling and trophic level kinetics (Pendleton and Ukler, 1960; Schultz and Klement, 1963; Reichle, 1967; Odum, 1965) originated with the concern about their translocation through food-chains leading to man (Odum, 1965; Cushing, 1970). The significance of the insect component in the cycling of radionuclides in aquatic ecosystems has been discussed by Polikarpov, 1967; Guthrie, 1969; and Reichle et al., 1970.

Radiocesium is translocated in ecosystems, thus the ecologist is provided in certain circumstances with a unique tool with which to study animal dispersion, foodweb relationships and productivity. Its biological half-life depends on the species and its temperature (Davis, 1963). Early application of cesium-137 was as a label for measuring feeding and food assimilation rates. Crossley (1966) estimated the daily rate of plant material consumed by insects living on the plants growing on a former lake bed contaminated with cesium-137 and other fission products. Guthrie and Brust (1969) used the nuclide to measure the assimilation rate of an aquatic hemipteran equiped with piercing-sucking mouthparts, Lethocerus americanus.

Guthrie and Brust (1969) stressed that the application of the radionuclide label to the estimation of the feeding rates of insects rested on the assumption that the rate of label uptake or elimination, required to calculate biological half-life, was reasonably constant

within a insect family (taxonimic family unit). One objective of the work to be reported in this thesis was to test the validity of this assumption. The second objective was to estimate the relative importance of the gut and anal papillae as routes for the uptake of radiocesium by mosquito larvae. The third objective was to apply steady-state conditions to the calculation of food assimilation by another species of aquatic hemipteran, in order to test if the value reported by Guthrie and Brust (1969) applied to more than one insect family.

# CHAPTER 2

# MATERIALS AND METHODS

## Experimental Organisms

### Culicidae (Diptera):

Five aedine species of mosquitoes, Aedes aegypti (L), Aedes atropalpus (Coquillett), Aedes dorsalis (Meiger), Aedes vexans (Meiger), and Aedes triseriatus (Say), were chosen to study the uptake and elimination of cesium-137. These were chosen for several reasons. First, mosquitoes are common around the world (Stone, Knight, & Stark, 1959). Second, aquatic dipterans make up some of the most important pest species in North America. The genus Aedes is one of the most important. Third, it has been suggested that aquatic insects could contaminate the surrounding environment with radionuclides when they emerge from habitats containing these materials (Peredel'skii and Bogatyrev, 1959).

### Notonectidae (Hemiptera):

Notonecta undulata, Say, the common backswimmer, was chosen as the species of aquatic hemipteran to be studied for two reasons. First, they are known to be a predator of mosquito larvae (Ellis and Borden, 1970), and are therefore at a higher trophic level than mosquito larvae. Secondly, they have piercing-sucking mouthparts. Only one previous attempt has been made to measure food consumption in a sucking insect by the radiocesium tracer method (Guthrie and Brust, 1969).

General Rearing Methods

The eggs of five aedine species of mosquitoes were supplied by Dr. R. A. Brust, Department of Entomology, University of Manitoba and the rearing methods employed were essentially those described by Brust (1968). The eggs were hatched in a solution of 100 milligrams of nutrient broth in 100 ml of distilled water for two hours. Groups of 100 first instar larvae were transferred to plastic rearing pans with lids (30 x 19 x 10 cm). The rearing medium consisted of 100 ml of distilled water at a temperature of 26°C. The larvae were fed a measured amount of Tetra Min Tube Food 66*. New food was added and the medium changed daily. Pupae were removed and placed in a container of distilled water inside an emergence cage. The relative humidity of 75 - 95% was maintained within the cage with a roll of moistened paper toweling.

The adults were offered honey solution ad libitum. Guinea pigs and/or mice were used as a means of blood feeding the female mosquitoes. Lighting conditions 16L:8D, were provided to stimulate mating. Moist filter paper in a funnel provided the surface for egg laying. The eggs were stored at 10°C for 7 days before hatching.

Rearing of N. undulata

Adult backswimmers were collected in the spring

*Tetra Kraft Werke Dr. rev. nat Baensch Melle, Western Germany.

soon after the ponds were free of ice. After identification, they were reared in the laboratory using the method described by Ellis and Borden (1969). The backswimmers were placed in a large aquarium with pond water and were fed tadpoles and mosquito larvae. A wire mesh in the water served as a site for egg attachment. After the eggs hatched, the nymphs were removed and placed in separate 100 ml beakers. Thus the feeding and growth could be observed individually. Each nymph was fed 10 larvae daily.

