THE EFFECTS OF CADMIUM COMPOUNDS ON REPRODUCTION AND GROWTH OF THE STABLE FLY, STOMOXYS CALCITRANS (LINNAEUS)

By

SIDNEY EDMUND KUNZ

Bachelor of Science
Texas A&M University
College Station, Texas
1958

Master of Science
Texas A&M University
College Station, Texas
1962

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of the Oklahoma State University
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for the degree of
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THE EFFECTS OF CADMIUM COMPOUNDS ON REPRODUCTION AND GROWTH OF THE STABLE FLY, STOMOXYS CALCITRANS (LINNAEUS)

D E Howell
Thesis Adviser

R R Walton

E D Bach

Robert D Morrison

N N Durham
Dean of the Graduate College

PREFACE

Entomologists must strive continually for new developments in order to combat the nemesis of insects affecting man and his crops. With this in mind, the author decided to investigate the effects of selected cadmium compounds on the growth and reproduction of the stable fly, Stomoxys calcitrans (Linnaeus). It was hoped that the results obtained might add to new control methods and that the experience gained would prepare the author for a career as a research entomologist.

The author wishes to express his appreciation to Dr. D. E. Howell for his guidance throughout this research program and in the preparation of this paper. Appreciation is also extended to Dr. R. R. Walton, Professor of Entomology; Dr. E. D. Besch, Professor of Veterinary Parasitology and Public Health; and Dr. R. D. Morrison, Professor of Mathematics, for their suggestions and criticisms in the preparation of this thesis.

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INTRODUCTION

The use of chemicals for insect control has received considerable criticism from many quarters during the past few years. Much of this criticism has been without basis or has been over emphasized. The net gain from the use of insecticides far outweighs the expressed criticism. Nevertheless, the entomologist must strive always for better and safer methods of insect control. A relatively new method for controlling insects is through the use of sterile insects to compete with normal individuals for mates. The success of this procedure has been evident in large areas of the southeastern and southwestern United States. The virtual elimination of the screw-worm, Cochliomyia hominivorax (Coquerel), has greatly affected livestock production and wildlife in these areas.

The use of chemicals has been investigated widely in a search for methods of inducing insect sterility. Numerous chemicals have been found to be effective against a variety of insect pests. A group of triphenyl tin compounds has been found to be effective in reducing the reproduction potential of several insects. Cadmium compounds have been known to cause sterility in a number of mammals. It was decided to investigate the potential of selected cadmium compounds against the stable fly, Stomoxys calcitrans (Linnaeus), as reproduction inhibitors. The results of these investigations are discussed in this thesis.

REVIEW OF LITERATURE

Intensive investigations have been conducted in the field of induced insect sterility during the past decade. Since 1960, research to determine the potential of chemosterilants as practical insect control agents has been reported in a large number of research publications. This interest was stimulated by the success of the screw-worm eradication program and by the demonstration that male and female house flies and other insects could be sterilized chemically.

In 1916 Runner observed that the cigarette beetle, Lasioderma serricorne (Fabricus), produced non-fertile eggs after exposure to roentgen rays. Müller (1927) noted that irradiation would induce mutations in Drosophila.

The possibility of introducing sterile insects into a natural population was first conceived by Knipling as early as 1938 (Bushland and Hopkins 1951). Through his encouragement, Bushland and Hopkins first succeeded in sterilizing the screw-worm, Cochliomyia hominivorax (Coquerel) by the use of x-rays.

The use of x-rays and gamma radiation sources proved to be successful in rendering the screw-worm male infertile. Baumhover et al. (1955) completely eliminated the natural population of screw-worms from the island of Curacao in the Caribbean in the first large scale field trials utilizing the sterile male technique. With this successful demonstration, the sterile male technique was put to test on a larger scale in the southeastern United States in 1958. By February 1959, this area

was declared free of the screw-worm (Smith et al. 1964).

With visions of greater success, scientists set out to eliminate the screw-worm from the southwestern areas of the United States. This eradication program was initiated in 1962 and by 1964 the area including Louisiana, Texas, Oklahoma and New Mexico was officially declared screw-worm free. Minor outbreaks have occurred since but certainly the devastation of screw-worm infestations has been greatly reduced compared to conditions existing before the start of the program.

The success of the eradication program caused intensified interest in the possible use of chemicals as chemosterilants in adaptations or extensions of the sterility approach to insect control as reported by Lindquist (1961). Knipling (1955) discussed the possibilities of insect control through the use of sterile males and in 1959 suggested theoretical advantages from the use of chemicals to induce sterility in a natural population. Sterilized insects cannot reproduce, thus, such effect is equivalent to killing the insects. Knipling (1962) also stated that the sterile insects compete with the normal insects to further decrease the chances for reproduction. Knipling (1960) noted that an insecticide that kills 90% of an insect population will reduce its reproduction potential to 10%, whereas a chemosterilant affecting 90% of the population will reduce this potential to one per cent.

Borkovec (1962) noted that, although the use of irradiation sources to sterilize insects has been proven as a successful technique, it also has some obvious limitations. It requires mass release of the sterilized males and this may often be undesirable and unfeasible. It requires a rather expensive, uniquely designed plant with specialized equipment to rear, irradiate and package the insects. Adequate trans-

portation systems are required to disperse the packaged sterilized insects often at distances far removed from the plant.

Weidhaas (1962) states that if sterilizing chemicals could be safely applied to natural populations, control or eradication could be realized by inducing sterility without the necessity of rearing and releasing large numbers of insects. Most of the more recent research effort has been directed towards developing chemically induced sterility.

Borkovec (1962) defines an insect chemosterilant as a chemical compound which, when administered to the insect, will deprive it of its ability to reproduce. Once a chemical is applied to an insect, the factors which should determine its effectiveness are (1) the rate of absorption, (2) rate of excretion, (3) rate of breakdown or detoxification, and (4) its accumulation and action at the effective site (Chamberlain and Hamilton 1964).

Many of the more basic questions about a given insect must be answered before the full potential of chemosterilants as practical control measures can be accurately assessed (Murvosh et al. 1964). Not only must the concentrations of chemical required to induce sterility be known but also what other physiological effects these chemicals have on the organism. Chemicals which produce marked deleterious side effects may prove relatively useless in the field. Knipling (1962) states that one of the basic requirements is that the sterilized males must be competitive for copulation with the wild males for the wild females.

LaBrecque et al. (1960) found 79 compounds of 200 screened showed deleterious effects when added to the larval medium of the house fly,

Musca domestica Linnaeus, but only ten affected the development of treated adults. LaBrecque (1961) showed that sterility similar to that caused by radiation was induced in both male and female house flies by three aziridinyl derivatives. Apholate was shown to cause permanent sterility when adult house flies were treated. These treatments did not affect the competitiveness of the males with normal males for females (LaBrecque et al. 1962a). Kilgore and Painter (1964) determined that apholate given to adult house flies resulted in the inhibition of DNA synthesis in eggs deposited by the treated insects.

Gouck (1964) demonstrated that apholate was effective against the house fly when pupae were dipped when one and two days of age. Adult development was prevented when pupae less than two hours old were treated with tepa or metepa. In field experiments, Gouck et al. (1963) reduced house fly egg hatch when a corn meal bait containing 0.75% apholate was supplied to the adults. Continuous treatments were more effective than periodic applications. LaBrecque et al. (1962b) eliminated house flies from an area in Florida field trials using aphoxide corn meal baits. Fye et al. (1965) screened 173 compounds against house flies and found 27 to be effective as chemosterilants.

Chang et al. (1964) found that hexamethylphosphoramide (HMPA) and hexamethylmelamine (HMM) were effective against male house flies as chemosterilants. Both compounds are structurally similar to tepa and tretamine but they differ in their low mammalian toxicity and their lack of alkylating properties. Chang and Borkovec (1964) demonstrated that at the ED_{50} (effective dosage) level, tepa was 4 times as effective as apholate and 12.5 times as effective as metepa when injected into male house flies.

Morgan and LaBrecque (1962) found that apholate administered to house flies inhibited but did not prevent ovarian development. Morgan and LaBrecque (1964) observed that the developed eggs of tepa treated ovaries of house flies began degeneration at 120 hours post treatment. The developed eggs of metepa treated flies did not show any signs of degeneration.

LaBrecque and Gouck (1963) tested P,P-bis(1-aziridinyl)-N-(p-methoxyphenyl)phosphinic amide, 5-fluoroörotic acid, and 1,4-piperazinediylbis[bis(1-aziridinyl)phosphine oxide] as sterilants against the house fly. These compounds induced sterility without apparent mortality in dosages ranging from 0.1% to 5.0%. Kilgore and Painter (1962) reduced the viability of house fly eggs when they fed a diet containing 5-fluorouracil 2-C^{14}.

Chamberlain and Hamilton (1964) found in comparative studies that the stable fly Stomoxys calcitrans (Linnaeus) absorbed a greater proportion of the applied dose of metepa than the screw-worm. Excretion by the screw-worm fly was twice as fast as that of the stable fly. Sterilization of the screw-worm fly required 3-18 times more metepa per gram of fly than that needed for the stable fly. Similar differences were noted for apholate (Chamberlain 1962, Harris 1962).

Chamberlain and Barrett (1964) used metepa to induce sterility in the screw-worm and stable fly. In comparative studies, the male screw-worm required 5.5 times as much toxicant per gram body weight as the stable fly. The female screw-worm required 18 times as much as the female stable fly.

Harris (1962) observed that the minimum effective dosage of apholate against stable flies, when both sexes were treated, was about 1.0

mg per fly. When only one sex was treated, complete sterility was not obtained at a 7-fold treatment. This investigator's data indicated that the stable fly males were more sensitive than the females to apholate. Aphoxide and methaphoxide were also effective. The principle effect of these compounds was the failure of the eggs to hatch. Egg production was not reduced except at high dosages.

Chamberlain (1962), using apholate, observed almost complete sexual sterility when the screw-worm was treated either in the larval, prepupal or adult stage. This treatment was generally more effective against the female than the male. Larval treatment caused a marked change in the shape and form of the resulting pupae and adults.

Crystal (1963) tested 29 compounds as sexual sterilants against the screw-worm. Twelve compounds were effective as topical applications, 26 as oral treatments. Some compounds only sterilized one sex while others were effective against both sexes. In these tests, males were more readily sterilized than females.

Crystal (1966) found that the screw-worm males were about 6.5 times more susceptible to a chemosterilant than females when treated with uredepa. Crystal and LaChance (1963) showed that five aziridinyl compounds were effective against screw-worms. Treatment with these compounds caused the induction of dominant lethal mutations which greatly reduced or eliminated the fertility of the test organism when treated at 24 hours of age. These aziridinyl compounds caused inhibition of oögenesis when treated flies were 0-4 hours of age.

Crystal (1964) demonstrated that the oral administration of 0.1% uredepa together with a folic acid antagonist was as effective as 1.0% uredepa alone in destroying the screw-worm female fertility. Uredepa

offered to both sexes orally in a single dose or in 5 equally divided daily fractions caused incomplete and complete sterility, respectively.

