# ALTERATIONS IN REPRODUCTION, GROWTH AND DEVELOPMENT OF <u>COCHLIOMYIA MACELLARIA</u> (FABR.) IN RESPONSE TO SELECTED ANTIMONY COMPOUNDS

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### PREFACE

Insecticides were born out of necessity, the necessity for man to escape the ravages of the insect-borne diseases and to more favorably compete for his food and fiber with those phytophagous species. It is generally felt today that in his tenacity to defeat the insect, man has unfavorably swung the balance of nature toward eventual disaster and the initial blame inevitably falls on the insecticide.

Few will dispute the claim that insecticides were a boon to mankind but indiscriminate use has greatly altered their initial benefits. Resistance to these chemicals has developed and our environment is becoming saturated with their residues. Whether this saturation eventually be proven detrimental to man and wildlife or not is moot; their buildup cannot continue.

The professional entomologist is faced with the necessity to develop new, environmentally safe yet effective methods of insect control. Such novel ideas as the sterilization techniques and biological control methods have given the impetus to develop even wider integrated control procedures. Fortunately the search for new, safe insect control methods is vigorously continuing.

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### INTRODUCTION

The effects upon many invertebrate species, especially the helminths, by various antimony compounds is well documented. With the exception of studies conducted at Oklahoma State University, however, few reports of the effects of these compounds on insects have been published. The previous research work at Oklahoma State was concerned primarily with the evaluation of cadmium salts as reproductive inhibitors and toxicants to various insects with some attention being given only two antimony compounds.

Because of the chemical proximity of antimony to cadmium and with prior knowledge of its effects upon reproductive inhibition in other invertebrates, it was a logical sequela to previous research to expand the work to include effects upon other insect species as well as inclusion of additional antimony compounds. Interest was aroused with the striking results reported by Abdel-Razig (1966) in work with triphenylantimony which indicated an increase in fecundity of <u>Musca domestica</u> L.

Reported here are the results of various tests using five antimony compounds at various concentrations to determine the alterations in reproduction, growth and development of the secondary screw-worm fly, <u>Cochliomyia macellaria</u> (Fabr.).

Selection of  $\underline{C}$ . macellaria as the test species was based on its ubiquity, importance as a disease vector and its close relationship to the primary screw-worm fly C. hominivorax (Coquerel).

#### LITERATURE REVIEW

## <u>Cochliomyia</u> macellaria (Fabr.)

The secondary screw-worm fly, <u>Cochliomyia macellaria</u> (Fabr.), is a secondary myiasis-producing species. As a myiasis-producer it is of minor importance, there being only 12 myiasis cases reportedly caused by <u>C. macellaria</u> as late as 1936 (Knipling and Rainwater 1937). It has, however, been incriminated as a carrier of pathogenic organisms. Greenberg et al. (1963) found <u>C. macellaria</u> harboring more <u>Salmonella</u> types than either <u>Musca domestica</u> or <u>Phormia regina</u>. In a subsequent work Greenberg (1971) listed 10 types of viruses, 33 species of bacteria and 7 protozoans that were harbored by <u>C. macellaria</u>.

The literature contains numerous reports of myiasis in man caused by <u>C</u>. macellaria but these undoubtedly came as a result of confusion of this fly with <u>Cochliomyia hominivorax</u> (Coquerel) (Coates 1914; Francaviglia 1914; Huber et al. 1914). Cushing and Patton (1933) in following up earlier work by Lahille (1915) reported that at least two species of this fly were confused. Hall (1948) reported 27 synonyms for the species. The confusion has apparently been satisfactorily resolved with <u>C</u>. <u>hominivorax</u> being the designation for the primary screw-worm fly responsible for myiasis of man and animals and <u>C</u>. <u>macellaria</u> the secondary screw-worm fly, a saprophagous species and only an occasional myiasis producer.

<u>C. macellaria</u> is widely distributed in both the Nearctic and Neotropical regions, from southern Canada throughout the United States; in Mexico; the West Indies, Central America, Chile and Argentina (Hall 1948). It was reported for the first time in Bermuda in 1955 where it was the most plentiful species of the summer filth fly populations comprising 70.5% of the trapped samples (Williams 1958). Of the flies trapped over offal at a Mexican slaughterhouse, <u>C. macellaria</u> accounted for almost one half of the total (Greenberg et al. 1963).

In the United States this species occurs in the southern parts of Florida and Texas throughout the year while never becoming abundant in the North and Northern Middle West (Hall 1948). Ten to fourteen broods per year are produced in southern Texas (Bishopp 1915). Generally their numbers increase from early spring until frost. Hall (1948) reported that a normal decrease in their numbers usually occurs during midsummer when the weather is hot and dry although observations in Stillwater, Oklahoma, indicate no such decrease occurs or if so it is imperceptible. The minimum temperature for adult activity is  $50-60^{\circ}$  F (Deonier 1940).

Bishopp and Laake (1921) found that adult <u>C</u>. <u>macellaria</u> would migrate as far as 8 miles in 24 hours and 10 miles in less than 48 hours. They observed a maximum migration of 15.1 miles. Quarterman et al. (1954) on the other hand found most of the marked individuals were trapped from 1-4 miles from the release point with the maximum distance travelled being 7.2 miles. The differences in these results were apparently due to the conditions under which the tests were conducted, the former involving a rural test environment and the latter an urban situation.

In nature <u>C</u>. <u>macellaria</u> feed upon a variety of foods including garbage refuse, fresh meat, carrion, animal feces, in wounds of living animals and the nectar of certain flowers such as wild parsnips (<u>Pastinaca sativa</u>) and <u>Aristolochia</u> spp. which gives off an odor resembling that of carrion (Hall 1948). LaBrecque et al. (1962) reported the flies seldom fed on a dry bait containing maize meal.

<u>C. macellaria</u> is frequently involved in the blowing of meat in shops and homes where they are abundant and sanitary conditions are lacking. It is obviously the common fly around market places and abattoirs in the American tropics (Greenberg et al. 1963).

Much of the life of this species is spent around carrion. The carcass and the vegetation around it may swarm with thousands of the adults. Baker and Schoof (1955) in tests of insecticides applied to carcasses reported dead adults (predominantly <u>C</u>. <u>macellaria</u>) often accumulated to a depth of one-half inch around the carcass.

Although <u>C</u>. <u>macellaria</u> larvae apparently kill infested animals the species is of no particular importance in areas where <u>C</u>. <u>homini-</u> <u>vorax</u> was not present except under conditions where unclean animals were made susceptible to severe larval infestation such as soiled wool, etc. (Knipling and Travis 1937).

The eggs of <u>C</u>. <u>macellaria</u> are deposited in a yellowish, rounded, irregularly flattened mass. They are loosely cemented together. The average number in a single mass varies greatly ranging from about 30 to as many as 250. The egg mass may be composite depositions from several females. On carrion the female attempts to hide the eggs within some body recess such as eye sockets, nasal and anal openings, in the posterior regions of the mouth or under lacerated skin in the case of a wound.

The method of egg laying on live animals is a characteristic used to differentiate <u>C</u>. <u>macellaria</u> and <u>C</u>. <u>hominivorax</u>. Whereas the former lays its eggs in a relatively haphazard mass, the latter's eggs are tightly cemented together and to the tissues of the host in a compact, shingle-like mass, the arrangement being peculiar to this species (Krull 1969).

The individual eggs of <u>C</u>. <u>macellaria</u> are approximately 1 mm long and 0.2 mm in diameter. They are smooth, glistening white when first deposited but take on a characteristic darkened appearance the nearer they get to the time of hatching. The egg is somewhat flattened anteriorly and more rounded posteriorly with a narrow dorsal longitudinal seam which extends around the micropyle anteriorly to form a cap. Due to the dorsal seam forming a much narrower band around the micropyle the cap of <u>C</u>. <u>macellaria</u> eggs appears much smaller than that of <u>C</u>. <u>hominivorax</u> (Laake et al. 1936).

Melvin (1934) reported the optimum temperature for hatching of <u>C. macellaria</u> eggs to be about  $89^{\circ}$  F. At this temperature the incubation period was 8.13 hours. Accordingly, he found that almost 33 hours were required for incubation at  $64^{\circ}$  F and that only a few eggs hatched much below this temperature. Ten percent hatched at  $59^{\circ}$  F and although incubation was complete in 6.73 hours at  $104^{\circ}$  F no hatch occurred 5 degrees above this. He observed that the incubation period for <u>C. macellaria</u> was about one half that required for <u>C. hominivorax</u> under the same conditions.

The larvae of <u>C</u>. <u>macellaria</u> are not unlike most other dipteran larvae. All instars are equipped with small brownish spines. The spines of the first instar have a single point at the apex while the second and third instars possess spines having two or occasionally three points.

With each successive molt following eclosion the larvae become larger and more robust. The cephaloskeleton of the first instar has a prominent anterodorsal projection on the pharyngeal sclerites and these projections join at their anterior extremeties. The labial sclerites consist of a pair of irregular elongate sclerites with a large number of small hooklets closely grouped anteriorly. The cephaloskeleton of the first instar is from 0.33-0.34 mm in length and as such is considerably larger than that of <u>C</u>. <u>hominivorax</u> (Laake et al. 1936). Anterior spiracles are absent as is the peritreme in the first instar.

The posterior spiracles of the second instar are more lightly pigmented and smaller than those of <u>C</u>. <u>hominivorax</u> and unlike <u>C</u>. <u>hominivorax</u> the tracheal trunks running anteriorly from the posterior spiracles are not pigmented. The length of the second instar cephalo-skeleton measures from 0.73-0.80 mm (Laake et al. 1936). There are two slits per posterior spiracle.

The cephaloskeleton of the third instar larva of <u>C</u>. <u>macellaria</u> have labial sclerites smaller than in <u>C</u>. <u>hominivorax</u>; the pharynx has longitudinal ridges, whereas in <u>C</u>. <u>hominivorax</u> it is smooth; pharyngeal sclerites are large and a pigmented strip of varying extent is found just above the dorsal cornua in more mature third instar larvae. The length of the cephaloskeleton is from 1.43-1.63 mm (Laake et al. 1936). There are three slits per posterior spiracle.

The larval durations under laboratory conditions are: first instar approximately 12 hours; second instar approximately 36 hours and the third instar approximately 48 hours.

Upon hatching the emerging larvae begin feeding voraciously, migrating considerably from one place to another whether upon the necrotic tissue in a wound of a live animal or within a carcass. They have no definite plan of attack in carcasses (Hall 1948). According to Deonier (1940) they are active when the internal temperature of a carcass reaches  $41-50^{\circ}$  F. In wounds the larvae of <u>C. macellaria</u> do not form the typical pocketlike injury characteristic of <u>C. hominivorax</u> but often do considerable migrating in the wool or hair around the wound (Laake et al. 1936). A carcass or larval medium will have a characteristic "honeycomb" appearance and will quickly dwindle under the impact of numerous feeders.

After attaining maturity in from 6 to 20 days, the larvae migrate from a carcass or in myiasis cases drop from the wound and burrow into the soil or otherwise crawl under some object to effect pupation. Laake et al. (1936) reported the results of experiments with <u>C</u>. <u>macellaria</u> and <u>C</u>. <u>hominivorax</u> to ascertain how deeply the larvae would penetrate into several types of soil. The weighted mean depth of penetration was 1.35 inches for clay, 1.41 inches for heavy black soil, and 1.76 inches for sand. It is of interest to note that <u>C</u>. <u>hominivorax</u> in every case penetrated 100 percent deeper and in one case (clay) as much as 6 times deeper (1.35 inches vs. 7.77 inches). In this regard <u>C</u>. <u>macellaria</u> is adversely affected by lack of oxygen. In circumstances where the oxygen supply is seriously limited death of the larvae is quite rapid.

The larvae of <u>C</u>. <u>hominivorax</u> may be preved upon by ants after leaving the host (Lindquist 1942). It would necessarily follow that where <u>C</u>. <u>macellaria</u> and <u>C</u>. <u>hominivorax</u> occur together the former would also be subject to attack by ants.

Baumhover (1963) pointed out that the larvae of <u>C</u>. <u>hominivorax</u> are susceptible to desiccation if exposed. In the total absence of covering on the other hand, <u>C</u>. <u>macellaria</u> will pupate. The resultant pupal case is quite thin and light colored as compared with the normal and the emergence capabilities are considerably depressed.

The pupae of <u>C</u>. <u>macellaria</u> are dark brown in color and are smaller and more elongated than those of <u>C</u>. <u>hominivorax</u> (Laake et al. 1936). They average approximately 32.25 mg in weight and are about 10 mm long and 4 mm wide. The pupal stage under laboratory conditions lasts approximately three days.

The adult <u>C</u>. <u>macellaria</u> has a deep greenish-blue metallic color and may readily be distinguished from species belonging to other genera of blowflies in the United States by the characteristic yellow, orange or reddish face, and the three dark longitudinal stripes on the dorsal surface of the thorax (Hall 1948). The species differs most obviously from <u>C</u>. <u>hominivorax</u> in having the anterior portion of the parafrontale clothed with pale yellowish hair instead of black hair. It is generally smaller and less heavily built and the male genital structures are decidedly different (Hall 1948).

The preoviposition period according to Bishopp (1917) is from 3 to 18 days. Observations during the course of this research indicate the mean preoviposition period to be 10.9 days.

After emergence from the puparium the adult pushes its way to the substrate surface and remains quiet displaying periodic flexions of the wings and appendages. Under laboratory conditions this usually takes about 4 hours.

Copulation begins approximately 2 days following emergence. The life span of adult flies is about 1 month.

Under controlled laboratory conditions the length of the life cycle of <u>C</u>. <u>macellaria</u> requires about one half the time as that of <u>C</u>. <u>hominivorax</u> (Laake et al. 1936).

#### Antimony

The element antimony is a naturally occurring brittle, crystalline, silvery-white metal (Stecher 1968). Its abundance on the earth's crust is low, 0.2 ppm (Manson 1952). Antimony ore is mined in China, Mexico, and Bolivia. The element shows distinct metallic properties as compared to arsenic but possesses nonmetallic properties as well (Stecher 1968).