## Cesium-137 Accumulation and Elimination

### Mosquito larvae

The same rearing procedure as already described was used except that the rearing medium contained carrier free $^{137}CsCl$ resulting in an activity of 10,000 $\pm$ 5% counts per minute (cpm)/ml of medium. The radioactivities of the rearing media will be included in the data for individual experiments.

To study cesium-137 elimination, larvae that had attained a cesium-137 body-burden were transferred to non-radioactive medium and food. The larvae were sampled at regular intervals to determine the decrease in cesium-137 activity.

### Mosquito Pupae

To measure the uptake of cesium-137 by pupae, late 4th instar non-radioactive larvae were placed in distilled water to pupate and minimize cross contamination. The

newly moulted pupae were placed in cesium-137 solution of approximately 1000 cpm/ml. Pupae of both sexes were sampled, washed, and total-beta counted (beta counted) every 24 hours. To reduce the likelihood that the radioactivity measured was due to surface contamination, the pupae sampled were washed and placed in a container of distilled water to allow the adults to emerge. These newly emerged adults as well as the pupal exuviae were beta counted.

Elimination was measured by transferring late 4th instar larvae reared in radiocesium to a container of distilled water where they were kept until pupation was completed. Again pupae were sampled, washed, and beta counted at 24 hour intervals. Samples of the emergence water were also beta counted.

Notonecta undulata (backswimmers)

Soon after the eggs hatched, individual backswimmer nymphs were transferred to 100 ml beakers. The accumulation of radiocesium was determined by feeding each backswimmer nymph ten cesium-137 labelled mosquito larvae. After feeding, the remains of the mosquito larvae, consisting mainly of the cuticle, were beta counted, and the amount of food consumed estimated. The radioactivity of each individual backswimmer was measured daily by gamma spectrometry prior to feeding.

The elimination rate was measured after the backswimmers had achieved a body-burden of $^{137}Cs$, by feeding

non-labeled mosquito larvae. The backswimmers were fed once a day, immediately after measuring their radiocesium content.

The same procedures were used for the adults.

Measurement of Cesium-137

Cesium-137 emits 0.52 MeV beta particles, and its metastable barium daughter product emits a 0.662 MeV gamma photon as the result of internal transition to ground state (Heath, 1964). Preliminary experiments using mosquito larvae, pupae and adults, and backswimmer nymphs and adults reared in the laboratory in local pond water, showed that the amounts of background cesium-137 were below the detection limits of the counting techniques employed. Since the change in counting rate attributable to an increase, or decrease, in cesium-137 was the parameter of interest, absolute counting was not required. Care was taken to ensure that all samples were counted under comparable geometry, hence relative counting was applicable.

The levels of radiocesium in the various samples including mosquito larvae, pupae, and adults, and the water in which they were reared, were measured by low level beta counting. Radiocesium levels in N. undulata nymphs and adults were measured by gamma spectroscopy. The principles and applications of these counting methods are described in reference texts and reports (Gatrousis and Crouthamel, 1960; Guthrie and Grummitt, 1963; Heath, 1964).

Individual mosquito larvae, pupae, and adults, were fastened at the center of stainless steel counting trays with a 1:10 collodoin-acetone mixture for beta counting. The trays were dried under heat lamps prior to counting. A Sharpe's Low Background Beta Counter, equipped with an automatic sample changer and a gas-flow end window detector, was used for all beta counting. The background of this equipment with a counting tray under the detector was 0.4 $\pm$ 0.05 (s.d.) counts per minute (cpm). The cesium-137 counting efficiency of the detector, determined by counting standard sources of comparable self-absorption and geometry was 42%.

The radiocesium level in N. undulata nymphs and adults was measured by a gamma spectrometer. The spectrometer system consisted of a 7.6 x 7.6 cm NaI (Tl) scintillation crystal coupled with a photomultiplier, housed in a Heath-type shield made of 15 cm thick steel and lined with OFC-grade copper sheeting (Heath, 1964), and a Nuclear Data 130 pulse height analyser. Individual back-swimmers were counted by placing them in a small sealed plastic vial with one drop of water. The vial containing the insect was placed on top of the scintillation crystal, and counted for 4 minutes. The net counting rate was determined by subtracting the background count rate from the gross counting rate. Background counting rate was determined by counting an empty vial containing one drop of water. As the change in count rate was the parameter of

interest, it was not necessary to determine the counting efficiency of the detector. The radiocesium activity of each backswimmer in cpm was obtained after subtracting the background and integrating the area under the 0.662 MeV photopeak.