Hair and Adkins (1964) successfully sterilized the face fly, Musca autumnalis DeGeer, with apholate and tepa by dipping 3 day old pupae in a 4% solution for 25 minutes. In diet treatments using apholate, Hair and Turner (1966) determined that mature face fly females displayed greater susceptibility than did immature females. This was explained by the fact that the older flies consumed twice as much food as the younger flies. When topical applications were employed, very little difference was indicated in the susceptibility of the two age groups.

Harris and Frazar (1966) induced sterility in adult male and female horn flies, Haematobia irritans (Linnaeus), using apholate, tepa, and an aziridinyl compound. Mating tests indicated that male horn flies sterilized with tepa were not as competitive as untreated males.

Dame et al. (1964) noted that apholate and tepa were effective sexual sterilants of Aedes aegypti (Linnaeus) larvae in laboratory tests but tepa failed in field trials after three days. Dame and Ford (1964) observed that A. aegypti males, when treated with apholate as larvae, were less competitive than when treated as adults. Results of these investigations also showed that tepa used either in the adult or larval stages appeared to induce a high degree of permanent sterility whereas apholate failed to do so in larval treatments.

These investigators were able to sterilize inseminated gravid females at less than one tenth of the normal dosage required. They stated that it appeared possible under these conditions that the sterilant apparently acted primarily on the sperm stored in the female spermathecae.

Dame and Schmidt (1964) found that metepa caused a severe reduction in the mating ability of Anopheles quadrimaculatus Say. Weidhaas and Schmidt (1963) noted that the use of chemosterilants offer an effective method of sterilizing males of A. aegypti without damaging their mating ability. A dosage of radiation sufficient to sterilize these males curtailed their competitiveness with normal males.

Weidhaas (1962) found that both sexes of A. quadrimaculatus were completely sterilized by contact with residual deposits of tepa. Rai (1964) treated A. aegypti with apholate and showed that greatly inhibited ovarian development resulted.

Schmidt et al. (1964) found that although the mating vigor of irradiated males of A. quadrimaculatus was severely reduced, the mating vigor of chemosterilized males appeared unaffected. The house fly was reported by LaBrecque et al. (1962a) to have shown an apparent increase in mating vigor following the chemosterilant treatment.

Mulla (1964) showed that the larval treatment of Culex pipiens quinquefasciatus Say with 0.001% apholate produced marked decreases in oviposition and also considerable sterility in adults. Higher concentrations produced almost total mortality in the subsequent metamorphic stages.

Altman (1963) demonstrated that in addition to the chemosterilant action of tepa on A. aegypti, it also affected the Plasmodium gallinaceum (Brumpt) organism. This treatment curtailed the development of P. gallinaceum and its transmission by the vector.

Smittle et al. (1966) treated German cockroaches, Blattella germanica (Linnaeus) with tepa. This treatment stopped spermatogenesis and caused the testes to atrophy. Tepa also caused abnormal oocyte

development and partial deterioration of the ovaries.

Lindquist et al. (1964) found that the boll weevil, Anthonomus grandis Boheman, could be sterilized with apholate using various treatment procedures. Data showed little, if any, differences in competitiveness of the treated males for females. Davich et al. (1965) found apholate to be less detrimental to the vigor of the sterilized boll weevil than radiation. But these investigators' data also showed that there was a marked reduction in the competitiveness of apholate-treated males when compared with untreated males. Studies completed in Louisiana demonstrated that the boll weevil could be successfully eradicated even when the sterile males used were low in mating vigor. Hedin et al. (1964) reduced the reproductive potential of the boll weevil by using apholate in artificial diet and with foliar treatments.

Shaw and Riviello (1965) demonstrated that the Mexican fruit fly, Anastrepha ludens (Loew), was effectively sterilized by dipping the pupae in a 5.0% aqueous solution of tepa for one minute. Actual sterilization occurred when the flies emerged on the puparium harboring the tepa residue. These investigators were able to obtain a high degree of control when sufficient numbers of the sterilized flies were released in mango groves.

Simkover (1964) noted the 2-Imidazolidione treated larval medium allowed normal development of Drosophila melanogaster Meigen but adults failed to emerge from the pupae. This chemical affected the growth and development of the immature stages of several other pests.

Ladd (1966) observed that topical applications of tepa, apholate and metepa appeared to effectively sterilize Japanese beetle, Popillia japonica Newman, adults. Four species of Diptera showed varying degrees

of reduced fecundity when sublethal doses of arsenic were included in their food (Pickett and Patterson 1963). A single application of lead arsenate resulted in a 98% reduction of pupae of Rhagoletis pomonella in field trials.

Henneberry et al. (1964) were able to completely sterilize Mexican bean beetle, Epilachna varivestis Mulsant, adults using 0.5% apholate. Hays and Cochran (1964) produced sterility in the plum curculio, Conotrachelus nenuphar (Herbst), by using aphamide but the treatment resulted in high mortality.

Harries (1961) demonstrated egg laying inhibition against the two-spotted spider mite, Tetranychus telarius (Linnaeus), by using certain antibiotics. Harries and Wiles (1966) were able to significantly reduce reproduction in the green peach aphid, Myzus persicae (Sulzer), by using certain antibiotics. Apholate and tepa inhibited reproduction in the pea aphid, Acyrthosiphon pisum (Harris) in studies conducted by Bhalla and Robinson (1966).

Howland et al. (1965) successfully sterilized both sexes of the cabbage looper, Trichoplusia ni (Hubner), when moths were fed apholate and tepa. Male moths were sterilized by lower dosages than females. Graham and Drummond (1964) screened 100 chemicals as chemosterilants against Boophilus annulatus microplus (Canestrini). Lindane, Telodrin, and 2-(diethoxyphosphinothioyloxy)-ethyl dimethylcarbamate were the most effective.

The EDTA (ethylenediamine tetraacetic acid) chelates of magnesium and iron were the most active in reducing the fecundity of the two-spotted spider mite, Tetranychus telaris, in investigations by Terriere and Rajadhycksha (1964). Cressman (1963) found several compounds to be

effective as sterilants against the citrus red mite, Panonychus citri (McGregor). Untreated females of the two-spotted spider mite mated with apholate treated males produced all male progeny. Females exposed to 2% apholate dip produced no viable eggs (Smith et al. 1965).

Swailes (1966) sterilized adults of the cabbage maggot, Hylemya brassicae (Bouche), by using apholate in a 10% sugar solution.

Observations by Feldmesser et al. (1962) indicated that the reproductive systems of the nematodes, Panagrellus sp. and Rhabdites sp., were affected by chemosterilants.

As with all chemical treatments, the natural selection pressures are making themselves known among chemosterilant investigations. Hazard et al. (1964) reported the first resistance of a test organism to a chemosterilant. Increased resistance to the sterilizing effects of apholate was observed in two laboratory colonies of Aedes aegypti.

Kenaga (1965) showed that triphenyl tin compounds were effective as reproduction inhibitors against certain test species. Several compounds were highly effective against the house fly and to a lesser extent against the confused flour beetle and German roach. Some of these tin compounds had a much wider margin of safety than the conventional aziridine compounds.

Parizek and Zahor (1956) observed that cadmium chloride administered subcutaneously to rats produced macroscopic lesions of the testes. At 2-4 hours post treatment, capillary stasis and edema of the interstitium could be observed microscopically. At 24-48 hours after injection, all testicular tissue was severely damaged. These investigators noted that no morphological lesions in the ovaries of female rats were observed.

Kar (1961) suggested that the male Rhesus monkey could be sterilized by a single intratesticular injection of cadmium chloride. The nature and sequence of changes evoked in the testes were comparable to those observed in rats. The net result of cadmium chloride was the complete destruction of the seminiferous epithelium. An eventual regeneration of the interstitium occurred after the initial phase of atrophy.

Meek (1959) treated young adult mice with a single dose of cadmium chloride producing extensive testicular damage. Total destruction of all tubular cells was noted within 96 hours. Macroscopic examinations of other organs at autopsy revealed no gross changes, only small lesions were found in histological sections of liver in limited cases.

Kar and Das (1962) found that the administration of cadmium chloride directly into the rat testes caused acute and irreversible destruction of the germinal epithelium. The minimum effective dose of the salt by this route was noted to be about 1/16th of the minimum dosage needed to evoke these changes when administered subcutaneously.

These authors also noted degenerative changes similar to those in rats in the testes of rabbits, goats and Rhesus monkeys following direct injection. The testes of rabbits appeared to be resistant to the effects of cadmium chloride when it was administered subcutaneously.

Gunn et al. (1961) postulated that testicular damage resulted from competition by cadmium for sites normally occupied by zinc. They noted that cadmium interferes with the amount of zinc taken up and that large doses of zinc salts can prevent, at least temporarily, the acute destructive effects of cadmium on the testes. Gunn et al. (1961) also stated that although cadmium produces permanent sterility in the male

rat that it does not alter the reproductive capacities of the female.

Parizek (1957) states that according to the most widely accepted theory, the toxic effect of cadmium is due to blocking of sulphydryl groups. It is interesting, however, that such a strong sulphydryl inhibitor as mercuric chloride does not affect the testes (Parizek and Zahor 1956). Parizek also states that cadmium probably affects spermatogenesis and that this effect may be counteracted with large amounts of zinc salts. This suggests competitive inhibition between these two elements. Kar et al. (1960) noted that the degenerative changes induced by cadmium in rat gonads can be prevented by zinc and selenium.

Kar and Pandoj (1963) were able to cause sterilization of male rats by scrotal inunction of cadmium chloride. Irreversible destruction of the seminiferous epithelium resulted from scrotal inunction using 20% or 40% cadmium chloride in aqueous or aqueous-glycerine medium.

Guthrie (1954) found cadmium anthranilate to be an effective ascaricide against Ascaris lumbricoides. Cadmium was retained in minute quantities in the kidneys, liver, lungs and spleen of the treated hogs.

Abdel-Razig (1966) observed that certain cadmium compounds delayed, reduced or prevented oviposition in house flies. The life span of treated flies was considerably shorter than untreated controls. Cadmium 2-hydroxyethyl mercaptide prevented oothecal production by American roaches but caused high mortality at effective levels.

METHODS AND MATERIALS

The Kerrville strain of stable flies, Stomoxys calcitrans, was used as the test organism throughout this investigation. Pupae of this strain were obtained from the United States Department of Agriculture laboratory at Kerrville, Texas where this strain has been reared continuously for the past twelve years.

Five cadmium compounds were selected as the chemicals to be used in this investigation. Three of the compounds, cadmium chloride, cadmium iodide, and cadmium acetate were readily water soluble. Cadmium succinate and 2-hydroxyethyl cadmium mercaptide, organic compounds, were slightly soluble in water. Cadminate, a commercially available fungicide, was used as the source of cadmium succinate. This compound was a wettable powder containing 60% cadmium succinate as its active ingredient. The 2-hydroxyethyl cadmium mercaptide was supplied by the Phillips Petroleum Company. The other three compounds were obtained as reagent grade materials from local sources.