Antimony forms many compounds that are similar to the salts of arsenic, such as  $\text{Sb}_2\text{S}_3$  and  $\text{Sb}_2\text{B}_5$ , both of which are solids;  $\text{SbH}_3$ , a very poisonous compound called stibine; all trivalent and pentavalent halides except  $\text{SbI}_5$ ; and thio salts.

The discovery of antimony is lost in antiquity, the first accurate description being made in 1604 by Thölde (Stecher 1968). Ancient records indicate that the Chinese used antimony preparations as drugs in very early times (Beveridge 1963). The ancients employed the element as a cosmetic and as a component of ointments for diseases of the eyes and skin. The responsibility for its popularity in the 16th century goes primarily to Paracelsus but its usage declined thereafter until tartar emetic's trypanocidal effect was demonstrated in 1908 (Beveridge 1963). In 1912 Vianna used tartar emetic successfully against leishmaniasis in Brazil, and in 1915 Di Christina and Caronia used it effectively against kala-azar in Italy.

Antimony is widely used in the manufacture of alloys, such as Britannia or Babbitt metal, hard lead, white metal, type set, bullets and bearing metal; in fireworks; for thermoelectric piles, blackening iron and coating metals (Stecher 1968).

Extensive use of trivalent antimony compounds in the treatment of all three types of schistosome infections has been going on for more than 40 years. DeWitt (1965) indicated that a series of closely spaced injections of antimony were more effective against <u>Schistosoma mansoni</u> in mice than the same or greater total amounts of the drug administered in fewer injections. The reason for the increased activity he concluded was that more effective antimony levels were produced when a large number of closely spaced injections were used.

Similarly, Khayyal et al. (1968) found the antimony level which is responsible for the schistosome paralysis and a subsequent hepatic shift soon after administration of a low level dose to be the same as the level found in parasites returning to the mesenteric veins at a longer period after the higher dose. This investigator suggested that the same level of antimony could be either paralyzing or pharmacologically inactivating the worms according to the length of time that had elapsed since drug administration.

Girgis et al. (1967) established that adult <u>Schistosoma</u> <u>mansoni</u> worms concentrate more antimony from a primary injection than from subsequent inoculations. Tarrant et al. (1971) reported that the drug penicillamine reduced the acute toxicity as well as the therapeutic activity of antimony potassium tartrate by slowing the uptake of antimony from the blood by both tissues and schistosomes.

In addition to dosage levels, Luttermost and DeWitt (1961) estimated that the antimony drug stibophen was 4 to 16 times more effective in eliminating schistosomes in mice fed semi-synthetic diets than from those fed a crude commercial ration.

Farid et al. (1968) reported the results of treatment against urinary schistosomiasis to be 82% effective by using antimony sodium tartrate. These results were superior to any reported prior to that time for other antimony drugs.

The effects of 2 antimony drugs on <u>Onchocerca volvulus</u> were reported to be productive only at or above the normal level of human tolerance (Duke 1968). The uncertain action of the compounds on <u>O. volvulus</u> and the accompanying toxic manifestations render them unsuitable in the treatment of onchocerciasis.

Mansour et al. (1967) concluded that antimonials were the treatment of choice for bilharziasis, a parasitic disease prevalent in many tropical and subtropical areas of the world.

Similarly, Maleki (1967) recommended the use of antimony compounds, injected intramuscularly, as the treatment of choice for leishmaniasis of the skin. On the other hand, Hart et al. (1969) reported pentavalent antimony compounds to be successful against late cutaneous leishmaniasis when administered intralesionally and Dotrovsky and Cohen (1967) reported treatment of late cutaneous leishmaniasis by simultaneous intralesional steroids and intramuscular antimony to be successful. Ciplea et al. (1966) recommended the use of antimony potassium tartrate in treatment of trichinosis. He found a 100% cure rate with this drug at the end of 5 weeks post-treatment.

Aviado et al. (1968) found a sodium antimony salt of astiban which was originally developed for chemotherapeutic reasons other than for malaria was capable of suppressing <u>Plasmodium berghei</u> parasitemia in mice.

El-Torsali (1968) reported success in the treatment of human filarial lymphedema with antimony preparations.

Intravenous injections of tartar emetic are used in the treatment of ulcerating granuloma of the pudenda, rhinoscleroma, lymphogranuloma venereum, and Calabar swellings (Manson 1950).

In addition to the parasitological applications, antimony has been used, though sparingly, in other areas of medicine. Cortes (1968) reported on the use of a complex of iodized antimony and pyrazalone in treatment of gastroenterological conditions. The results were excellent or very good in all cases but one and no side effects were observed with the administered doses. The hydrated form of antimony potassium tartrate has been used as a medicinal emetic (Weast 1969).

A considerable amount of research concerning the action of antimony and its effects upon the body has been conducted within the last few years. Goodwin and Page (1943) determined that mice excreted 29.8% and 34.8% of the antimony contained in a dose of 6 mg tartar emetic/kg body weight, after 1 and 2 days respectively, when administered intraperitoneally. However, when a dose of 3.8 mg/kg was injected intravenously, the investigators found the excretion amounts over the same periods of time to be 61.5 and 68.5% respectively. Antimony is excreted chiefly in the urine (Rowland 1968).

In their work with dogs, Brady et al. (1945) found that after an intravenous injection of one dose of tartar emetic there was an initial rapid increase of antimony in the blood during the first hour following injection then the removal slowed for the next 4-16 hours. In some cases a secondary rise in the blood at 24 or 36 hours was apparent. An examination of the tissues showed the highest concentration in the liver with the thyroid and parathyroid ranked next. Rowland (1968) in work with <sup>124</sup>Sb-labelled sodium antimony dimercaptosuccinate administered intraperitoneally to mice confirmed the concentration of antimony in the liver and suggested that an active transport mechanism was involved in its entry into as well as loss from the organ. In subsequent investigations, Rowland (1968) reported that the uptake of antimony by the liver was rapid but loss from that organ was slower than from the rest of the body. Fifty to sixty percent had left the liver by 24 hours. This apparently disagrees with the findings of Mansour et al. (1967) which stated that the biological half-life of antimony in the liver is given as thirty-eight days. These investigators point out, however, that this figure is derived from indirect data and does not represent a true long term value. Smith (1969) measured the uptake of <sup>124</sup>Sb-labelled antimony potassium tartrate by mouse liver slices and found a high tissue/medium concentration ratio. Antimony uptake was not influenced by lack of oxygen, potassium, dinitrophenol or sodium arsenate.

In guinea pigs given trivalent antimony in doses of 2.5 and 0.25 mg/kg, Osintseva et al. (1966) found there were disturbances of the

functional state of the liver and the thyroid gland as determined in histochemical examination and in observations with the aid of radio-isotopes.

Amer et al. (1969) studied the effects of four antischistosomal drugs, including stibophen and tartar emetic, on the metabolism of kynurenine and found the antimony-containing drugs produced inhibition of both kynureninase and kynurenine transaminase.

Several studies in man and in experimental animals have shown that maximum blood levels of antimony occur within a few minutes following injection and that the concentration drops very rapidly to a low level within a few hours. Bartter et al. (1947) conducted a study of the fate of radioactive tartar emetic administered to human subjects and demonstrated the extreme rapidity with which removal of the drug from the blood takes place. Similarly, Rowland (1968) found that the antimony derived from astiban disappeared from the blood during the first 2 hours following injection. In another study Rowland (1968) determined the blood concentration of antimony expressed as antimony potassium tartrate at periods of 15 minutes to 21 days after doses of varying sizes were given intraperitoneally to mice. He concluded that the relationship between blood concentration (y), dose (d) and time (t) was  $y = Bd^{\alpha 1} t^{\alpha 2}$  and small yet significant interaction between dose and time was demonstrable. A further experiment confirmed the linear relationship but failed to prove the presence of interaction. Accordingly, DeWitt (1965) stated there was evidence that the peak blood level concentration may not correlate with the size of the administered dose. Antaki (1952) estimated the antimony blood levels in patients receiving daily stibophen injections and found the level of antimony in the blood was influenced little by the total amount given in 10 successive days although marked individual variation existed due probably to impaired excretion.

Abdel-Meguid et al. (1967) determined that tartar emetic, stibophen, and astiban caused a significant progressive decrease in the percent oxygen saturation of the arterial blood of the dog when given in a single dose equivalent to the individual dosage prescribed for man in antibilharzial therapy. This drop in percent oxygen saturation of the blood was evident 10 minutes after injection of tartar emetic but was more delayed with the other two compounds.

Industrial exposure to antimony dust may lead to irritation of the respiratory tract resulting in rhinitis, laryngitis, tracheitis, bronchitis and pneumonitis (von Oettingen 1958). Gudyovskii (1967) conducted an experiment with rats given intratracheal doses and inhalation treatment with metallic antimony for 2-4 hours per day for 6 months. The antimony dust caused profound morphological changes in the pulmonary parenchyma and the air passage walls. Specifically the antimony dust caused the development of focal endogenous lipids pneumonia. The seriousness as well as the speed of development of the pathological conditions in the lungs was dependent upon the antimony compound, concentration of the dust, the extent of physical stress and duration of exposure to the dust. The investigator determined that the greatest degree of pathology was exerted by metallic antimony dust followed by the trivalent antimony compounds. The least biological activity was produced by the pentavalent antimony.

As pointed out by Cooper and Pendergrass (1968), data concerning the lung pathology and chemistry of antimony in humans are lacking.

They stated that antimony pneumoconiosis appeared to be a benign process similar to that seen in siderosis, stannosis and baritosis.

Schroeder (1970) advised that although antimony in the air is not presently a real hazard to human health it does need careful control. Murthy et al. (1971), however, in determining levels of various trace elements in institutional total diets found the amount of antimony to be higher than cadmium or chromium and only slightly lower than cobalt or manganese.

Antimony has been shown to have a marked effect upon the mammalian heart. Cotten and Logan (1966) reported that antimony potassium tartrate and sodium antimony dimercaptosuccinate increased the heart rate of dogs progressively during the course of this study following repeated daily injections. Dogs receiving tartar emetic died after 3 to 5 days of treatment. Geometrically increasing doses of the compound administered to anesthetized dogs lowered the blood pressure and increased the heart rate progressively. Rowland (1968) determined the antimony concentrations in the heart muscle of mice and found it to be low 2 hours after injection following the blood level concentrations. Aviado et al. (1968) reported that WR7035 (a sodium antimony salt of astiban) increased the excitability of the atrial muscle and decreased cardiac output. Sapire and Silverman (1970) reported a severe case of myocardial involvement due to antimonial therapy. Von Oettingen (1958), however, stated that although there may be changes in the electrocardiogram during the therapeutic use of antimony compounds, they are largely of a transient nature and are not indicative of cardiac damage or serious impairment of the cardiac functions. Baetjer (1969) found that antimony produced a fall in the heart rate until an hour before death

when the rate increased sharply. Interestingly, she found in dehydrated mice that death from antimony injections occurred more rapidly and mortality was significantly higher than in the nondehydrated animals. It was also found in this study that exposure of rats and mice to high environmental temperatures,  $94^{\circ}$  F for 48 hours preceding and one week following injections of antimony, significantly increased their susceptibility to antimony toxicity. Similarly, Smith (1969) found the uptake of  $^{124}$ Sb-labelled antimony potassium tartrate by mouse liver slices to be reduced at low temperatures.

The toxicity of some of the antimony compounds may be quite high as can be seen from the  $LD_{50}$  values (Hawking 1963):

Compound	LD <sub>50</sub> (in mg/kg)		
A. + *	Intraveneous	<u>Intramuscular</u>	<u>0ra</u> 1
Antimony Potassium Tartrate	18.2	17.2	600
Antimony Sodium Tartrate	23.0		'
Stibophen	210	141	
Sodium Antimony Gluconate	56		
Stibamine		251	

Schroeder et al. (1968) in a study of the effects on growth, survival and tissue levels of several compounds incorporated into the diet found increments of antimony accumulated in the soft tissues. The element was also associated with a decrease in life span of most female mice and there was a suppression of growth in the older animals. In a subsequent study, Schroeder (1970) found innate toxicity in terms of life span and longevity with groups of rats given antimony in drinking water.

Girgis et al. (1967) in studying the chemotherapy of <u>Schistosoma</u> <u>mansoni</u> infected hamsters using stable and <sup>124</sup>Sb-labelled tartar emetic and astiban found that the female worms showed higher antimony concentrations than the male worms treated under identical conditions. The retention of the antimony by female worms was increased with subsequent inoculations. Malokhia and Smith (1968) found similar results with mice infected with <u>S. mansoni</u>. They could detect no localization of antimony in the female worms though in the males there appeared to be some concentration in the testes.

Duke (1968) reported that antimony had a lethal or sterilizing action on some or all adult female <u>Onchocerca volvulus</u> in tests against that organism.

Belyaeva (1967) in studies made with female rats on the effects of antimony on the reproductive function found the metal disturbed the reproductive function whether administered intraperitoneally or given as a dust. These disturbances were manifested in infertility and a reduction in the number of offspring produced and came as a result of disturbed ovogenesis in the ovaries. In a subsequent report Belyaeva (1969) found on examination of female workers in an antimony plant that they had a greater incidence of gynecological afflictions than shown by a control group (77.5% as compared to 56.0%). These workers showed more susceptibility to early interruption of pregnancy, particularly to spontaneous late abortions. Although no postnatal weight differences of the newborn infants could be detected, by the age of 3 months the plant workers' infants were perceptibly lagging behind the

control group in their weight. This lag was quite apparent by the time the infants reached 1 year. Antimony was detected in all female work<sub>7</sub> ers of the plant (0.5-20 mg%) in the blood, urine, milk, placenta and amniotic fluid.

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Bourgeois and Bueding (1971) by use of an intra vitam staining method, were able to detect damage to the reproductive organs of female <u>Schistosoma mansoni</u> within 5 minutes, 5-6 hours, 12 hours, and 3 days following administration of antimony potassium tartrate.