Removal of Anal Gills (Papillae)

Several methods have been described in the literature for the removal of tha anal gills (Wigglesworth, 1938; Stobbart, 1964). However, the description of these methods is not very precise, so that some experimentation was required to determine the most effective method.

The anal gills were removed chemically with solutions of NaOH or $AgNO_3$. In this process, fourth instar larvae were exposed to different concentrations of each chemical for varying lengths of time. The NaOH and $AgNO_3$ solutions ranged from 0.1M to 0.5M and exposure times varied from 0.5 to 5.0 minutes.

After the treatments, the larvae were immediately washed with distilled water and placed in non-labeled rearing medium. The larvae were examined after 48 hours to determine which treatment most successfully removed the anal gills and caused the least mortality. Ninety per cent survival after 48 hours was considered acceptable. The larvae were examined under a dissecting microscope to determine the success of degilling. Only completely degilled larvae, those which had four healed scars in place of the gills, were used.

After two experiments with chemically degilled larvae, a surgical method was used. This method proved to be the most successful in the survival of larvae and complete removal of the anal gills. The gills were removed under a dissecting microscope using minuten nadeln. The larvae were held for 24 hours to recover from the stress of the procedure, before being placed in a cesium-137 medium. This allowed for the complete healing of the surgical wounds.

Carbon Particle Uptake

An experiment was carried out to determine if larval feeding was affected by the degilling procedure. Inert substances, such as latex and carbon particles, that are visible in the gut have been used to measure feeding rates (Dadd, 1968). In experiments reported here, carbon particles were used to compare the feeding rates of normal and degilled larvae. The system used for quantifying the amount of carbon in the gut is illustrated in Figure 4.

Statistical Analysis

All radioactivities and insect weights are reported as means $\pm$ one standard deviation (s.d.). The number of observations (n) in each mean ($\bar{x}$) is also shown. In the graphs the logarithm of $^{137}Cs$ activity (dependent variable) is linearly regressed on time (the independent variable) and a line is fitted to the points by the least-squares method. Pertinent statistics of these regressions

are given in the appropriate Appendix. Students 't' test was used to determine the significance of any differences between regression coefficients or means, $p = 0.05$.

# CHAPTER 3

# ACCUMULATION AND ELIMINATION OF CESIUM-137 BY MOSQUITOES

## Introduction

Extensive use of radioisotopes has been made in entomology. Crossley (1966) and Reichle (1967) used radioisotope uptake and elimination rates to estimate material and energy flow in terrestrial food chains. Pendleton (1957, 1962) has included insects in his studies of cesium-137 accumulation by various components of aquatic communities. Crossley and Schnell (1961) measured the elimination of cesium-137 and strontium-90 by grasshoppers. Getsova and Volkova (1962) studied uptake of radioisotopes by insects reared in contaminated ponds.

Radioisotope studies with mosquitoes have also been quite extensive. Several radionuclides have been used to 'tag' mosquitoes in order to study their dispersal patterns (O'Brien and Wolfe, 1963). Several authors (Bruce-Chwatt and Hayward, 1956; Hassett and Jenkins, 1951; Quraishi, 1968) have studied the uptake of phosphorus-32 by mosquitoes, and the accumulation and elimination of cesium-137 by A. aegypti has been reported by Guthrie (1969) and by Guthrie and Burzynski (1972).

## Results and Discussion

### Accumulation of Cesium-137 by Mosquito Larvae

The accumulation of radiocesium by A. aegypti larvae was performed for comparison with the other aedine species, as well as to compare the results of this radiolabeling technique with that of Guthrie (1969). Groups of one hundred newly hatched larvae were reared in medium containing 10,000 cpm/ml of carrier free cesium-137 at 26°C until pupation. Samples of ten larvae of each species were taken at least twice in each instar and beta counted. The mean radioactivity of larval instars 1 to 3 increased uniformly (Phase 1), then leveled off during the fourth instar (Phase 2). The results are given in Appendix A, Tables A-1 to A-5. Figure 1, a plot of the logarithm of cesium-137 activity versus time in hours, summarizes these two phases in the uptake by the five species. The best fit line for each phase was obtained by the least-squares method. The rate at which the larvae doubled their body-burden of cesium-137, $T_d$ (doubling time), was different for the two phases. The slope of the least-squares fitted line is a measure of the rate of cesium-137 uptake. The doubling times of the two phases for each species are shown in Table 1.