Rearing of Stable Flies

Adult stable flies were maintained in screen wire cages. Cages consisted of a flat bottom made from 1"x6"x18" lumber. The back of the cage was made of 1 inch lumber approximately 12 inches in diameter. Galvanized screen wire was attached to the bottom and back wood supports making a circular enclosure (Figure 1). A muslin sleeve was attached to the front of the cage to provide an entrance. A cage of this size

was adequate to confine approximately 1500 flies during the adult stage. Flies were maintained in the laboratory at 78-84 F and 40-50% relative humidity. Overhead fluorescent lighting was controlled by a time clock programmed to provide 12 hours of light and 12 hours of darkness.

One gallon, wide-mouth jars were converted to battery jars which were used for the larval rearing. Jars were modified by cutting off the upper two inches of each jar, thus making it easier to perform the necessary manipulations (Figure 2).

The larval rearing technique was a modification of the Campau et al. (1953) procedure. The larval medium consisted of the standardized C.S.M.A.[1] medium mixed with wood shavings at a 2:1 ratio. Enough water containing 1% calcium propionate was added to the dry mix to thoroughly wet the medium, yet not enough to cause excess moisture to collect in the bottom of the jar.

Wood shavings from a planing mill and calcium propionate were added to retard mold formation in the rearing medium. Approximately 0.25 ml of eggs were added to the medium. This consisted of approximately 1500-1800 eggs.

About four days after seeding, the top layer of medium was gently stirred to prevent crusting. The larvae were left undisturbed for the remainder of the larval cycle. Pupation began on the 10th or 11th day and was usually completed by the 12th day after "seeding". Any larvae left after this time were discarded.

Full grown larvae ready to pupate congregated around the upper edges of the battery jar about one inch below the level of the medium.

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[1]Made by the Ralston Purina Company, St. Louis, Missouri.

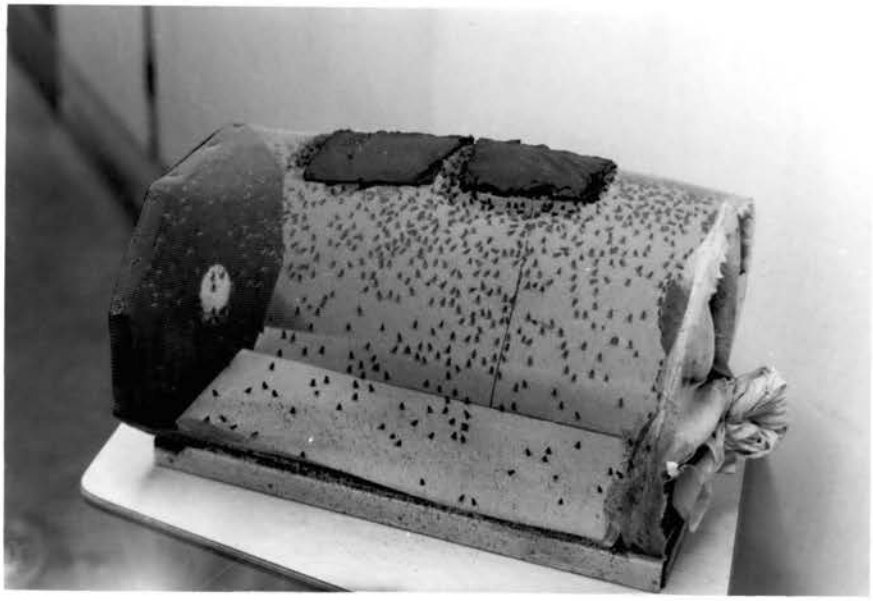

Figure 1. Colony Cage for Adult Flies

Figure 2. Battery Jar for Larval Rearing and Egging Dish

Pupation occurred at this location making it easy to "spoon" the pupae from the jar. This could be done with very little accompanying debris. Pupae that could not be removed in this manner were discarded.

Pupae were allowed to dry overnight on a screen and then were placed in 4 oz. Dixie cups. The cups were either placed in the large colony cages and held for emergence or in smaller holding cages from which they could easily be manipulated as test organisms (Figure 3).

Adults began to emerge 4 days after pupation but most emergence occurred 5-6 days later. Adult flies in colony cages were fed citrated bovine blood. The blood was allowed to absorb into 4 inch squares of sterilized absorbent cotton. Each square was separated longitudinally thus making two feeding surfaces and then placed on the cages. Flies fed readily on the blood at refrigerated temperatures. They were fed once daily throughout the adult period. Females began to oviposit as early as 5 days after emergence. Peak egg deposition did not occur until 7-9 days post emergence. Thus the total time required from egg to egg ranged from 20-25 days.

Black cotton cloth wrapped around a ball of wet cotton placed in a petri dish was used as an oviposition site. This dish was placed in the cage and allowed to remain overnight. Eggs collected were used to seed the larval medium in battery jars. Cages of flies of adequate age were egged on alternate days to maintain the necessary number of flies as colony stock and as test organisms.

Testing Procedures

Test Cages. Cylindrical cages to contain the flies during testing periods were 1.5 inches in diameter and 2.5 inches long (Figure 4.) Ends of the cages which provided the framework were made of 60 mm

Figure 3. Emergence Cage for Test Flies

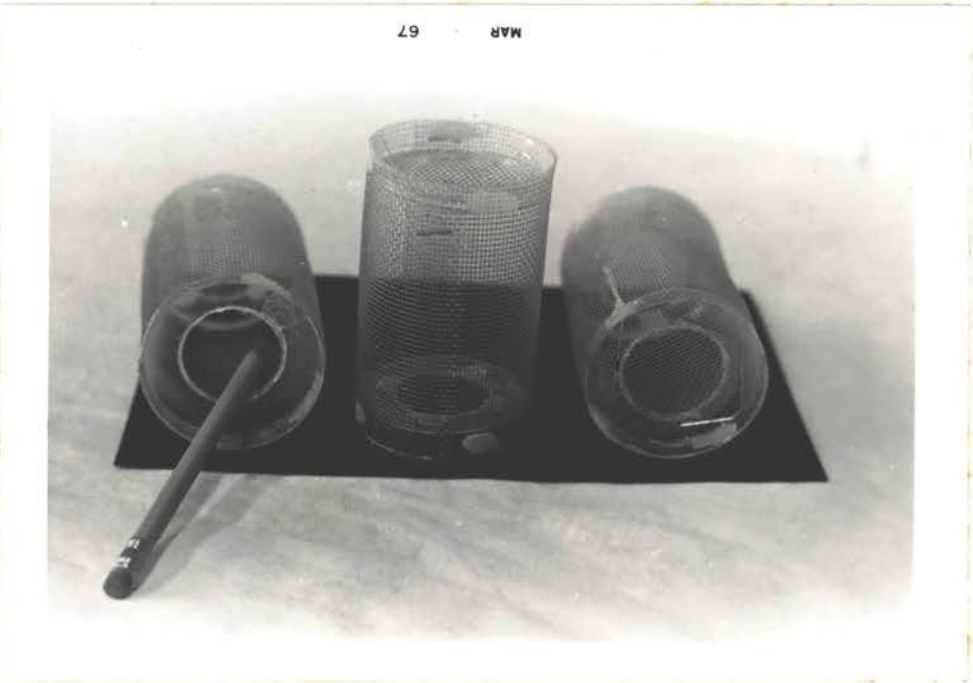

Figure 4. Lumite Test Cages Used in Investigations

plastic petri dishes. The centers of the petri dishes were removed to provide access to the cage and to minimize the area for waste material to collect within the cage. The cages were covered with Lumite[2], a saran screen material. The openings in the petri dishes were also covered with this material. One end was completely secured with a plastic cement, while the other end was glued to one side only to provide access to the cage. This end was held in place with a reshaped paper clip which provided enough spring to secure the cage door.

These cages were lightweight, easily cleaned, and rustproof. After the completion of a test, the cages were cleaned by soaking them in a detergent solution overnight. Cages were rinsed, allowed to dry, and readied for the next trial.

Multiple Feeding Test. Adult feeding tests were conducted in the Lumite cages. Flies were allowed to emerge in the small holding cages for a 12-18 hour period, then anesthetized with CO_2 and sexed. Five males and females were placed in each cage. After the flies had recovered from the anesthesia, each cage was checked for mortality which may have occurred due to the anesthesia or to the handling procedure. Any dead flies were removed and replaced.

Three to four hours after being placed in the cages, flies were treated with a candidate chemical. The chemicals were incorporated with the citrated bovine blood at the desired treatment levels. The test chemicals in the blood diet were allowed to absorb into ½ inch squares of sterilized absorbent cotton. One square was placed on each cage (Figure 5). Each treatment was replicated six times and untreated

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[2]Made by the Lumite Division, Chicopee Manufacturing Corporation of Georgia, 40 Worth Street, New York 13, New York.

controls were used for comparisons.

The blood pads were allowed to remain on the cages overnight except after the egging portion of the test started, when the diet pads were removed from the cages after 30-45 minutes. This provided adequate feeding time and prevented oviposition on the blood pads rather than the oviposition sites. Flies in the multiple treatment test were treated daily throughout the test period with various cadmium compounds.

At six days of age, the flies were placed on oviposition sites for the first time. Oviposition sites consisted of approximately 1 inch squares of sponge covered with black cotton cloth. These covered sponges were saturated with water and put in a petri dish which was placed beneath each cage of flies. The test cages were placed on their sides on a screen of ¼ inch hardware cloth over the egging dishes (Figure 6). This separated the cages from the egging surface sufficiently to allow most of the eggs to be deposited on the egging cloth rather than adhering to the cages. Flies were allowed to oviposit daily for 14-20 consecutive days during the test period. Egg counts were made daily.

Mortality counts were made daily after the feeding period and prior to placement over the egging dishes. Daily counts insured accuracy in determination of the number of eggs laid per day per female and in determining the longevity of the flies.

All dead flies were removed daily. Dead flies could easily be moved over the "door" on the cage, the clip removed, and the dead fly allowed to drop out. The engorged flies in the cage were docile enough to allow for easy removal of dead flies.