Many antimony compounds have been applied experimentally as insecticides including the arsenite, arsenate, lactate, oxide, oxychloride, sulfide, antimony potassium citrate, antimony calcium tartrate and antimony potassium tartrate (Shepard 1951).

By 1890 antimony potassium tartrate mixed with flour was recommended for poisoning the cotton leafworm, <u>Alabama argillacea</u> and in 1916 it became a component of poison ant baits (Shepard 1951).

Gilmore and Milam (1933) found antimony potassium tartrate to have promise as the toxic component of baits containing sugar sirup and isoamyl salicylate to attract the moths of the tomato and tobacco hornworms, <u>Protoparce</u> spp. Burdette (1934) then began applying this compound at an effective strength of 1.5 to 2 pounds in 50 gallons of sweetened water to kill corn earworm moths. At higher concentrations a diarrheal condition developed in the moth which caused reduced mortality due probably to increased rate of excretion.

Before the widespread usage of DDT, antimony potassium tartrate sprays were developed for control of the citrus thrips, <u>Scirtothrips</u> citri; the onion thrips, Thrips tabaci; for red spiders in greenhouses,

and in the Canal Zone, Florida and Australia for control of fruit flies (Shepard 1951).

Nelson and Weigel (1939) developed a spray to control gladiolus thrips, <u>Taeniothrips simplex</u> which contained antimony potassium tartrate, brown sugar and water.

Eckert (1940) determined that the minimum lethal dose of antimony potassium tartrate for the honey bee was between 3 and 6 micrograms per bee.

Studies of the effects of antimony on the reproductive organs of insects have been quite limited. Bosworth (1969) found no effects upon the reproductive organs of Culex quinquefasciatus Say by antimony potassium tartrate when the compound was incorporated into the larval rearing water. Abdel-Razig (1966) reported an inherent toxicity of triphenylantimony to Blatella germanica when fed in the diet at concentrations of 1.0, 2.0, and 5.0%. Simultaneous studies with the house fly, Musca domestica, however, revealed that the same concentrations of triphenylantimony fed to newly emerged flies increased both their fertility and fecundity. There were no adverse effects to the longevity of the treated insects. Rogers and Howell (1971) reported that antimony potassium tartrate and triphenylantimony were less toxic to in vitro engorging neonymphs of the common fowl tick, Argas radiatus (Oken) at the 0.001 and 0.0001% concentration levels (65.2-70.6% survival rate) than at concentrations of 0.01, 0.075, and 0.133% (6.7% survival rate). Adult ticks inuncted with a lanolin mixture containing triphenylantimony showed a decrease in the mean number of ova deposited by more than 50% and a decreased fertility rate of those produced by 19.1%. Histological examinations showed apparent gametic damage from

all concentrations of the triphenylantimony inunctum. The males showed that about 50% of their spermatids were wrinkled and shrunken and the females contained ootids that displayed cytoplasmic splitting and clumping.

### The Test Compounds of Antimony

<u>Antimony Barium Tartrate</u>. This compound is a white fluffy precipitate or powder developed by the Bureau of Animal Industry, Zoological Division, of the United States Department of Agriculture for treatment of gapeworm infections in birds. The birds are treated with the dust of this compound for a maximum of 15 minutes. Its chemical formula is given as  $Ba[(Sb0) C_4H_4O_6]_2 \cdot x H_2O$  (?) (Stecher 1968).

Antimony Potassium Tartrate. This compound is variously called tartar emetic, tartrated antimony, tartarized antimony and potassium antimonyl tartrate. Produced as a transparent powder, it is quite poisonous as indicated by the  $LD_{50}$  values mentioned previously. The chemical formula is  $C_4H_4K0_7Sb$  with antimony comprising 37.47%. It is readily soluble in water (Stecher 1968).

<u>Antimony Sodium Tartrate</u>. As a hygroscopic powder, this compound is readily soluble in 1.5 parts water. The antimony content of (SbO)  $NaC_4H_4O_6$  is in excess of 39.0% (Stecher 1968).

<u>Stibophen</u>. This compound has over 10 common or trade names, the most widely used being Fuadin<sup>®</sup>. It was introduced in 1929 for the treatment of schistosomiasis in Egypt (Manson 1950). The chemical formula is  $C_{12}H_4Na_50_{16}S_4Sb \cdot 7 H_20$  which contains not less than 15.6 and not more than 16.0% of trivalent antimony, calculated on a

moisture-free basis. It is prepared in fine crystals and is readily soluble in cold water (Stecher 1968).

<u>Triphenylantimony</u>. Triphenylantimony, variously called antimonyl triphenyl and triphenyl stibine, is a white crystalline material having a chemical formula of  $Sb(C_6H_5)_3$ . It is insoluble in water but very soluble in other organics such as benzene and ether (Lange 1949).

### MATERIALS AND METHODS

### Cochliomyia macellaria (Fabr.)

A parent fly colony was established from wild flies trapped at the canine experiment kennels near the entomology insectary in Stillwater, Oklahoma, using dead fish as an attractant. A number of <u>C</u>. macellaria (Fabr.) adults were brought into the laboratory and housed in cylindrical type cages (Figures 1, 2). They were maintained on a diet of granulated sugar. Each cage was provided with a constant water source using 200 ml Erlenmeyer flasks into which rolled paper toweling was inserted. The flasks were filled daily. The colony was maintained under laboratory conditions of  $26.7^{\circ}$  C  $\pm 2.8^{\circ}$  C and an environmental relative humidity ranging from about 40-70%. A constant light source provided 12 hours light and 12 hours darkness.

Adult testing was conducted in a large one unit, 132 cm x 132 cm test battery consisting of 25 individual test cages each of which measured 52 cm deep x 26.4 cm wide (Figure 3). The batteries were mounted on casters for easy movement and covered in front with plastic screen. A removable plexiglass strip 1 inch wide at the back of each bank of test cages allowed view from the back.

Several oviposition materials were tried in an effort to keep down unpleasant odors resulting from decomposition. Frings (1947) suggested the use of commercial kibbled dog biscuits both as an ovipositional medium and larval medium; however, this material proved unsuccessful

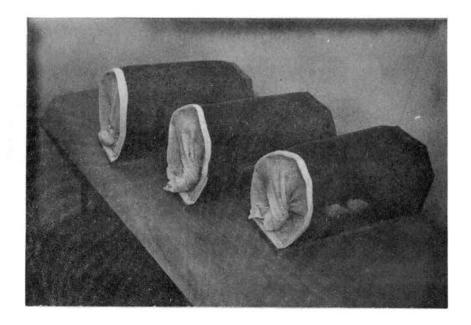


Figure 1. Cylindrical type cages used for housing the parent colony of <u>C</u>. <u>macellaria</u>.

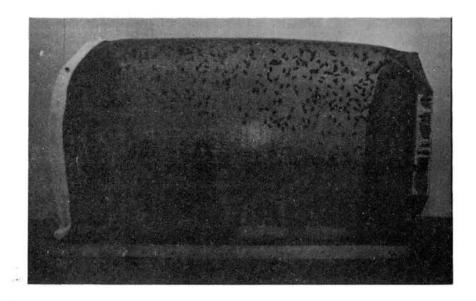


Figure 2. An individual parent colony cage showing method of watering and feeding.

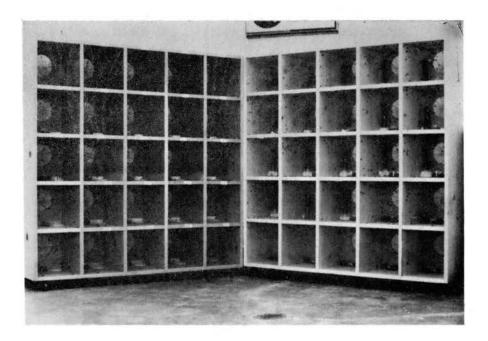


Figure 3. Test batteries consisting of 25 individual test cages.

for use with this species. Commercially prepared ground horse meat (Hill's) was selected to serve both as an ovipositional medium and protein source for the adult flies as well as a larval medium. This is a modification of the medium described by Graham and Dudley (1959).

Seven-day-old adult flies were offered freshly thawed ground horse meat in a standard type 80 x 40 mm crystallization dish (Figure 4). The material began to decompose and emit unpleasant odors in about 48 hours. Eggs were usually laid underneath the medium or within indentations or crevices on the surface which offered some degree of protection.

If the eggs were to be used for test purposes they were removed from the ground horse meat by forceps and placed in a 2% NaOH solution for 15 to 20 minutes. This separated the eggs from each other and from traces of meat. The NaOH was decanted from the eggs and they were rinsed in 70% ethyl alcohol. This alcohol was then poured off and an additional aliquot of 70% ethyl alcohol was added and allowed to stand for 15 minutes. The eggs were withdrawn from the ethanol by a tapered pipette calibrated to deliver a given number.

Following oviposition the medium containing the recently oviposited eggs was transferred to glass gallon jars filled to one-fourth capacity with damp wood shavings (Figure 5). From 85 to 170 grams of horse meat were placed in the jar with the eggs, the amount depending upon the number of eggs that had been oviposited. Each jar contained approximately 5,000 larvae. Approximately 170 grams of horse meat were provided daily to the larvae in each jar for the first two instars and approximately 226 grams were provided for the final instar.

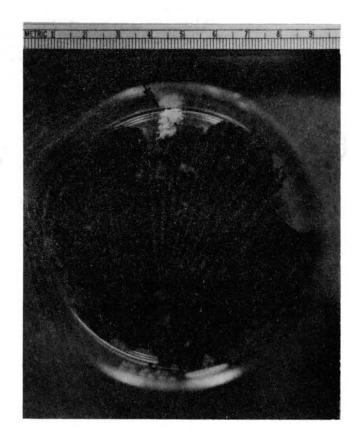


Figure 4. Oviposition medium with egg mass exposed to show its relative size.

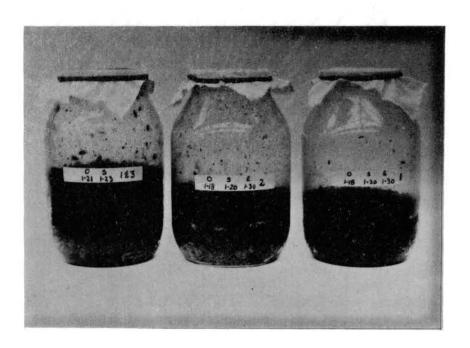


Figure 5. Jars used for larval rearing.

When the fully grown larvae are ready to pupate their migrating activity becomes quite pronounced and movement begins around the sides of the larval container. At this time the jars were filled to about one-half capacity with damp wood shavings. This provided a cool, drier area into which the larvae migrated rapidly to effect pupation.

Pupae were normally floated out of the larval medium by placing the contents of the larval containers into a porcelain enameled metal pan and running tepid tap water over it. The pupae float while the wood shavings readily sink (Figure 6). The pupae were then dipped off the water with a strainer and placed back into the cleaned glass container from which they came. Some cultures were allowed to emerge within their larval and pupal medium and no observable differences could be detected between these and the ones that had been floated out.

The newly emerged flies crawl up the sides of the glass container to harden their exoskeletons. For those emerging from the old larval medium adequate humidity is normally present so that the wings are fully formed. However, damp paper toweling was added in with those pupae that had been floated free of the medium to insure adequate humidity for proper wing development. As the flies emerged, hardened their exoskeletons and blew their wings out, they were periodically allowed to escape into the adult holding cages, or into trap cages if they were to be used for test purposes (Figure 7).

# Methods of Inactivation

Several methods were tried in an effort to inactivate adult flies. Carbon dioxide gas was an excellent material to effect knock-down but when it became necessary to keep them under for prolonged periods,



Figure 6. Pupae floated in an enamel pan showing the method of recovery from the larval and pupal medium.

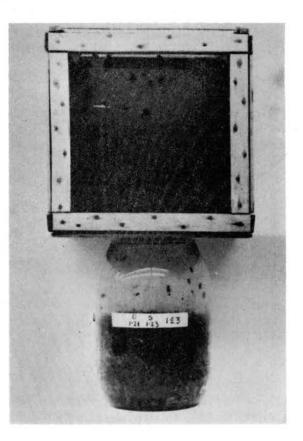


Figure 7. Trap cages showing recovery of emerging flies to be used for test purposes. mortality was high. Ethyl ether was also an excellent knock-down material but mortality was higher even for shorter periods of time.

Cold was the method of choice and proved to be as effective as the others in knock-down, with a faster recovery rate and little to no mortality. Inactivation was accomplished by placing a trap cage containing the adult flies in a refrigerator freezing compartment for about 3-5 minutes during which time the flies were totally inactivated. They were then brushed into a porcelain pan nested in another larger pan that had been filled with crushed ice, rock salt and water. One such set-up could be used to inactivate flies over a period of about 10 hours.

#### Treatments

The compounds tested were reagent grade antimony potassium tartrate, antimony sodium tartrate, antimony barium tartrate, triphenylantimony and stibophen. The first three compounds were purchased from The British Drug Houses, Laboratory Chemicals Division, Poole, England. The triphenylantimony was purchased in the United States from Fischer Scientific Co. and the stibophen (Fuadin<sup>®</sup>) was provided courtesy of Dr. F. C. Nachod of the Sterling-Winthrop Research Institute of Rensselaer, New York.

These compounds were administered to the flies by: (1) adult feeding of the solid compound; (2) injection of an aqueous solution of the compound into adult flies; (3) dusting the adults with the undiluted compounds; (4) dipping the puparium containing a pupa in aqueous solutions of the compounds; (5) inuncting puparia containing pupae with a triphenylantimony-lanolin mixture; (6) dipping eggs in an aqueous

solution of antimony potassium tartrate; (7) feeding larvae the solid compound incorporated into the larval medium; and (8) dipping 1st and 2nd larval instars in aqueous solutions of the compounds.

### Adult Feeding

This procedure involved the incorporation of the solid antimony compounds into a mixture with granulated sugar. The concentrations of chemicals used ranged from a 0.01% weight/weight ratio to 5.0%.