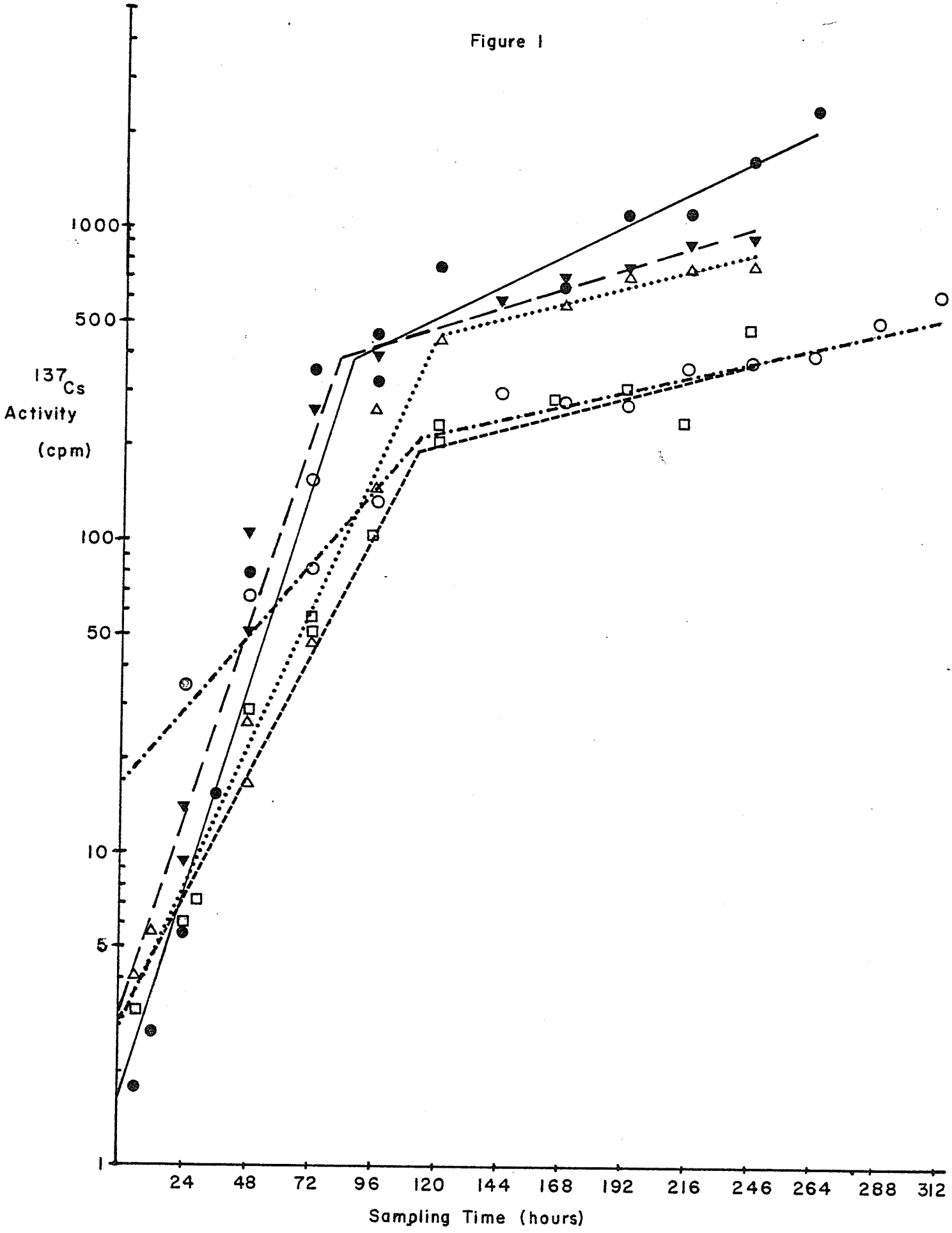
Figure 1

Accumulation of $^{137}Cs$ by five aedine species of mosquito larvae reared at 26°C and sampled at successive periods. The regression lines of activity of the larvae for the two uptake phases are shown.

●- A. aegypti ▼- A. atropalpus □- A. dorsalis

○- A. triseriatus △- A. vexans

Figure 1


1000
500
137Cs
Activity
(cpm)
100
50
10
5
1
24
48
72
96
120
144
168
192
216
246
264
288
312
Sampling Time (hours)