Composite samples of eggs collected from each treatment were used

Figure 5. Test Cages With Feeding Pads in Place

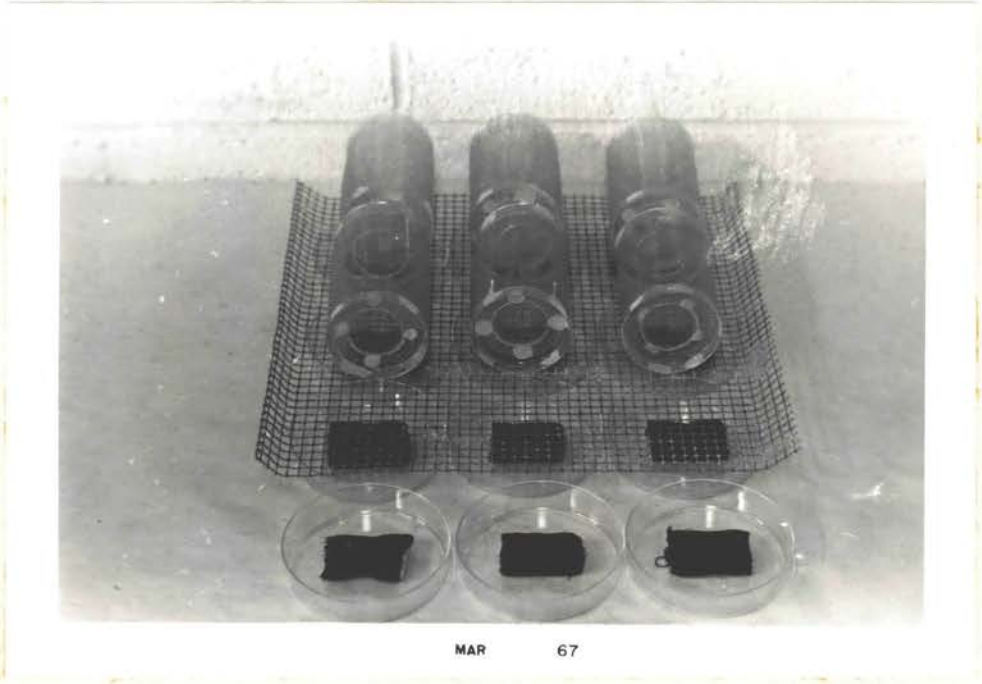

Figure 6. Cages in Place Over Egging Dishes

to seed rearing medium to determine the fertility of the eggs. A daily sample of eggs was taken and placed on medium in 15 dram plastic vials. After 4-6 days, checks were made to determine the percent hatch as compared to the hatch of eggs collected from untreated controls.

Single Feeding Test. Flies were allowed to emerge over a 6 hour period, were sexed and caged as in the multiple feeding test. The shorter emergence time was used to insure a group of flies of the same age. After caging, about 9 hours elapsed before treatment. This period allowed flies to recuperate from the anesthesia and to complete development of their mouthparts to insure that all flies would be capable of feeding.

Flies were treated before they reached 18 hours of age with various concentrations of all cadmium compounds listed. Treatments were made with all compounds during any one test period. All treatments were replicated six times and untreated controls were used for comparison. The feeding pads containing the cadmium compounds were allowed to remain on the cage overnight. After the initial treatment, all feeding was on untreated diet.

A different, less permanent type cage was used for this test. The Lumite cages were not used to avoid jeopardizing other tests that were being conducted simultaneously. Half pint ice cream cartons with both ends removed and replaced with fine meshed nylon netting, were used as cages (Figure 7). These cages were satisfactory, but were not as convenient to work with. Egging procedures were similar to those used with the Lumite cages. Each cage was placed directly over the egging dish but did not require the hardware cloth separator.

Feeding procedures, egging procedures, and mortality counts were

conducted the same as in other tests.

Larval Diet Treatments. Cadmium acetate, cadmium chloride, and cadmium succinate were incorporated in a mixture of one part C.S.M.A. medium to one part wood shavings by volume to determine their effect in larval diets. Known concentrations of each of the chemicals in 1% calcium propionate-water solution were added to known amounts of diet to determine the amount of chemical by weight in each treatment.

Fifteen dram plastic vials were used as rearing containers. The center of the plastic snap cap was removed. The top of the vial was covered with a single thickness of muslin held in place by the snap-on cap. The vial contained adequate food for the development of 10-15 stable fly larvae. The clear plastic vial allowed easy observation of the larval development without disturbing the diet.

Each vial was filled about two thirds full with the treated diet. Eggs obtained from the laboratory colony, less than 4 hours of age, were used to seed the treated diet. Twelve eggs were placed on small squares of paper toweling to insure an accurate count. Eggs were placed into each vial and additional diet was then placed on top of the eggs to prevent drying before hatching. Each treatment was replicated six times.

After the vials were seeded and the tops put in place, they were placed in an aquarium (Figure 8). Moist paper toweling, supported by a hardware cloth screen, was maintained about ½ inch above the vial tops. This prevented the diet in the containers from drying and crusting over. Development of the larvae was allowed to continue undisturbed. Pupation occurred in the vial. Pupae were not removed but allowed to emerge in the vials. As emergence occurred the flies were anesthetized,

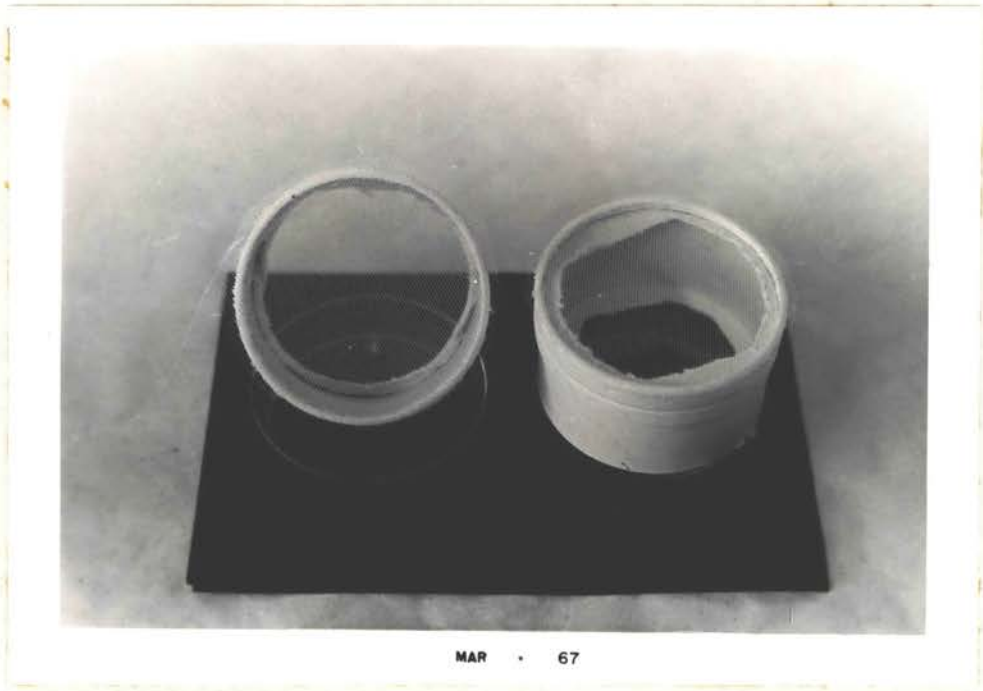

Figure 7. Cages Made From Ice Cream Cartons

Figure 8. Larval Rearing Vials Used in Investigations

sexed, and placed in the Lumite test cages. If desired, early emerging flies could be fed on cotton pads placed on the muslin tops of the plastic vials. By feeding early emerging flies, transfer of flies could be delayed until the peak emergence occurred. In tests where emergence was delayed and occurred over several days, flies were transferred daily.

Emergence times were recorded. Adult flies were maintained on untreated bovine blood. Six days following emergence, flies were placed over egging dishes as previously described. Egg counts and mortality counts were made daily.

Pupal Dipping Tests. Stable fly pupae were dipped in various concentrations of selected cadmium compounds to determine their effectiveness on this stage of the life cycle. Pupae 2-3 days of age were used for these tests. Tests were conducted with each of the three water soluble compounds. The chemicals were dissolved in a 15% dimethyl sulfoxide-water solution. This provided enough water in the solution to completely dissolve the technical material. The DMSO provided the vehicle by which the cadmium compounds were transported into the puparia.

Various concentrations were made of each chemical. Pupae were placed in these concentrations and allowed to remain for 10 and 20 minute exposures. Each treatment at each time of exposure was replicated five times. After the respective exposure time had elapsed, the chemical solution was poured off and the pupae were placed on paper toweling to remove any excess moisture. The pupae remained on the toweling overnight.

The treated pupae were placed in small closed plastic pill cups

(Figure 9). Upon emergence, the flies were anesthetized lightly to allow sexing and transferred to Lumite test cages. Adult flies were fed untreated bovine blood daily throughout the test period. Egging procedures as outlined before were started on the sixth day following emergence. Egging data and mortality counts were determined as for the preceding tests.

Repellent Studies. Cadmium chloride was used to determine if this compound possessed repellent properties when applied to oviposition sites. Seven to nine day old flies were used in these tests. Flies were released into a cage containing treated egg pads. The circular cage was mounted on a turntable which rotated at 3 rpm.

Egging pads soaked in various concentrations of the cadmium chloride solutions were offered as oviposition sites. Untreated pads were used for comparisons. Flies were allowed to remain in the turntable cage for a six hour period. Each treatment was replicated two times with all treatments present at one time. At the conclusion of the test period, the egging dishes were removed. Egg counts were made to determine preferences.

Histological Studies. Cadmium acetate treated females were collected daily throughout the multiple feeding test and preserved in modified Kahles solution. This consisted of 30 ml distilled water, 15 ml 95% ethyl alcohol, 6 ml formalin, 1 ml glacial acetic acid, and 1 ml glycerine.

Ovaries of the treated flies were dissected out and stained with a fast green stain. After staining, the ovaries were placed on a slide in Hoyers mounting medium. With the cover slip in place, gentle pressure was applied and a squash preparation of the ovary resulted. This allowed for adequate examination of the ovaries.

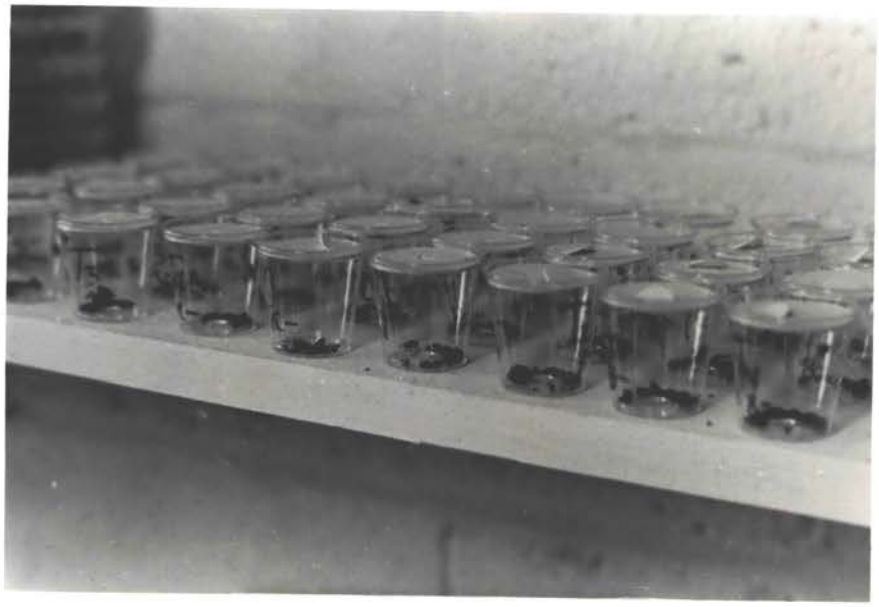

Figure 9. Pill Cups Holding Treated Pupae for Fly Emergence

RESULTS AND DISCUSSION

The results of various types of treatments using cadmium compounds against the stable fly will be reported in this section. The experimental data for each of the tests conducted has been included in the appendix in tabular form as listed in the table of contents.