The desired amount of antimony compound was weighed and thoroughly mixed with the proper amount of granulated sugar in the bottom portion of a standard 100 x 20 mm plastic petri dish. All of the tested compounds were of the same color as granulated sugar and all were generally of the same texture so a thorough mixing of the two materials was necessary to insure uniformity.

Twenty-five adult females and 25 adult males, all less than 24 hours old, were placed into test cages and provided the test mixture and a supply of water. Three replicates of each concentration were made and a control received granulated sugar only as a food source. Checks for mortality were made daily.

At the end of 7 days, ground horse meat was provided to serve as an oviposition medium as well as a protein source and daily checks were made thereafter for oviposition. When oviposition was accomplished, the number of eggs produced was estimated then placed into quart mason jars filled to one-fourth capacity with damp wood shavings and covered with unbleached muslin secured with rubber bands. The developing larvae were provided with from 85 to 170 grams of ground horse meat daily depending upon the number of developing larvae in the jar. They were allowed to go through the larval and pupal stages and emerge as adults. The adults were allowed to die and then counted.

# Adult Injection

This treatment method necessitated the use of two persons and involved the injection of varying concentrations of the candidate compounds into the abdomen of newly emerged adult flies (Figure 8).

Ten females and 5 males all less than 24 hours old were injected through a ventral posterior intersegmental membrane of the abdomen with the three readily-soluble antimony compounds: antimony potassium tartrate, antimony sodium tartrate and stibophen. Attempts were made to insure that each test insect received the injection at about the same position. The test compounds were injected with a microliter syringe equipped with a 2 inch, 32 gauge needle (Popper & Sons, Inc., N. Y., N. Y.). Each insect received 1 microliter. The concentration of test compound in distilled water varied from a weight/volume ratio of 0.01% to 5.0%. A control group was injected with distilled water only and a check received no compound injection although the cuticle was punctured and the needle inserted as in the test groups.

This test method has been used recently to determine the utilization of injected glucose by the tsetse fly and stable fly (Nayar and Handel 1972).

Following injection the flies were placed into test cages and provided with granulated sugar and a water source. They were checked daily for mortality. At the end of 7 days post-treatment the flies were offered an oviposition medium of freshly thawed ground horse meat. Upon oviposition the medium was removed and the number of eggs

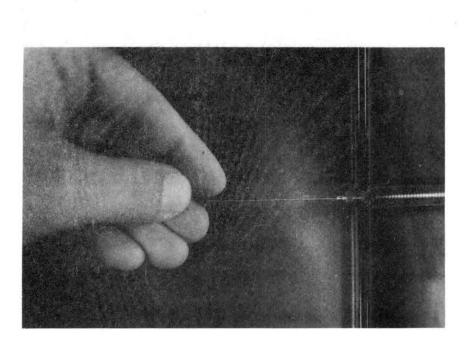


Figure 8. The adult injection treatment showing the microsyringe, the method of holding the fly and the point of insertion of the needle. estimated. The eggs were then placed into quart mason jars filled to one-fourth capacity with damp wood shavings, covered with unbleached muslin and secured with rubber bands. The developing larvae were provided with ground horse meat until pupation. The number of emerging adults was determined.

### Adult Dusting

Adult flies, less than 12 hours old, were allowed to walk on undiluted thin layers of the antimony compounds applied to the sides of wide mouth quart mason jars. After being exposed to the test compounds for 5, 15 or 30 minutes, excess dust adhering to the flies was removed by allowing them to walk around in a clean jar. They were then placed in a holding jar containing moistened filter paper to further remove the dust.

The treated flies were transferred to test cages and provided with granulated sugar and water. Mortality checks were made at 24, 36, 48, 72 and 96 hours, then daily thereafter.

Oviposition medium was provided on the 7th day post-treatment and the number of eggs and their hatchability was checked. Two replicates of each compound were made and a control received no treatment.

# Pupal Dipping

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The three readily-soluble compounds, antimony potassium tartrate, antimony sodium tartrate and stibophen, were used in this treatment. Volume/volume concentrations of test compound and distilled water were 0.05, 1.0, 2.0, 5.0 and 10.0%. A control group was dipped in distilled water only. <u>C. macellaria</u> (Fabr.) puparia containing pupae approximately 24 hours old were wrapped in gauze squares and completely submerged in the test solutions for 5, 15, or 25 minutes. They were then removed and placed on filter paper until dry. The dried puparia were then placed into pint food cartons, maintained at  $26.7^{\circ}$  C  $\pm 2.8^{\circ}$  C and allowed to emerge. Observations on the newly emerged flies were made to determine if any gross morphological malformations had resulted from the treatment. The number of successfully emerging adults was recorded and the development time was calculated for each test group.

No attempt was made to determine the number of offspring produced by the treated individuals but only if they were capable of producing fertile eggs.

## Pupal Inunction

The compound tested was triphenylantimony using lanolin to serve both as a solvent and a carrier. The remaining antimony compounds with the exception of antimony barium tartrate are readily soluble in water and the waxy nature of the pupal case would not allow any degree of absorption.

Randomly selected puparia were floated from the pupation media as previously described, washed 3 times in tap water, and spread on paper toweling and allowed to dry overnight.

Weights of triphenylantimony providing concentrations of 0.05, 0.10, 0.50, 1.0 and 1.5% were thoroughly mixed with 2.0 grams of lanolin and allowed to stand overnight (about 12 hours). The test batches were mixed again immediately prior to inunction.

Two replicates of each concentration were made and a control group was inuncted with lanolin only. A check against the lanolin was made in which the pupae received no treatment.

A thin line of the test mixture was drawn around the pupal case in a ring roughly corresponding to the first two abdominal segments with the broad end of a flat toothpick (2.3 mm). The treated puparia were then placed onto filter paper that had been pre-cut to fit into a 250 ml jar and just resting on damp wood shavings that filled the jar to one-half capacity. They were covered with a muslin cloth top and maintained at  $26^{\circ}$  C  $\pm 2.8^{\circ}$  C until emergence. The only exception to this treatment was that one group received complete inunction; that is, the lanolin based triphenylantimony completely covered the pupal case.

The mean weight of the treated puparia containing pupae was 33 mg with the largest being 35 mg and the smallest 30 mg and each received approximately 12 mg of the triphenylantimony and lanolin mixture.

As the adults emerged they were placed in test cages and maintained on granulated sugar and water until 7 days post-emergence at which time they were offered horse meat as an ovipositional medium. Postemergence mortality was recorded daily and the number of eggs deposited was determined.

# Larval Feeding

The effects of antimony compounds upon the growth, development, and offspring production capabilities of <u>C</u>. <u>macellaria</u> (Fabr.) larvae were determined by feeding the test compounds incorporated into the larval medium. Mixtures of the test compounds and ground horse meat were prepared in weight/weight concentrations of 0.01, 0.05, 0.10, 0.50, 1.0, and 5.0%. The ground horse meat was placed in a standard Sunbeam blender and the test compounds were blended in. The mixture was placed on top of damp wood shavings filling about one fourth of a quart mason jar. Fifty 1st and 2nd (mostly 2nd) instar larvae were placed in each of the quart jars, covered with unbleached muslin secured with rubber bands. The larval jars were maintained under laboratory conditions. Two replicates of each compound were made and a control received no antimony compound but was handled in the same manner otherwise.

The treated larvae were observed daily for growth and development. They were carried through the pupal stage and observations were made at the time of adult emergence to determine if any resulting morphological effects could be detected. The successfully emerging adults were placed in test cages and provided with granulated sugar and drinking water. Post-emergence mortality was recorded daily.

On the 7th day post-emergence the adult flies were provided with ground horse meat on which to oviposit and the number of eggs produced and their percent hatch was determined.

# Larval Dipping

The larval dipping treatment was conducted with the three readilysoluble compounds, antimony potassium tartrate, antimony sodium tartrate and stibophen. Each compound was tested in distilled water weight/volume concentrations of 0.05, 0.10, 1.0, 5.0, and 10.0%. A control group of distilled water only was used and a check against the distilled water received no treatment.

The stibophen which is readily soluble in cold water was mixed at  $5^{\circ}$  C and treatment was accomplished when the temperature reached  $20^{\circ}$  C.

First and 2nd instar larvae were randomly selected from the maintenance colony and placed into 200 ml beakers containing the test solution and allowed to remain for 5, 15 or 30 minutes. At the end of each time period the test compound and larvae were poured through a funnel holding filter paper and the larvae were counted from the filter paper.

The treated larvae were placed into wide mouth quart mason jars filled to one-fourth capacity with damp wood shavings, covered with unbleached muslin and secured. They were maintained under laboratory conditions for 72 hours at which time the remaining medium was thoroughly searched for 3rd instar larvae. The number of 3rd instar larvae was recorded and they were replaced in the development jars. At the time of pupation the medium was again searched for pupae. The number of pupae was recorded and again replaced in the rearing jars.

The successfully emerging adults were allowed to emerge into test cages and provided granulated sugar and drinking water. Mortality was recorded daily. On the 7th day post-emergence the flies were offered oviposition medium of ground horse meat and the number of eggs resulting was determined.

## Egg Dipping

The effects of aqueous solutions of antimony potassium tartrate on <u>C. macellaria</u> (Fabr.) eggs were studied by dipping the eggs in distilled water containing the compound for periods ranging from 1 minute to 25 minutes. All eggs had been oviposited for less than 12

hours at the time of treatment. Eggs were dipped in test concentrations of 0.5, 0.4, 0.3, 0.2, 0.1 and 0.05%. One group was dipped in distilled water only to serve as a control while a check against the distilled water received no treatment.

At the end of each time period each group of eggs was removed from the test solutions and allowed to dry on filter paper. Each group was then placed onto about 14 grams of ground horse meat which was placed into a 225 ml capacity jar containing wood shavings and covered with unbleached muslin.

The number of hatched eggs was determined by counting the resulting larvae after incubating for 48 hours. These larvae were carried through to adulthood and allowed to oviposit. The number of offspring resulting was determined.

Due to the high number of uncontrollable variables inherent in this type of test no other antimony compounds were tested in this manner.

# Histological studies

Histological sections of both male and female reproductive systems were made to detect any morphological damage induced by the antimony compounds. After oviposition was completed in a test or control group, randomly selected members of each sex were removed and their gonads were dissected out in a normal saline solution. The gonads were fixed in Carnoy's fixative for 1 hour (Humason 1962), washed in 70% ethyl alcohol and dehydrated up the alcohol series. They were infiltrated in thin and thick celloidan each for 2 days. The gonads were removed from the celloidan, mounted on a glass slide with another aliquot of thick celloidan and dried in chloroform for 30 minutes.

The gonadal tissue was chipped out of the celloidan block, retaining a portion immediately around the tissue, and embedded in medium paraffin.

The double-embedded tissue was serially sectioned at 6 to 10 microns and mounted on microscope slides. The mounted sections were stained with hematoxylin and eosin, run through the alcohol series and mounted with Permount.

### RESULTS AND DISCUSSION

#### Adult Feeding

The effects on mortality, fecundity and fertility of <u>C</u>. <u>macellaria</u> adults fed the selected antimony compounds are presented in Tables 1-5. Fecundity is represented graphically in Figure 9. Antimony potassium tartrate proved to be the most toxic (22.2% mean mortality) of the compounds followed in order by stibophen, antimony sodium tartrate, triphenylantimony and antimony barium tartrate.

Total mortality caused by antimony potassium tartrate showed a direct linear relationship with concentration (Table 1). The changes in antimony sodium tartrate toxicity did not become apparent until the 5.0% concentration level was reached, with no significant difference (5% level) in toxicity experienced between the lower levels. At the 5.0% concentration level, however, the effects were marked with a significant increase in total mortality from 16.0% to 59.3% (Table 2).

Triphenylantimony showed a similar toxicity response but the changes at the highest levels were not as profound as with antimony sodium tartrate (Table 3). These results do not agree with those of Abdel-Razig (1966) who reported zero mortality of adult house flies fed a diet containing 5.0% triphenylantimony in powdered milk and sugar. <u>C. macellaria</u> experienced a 46.0% mortality rate at the 5.0% triphenylantimony level and it could be postulated that a substantial increase in dosage percentages would similarly increase mortality. Two

Per Cent	M	Mortality <sup>a</sup>			Per Cent	Eggs Per	% Egg Reduction From
Conc.	М	F	Т	Eggs <sup>a</sup> Produced	Hatch <sup>a</sup>	Female	Control
.01	1.3	6.7	4.0	4806	96.3	68.7	-10.1
.05	5.3	9.3	7.3	4925	97.0	71.3	-6.7
. 10	4.0	4.0	4.0	4442	95.4	61.7	-19.2
1.0	21.3	6.7	14.0	1697	96.4	24.2	-68.3
2.0	81.3	24.0	52.7	1287	93.7	22.6	-70.4
5.0	100	90.7	95.3	0	-	0.0	-100.0
Control <sup>a</sup>	8.0	14.7	11.3	4816	96.5	76.4	-
x <sup>b</sup>	35.6	23.6	29.6	2859.5	95.8	50.8	-33.5

Table 1. Effects on mortality, fecundity and fertility of  $\underline{C}$ . <u>macellaria</u> adults fed antimony potassium tartrate in mixtures with granulated sugar.

<sup>a</sup>Total for all replicates

Per Cent Conc.	M	Morta F	lity <sup>a</sup> T	Eggs <sup>a</sup> Produced	Per Cent Hatch <sup>a</sup>	Eggs Per Female	% Egg Reduction From Control
.01	20.0	18.7	19.3	4254	96.3	63.5	-16.9
.05	21.3	5.3	13.3	4621	90.2	64.1	-16.1
.10	18.7	6.7	12.7	4398	89.9	61.1	-20.0
1.0	13.3	12.0	12,7	469	28.0	7.1	-90.7
2.0	22.7	9.3	16.0	0	-	0.0	-100.0
5.0	70.7	48.0	59.3	0	-	0.0	-100.0
Control <sup>a</sup>	8.0	14.7	11.3	4816	96.5	76.4	-
⊼ <sup>b</sup>	27.8	16.7	22.2	2290.3	76.1	35.8	-53.1

Table 2. Effects on mortality, fecundity and fertility of  $\underline{C}$ . <u>macellaria</u> adults fed antimony sodium tartrate in mixtures with granulated sugar.