Multiple Treatment Tests

Preliminary treatments were conducted with each of the cadmium compounds to determine the desired treatment levels. Cadmium acetate was fed in the blood diet at concentrations of 0.0090, 0.0060, 0.0045 and 0.0030%. Mortality for all treatments was less than 10% at 24 hours after the initial treatment. However, at 72-96 hours after the start of the experiment mortality increased considerably. On the 6th day at the start of the egging period, only 43% of the females remained alive in the highest treatment. This high degree of mortality occurred to a lesser extent throughout the other treatments during the test period.

An average of 0.80 eggs per female per day was oviposited by the flies receiving the 0.0090% treatment as compared with 21.2 eggs produced by the untreated controls (Table I). All treatments showed a definite reduction in the number of eggs produced as compared with the controls. As an indication of the reduction in the longevity of the treated flies, the treatments at the 0.009 and 0.006% concentrations had a total of 86 and 195 females respectively contributing to the

total egg production. This compared with 403 egg days (ED)[1] by the untreated flies.

TABLE I

EFFECTS OF CADMIUM ACETATE APPLIED IN MULTIPLE FEEDINGS ON EGG PRODUCTION IN STABLE FLIES

Per Cent Conc.	Eggs/female/day	Per Cent Reduction	No. egg days
.0090	.8	96.3	86
.0060	5.8	72.7	195
.0045	12.7	40.9	353
.0030	13.8	35.0	394
Controls	21.2	----	403

There was a definite retardation of egg laying at the two higher concentrations of cadmium acetate as indicated by the fact that oviposition was delayed 7 and 5 days past the start of the normal egging period. Although egg production varied greatly from day to day, the maximum was reached on the 14th egging day for all treatments except the highest concentration as compared to the 7th day in the controls. Day to day variation in egg production normally occurs as noted by Killough and McKinstry (1965).

All of the flies in one replicate and probably some in other replicates failed to oviposit throughout the 20 day egging period at the 0.006% concentration. Examination of the ovaries of similarly treated flies showed that the ovaries were much smaller in size as

[1]Hereafter this will be referred to as the number of egg days (ED) where, if one female is present for 20 days she would contribute 20 egg days to the total.

compared to those of the untreated controls (Figure 10).

Microscopic examination showed that none of the ovaries removed from the treated flies had produced fully developed eggs. Ovaries of untreated flies contained fully developed eggs after six days of age as would be expected. Oviposition would normally be expected to start at this age.

None of the treated flies which were checked showed ovarian development beyond that of the four day old untreated flies. Limited oocyte formation was evident in all of the ovaries removed from the 6-10 day old flies. In most cases, the developing oocytes could be distinguished from the nurse cells in the egg chambers. Usually nurse cells were clearly defined. The ovaries of a 16 day old fly treated at the 0.009% level showed the same type response.

Not all of the treated flies, as was noted earlier, were completely inhibited from egg laying. It is assumed that the threshold for sterility and mortality are too similar to be clearly separated. The flies from which the ovaries were removed for the ovarian development observations were younger (4-6 hours of age) than most of the test flies when first treated. Due to the fact that this may be the threshold treatment for sterility, the younger flies may have been more seriously affected than the older flies (12-24 hours) which may explain the differences in response to the same treatment. These results could be explained by treating flies of the same age group at hourly intervals to determine the time at which treatment would be most effective. Testing in this manner also would determine whether single or multiple treatments are necessary to obtain this type of response.

A test was conducted to confirm the apparent inhibition of ovarian

Figure 10. Reduced Size of Treated Ovary (Left) Compared With Untreated Ovary Showing Fully Developed Eggs (Approx. 150X)

development discussed above. Females were treated with multiple feedings of cadmium acetate at the 0.006% concentration. All of the flies which survived the treatment were examined and none showed signs of normal ovarian development. All of the ovaries showed a response similar to those examined from the initial test.

It could not be determined whether the response obtained was selective for the ovaries or whether growth inhibition was due to the more general toxic effect. Although the ovarian development of the flies which failed to reproduce was not checked at the termination of the egging period, their extended life expectancy tends to indicate that the damage may have been selective for the ovaries. The delay in initial oviposition when compared with that of the controls, as noted earlier, is also an indication that ovarian development of those flies ovipositing was delayed although not completely inhibited in those flies receiving the 0.006% treatment.

Cadmium chloride was fed at concentrations of 0.0060, 0.0045, and 0.0030%. Initial mortality was low for the first 48 hours after the treatment at all concentrations. Again as the treatment progressed, the mortality increased considerably at the highest concentration with only 47% of the females remaining in the test at the start of the egging period.

An average of 8.7 eggs per female per day was oviposited at the highest concentration as compared with 18.0 for the controls. Total production was reduced 51.7% from the untreated controls (Table II). A total of 121 egg days contributed to the total production at the highest concentration as compared with 477 for the controls. Egg production was retarded but not as much as that observed in the cadmium

acetate treatment. Peak production occurred at 11, 12, and 3 days for the respective treatments after egging started as compared to 3 days for the controls.

TABLE II

EFFECTS OF CADMIUM CHLORIDE APPLIED IN MULTIPLE FEEDINGS ON EGG PRODUCTION IN STABLE FLIES

Per Cent Conc.	Eggs/female/day	Per Cent Reduction	No. egg days
.0060	8.7	51.7	121
.0045	8.6	52.3	261
.0030	13.7	23.9	491
Controls	18.0	----	477

Cadmium succinate was used in treatments at concentrations of 0.0090, 0.0060, and 0.0045%. Mortalities after 24 hours were higher than any of the other compounds tested at the two highest concentrations. By the start of the egging period at six days, the mortality of the 0.0090% treatment was in excess of 95%. Due to the high mortality obtained, this portion of the test was discontinued. For the remaining two treatments, 37% and 80% of the females were alive at the start of the egging period for the respective treatments as compared with 93% for the controls.

An average of 7.2 eggs per female per day was oviposited in the 0.006% treatment as compared with 22.6 for the controls; a reduction of 68% (Table III). A total of 163 egg days contributed to this production as compared with 437 for the controls. Again, when the longevity of the flies approximated that of the controls, the per cent

reduction of the treated flies was reduced greatly. The peak production for the treated flies was at 15 days as compared with 10 days for the controls.

TABLE III

EFFECTS OF CADMIUM SUCCINATE APPLIED IN MULTIPLE FEEDINGS ON EGG PRODUCTION IN STABLE FLIES

Per Cent Conc.	Eggs/female/day	Per Cent Reduction	No. egg days
.0060	7.2	68.2	163
.0045	13.9	38.5	380
Controls	22.6	----	437

After preliminary testing, cadmium iodide was used at concentrations of 0.0060, 0.0045, 0.0030, and 0.0015 per cent. Initial mortalities were low but by the time the egging portion of the test was started, fewer than 50% of the females remained alive in the highest treatment in this test. The remaining concentrations compared more favorably with the controls throughout the test.

An average of 8.9 eggs per female per day was recorded for the highest treatment compared with 16.9 for the controls. As an indication of the treatment effect on the longevity of the flies, 127 egg days contributed to the total production as compared to 332 for the controls. This test was terminated after 14 egging days. A total reduction of 47.4% was obtained using the 0.0060% concentration when compared to results obtained for the controls (Table IV). The remaining treatments resulted in less than a 15% reduction. This response was considerably below those recorded from the other chemicals tested

at similar concentrations.

TABLE IV

EFFECTS OF CADMIUM IODIDE APPLIED IN MULTIPLE FEEDINGS ON EGG PRODUCTION IN STABLE FLIES

Per Cent Conc.	Eggs/female/day	Per Cent Reduction	No. egg days
.0060	8.9	47.4	127
.0045	14.9	11.9	300
.0030	14.4	14.8	252
.0015	16.4	<1.0	374
Controls	16.9	----	332

These chemicals were not tested simultaneously and, therefore, cannot be statistically compared. Summarization of data obtained using cadmium iodide showed the least differences between the chemical treatments and the untreated controls. It was apparent from preliminary investigations that solutions of cadmium iodide became less toxic when formulations were made from a stock solution which was several days old. As a result of these findings, all treatments of all tested chemicals were formulated daily. It was not determined why the cadmium iodide lost potency in a stock solution.

Cadmium 2-hydroxyethyl mercaptide was found to be considerably less toxic than the other materials tested in the multiple feeding tests. Concentrations of 0.018, 0.012, 0.009 and 0.006% were used in this test. At the highest concentration, the initial mortality was low but at the start of the egging period, fewer than 50% of the females remained alive. A larger percentage of the females remained

alive in the other concentrations at the beginning of the egging period but were reduced considerably as the test progressed.

An average of 4.8 eggs per fly per day was produced at the 0.018% concentration, compared to 15.9 for the controls. This was a total reduction of 69% when compared to the controls. Only 87 egg days contributed towards the total production compared with 532 for the controls (Table V). A higher concentration of cadmium 2-hydroxyethyl mercaptide was tolerated by the treated flies; however, this compound did not produce an effect greater than any of the other chemicals tested.

TABLE V

EFFECTS OF CADMIUM 2-HYDROXYETHYL MERCAPTIDE APPLIED IN MULTIPLE FEEDINGS ON EGG PRODUCTION IN STABLE FLIES

Per Cent Conc.	Egg/female/day	Per Cent Reduction	No. egg days
.0180	4.8	69.9	87
.0120	9.6	39.7	250
.0090	7.5	52.9	331
.0060	13.8	13.3	497
Controls	15.9	----	532

Throughout the foregoing tests, observations were made to determine if any apparent repellency existed to the various compounds as reported by Abdel-Razig (1966). No repellent action was noted for any of the compounds tested at any of the concentrations used. There was some reluctance to feed but only at the time of the initial feeding. This response may have been due to the effects of the anesthesia and

the age of the flies rather than to the characteristics of the cadmium materials. Hair and Turner (1966) noted differences in the feeding activity of newly emerged and older face flies.

There was no evidence obtained which indicated that these compounds affected the fertility of the female or male flies. No reduction in the fertility of eggs collected from flies receiving any of the treatments was observed. Even where only a few eggs resulted from the higher concentrations, the per cent hatchability compared favorably with the per cent obtained from the untreated controls.

Although the initial number of flies used in each test was equal, the eggs per female per day values recorded for the multiple feeding tests were obtained from an unequal number of flies within each replicate. The unequal number of flies within the replicates may have been caused by several factors so it was not considered feasible to make a statistical analysis on the egg laying response. These chemicals, especially at the higher concentrations, are too toxic to this species to obtain valid data without greatly reducing the longevity of the flies. Data indicated that if the concentrations were reduced to eliminate the high mortality, no significant differences would result in egg production.