<sup>a</sup>Total for all replicates

Per Cent		Mortality <sup>a</sup>			Per Cent	Eggs Per	% Egg Reduction From
Conc.	М	F	Т	Eggs <sup>a</sup> Produced	Hatch <sup>a</sup>	Female	Control
.01	6.7	2.7	4.7	4380	94.0	60.8	-20.4
.05	6.7	5.3	6.0	4159	98.0	58.6	-23.3
.10	6.7	9.3	8.0	3877	97.6	55.4	-27.5
1.0	6.7	4.0	5.3	4345	93.7	60.3	-21.1
2.0	18.7	16.0	17.3	4784	98.6	73.6	-3.7
5.0	57.3	34.7	46.0	2890	98.6	54.5	-28.7
Control <sup>a</sup>	8.0	14.7	11.3	4816	96.5	76.4	<b></b>
<b>x</b> <sup>b</sup>	16.8	12.0	14.4	4072.5	96.8	60.6	-20.7

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Table 3. Effects on mortality, fecundity and fertility of  $\underline{C}$ . <u>macellaria</u> adults fed triphenylantimony in mixtures with granulated sugar.

<sup>a</sup>Total for all replicates

Per Mor Cent		lortality	a	Eggs <sup>a</sup>	Per Cent	Eggs Per	% Egg Reduction From
Conc.	М	F	Т	Produced	Hatcha	Female	Control
.01	2.7	10.7	6.7	4781	93.0	69.3	-9.3
.05	25.3	28.0	26.7	2291	94.7	41.7	-45.4
.10	21.3	45.3	33.3	837	92.8	27.9	-63.5
1.0	36.0	30.7	33,3	1567	92.0	27.9	-63.5
2.0	34.7	13.3	24.0	1732	88.6	26.6	-65.2
5.0	33.3	20.0	26.7	1544	89.8	25.9	-66.1
Control <sup>a</sup>	8.0	14.7	11.3	4816	96.5	76.4	-
⊼ <sup>b</sup>	25.6	24.7	25.1	2125.3	91.8	38.1	-50.1

Table 4.	Effects	on mortality,	fecundity and fert	ility of C.
macellari	a adults	fed stibophen	in mixtures with g	granulated sugar.

<sup>a</sup>Total for all replicates

 $^{\rm b}{\rm Mean}$  for all concentrations excluding control

Per	Mo	ortality <sup>a</sup>		Eggs <sup>a</sup>	Per Cent	Eggs Per	% Egg Reduction From
Cent Conc.	М	F	Т	Produced	Hatch <sup>a</sup>	Female	Control
.01	10.7	10.7	10.7	3645	96.8	52.8	-30.9
.05	12.0	4.0	8.0	4593	98.9	62.9	-17.7
.10	9.3	4.0	6.7	5881	98.9	81.7	+6.9
1.0	8.0	10.7	9.3	2883	96.3	42.3	-44.6
2.0	9.3	6.7	8.0	4519	94.2	64.6	-15.4
5.0	14.7	6.7	10.7	960	90.7	13.7	-82.1
ontrol	a 8.0	14.7	11.3	4816	96.5	76.4	-
<b>⊼</b> b	10.7	7.1	7.8	3738.5	95.9	53.3	-30.2

Table 5. Effects on mortality, fecundity and fertility of <u>C</u>. <u>macellaria</u> adults fed antimony barium tartrate in mixtures with granulated sugar.

<sup>a</sup>Total for all replicates

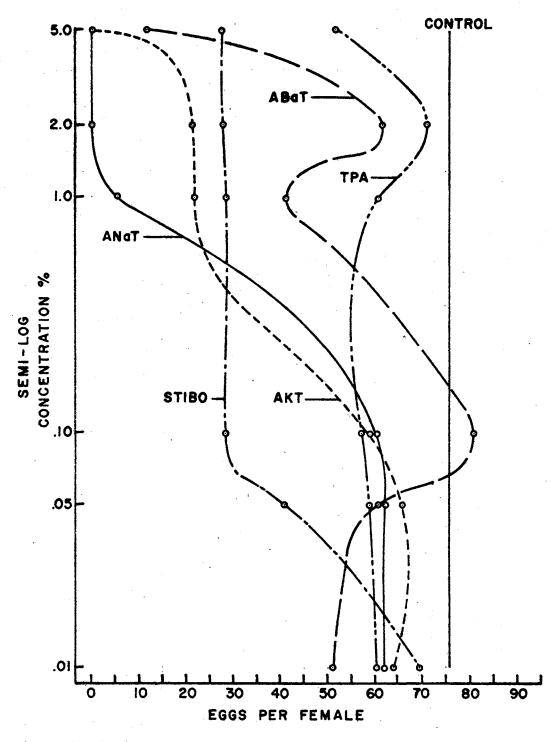


Figure 9. Number of eggs per female resulting from adults fed antimony barium tartrate (ABaT), antimony potassium tartrate (AKT), antimony sodium tartrate (ANaT), stibophen (STIBO) and triphenylantimony (TPA).

possible explanations for this disparity could be (1) inherent species resistance to the compound, or (2) the possible introduction of a trace element antagonistic to triphenylantimony by incorporation of powdered milk into the diet. Kar et al. (1960), Supple (1961), and Powell et al. (1964), among others, demonstrated the antagonistic behavior of various compounds to cadmium which ameliorated gonadal damage resulting from that element. It would therefore logically follow that compounds or elements exist that would demonstrate a similar antagonism to triphenylantimony although no such compounds have been reported. Additional research would be necessary in order to clarify these points.

Although stibophen showed relatively high toxicity percentages there were no obvious differences between concentrations above the 0.01% level (Table 4). Similarly, no significant differences in percentage mortality between all levels of antimony barium tartrate were observed (Table 5).

Antimony sodium tartrate was the only compound that had an obvious effect on the hatch of the eggs resulting from treated adults (Table 2). The mean percent hatch of 76.1% was due primarily to the 28.0% observation experienced at the 1.0% compound level. It would be interesting to know what effect the compound might have at a higher concentration but in these tests no eggs were produced above the 1.0% level. All compounds showed lower mean percent hatch than the control but in no case were the differences significant.

Fecundity of the test insects receiving antimony treatment followed generally the same pattern as toxicity. Eggs per female, based on the total offspring produced and the number of surviving females, were lowest for antimony sodium tartrate (mean of 35.8 eggs

per female), followed closely by stibophen (mean of 38.1), antimony potassium tartrate (mean of 50.8) and antimony barium tartrate (mean of 53.3) (Figure 9). Triphenylantimony allowed the greatest number of offspring per female at a mean of 60.6 eggs per female.

The percent egg reduction from the control with antimony potassium tartrate, as with toxicity, showed a direct linear relationship with concentration although not as pronounced as in the former case, ranging from a low of -6.7% at the 0.05% concentration level to a high of -100% at the 5.0% level. There was no significant difference between levels 0.01%, 0.05% and 0.10% nor between the 1.0% and 2.0% concentrations, however, significance was evident between the lower 3 concentrations, the higher 2 concentrations (1.0% and 2.0%) and the highest concentration (5.0%).

There was no significant difference between the first 3 levels (0.01%, 0.05%, 0.10%) of antimony sodium tartrate but significance appeared between these and the remaining 3 levels of concentration. The sharp increase between the 0.10% concentration and the 1.0% concentration would be expected with a compound possessing sterilizing capabilities since there was a 10 fold increase in concentration of the compound. Stibophen showed no significant differences between concentrations above the 0.01% concentration level although all percent egg reductions from the control were high (Table 4).

A great deal of variation in percent egg reduction from the control was apparent with antimony barium tartrate ranging from a difference of +6.9% at the 0.10% concentration level to -82.1% at the 5.0% concentration with a resultant mean of -30.2% (Table 5). The 0.10% concentration showed a significant difference from the control; the

0.5% concentration was not significant nor was the 0.10% concentration, then an abrupt upswing brought back significance at the 1.0% level, back down at the 2.0% concentration then a sharp increase to significance again at the 5.0% concentration. No explanation can be offered for the wide variability.

Percent egg reduction from the control with triphenylantimony on the other hand showed very little variability in the pattern with no significance existing between all concentration levels. All concentrations with the exception of the 2.0% level followed a relatively uniform pattern resulting in a mean reduction from the control of -20.7%. These data are again in disagreement with the results obtained by Abdel-Razig (1966). He reported that house flies fed a diet containing 2.0% triphenylantimony produced 30% more eggs than the controls. <u>C. macellaria</u> produced 3.7% fewer eggs than the controls when fed 2.0% triphenylantimony. It is interesting to note, however, that this level (2.0% concentration) was the only one which did not fit the uniform pattern alluded to previously. Experimental error could account for some of the variability in results but probably not to account for the total difference from -3.7% for <u>C. macellaria</u> to +30.0% for <u>M. domestica</u>.

Histological preparations indicated that the oocytes from those females receiving 2.0 and 5.0% concentrations of antimony potassium tertrate and antimony sodium tartrate apparently never reached maturity. The oocytes from those treated females were quite small as compared to those of the controls and the other treated flies except for the 5.0% concentration of antimony barium tartrate. The oocytes from the flies treated with 5.0% antimony barium tartrate were intermediate

in size between those treated with the higher concentrations of antimony potassium tartrate and antimony sodium tartrate and the controls.

The nurse cells were apparent, at all stages of vitellogenesis and maturation of the oocyte, and could be seen in various stages of degeneration as expected in the controls and at all concentrations of stibophen, triphenylantimony and antimony barium tartrate. In the 2.0 and 5.0% concentrations of antimony potassium tartrate and antimony sodium tartrate, however, the nurse cells were quite large in the first egg chamber indicating that growth of the oocyte had stopped and no eggs were produced as a consequence.

At the higher concentrations of antimony potassium tartrate and antimony sodium tartrate only the oocyte in the first egg chamber was apparent; no development in the second or third egg chambers was observed. These observations are apparently much like those made by La Chance and Leverich (1968) with <u>C</u>. <u>hominivorax</u> treated topically with an alkylating agent. Wright et al. (1971) found a similar situation in that a steroid, 20-hydroxyecdysone inhibited ovarian maturation in the stable fly when given by mouth. They found the egg chambers of both treated and untreated flies grew at a similar rate during the previtellogenic period but the egg chambers in the treated flies abruptly stopped growth thereby rendering those females sterile.

That oocyte growth stopped was further suggested by the thickness of the tunica propria of the ovarioles which is initially quite thick but becomes stretched and very thin as the oocyte develops. Sections at the higher concentrations of antimony potassium tartrate and antimony sodium tartrate revealed that the tunica propria remained

quite thick around each ovariole apparently indicating that little growth of the oocyte had occurred.

Obviously in order to determine the exact nature of these antimony compounds' action on ovarian growth, additional research is necessary.

### Adult Injection

The effects of antimony compounds injected into the test insect are illustrated in Tables 6-8. Antimony potassium tartrate proved to be the most toxic of the three tested compounds with a mean mortality of 64.7% as compared with 21.5% for the control group and 0.0% for the check (Table 6). In this regard it is important to note that a significant difference existed between the control group which received injections of distilled water and the check which was punctured but did not receive an injection.

Stibophen was second to antimony potassium tartrate in displaying toxic effects (Table 7). Total mean mortality was only slightly lower for stibophen (52.7%) than for antimony potassium tartrate and as with this compound was significantly higher than for the control or check.

Antimony sodium tartrate was the least toxic of the tested compounds but the differences in mortality were not appreciable between any of the three compounds (Table 8) (Figure 10).

The rapid onset of the toxic effects of antimony potassium tartrate and antimony sodium tartrate were striking as shown by day one post-treatment mean mortality percentages of 47.8% and 38.4% respectively. Stibophen toxicity, on the other hand, was more gradual with only a 4.0% post-emergence mean mortality on day one and increasing to a final day twelve post-emergence mortality mean of 52.7%.

	Percent Concentration							
	.01	.10	.50	1.0	5.0	Control	Check	
Post- Treatment Mortality Per Day %	Y				i			
1	0.0	0.0	66.6	85.7	86.7	0.0	0.0	
2	0.0	0.0	6.7	0.0	0.0	0.0	0.0	
3	0.0	0.0	0.0	0.0	6.6	0.0	0.0	
4	0.0	12.5	6.7	0.0	0.0	0.0	0.0	
5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
7	0.0	0.0	0.0	7.1	0.0	7.2	0.0	
8	0.0	6.2	0.0	0.0	0.0	0.0	0.0	
9	6.6	6.2	0.0	0.0	0.0	0.0	0.0	
10	6.6	0.0	0.0	0.0	0.0	0.0	0.0	
11	0.0	0.0	6.7	0.0	0.0	0.0	0.0	
12	0.0	6.2	6.7	0.0	0.0	14.3	0.0	
Total Mortality % <sup>a</sup>								
М	0.0	12.5	26.7	35.7	33.3	14.3	0.0	
F	13.2	18.6	66.7	57.1	60.0	7.2	0.0	
<u> </u>	13.2	31.1	93.4	92.8	93.3	21.5	0.0	
Total Offspring								
Eggs	729	461	0	0	0	844	1133	
Adults	708	447	0	0	0	828	1130	
<u>% Hatch</u>	97.1	96.9		-	<b></b>	98.1	99.7	
Eggs/F	91.1	57.6	0.0	0.0	0.0	93.8	125.8	

Table 6. Post-treatment mortality per day, total offspring, percent hatch and number of eggs per female of adults injected with aqueous solutions of antimony potassium tartrate.