Single Feeding Tests

It was noted that the high mortality recorded at the higher treatments in all of the multiple feeding tests generally occurred 48-96 hours after the initial feeding. Single treatments with the higher concentrations of all of the compounds tested were used to determine whether the high mortalities could be avoided without reducing the

effectiveness of the test materials. Some of these treatments were probably as effective as those in the multiple feeding tests but high mortality was also present.

Cadmium acetate treatments were made at concentrations of 0.012 and 0.009%. Mortalities were too high at both concentrations at the beginning of the egging period to continue with this test. Cadmium succinate treatments were made at concentrations of 0.009 and 0.006%. These single treatments were probably as effective as the lower treatments used in the multiple feeding tests. Only 47 and 63% of the females remained in test at the beginning of the egging period for the respective treatments. An average of 7.1 eggs per female per day was oviposited at the highest treatment as compared with 19.5 eggs per untreated female (Table VI).

TABLE VI

EFFECTS OF CADMIUM COMPOUNDS APPLIED IN SINGLE FEEDINGS ON EGG PRODUCTION IN STABLE FLIES

Compound	Per Cent Conc.	Eggs/females/day	Egg Days
Cadmium succinate	0.009	7.1	130
	0.006	12.8	186
Cadmium mercaptide	0.024	10.7	103
	0.018	11.7	176
	0.012	14.2	270
Cadmium iodide	0.012	11.9	140
	0.009	12.8	192
Cadmium chloride	0.009	8.6	60
	0.006	9.7	188
	Controls	19.5	282

Cadmium 2-hydroxyethyl mercaptide was used at concentrations of 0.024, 0.018 and 0.012%. Females remaining in test at the start of the egging period ranged from 37 to 90% of the original number as compared to 93% for the controls. An average of 10.7 eggs per female per day was oviposited as compared to 19.5 for the untreated controls.

Cadmium iodide treatments were made at concentrations of 0.012 and 0.009%. This treatment resulted in an average egg production of 11.9 eggs per female per day as compared to 19.5 for the untreated controls. Females remaining in test at the beginning of the egging period represented 47 and 67% of the original number for the respective treatments as compared with 97% for the untreated controls.

Cadmium chloride treatments were made at concentrations of 0.009 and 0.006%. This was probably the most toxic compound of the four tested at the 0.009% level as only 20% of the original number of females remained in test at the beginning of the egging period. Averages of 8.6 and 9.7 eggs per female per day were produced by females treated with these respective concentrations as compared with 19.5 for the untreated controls.

The reductions in egg production from these single treatment tests is another indication that these compounds are possibly most effective against the ovarian development of the younger flies. It appears that the chemical which the treated flies received after 3 or 4 days of age in the multiple feeding tests added more to the toxic effect than to the ovarian inhibition.

Larval Treatment Tests

Various concentrations of cadmium acetate, cadmium chloride, and cadmium succinate were used to treat larval media (Table VII). No

emergence resulted at the highest treatments for cadmium acetate nor for the two highest treatments of cadmium chloride or cadmium succinate. From the data obtained there appeared to be some reduction in egg laying of the resulting flies at the 0.008% treatment for both cadmium acetate and cadmium succinate. No appreciable reduction was observed at the other treatment levels for any of the three compounds tested when compared to the untreated controls.

TABLE VII

EFFECTS OF CADMIUM COMPOUNDS WHEN ADDED TO LARVAL MEDIA ON EGG PRODUCTION IN STABLE FLIES

Compound	Per Cent Conc.	No. eggs/female/day
Cadmium acetate[1]	.0330	No emergence
	.0080	13.8
	.0040	20.3
	.0008	19.8
	.0004	19.4
	Controls	18.6
Cadmium chloride[2]	.0330	No emergence
	.0165	No emergence
	.0080	18.9
	.0040	23.8
	.0008	30.2
	Controls	28.4
Cadmium succinate[2]	.0330	No emergence
	.0165	No emergence
	.0080	10.6
	.0040	19.5
	.0008	19.3
	Controls	21.0

[1]Based on 20 egging days.

[2]Based on 8 egging days.

A considerable difference was observed in the size of the larvae exposed to the higher concentrations for each of the compounds tested (Figure 11). The larvae at the 0.0330 and 0.0165% treatments were not prevented from feeding as noted by the material present in the gut. The smaller larvae were sluggish and did not move about in the medium as did those of the lower treatments. After 5-6 days the larvae in the 0.0330 and 0.0165% treatments succumbed to the toxic effects of the chemical.

Emergence was somewhat delayed for the 0.008% treatment and occurred over a 4-6 day period. Emergence for the other treatments was similar to those of the untreated controls. No appreciable difference was noted in the longevity of the females in the egging portion of the test when compared to the controls. The sex ratio's of the resulting flies were all within the expected 40:60-60:40 range. The larva should be the most susceptible to treatment since early ovarian development occurs in this stage. Apparently the toxicity of the chemicals tested manifest their lethal effect before an affect can be measured on the ovarian development. The chemicals in these tests were not as effective in reducing the egg production as in the adult treatments.

Pupal Dipping Treatments

Pupae 2-3 days of age were dipped in 1.0, 0.5, 0.1 and 0.05% concentrations of cadmium chloride or cadmium acetate. Pupae were exposed to this treatment for 10 or 20 minute time periods.

Neither of the compounds at any concentration or time period tested showed any apparent effect on the egg production of the resulting females (Table VIII). This test was terminated after 10 egging

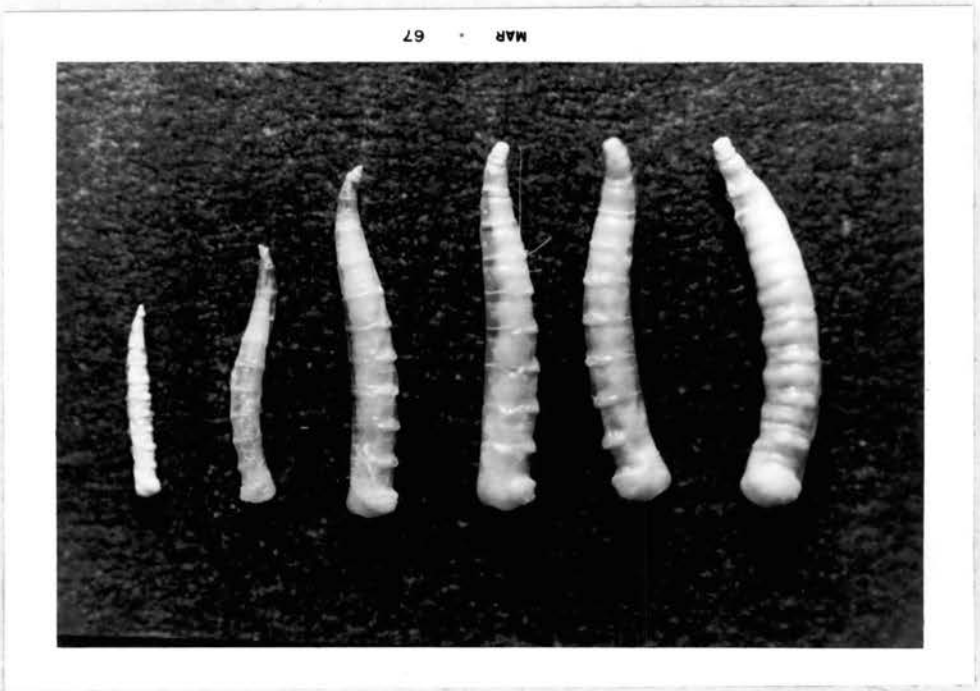

Figure 11. Size Variation of Stable Fly Larvae (Same Age) Resulting From Cadmium Treated Media With Highest Concentration at Left and Untreated Control at Right

days because of no apparent effect on egg production.

TABLE VIII

EFFECTIVENESS OF TWO CADMIUM COMPOUNDS AS PUPAL DIPS ON EGG PRODUCTION AGAINST THE STABLE FLY

Compound	Per Cent Conc.	Exposure Time	Eggs/female/day
Cadmium chloride	1.0	10 min.	17.6
	0.5	"	20.5
	0.1	"	23.2
	0.05	"	18.2
	Controls	"	17.3
	1.0	20 min.	20.4
	0.5	"	23.7
	0.1	"	21.2
	0.05	"	19.1
	Controls	"	18.4
Cadmium acetate	1.0	10 min.	21.6
	0.5	"	20.9
	0.1	"	26.0
	0.05	"	21.2
	Controls	"	17.3
	1.0	20 min.	22.8
	0.5	"	15.6
	0.1	"	22.4
	0.05	"	17.9
	Controls	"	18.4

The per cent DMSO was increased from 15 to 25% in order to get more penetration by the solvent and toxicant. At the increased percentage, the wings of the emerging flies were damaged (Figure 12). This damage resulted from the effects of the solvent rather than the

Figure 12. Damaged Wings of Stable Flies Resulting From Dimethylsulfoxide Treatment

cadmium compounds as indicated from the response when pupae were dipped in the solvent alone. Wing damage ranged from a slight curl at the tips to almost total lack of development. The per cent of damage was in excess of 50% when pupae were dipped for 20 minutes.

Considering the apparent damage to the ovaries of the newly emerged flies, as discussed in the multiple feeding test, the late pupal stage could be ideal for treatment. A suitable solvent to allow penetration of the cadmium compound into the puparium is needed. The cadmium compounds used in these investigations are not readily soluble in acetone, the solvent widely used with the more common chemosterilants for pupal treatment. The use of the proper solvent as a carrier would be an easy way of applying the treatment to large numbers of flies.

Repellent Test

Duplicate oviposition sites, treated with cadmium chloride at concentrations of 5.0, 1.0 and 0.1%, were offered to 11 day old flies. After six hours exposure, egg counts were made to determine if this compound showed repellent properties. There was only a slight reduction in the number of eggs deposited at the highest treatment (Table IX). The number of eggs oviposited in all the treatments exceeded those on the untreated oviposition sites. This was probably due to random selection and by increasing the number of replicates this difference could probably be overcome. The strong repellent action was definitely not present, even at the 5.0% treatment, as reported by Abdel-Razig (1966).

TABLE IX

EFFECTIVENESS OF CADMIUM CHLORIDE AS A REPELLENT WHEN TREATING OVIPOSITION SITES

Per Cent Conc.	No. eggs deposited
5.0	1210
1.0	2247
0.1	2510
Controls	1477

SUMMARY AND CONCLUSIONS

Effects On Egg Production Of Multiple Cadmium Feedings

Various concentrations of cadmium acetate, cadmium chloride, cadmium iodide, cadmium succinate and cadmium 2-hydroxyethyl mercaptide were administered to stable flies, Stomoxys calcitrans, in daily feedings. There was a definite reduction in the number of eggs laid by the females treated with the higher concentrations. These high concentrations also caused high mortality and as the mortality rates were reduced in the lower treatments, the reduction in egg production was not as great.