<sup>a</sup>Male, female and total mortality

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<u></u>	Percent Concentration							
· · · · · · · · · · · · · · · · · · ·	.01	.10	.50	1.0	5.0	Control	Check	
Post- Treatment Mortality Per Day %								
1	0.0	0.0	0.0	0.0	20.0	0.0	0.0	
2	7.2	5.9	0.0	0.0	53.2	0.0	0.0	
3	0.0	23.5	26.6	61.5	6.7	0.0	0.0	
4	0.0	5.9	6.6	15.4	0.0	0.0	0.0	
5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
6	0.0	0.0	0.0	0.0	6.7	0.0	0.0	
7	0.0	0.0	0.0	0.0	6.7	7.2	0.0	
8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
11	0.0	0.0	6.6	0.0	0.0	0.0	0.0	
12	0.0	0.0	13.2	7.7	0.0	14.3	0.0	
Total Mortality % <sup>a</sup>								
M	0.0	11.8	20.0	23.1	33.3	14.3	0.0	
F	7.2	23.5	33.3	61.5	60.0	7.2	0.0	
<u> </u>	7.2	35.3	53.3	84.6	93.3	21,5	0.0	
Total Offspring								
Eggs	640	902	298	0	0	844	1133	
Adults	640	900	294	0	0	828	1130	
<u>% Hatch</u>	100.0	99.8	98.7			98.1	99.7	
Eggs/F	71.1	90.2	49.7	0.0	0.0	93.8	125.8	

Table 7. Post-treatment mortality per day, total offspring, percent hatch, and number of eggs per female of adults injected with aqueous solutions of stibophen.

<sup>a</sup>Male, female and total mortality

	<u> </u>	- , <u>, ,</u> ,, , , ,, ,,		***********				
	Percent Concentration							
	.01	.10	.50	1.0	5.0	Control	Check	
Post Treatment Mortality Per Day %								
1	0.0	6.6	46.6	53.3	85.7	0.0	0.0	
2	7.2	6.6	6.7	13.3	0.0	0.0	0.0	
3	21.4	0.0	0.0	0.0	0.0	0.0	0.0	
4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
5	0.0	0.0	0.0	0.0	0.0	0,0	0.0	
6	7.2	0.0	0.0	0.0	0.0	0.0	0.0	
7	0.0	0.0	0.0	0.0	0.0	7.2	0,0	
8	0.0	6.6	0.0	0.0	0.0	0.0	0.0	
9	0.0	6.6	0.0	0.0	0.0	0.0	0.0	
10	0.0	6.6	0.0	0.0	0.0	0.0	0.0	
11	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
12	21.4	0.0	0.0	6.6	14.3	14.3	0.0	
Total Mortality % <sup>a</sup>								
M	14.4	15.4	13.3	33.2	35.7	14.3	0.0	
F	42.8	23.1	40.0	40.0	64.3	7.2	0.0	
T	57.2	38.5	53.3	73.2	100	21.5	0.0	
Total Offspring								
Eggs	446	742	488	125	0	844	1133	
Adults	440	736	421	85	0	828	1130	
<u>% Hatch</u>	98.7	99.2	86.3	68.0	_	98.1	99.7	
Eggs/F	110.5	148.4	122.0	31.3	0.0	93.8	125.8	

Table 8. Post-treatment mortality per day, total offspring, percent hatch, and number of eggs per female of adults injected with aqueous solutions of antimony sodium tartrate.

<sup>a</sup>Male, female and total mortality

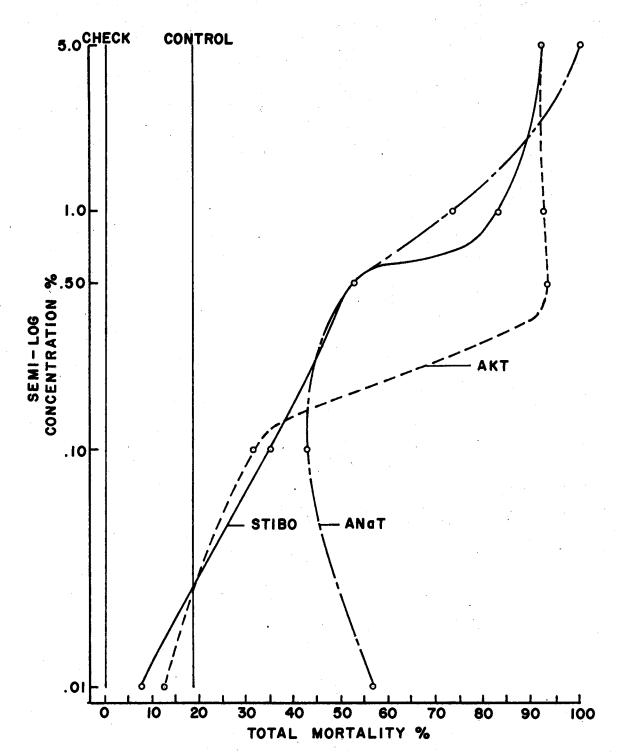


Figure 10. Percent total mortality of <u>C</u>. <u>macellaria</u> adults injected with antimony potassium tartrate (AKT), antimony sodium tartrate (ANaT), and stibophen (STIBO).

Toxicity to antimony sodium tartrate appeared to be independent of concentration throughout the lower 4 concentrations but mortality reached 100% at the 5.0% concentration. Stibophen toxicity was gradual after an initial 7.2% mortality experienced at the 0.01% concentration and followed a rather smooth linear increase to 93.3% at the 5.0% concentration level. The increase in mortality was rather smooth with antimony potassium tartrate at the two lowest concentration levels (13.2% and 31.1%) but swung upward sharply at the 0.50% concentration and remained at this high percentage up to and including the 5.0% concentration.

As a result of these studies one can conclude that in all three compounds and at all concentrations except stibophen at the 0.10% concentration female mortality exceeded male mortality. These findings do not agree with observations from other concurrent studies conducted with these compounds nor do they agree with Rockstein (1957) who observed that female house flies live longer than males of that species. Several possible explanations can be advanced to explain this phenomenon. Activity of the female is much greater than that of the male during the adult stage and she can be observed in a much more active state while searching for a suitable oviposition site. Davis and Fraenkel (1940) reported an increase in the respiratory rate of 30-50 times during flight of the blowfly Lucilia sp. and a 50-100 fold increase in metabolic rate is known to occur in this species upon the initiation of flight (Sacktor 1970). With an increase in metabolic activity comes a concomitant increase in body temperature (Chapman 1969) and it has been shown that in rats and mice an increase in

temperature significantly increases their susceptibility to antimony toxicity (Baetjer 1969).

Similarly, Baetjer (1969) found in dehydrated mice that death from antimony injections occurred more rapidly and mortality was significantly higher than in the nondehydrated animals. The increased metabolic activity during the search for a suitable oviposition site as well as an increased utilization of water during vitellogenesis would conceivably dehydrate the female insect to a greater degree than the male with a subsequent increase in antimony toxicity.

Yet another conceivable explanation is the female's elaboration of  $Na^+$  and  $K^+$  ions into the developing ova. That is, these ions entering the developing gonadal tissue in conjugation with antimony are possibly detached from the latter ion releasing it back into circulation to be picked up by the malpighian tubules and eliminated. This would effectively increase the concentration of antimony within the female since no such degree of elaboration occurs in the male. Antimony toxicity is similar to that of arsenic (Shepard 1951) and an increase in concentration would lead to an increased uptake by the organs of excretion so that epithelial lining erosion would be more pronounced.

Although these explanations may be possible no clear-cut data have been found to verify the assumptions and additional research is clearly called for.

The only significant decrease in fertility of <u>C</u>. <u>macellaria</u> eggs was observed with antimony sodium tartrate at the 1.0% concentration levels. All other concentrations in all three compounds had no effect upon fertility when compared to the controls.

Fecundity was significantly reduced in all of the tested compounds with reduction in offspring being most severe with antimony potassium tartrate. Total sterilization occurred at the 0.50% level and remained at zero throughout the increasing concentration levels (Figure 11). Stibophen produced total sterilization only at the 1.0% and 5.0% concentrations while antimony sodium tartrate produced zero eggs per female only after reaching the 5.0% concentration and this was due to total mortality at this level.

While the decrease in eggs per female is linear with antimony potassium tartrate treatment and to a lesser degree with stibophen, the decrease in eggs per female with antimony sodium tartrate treatment is erratic showing an increase over the control and check at the 0.10% concentration. This increase was due to contamination of the oviposition media by sarcophagid eggs from fertilized sarcophagid females that gained entrance to the test cages and oviposited successfully.

### Adult Dusting

Results of the dusting experiments on <u>C</u>. <u>macellaria</u> adults are presented in Table 9. These results are somewhat confusing in that treated adults in all antimony compound tests at the 5 minute and 15 minute exposure times experienced a lower rate of mortality than the controls. Only at the 30 minute exposure time with antimony potassium tartrate and triphenylantimony did treated fly mortality exceed that of the untreated flies.

These results would tend to suggest that some therapeutic value was derived from the dust treatment, however, it should be noted that

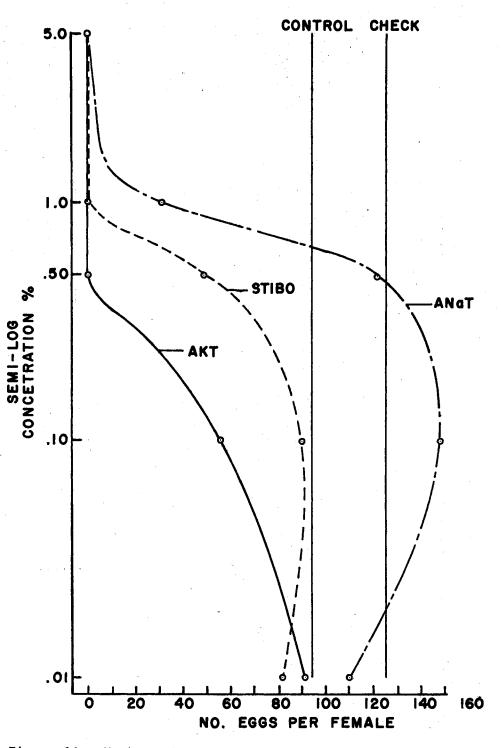


Figure 11. Number of eggs per female resulting from adult injection of antimony potassium tartrate (AKT), antimony sodium tartrate (ANaT), and stibophen (STIBO).

Test Com-	Hrs. Foll		Percen lortali			No. Eg Produc			No. Eg Hatche	gs d	P	ercent Hatch			Eggs Pe Female	er i
pound	Trt.	-5ª	15	30	5	15	30	5	15	30	5	15	30	5	15	30
	24	0.0	0.0	0.0	0	0	0	-			-	-	_	-	_	
	36	4.0	4.0	8.0	0	0	0	-	-	-	-	-	-	-	-	-
ANaT <sup>C</sup>	48	0.0	0.0	0.0	0	0	0	-	-	-	-	-	-	-	-	-
	72	0.0	0.0	0.0	0	0	0	-	. 🖛	-	-	-	-	-	-	-
	96	0.0	0.0	0.0	0	0	0	-	-	-	-	-		-	-	-
Tot	als <sup>b</sup>	25.0	36.0	24.0	1162	1380	1451	1061	1288	1301	91.3	75,.8	89.6	290.5	388.0	85.3
	24	0.0	0.0	0.0	0	0	0	-	-	-	-	-	-		-	-
	36	0.0	1.5	17.2	0	0	0	-	-	-	-	-	-	-	-	-
акт <sup>ф</sup>	48	0.0	0.0	9.4	0	0	0	-	-	-	-		-	-	-	-
	72	0.0	0.0	0.0	0	0	0	-	-	-	-	-	-	-	-	-
	96	0.0	0.0	0.0	0	0	0	-		-	-	-	-	. –	-	-
To	tals	33.3	1.5	39.1	517	2076	1183	409	1879	1108	79.1	90.5	93.6	86.2	103.8	107.5
	24	0.0	0.0	0.0	0	0	0	-	-	-	-	- ,	-	-	-	· _
	36	0.0	0.0	0.0	0	0	0		-	-	-	-	-	-	-	-
трае	48	0.0	0.0	0.0	0	0	0	-	-	-	-	-	-	-	-	-
	72	0.0	0.0	0.0	0	0	0	-	-	-	-	-	-	-	-	-
	96	0.0	0.0	0.0	0	0	0	-	-	-	-	-	-	-	-	-
To	tals	11.4	26.0	44.6	579	398	732	579	357	690	100	89.9	94.2	96.5	99.5	106.4

Table 9. Post-treatment mortality, fertility and fecundity of <u>C</u>. <u>macellaria</u> adults exposed to dusts of antimony compounds.

Table 9. (Continued)

Test Com-	Hrs. Foll		Perce Mortal			No. Eg Produc	ed		No. E Hatch	ed		Perce Hatc	h		Eggs Fema	le
pound	Trt.				5	15	30	5	15	30	5	15	30	5		
<u></u>	24	0	0	0	0	0	. 0	_	-		-	-	_	-	-	-
	36	0	0	0	0	0	0	-	-	-	-	-	-	-	-	. –
ABaTf	48	0	0	0	0	0	0	<b>-</b>	-	-	-	-	-	-		-
	72	0	0	0	0	0	0	-	-	-	-	-	-	-	-	-
	96	0	0	0	0	0	0	-	- <b>-</b>	-	-		-	-	-	-
Tota	als	2.7	11.6	3.2	952	1763	2552	877	1688	2375	92.1	96.7	93.1	158.6	332.6	150.1
	24	0	0	0	0	0	0	-	-	-	-	-	-	-	-	-
	36	4.0	0	2.6	0	0	0	-	-	-	-	-		-	-	-
STIBO <sup>g</sup>	48	0	0	5.3	0	0	0	-	_	-	-		-	-	-	-
	72	0	0	0	0	0	0	-	-	-	-	. <b>-</b>	-	-	-	-
	96	0	0	0	0	0	0	•	-	-	-	-	-	-	-	-
Tota	als	8.0	9.0	15.8	730	1174	2010	730	1174	1980	100	100	98.5	182.5	146.8	118.2
	24	7.3	10.0	5.3	0	0	0	-	-	-	-	-	-	• –	-	-
	36	18.3	32.5	17.1	0	0	0		-	-	-	-	-	-	-	-
Con-	48	1.8	0.0	0.0	0	0	0	-	•••	-		-	-	-	-	-
trol	72	0.9	1.3	00	0	- 0	0	-	-	-	-		-	-	-	-
	96	1.8	0.0	0.0	0	0	0	-	-	-		-	-	-	-	-
Tota	als	44.9	56.3	28.6	1621	2210	1288	1621	2210	1288	100	100	100	55.8	122.8	163.3

<sup>a</sup>Exposure time in minutes <sup>C</sup>Antimony Sodium Tartrate <sup>e</sup>Triphenylantimony <sup>D</sup>Total includes first 96 hours plus time remaining until owiposition <sup>d</sup>Antimony Sodium Tartrate fAntimony Barium Tartrate <sup>g</sup>Stibophen untreated adult mortality was extremely high during these tests which could render the results invalid.