Studies made of ovaries dissected out of cadmium acetate treated flies indicated a definite inhibition of ovarian growth. In untreated controls of the same age, fully developed eggs were present whereas none were present in the treated flies.

The cadmium iodide treatments were probably the least effective. The cadmium 2-hydroxyethyl mercaptide treatment was less toxic than the other materials but it was no more effective, even at higher concentrations, than the other compounds tested.

It appears that the age of the fly at the time of the initial treatment is critical in order to prevent ovarian development. For all of the compounds tested, the effective levels for sterility and the lethal dose were too similar to be clearly separated. Whether the response to these treatments was selective for the ovaries or whether the growth inhibition was due to the more general toxic effect was not ascertained.

No apparent repellent action of the cadmium compounds was observed at the concentrations tested to the feedings of the stable flies. There was no evidence that any of these compounds affected the fertility of either sex. At the higher concentrations, these chemicals are too toxic to S. calcitrans to obtain valid data without greatly reducing the longevity of the flies. It was demonstrated that no significant differences would result in the egg production if the concentrations were reduced to eliminate the high mortality.

Single Feeding Effects

Single treatments were made of all five of the cadmium compounds used in the multiple feeding tests. This test was conducted to determine if the high mortality encountered in the multiple feeding tests could be eliminated yet not lose the effectiveness of the compounds. Higher concentrations were used along with the higher treatments used in the multiple feeding tests.

The high mortality rates were not overcome. Some of these treatments appeared to be as effective as the multiple feeding treatments. It is postulated that these compounds would be most effective against the younger flies. Treating the flies after 3-4 days of age added more to the toxic effect than to the effect on the ovarian development.

Larval Treatment Effects

Cadmium acetate, cadmium chloride, and cadmium succinate were incorporated in the larval diet to determine their effectiveness as ovarian development inhibitors. No emergence occurred in the higher treatments. There was a reduction in the size of the larvae at the higher treatments but all died at 5-6 days of age as a result of the

toxicity of the chemicals. Emergence was delayed when some of the other treatments were used but there was very little reduction of egg production by the flies treated with any of the chemicals tested when compared to the untreated controls. There was no variation from the expected sex ratios of the emerging flies.

The larval stage would be expected to be a critical stage in the development of the ovaries but again the toxic effects were manifested before an effect on the ovarian development could be measured. This treatment was not as effective as either of the adult treatments.

Pupal Dipping Effects

Pupae 2-3 days of age were dipped in dimethyl sulfoxide-water solutions of cadmium chloride and cadmium acetate. Neither of these compounds showed any appreciable effect on the egg production of the resulting females. The late pupal stage should be susceptible to this treatment in view of the apparent effect on the younger adult flies. With a suitable solvent this could possibly be an effective and efficient method of treatment as large numbers could be readily treated.

Repellent Effects

No repellency was noted when egging sites were treated with up to 5.0% concentrations of cadmium chloride.

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APPENDIX

TABLE X

DAILY OBSERVATIONS OF STABLE FLY EGG PRODUCTION AS AFFECTED BY MULTIPLE CADMIUM ACETATE FEEDINGS

Per Cent Conc.	Days 1	2	3	4	5	6	7	8	9	10
0.0090	0(13)*	0(11)	0(10)	0(10)	0(9)	0(9)	69(7)	0(7)	0(6)	0(4)
0.0060	0(19)	0(18)	0(16)	0(12)	0(12)	20(11)	196(11)	0(11)	154(10)	134(10)
0.0045	0(23)	306(22)	165(22)	0(22)	152(22)	241(22)	440(22)	99(20)	400(18)	234(18)
0.0030	0(25)	673(25)	329(25)	0(24)	0(24)	568(24)	672(24)	152(23)	405(21)	171(21)
Controls	378(29)	1007(29)	435(29)	348(27)	0(27)	1048(27)	1295(27)	232(26)	943(25)	591(25)

*No. eggs (no. females)

Per Cent Conc.	11	12	13	14	15	16	17	18	19	20	Totals
0.0090	-------	-------	------	------	------	------	------	------	------	------	69(86)
0.0060	72(10)	5(10)	39(9)	312(8)	0(7)	142(7)	0(7)	66(7)	0(7)	------	1140(195)
0.0045	356(18)	251(18)	99(18)	698(18)	184(17)	120(17)	534(17)	194(17)	0(15)	------	4483(353)
0.0030	213(21)	65(21)	111(20)	875(20)	168(20)	347(20)	341(19)	362(17)	0(17)	------	5449(394)
Controls	317(25)	87(23)	164(20)	706(18)	178(18)	247(13)	410(13)	148(10)	0(8)	------	8534(403)

TABLE XI

DAILY OBSERVATIONS OF STABLE FLY EGG PRODUCTION AS AFFECTED BY MULTIPLE CADMIUM CHLORIDE FEEDINGS

Per Cent Conc.	Days 1	2	3	4	5	6	7	8	9	10
0.0060	0(14)*	0(12)	83(10)	0(6)	0(5)	75(5)	27(5)	79(5)	30(5)	31(5)
0.0045	0(22)	0(20)	158(20)	164(17)	197(16)	212(16)	241(16)	92(16)	120(14)	45(13)
0.0030	0(29)	295(29)	721(29)	479(29)	649(29)	429(29)	615(28)	323(28)	315(26)	110(26)
Controls	512(29)	607(29)	1161(29)	472(29)	507(29)	992(29)	310(28)	484(28)	850(28)	90(28)

*No. eggs (no. females)

Per Cent Conc.	Days 11	12	13	14	15	16	17	18	19	20	Totals
0.0060	281(5)	75(5)	77(5)	3(5)	77(5)	108(5)	0(5)	135(5)	58(5)	19(4)	1048(121)
0.0045	148(11)	227(11)	3(11)	212(10)	55(9)	45(9)	99(9)	0(7)	0(7)	127(7)	2242(261)
0.0030	444(26)	461(25)	304(24)	248(22)	238(21)	221(19)	332(19)	300(15)	59(14)	207(14)	6740(491)
Controls	194(27)	494(23)	226(22)	482(21)	181(18)	66(17)	447(17)	163(17)	240(17)	383(12)	8595(477)

TABLE XII

DAILY OBSERVATIONS OF STABLE FLY EGG PRODUCTION AS AFFECTED BY MULTIPLE CADMIUM SUCCINATE FEEDINGS

Per Cent Conc.	Days 1	2	3	4	5	6	7	8	9	10
0.0060	0(11)*	0(11)	0(10)	0(10)	0(9)	86(9)	0(9)	53(9)	34(9)	135(9)
.0045	0(24)	61(23)	296(21)	311(21)	379(20)	302(19)	0(19)	214(19)	543(19)	78(19)
Controls	99(28)	1350(28)	68(28)	280(28)	1730(28)	478(27)	58(26)	1212(26)	1010(26)	76(23)

*No. eggs (no. females)

Per Cent Conc.	Days 1	2	3	4	5	6	7	8	9	0	
0.0060	109(9)	53(8)	0(8)	131(8)	45(7)	127(6)	128(6)	65(5)	47(5)	161(5)	1174(163)
.0045	350(18)	495(18)	0(18)	516(18)	354(18)	216(18)	279(17)	328(17)	162(17)	406(17)	5290(380)
Controls	455(19)	384(19)	226(19)	271(19)	560(19)	723(18)	72(18)	249(17)	446(16)	143(15)	9890(437)

TABLE XIII

DAILY OBSERVATIONS OF STABLE FLY EGG PRODUCTION AS AFFECTED BY MULTIPLE CADMIUM IODIDE FEEDINGS

Per Cent Conc.	Days 1	2	3	4	5	6	7	8	9	10
0.0060	0(12)*	0(11)	75(11)	0(11)	64(9)	86(9)	0(8)	50(8)	411(8)	0(8)
0.0045	70(24)	114(22)	54(22)	550(22)	371(21)	461(21)	237(21)	276(21)	1019(21)	99(21)
0.0030	0(23)	228(20)	254(20)	112(19)	382(19)	424(19)	64(19)	361(19)	891(17)	78(17)
0.0015	146(29)	550(28)	595(28)	357(27)	346(27)	585(27)	165(26)	495(26)	1481(26)	20(26)
Controls	396(28)	397(27)	289(27)	505(27)	607(27)	367(26)	243(25)	450(24)	1166(24)	119(21)

*No. eggs (no. females)

Per Cent Conc.	Days 11	12	13	14	Totals
0.0060	188(8)	175(8)	46(8)	48(8)	1141(127)
0.0045	362(21)	616(21)	243(21)	10(21)	4482(300)
0.0030	239(17)	350(17)	245(15)	0(14)	3628(252)
0.0015	454(25)	603(25)	349(25)	0(25)	6144(374)
Controls	310(20)	462(20)	238(20)	75(18)	5624(332)

TABLE XIV

DAILY OBSERVATIONS OF STABLE FLY EGG PRODUCTION AS AFFECTED BY MULTIPLE CADMIUM 2-HYDROXYETHYL MERCAPTIDE FEEDINGS

Per Cent Conc.	Days 1	2	3	4	5	6	7	8	9	10
0.018	0(12)*	0(10)	0(8)	0(6)	0(5)	68(4)	0(4)	0(3)	0(3)	0(3)
0.012	0(21)	0(16)	0(15)	0(13)	127(13)	272(13)	79(13)	333(13)	55(12)	25(11)
0.009	116(26)	66(23)	57(22)	0(20)	24(20)	599(20)	44(19)	195(19)	182(19)	62(17)
0.006	477(26)	213(26)	284(26)	338(26)	29(26)	745(26)	467(26)	272(26)	284(26)	209(26)
Controls	555(29)	245(29)	609(29)	579(29)	103(29)	620(29)	635(29)	785(29)	457(29)	224(29)

*No. eggs (no. females)

Per Cent Conc.	Days 11	12	13	14	15	16	17	18	19	20	Totals
0.018	126(3)	0(3)	50(3)	0(3)	58(3)	0(3)	0(3)	115(3)	0(3)	0(2)	417(87)
0.012	257(11)	21(11)	248(11)	159(11)	288(11)	0(11)	0(11)	377(11)	149(11)	0(11)	2398(250)
0.009	393(16)	0(14)	234(14)	121(14)	179(13)	0(13)	0(13)	218(12)	0(10)	0(8)	2490(331)
0.006	1065(26)	147(25)	798(24)	484(24)	152(24)	0(24)	111(23)	761(23)	115(22)	124(22)	6848(497)
Controls	795(29)	78(27)	310(26)	492(24)	332(23)	0(23)	158(23)	815(23)	514(22)	135(22)	8491(532)

TABLE XV

DAILY OBSERVATIONS OF STABLE FLY EGG PRODUCTION AS AFFECTED BY SINGLE CADMIUM SUCCINATE FEEDING

Per Cent Conc.	Days 1	2	3	4	5	6	7	8	9	10	Totals
0.009	0(14)*	0(14)	0(14)	0(14)	147(13)	137(13)	125(12)	60(12)	439(12)	13(12)	923(130)
0.006	0(19)	0(19)	8(19)	132(19)	721(19)	177(19)	403(18)	150(18)	670(18)	169(18)	2392(186)
Controls	0(28)	455(28)	659(28)	316(28)	1638(28)	358(28)	589(28)	441(28)	883(28)	321(28)	5660(280)