# Pupal Dipping

Results of tests on emergence capabilities, fertility and development time of <u>C</u>. <u>macellaria</u> pupae dipped in aqueous solutions of three antimony compounds are presented in Tables 10-12.

Antimony sodium tartrate was the most effective of the compounds in retarding development with emergence percentage means of 82.4%, 81.6% and 84.0% at 5, 15 and 25 minutes dipping time respectively (Table 10). Although these figures represented a decrease in emergence from the controls there were no substantial changes apparent.

Stibophen and antimony potassium tartrate produced essentially identical results on emergence capabilities (Tables 11, 12). As with antimony sodium tartrate, deleterious effects upon emergence capabilties were independent of dipping time.

As the data clearly indicate (Table 10) antimony sodium tartrate was more effective in reaching the developing pupae within the puparia as indicated by the number of adults displaying observable malformations. These malformations appearing in the 2.0%, 5.0% and 10.0% concentrations included twisted wings and soft malformed bodies with cuticles which apparently could not be dried and hardened properly. Although the number of the malformed individuals is reflected in the total emerging figures they were not able to grow or develop and died within 2-3 days.

The obvious differences in penetration ability could be due in part to the differences in molecular weights of these compounds.

· · · · · · · · · · · · · · · · · · ·		. Pu reat	•		. Adul merged		Emergence				Adults mplete elopeo	ely	Pro	ggs <sup>1</sup> odu F–I	ced	Re	Days <sup>c</sup> quire	: ed
Time (Min.)	5	15	25	5	15	25	5	15	25	5	15	25	5	15	25	5	15	25
Percent Conc.						• • •												
.05	25	25	25	24	25	25	96	100	100	0	0	0	F	F	F	3.0	3.0	3.0
1.0	25	25	25	23	24	25	92	96	100	0	0	0	F	F	F	3.0	3.0	3.0
2.0	25	25	25	23	25	25	92	100	100	0	3	3	F	F	F	3.0	3.0	3.0
5.0	25	25	25	21	17	18	84	68	72	5	10	6	F	F	F	3.5	4.0	3.5
10.0	25	25	25	12	11	12	48	44	48	3	2	4	F	F	F	4.0	4.0	4.0
Control	25	25	25	25	25	25	100	100	100	0	0	0	F	F	F	3.0	3.0	3.0
x <sup>d</sup>	25	25	25	20.6	20.4	21.0	82.4	81.6	84.0	1.6	3.0	2.6	-		-	3.3	3.4	3.3

Table 10. Effects on emergence capabilities of  $\underline{C}$ . <u>macellaria</u> pupae dipped in aqueous solutions of antimony sodium tartrate.

<sup>a</sup>Includes any observable malformations

<sup>b</sup>Fertility or infertility of eggs resulting from subsequent matings; both sexes received treatment

<sup>C</sup>Days from treatment to emergence

d<sub>Mean</sub> excluding control

			Pupae No. Adults ated Emerged					Percent mergenc		No. A Incom Deve	ip]e	etely	P۲	ggs rodu F-II	uced	R	Days lequir	
ime (Min	.)5	15	25	5	15	25	5	15	25	5	15	25	5	15	25	5	15	25
Percent Conc.																		
.05	25	-25	25	23	25	23	92	100	92	0	0	0	F	F	F	3.0	3.0	3.0
1.0	25	25	25	25	24	25	100	96	100	0	0	0	F	F	F	3.0	3.0	3.0
2.0	25	25	25	24	24	25	96	96	100	0	0	0	F	F	F	3.0	3.0	3.0
5.0	25	25	25	23	25	24	92	100	96	0	0	0	F	F	F :	3.5	3.0	3.0
10.0	25	25	25	24	24	25	96	96	100	0	0	0	F	F	F	3.0	3.0	3.0
Control	25	25	25	25	25	24	100	100	96	0	0	0	F	F	F	3.0	3.0	3.0
$\bar{x}^{d}$	25	25	25	23.8	24.4	24.4	95.2	97.6	97.6	0	0	0	_	-	-	3.1	3.0	2.9

Table 11. Effects on emergence capabilities of  $\underline{C}$ . <u>macellaria</u> pupae dipped in aqueous solutions of stibophen.

<sup>a</sup>Includes any observable malformation

<sup>b</sup>Fertility or infertility of eggs resulting from subsequent matings; both sexes received treatment

<sup>C</sup>Days from treatment to emergence

<sup>d</sup>Mean excluding control

		. Pu reat			Adult mergeo			Perce		No. Adult Incomplet Develope	ely	Pro	gs <sup>b</sup> duc -IF	ed	Re	Days <sup>C</sup> quire	d
Time (Min.	) 5	15	25	5	15	25	5	15	25	5 15	25	5	15	25	5	15	25
Percent Conc.							- , <i>, , , , , , , , , , , , , , , , , ,</i>			<u> </u>					<u> </u>		
.05	25	25	25	19	25	25	76	100	100	0.0	0	F	F	F	3.0	3.0	3.0
1.0	25	25	25	25	25	24	100	100	96	0 0	0	F	F	F	3.0	3.0	3.0
2.0	25	25	25	23	25	25	92	100	100	0 0	0	F	F	F	3.0	3.0	3.0
5.0	25	25	25	25	25	24	100	100	96	0 1	0	F	F	F	3.5	3.0	3.0
10.0	25	25	25	25	23	22	100	100	100	03	3	F	F	F	3.0	3.5	3.5
Control	25	25	25	25	25	25	100	100	100	0 0	0	F	F	F	3.0	3.0	3.0
<b>x</b> <sup>d</sup>	25	25	25	23.4	24.6	24.0	93.6	100	98.4	0.80	.60	-	-	-	3.1	3.1	3.1

Table 12. Effects on emergence capabilities of  $\underline{C}$ . <u>macellaria</u> pupae dipped in aqueous solutions of antimony potassium tartrate.

<sup>a</sup>Includes any observable malformations

<sup>b</sup>Fertility or infertility of eggs resulting from subsequent matings; both sexes received treatment

<sup>C</sup>Days from treatment to emergence

<sup>d</sup>Mean excluding control

Stibophen with a high molecular weight of 895.27 (Stecher 1968) would probably not be expected to penetrate the puparium unless some action of the compound altered the integrity of the case. To a greater degree antimony potassium tartrate with a molecular weight of 329.92 (Stecher 1968) would be expected to penetrate the pupal case more easily than stibophen, and antimony sodium tartrate, molecular weight 308.83 (Stecher 1968), would be expected to penetrate more easily than the other two compounds using molecular weights only as a basis for comparison.

Smith et al. (1964) considered pupal age to be a critical factor in chemosterilant action at this stage, however, all pupae used in these tests were of the same age so no detectable differences in ageinfluenced emergence capabilities or fertility would be expected.

No attempts were made to determine the number of offspring produced by the adults resulting from treated pupae. Adults from all concentration levels at all times produced fertile eggs indicating that although some degree of reproductive decrease may have been possible it was in no case total.

The time required for development of the pupae was influenced appreciably only by antimony sodium tartrate. The increase in time of pupal development was independent of dipping time but a definite relationship with concentration levels was established. At the 5.0% level the time required from treatment to emergence for all times was 3.6 days and at the 10.0% level 4.0 days were required as compared to 3.0 days for the control.

### Pupal Inunction

The results on emergence capabilities, post-emergence mortality and offspring production from adult flies resulting from puparia that received inunction with a triphenylantimony-lanolin mixture are presented in Table 13 and graphically in Figures 12 and 13.

The data indicate that the lanolin which was used as a solvent and carrier for the triphenylantimony was not toxic to the developing pupae when applied alone and in the same manner as applications for the test compound mixtures. The lanolin-only inunction produced an 80% emergence rate as compared to a mean of 82.4% for the lanolin-triphenylantimony mixture and 76.0% for the control (Figure 12).

When the pupal case received total inunction with lanolin-only, however, no emergence was observed. Lanolin is a highly viscous material and under warm conditions spreads to fill all depressions on the pupal case. Since the pupa is in a closed system for all metabolites except gases (Bodnaryk 1971), this oily covering undoubtedly served as an effective barrier to the transport of oxygen into the developing pupa and death came as a result of asphyxiation.

The data indicate that some amounts of the antimony compounds were effectively transported through the pupal case and absorbed by the developing pupae as shown by the post-emergence percentage figures. At all concentrations mortality was higher than the lanolin-only inunction or the controls (Table 13).

With increasing triphenylantimony concentration came a concurrent reduction in the number of offspring per female (Figure 13).

Mean weights of test pupae are included in Table 13 for the reader's interpretation. Spates and Hightower (1970) reported a

TPA Conc. %	No. Treated	Emer genc No.	es		ergence ality %	Offsp <u>Produ</u> Total		Mean Wgts. of Test Pupae (g)
.05	25	23	92	3	13.0	1257	114.3	.034
.10	25	21	84	4	19.0	770	77.0	.032
.50	25	19	76	3	15.8	571	63.2	.034
1.0	25	18	72	5	27.8	550	61.1	.031
1.5	25	22	88	5	22.7	466	42.4	.034
Lanolin only	25	20	80	1	5.0	845	84.5	.030
Lanolin only comp. inunction	25	0	0	-	-	0	0.0	.032
Control	25	19	76	1	5.3	780	88.9	.033

Table 13. Effects on emergence capabilities, post-emergence mortality, and offspring production, of adult flies resulting from puparia receiving triphenylantimony lanolin mixture inunction.

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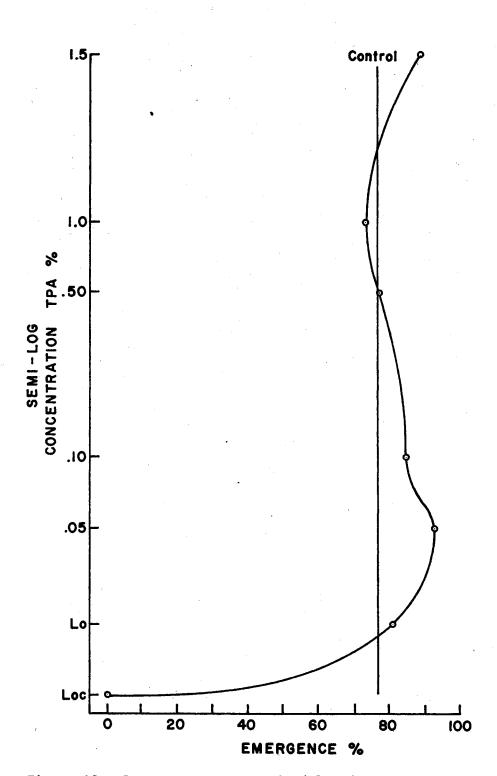


Figure 12. Percent emergence of adults from puparia that received triphenylantimony lanolin mixture inunction, lanolin only (Lo) and lanolin only complete (Loc) inunction.

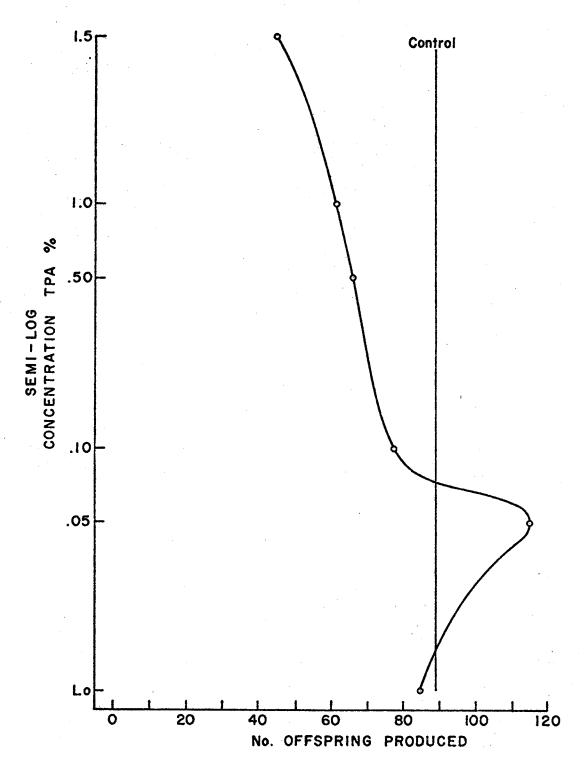


Figure 13. Number of offspring produced by females resulting from puparia that received triphenylantimony lanolin mixture inunction.

definite relationship existed between size and mating capability in <u>Cochliomyia hominivorax</u> and preliminary tests would have been necessary to determine if the same relationship occurred in <u>C</u>. <u>macellaria</u>. Three of the pupal test groups receiving triphenylantimony-lanolin inunction were identical in mean weights (.034 grams) and the triphenylantimony concentration levels of these test pupae were at both extremes (0.05% and 1.5%) as well as the middle (0.50%) level. The heaviest pupae therefore produced the greatest number of eggs per female; a number of eggs per female midway between the extremes; and the fewest number of eggs per female. Therefore if it can be assumed that a size-offspring relationship does exist in <u>C</u>. <u>macellaria</u> as in <u>C</u>. <u>hominivorax</u> then triphenylantimony was responsible for the decreased egg production.