*No. eggs (no. females)

TABLE XVI

DAILY OBSERVATIONS OF STABLE FLY EGG PRODUCTION AS AFFECTED BY SINGLE CADMIUM 2-HYDROXYETHYL MERCAPTIDE FEEDING

Per Cent Conc.	Days 1	2	3	4	5	6	7	8	9	10	Totals
0.0240	0(11)*	0(11)	0(11)	0(11)	402(11)	52(11)	146(10)	104(9)	260(9)	148(9)	1104(103)
0.0180	0(18)	0(18)	0(18)	73(18)	563(18)	126(18)	301(17)	362(17)	289(17)	347(17)	2061(176)
0.0120	0(27)	0(27)	55(27)	129(27)	1269(27)	112(27)	812(27)	305(27)	790(27)	356(27)	3828(270)
Controls	0(28)	455(28)	659(28)	316(28)	1638(28)	358(28)	589(28)	441(28)	883(28)	321(28)	5660(280)

*No. eggs (no. females)

TABLE XVII

DAILY OBSERVATIONS OF STABLE FLY EGG PRODUCTION AS AFFECTED BY SINGLE CADMIUM IODIDE FEEDING

Per Cent Conc.	Days 1	2	3	4	5	6	7	8	9	10	Totals
.0120	0(14)*	0(14)	27(14)	0(14)	461(14)	169(14)	425(14)	42(14)	454(14)	83(14)	1667(140)
.0090	0(20)	0(20)	50(20)	98(20)	715(19)	333(19)	468(19)	318(19)	291(19)	186(19)	2458(192)
Controls	0(29)	609(29)	412(29)	244(29)	1462(29)	377(28)	295(28)	825(28)	401(28)	696(26)	10981(563)

*No. eggs (no. females)

TABLE XVIII

DAILY OBSERVATIONS OF STABLE FLY EGG PRODUCTION AS AFFECTED BY SINGLE CADMIUM CHLORIDE FEEDING

Per Cent Conc.	Days 1	2	3	4	5	6	7	8	9	10	Totals
.0090	0(6)	0(6)	0(6)	0(6)	79(6)	193(6)	42(6)	0(6)	183(6)	16(6)	513(60)
.0060	0(18)	0(18)	11(18)	53(18)	417(18)	487(18)	152(15)	301(15)	314(15)	90(15)	1826(188)
Controls	0(29)	609(29)	412(29)	244(29)	1426(29)	377(28)	295(28)	825(28)	401(28)	696(26)	10981(563)

*No. eggs (no. females)

TABLE XIX

DAILY OBSERVATIONS OF STABLE FLY EGG PRODUCTION AS AFFECTED BY CADMIUM ACETATE TREATED LARVAL MEDIUM

Per Cent Conc.	Days 1	2	3	4	5	6	7	8	9	10
.0330	No emergence									
.0080	0(24)*	0(24)	566(24)	254(22)	188(20)	134(20)	274(19)	270(19)	339(19)	458(19)
.0040	1118(29)	538(29)	881(29)	957(29)	425(29)	1016(29)	238(28)	771(28)	428(28)	346(27)
.0008	1608(36)	779(36)	914(36)	1107(36)	869(36)	683(35)	318(35)	344(34)	1000(32)	383(32)
.0004	804(32)	444(32)	1273(32)	575(32)	445(32)	704(32)	171(32)	489(31)	705(30)	909(29)
Controls	916(32)	886(32)	963(32)	831(32)	823(31)	200(30)	112(30)	212(30)	514(30)	812(28)

Per Cent Conc.	Days 11	12	13	14	15	16	17	18	19	20	Totals
.0080	204(19)	802(19)	292(19)	189(17)	460(17)	390(16)	65(16)	202(14)	0(13)	0(13)	5137(373)
.0040	723(25)	586(24)	142(23)	238(20)	642(20)	69(16)	31(14)	273(13)	0(12)	25(12)	9438(464)
.0008	835(31)	736(31)	369(28)	375(27)	461(27)	258(23)	131(19)	327(19)	246(18)	24(16)	11819(597)
.0004	719(29)	860(28)	466(28)	241(26)	456(26)	375(23)	216(22)	261(21)	648(20)	34(19)	10795(556)
Controls	557(27)	754(26)	455(26)	105(26)	295(26)	545(21)	82(17)	90(17)	592(16)	116(15)	9854(530)

*No. eggs (no. females)

TABLE XX

DAILY OBSERVATIONS OF STABLE FLY EGG PRODUCTION AS AFFECTED BY CADMIUM CHLORIDE TREATED LARVAL MEDIUM

Per Cent Conc.	Days 1	2	3	4	5	6	7	8	Totals
.0330	No emergence								
.0165	No emergence								
.0080	269(25)*	504(25)	249(24)	752(24)	496(24)	95(24)	770(24)	542(24)	3677(194)
.0040	330(30)	616(30)	329(30)	1121(29)	1254(29)	102(29)	1293(29)	557(29)	5602(235)
.0008	932(25)	586(23)	853(23)	673(22)	1174(22)	19(22)	492(22)	802(22)	5471(181)
Controls	1148(32)	860(31)	705(31)	1203(30)	1077(30)	407(30)	661(30)	864(30)	6925(244)

*No. eggs (no. females)

TABLE XXI

DAILY OBSERVATIONS OF STABLE FLY EGG PRODUCTION AS AFFECTED BY CADMIUM SUCCINATE TREATED LARVAL MEDIUM

Per Cent Conc.	Days 1	2	3	4	5	6	7	8	Totals
.0330	No emergence								
.0165	No emergence								
.0080	0(13)*	174(13)	36(13)	216(13)	302(13)	0(13)	95(13)	281(13)	1104(104)
.0040	648(28)	246(26)	424(26)	461(26)	1008(26)	254(26)	289(26)	766(26)	4096(210)
.0008	601(21)	104(20)	326(20)	611(20)	600(20)	7(20)	323(20)	535(20)	3107(161)
Controls	1169(33)	719(33)	281(32)	519(32)	1179(31)	293(31)	259(31)	919(31)	5340(254)

*No. eggs (no. females)

TABLE XXII

DAILY OBSERVATIONS OF STABLE FLY EGG PRODUCTION AS AFFECTED BY CADMIUM CHLORIDE WHEN APPLIED AS A PUPAL DIPPING TREATMENT FOR 10 AND 20 MINUTE EXPOSURES

Per Cent Conc.	Exp. Time	Days 1	2	3	4	5	6	7	8	9	10	Totals
1.0	10 min.	613(14)*	115(14)	58(14)	89(14)	332(14)	408(14)	166(14)	193(14)	352(14)	145(14)	2471(140)
	20 min.	431(10)	62(10)	134(10)	338(10)	201(10)	236(10)	326(10)	83(9)	177(9)	0(9)	1988(97)
0.5	10 min.	430(11)	148(11)	178(11)	331(11)	163(11)	253(11)	235(11)	244(11)	268(11)	6(11)	2256(110)
	20 min.	439(12)	350(12)	170(12)	346(12)	186(12)	450(11)	251(11)	184(11)	246(11)	108(11)	2730(115)
0.1	10 min.	277(11)	329(11)	70(11)	313(11)	305(11)	587(11)	115(11)	121(11)	380(11)	56(11)	2553(110)
	20 min.	347(9)	302(9)	126(9)	287(9)	107(9)	87(8)	176(8)	263(8)	102(8)	6(8)	1803(85)
Control	10 min.	363(12)	235(12)	162(12)	127(12)	240(12)	159(11)	209(11)	152(10)	282(10)	14(10)	1943(112)
	20 min.	265(13)	104(13)	139(13)	442(13)	191(13)	371(13)	90(13)	472(13)	307(13)	0(13)	2389(130)

*No. eggs (no. females)

TABLE XXIII

DAILY OBSERVATIONS OF STABLE FLY EGG PRODUCTION AS AFFECTED BY CADMIUM ACETATE WHEN APPLIED AS A PUPAL DIPPING TREATMENT FOR 10 AND 20 MINUTE EXPOSURES

Per Cent Conc.	Exp. Time	Days 1	2	3	4	5	6	7	8	9	10	Totals
1.0	10 min.	810(15)*	132(15)	172(15)	205(15)	200(15)	450(15)	405(15)	374(14)	267(14)	178(14)	3171(147)
	20 min.	499(12)	165(11)	87(11)	314(11)	110(11)	524(11)	349(11)	251(10)	165(10)	0(10)	2464(108)
0.5	10 min.	422(14)	321(14)	156(14)	186(14)	199(14)	615(14)	315(14)	110(14)	513(14)	97(14)	2934(140)
	20 min.	162(10)	162(10)	346(10)	156(10)	237(10)	255(10)	0(10)	86(9)	109(9)	0(9)	1513(97)
0.1	10 min.	404(10)	169(10)	223(10)	202(10)	496(10)	194(10)	228(10)	351(10)	339(10)	0(10)	2606(100)
	20 min.	893(19)	107(19)	468(19)	304(19)	481(19)	497(19)	447(19)	661(19)	343(19)	47(19)	4248(190)
Control	10 min.	363(12)	235(12)	162(12)	127(12)	240(12)	159(11)	209(11)	152(10)	282(10)	14(10)	1943(112)
	20 min.	265(13)	104(13)	139(13)	442(13)	191(13)	371(13)	90(13)	472(13)	307(13)	0(13)	2389(130)

*No. eggs (no. females)

VITA

Sidney Edmund Kunz

Candidate for the Degree of

Doctor of Philosophy

Thesis: THE EFFECTS OF CADMIUM COMPOUNDS ON REPRODUCTION AND GROWTH OF THE STABLE FLY, STOMOXYS CALCITRANS (LINNAEUS)

Major Field: Entomology

Biographical:

Personal Data: Born in Fredericksburg, Texas, December 24, 1935, the son of Theodore and Louise Kunz.

Education: Graduated from Tivy High School, Kerrville, Texas, 1954. Received Bachelor of Science Degree, Texas A&M University, College Station, Texas, May, 1958; received Master of Science Degree, Texas A&M University, College Station, Texas, January, 1962; completed requirements for the Doctor of Philosophy Degree in May, 1967.

Professional Experience: Student Trainee, USDA, ARS, ERD, Kerrville, Texas, summers 1956, 1957; Biological Aide, USDA, ARS, ERD, Kerrville, Texas, May-December 1958; Lieutenant, U. S. Army, Ft. Bliss, Texas, 1958-1960; Research Assistant, Texas A&M University, College Station, Texas, 1960-1961; Survey Entomologist, Oklahoma State University, 1961-1964; Assistant Extension Entomologist, Oklahoma State University, 1964-1967.

Organizations: Entomological Society of America, Phi Sigma, Alpha Zeta, Sigma Xi.