### Larval Feeding

The results of tests on emergence capabilities, oviposition, hatching and mortality of 2nd instar larvae fed on medium containing the various antimony compounds are presented in Tables 14-18.

As shown in Figure 14 the effects on emergence capabilities were most pronounced for triphenylantimony with a mean emergence percentage of 40.7%. Antimony sodium tartrate was next in degree of influence with a mean emergence percentage of 41.8%. Antimony potassium tartrate and antimony barium tartrate were close in their effects upon emergence allowing means of 48.5% and 46.8% respectively. Stibophen showed no effect when mean emergence percentages were compared with the control. Only at the 5.0% concentration did the percentage emergence fall below that of the control, all other levels being greater than the control.

Table 14. Effects on emergence capabilities, oviposition, hatching and post-emergence mortality of stages resulting from 2nd instar larvae fed on medium containing triphenylantimony.

	Ni	0.		Er	nergen	ce			ning at sition	ng at tion						Per	cent Ha	itch	Ε	ggs Per	F		st-Emer Mortali Percen	ťy
Percent		ated		No.		Perce	nt	M/1	<u>F</u>								•							
Conc.	. R <sub>1</sub>	R <sub>2</sub>	R <sub>1</sub>	R2	R <sub>1</sub>	R <sub>2</sub>	x	R <sub>1</sub>	R <sub>2</sub>	R <sub>1</sub>	R <sub>2</sub>	x	R <sub>1</sub>	R <sub>2</sub>	x	R <sub>1</sub>	R <sub>2</sub>	x	R <sub>1</sub>	R <sub>2</sub>	x	R <sub>1</sub>	<sup>R</sup> 2	x
.01	50	50	40	38	80	76	78	20/18	4/4	1335	410	872.5	1293	410	851.5	96.9	100	98.5	74.2	102.5	88.4	5.0	78.9	41.9
.05	50	50	42	40	84	80	82	20/19	17/15	407	682	544.5	400	636	518.0	98.3	93.3	95.8	21.4	45.5	33.5	7.1	20.0	13.6
.10	50	50	45	39,	90	78	84	1/6	2/2	182	0 ·	91.0	179	0	89.5	98.4	0	49.2	30.3	0	15.2	84.7	89.7	87.2
.50	50	50	0	0	0	.0	0	0/0	0/0	, · · 0	0	0.0	· 0	0	0.0	0	0	0.0	0	0	0.0	-	-	-
1.0	50	50	Ō	0	0	0	0	0/0	0/0	0	0	0.0	0	0	0.0	0	0	0.0	0	0	0.0	: -	-	-
5.0	50	50	0	0	0	0	0	0/0	0/0	0	0	0.0	0	0	0.0	0	0	0.0	0	0	0.0	-	-	
Control	50		32	-	64	-	64	15/17	-	· 1870	-	1870	1865	-	1865	99.7	-	99.7	110.0	-	110.0	0.0	-	0.0

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Table 15.	Effect on	emergence cap	pabilities, c	oviposition,	hatching and p	ost-emergence mortality of
stages res	ulting from	1 2nd instar 1	larvae fed on	n medium cont	taining antimon	y sodium tartrate.

	Nc	n.		Er	nergen	ce		Rema Ovip	ults ining at osition ime	ning at ition Offspring Perc					cent H	atch	E	ggs Per	<u>F.</u>	Mo	-Emerg rtalit ercent	у		
Percent		ated	<u>N</u>	0.		Perce	nt	<u> </u>	<u>/F</u>						<u> </u>									
Conc.	R <sub>1</sub>	<sup>R</sup> 2	R <sub>1</sub>	R <sub>2</sub>	R <sub>1</sub>	R <sub>2</sub>	x	R <sub>1</sub>	R <sub>2</sub>	R <sub>1</sub>	R <sub>2</sub>	x	R <sub>1</sub>	R <sub>2</sub>	x	R <sub>1</sub>	R <sub>2</sub>	x	R <sub>1</sub>	R <sub>2</sub>	x	R <sub>1</sub>	<sup>R</sup> 2	ž
.01	50	50	41	39	86	78	80	24/12	24/12	1019	1104	1062	969	1104	1037	95.1	100	97.6	78.4	92.0	88.5	12.2	7.7	9.9
.05	50	50	46	33	92	66	79	17/29	20/21	1205	1495	1350	1155	1495	1325	95.8	100	97.9	41.6	71.2	54.0	0	2.4	1.2
.10	50	50	43	46	86	92	89	21/16	24/20	915	993	954	915	993	954	100	100	100	50.9	49.7	53.Q	13.9	4.3	9.1
.50	50	50	2	1	4	2	3	1/1	0/0	0	0	0	0	0	0	0.	0	Ö	0	0	0	0	100	50.0
1.0	50	50	0	0	0	0	0	0/0	0/0	0	0	0	0	0	0	0	0	0	0	0	0	-	-	-
5.0	50	50	0	. 0	0	0	0	0/0	0/0	0	0	· · 0	0	0	0	0	0	0	0	0	0		-	-
Control	<del>5</del> 0	50	32	-	64	-	64	<b>15/</b> 17	-	1870	-	1870	1865	-	1865	99.7	-	99.7	110.0	-	110.0	0	-	0.0

Table 16. Effects on emergence capabilities, oviposition, hatching and post-emergence mortality of stages resulting from 2nd instar larvae fed on medium containing antimony potassium tartrate.

N			Eme	ergenc	e		Remai Ovipo:	ning at sition	n .					Per	cent Ha	tch	E	ggs Per	F	M	ortaliț	у	
		No	<b>.</b>	P	ercen	t	M/	F	Eggs Adults														
R <sub>1</sub>	R <sub>2</sub>	R <sub>1</sub>	R <sub>2</sub>	R <sub>1</sub>	R <sub>2</sub>	x	R <sub>1</sub>	R <sub>2</sub>	R <sub>1</sub>	R <sub>2</sub>	x	R <sub>1</sub>	R <sub>2</sub>	x	R <sub>1</sub>	R <sub>2</sub>	x	R <sub>1</sub>	R <sub>2</sub>	x	R <sub>1</sub>	R <sub>2</sub>	x _
50	50	44	48	88	96	92	24/14	25/13	564	704	634	544	604	574	96.4	98.5 <sub>.</sub>	97.5	40.3	54.2	47.3	2.3	20.8	11.6
50	50	47	44	94	88	91	20/25	19/22	749	1172	961	748	1170	959	99.9	99.9	99.9	29.9	53.3	41.6	<b>4.3</b>	6.8	5.6
50	50	41	44	82	88	85	23/15	21/19	835	1269 <sup>.</sup>	1052	795	1258	1027	95.2	99.1	97.2	55.7	66.8	61.3	7.3	9.1	8.2
50	. 50	20	3	40	6	23	9/11	1/2	. 861	0	431	861	0	431	100	0	50	78.3	0	39.2	0	0	0.0
50	50	0	0	0	0	0	0/0	0/0	0	0	0	Ò	0	0	0	0	0	0	0	0		-	-
50	50	0	0	0	0	0	0/0	0/0	0	0	0	0	0	0	Ū	0	0	. 0	0	· 0	· _	-	-
50	•	32	-	64	-	64	15/17	-	1870	-	1870	1865	-	1865	99.7	-	99.7	110.0	-	110.0	Q	-	0.0
	<u>Trea</u> R <sub>1</sub> 50 50 50 50 50 50	1         2           50         50           50         50           50         50           50         50           50         50           50         50           50         50           50         50           50         50           50         50           50         50	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	Treated         No.         Percent           R1         R2         R1         R2         R1         R2           50         50         44         48         88         96           50         50         47         44         94         88           50         50         41         44         82         88           50         50         20         3         40         6           50         50         0         0         0         0           50         50-         0         0         0         0	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	Remaining at Oviposition TimeOffspringPerNo.Emergence $M/F$ EggsAdultsR1R2R1R2 $\overline{x}$ $R_1$ R2 $\overline{x}$ $R_1$ $R_2$ $\overline{x}$ $\overline{x}$ $R_1$ $\overline{x}$ $\overline{x}$ $\overline{x}$ $\overline{x}$ $\overline{x}$	Remaining at OvipositionOffspringPercent HarNo.EmergenceM/FEggsAdultsTreatedNo.PercentM/FEggsAdultsR1R2R1R2 $\overline{X}$ R1R2 $\overline{X}$ 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<td>Remaining at Oviposition TreatedPercentRemaining at Oviposition TimeOffspringPercent HatchEggs Per FPercent HatchFPercent HatchPercent Percent PercentNo.PercentM/FEggsPercentM/FPercent<th< td=""><td>No.       Emergence       No.       Percent       <math>M/F</math>       Eggs       Percent       <math>Adults</math>       Percent Hatch       Eggs Per F       Post-Emerge Mortality Percent         <math>R_1</math> <math>R_2</math> <math>R_1</math> <math>R_2</math> <math>\bar{x}</math> <math>R_1</math></td></th<></td>	Remaining at OvipositionNo.Emergence $M/F$ EggsAdults $R_1$ $R_2$ $R_1$ $R_2$ $\bar{x}$ $R_1$ $R_2$ $\bar{x}$ $R_1$ $R_2$ $\bar{x}$ $R_1$ $R_2$ $R_1$ $R_2$ $\bar{x}$ $R_1$ $R_2$ $\bar{x}$ $R_1$ $R_2$ $\bar{x}$ $R_1$ $R_2$ $\bar{x}$ 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Mortality Percent $R_1$ $R_2$ $R_1$ $R_2$ $\bar{x}$ $R_1$

Table 17. Effects on emergence capabilities, oviposition, hatching and post-emergence mortality of stages resulting from 2nd instar larvae fed on medium containing antimony barium tartrate.

	N	).	<u> </u>	<u> </u>	ergen	ce		Rema	ults ining at osition ime	g at ion <u>Offspring J</u> Eggs <u>Adults</u>							cent Ha	tch	Ε.	ggs per	F	Mo	-Emergen rtality ercent	
Percent		ited	<u>_N</u>		<u>P</u>	ercen	t	M	<u>/F</u>		Eggs			Adults	·									
Conc.	R <sub>1</sub>	R <sub>2</sub>	R <sub>1</sub>	R2	R <sub>1</sub>	R <sub>2</sub>	x	R <sub>1</sub>	R <sub>2</sub>	R <sub>1</sub>	R <sub>2</sub>	x	R <sub>1</sub>	R <sub>2</sub>	x ·	R <sub>1</sub>	<sup>R</sup> 2	x	R <sub>1</sub>	R <sub>2</sub>	x	R <sub>1</sub>	R <sub>2</sub>	x
.01	50	50	34	45	68	<b>90</b> ,.	79	16/16	26/19	841	1378	1109.5	841	1300	1070.5	100	94.3	97.2	52.6	72.5	62.6	5.9	0.0	2.9
.05	50	50	38	36	76	72	74	22/16	17/19	641	586	598.5	630	528	579	98.3	90.1	92.2	40.1	30.8	35.5	0.0	0.0	0.0
. 10	50	50	44	50	88	100	94	19/22	25/25	457	524	490.5	452	517	482.0	98.9	98.7	98.8	20.8	20.9	20.9	6.8	0,0	3.4
.50	50	50	12	10	24	20	22	5/7	2/8	149	157	153.0	149	150	149.5	100	95.5	97.8	21.3	19.6	20.5	0.0	0.0	0.0
1.0	50	50	9	3	18	6.	12	8/1	1/1	0	<u>_</u> 0	0	0	0	0	ò	0	0	0	0	0	ò.0	33.3	16.7
5.0	50	50	0	0	0	0	0	0/0	0/0	0	0	0	0	0	0	0	0	0	0	0	0		-	-
Control	. 50	50	32	_ '	64	-	64	15/17	-	1870	-	1870	1865	-	1865	99.7	-	99.7	110.0	•	110.0	0.0		0.0

Table 18. Effects on emergence capabilities, oviposition, hatching and post-emergence mortality of stages resulting from 2nd instar larvae fed on medium containing stibophen.

	N	D.		_ £	nergen	ce			ning at sition	ig at ion				cent Ha	tch		Eggs Per	F	Moi	-Emerg rtalit ercent	y			
Percent		ated	N	o	_ P	ercen	t	<u> </u>	F				s											
Conc.	R <sub>1</sub>	R <sub>2</sub>	R <sub>1</sub>	R <sub>2</sub>	R <sub>1</sub>	R <sub>2</sub>	x	R <sub>1</sub>	R <sub>2</sub>	R <sub>1</sub>	R <sub>2</sub>	x	R <sub>1</sub>	<sup>R</sup> 2	x	R <sub>1</sub>	R <sub>2</sub>	x	R <sub>1</sub>	R <sub>2</sub>	x	R <sub>1</sub>	R <sub>2</sub>	x
.01	50	50	43	39	86	78	82	19/24	21/17	3060	2574	2817	2910	2373	2642	95.1	92.2	95.2	127.5	151.4	139.5	0.0	2.6	1.3
.05	50	50	45	42	90	84	87	21/23	20/19	1740	2248	1994	1665	2198	1932	95.7	97.8	96.8	75.7	118.3	97.0	2.2	7.1	4.7
. 10	<b>50</b> '	50	35	41	70	82	76	16/19	19/21	1807	<sup>.</sup> 896	1352	1782	871	1327	98.6	97.2	97.9	95.1	42.7	68.9	0.0	2.4	1.2
.50	50	50	43	46	86	92	89	24/19	21/25	1663	3876	2770	1659	3807	2733	99.8	98.2	99.0	87.5	155.0	121.3	0.0	0.0	0.0
1.0	50	50	40	30	80	60	70	17/21	16/13	2340	2282	2311	2325	2257	2291	99.4	98.8	99.1	111.4	175.5	143.5	5.0	3.3	4.2
5.0	50	50	17	14	34	28	31	11/6	9/5	311	299	305	300	291	. 296	96.5	97.3	96.9	51.8	.59.8	55.8	0.0	0.0	0.0
Control	50	50	32	-	64	-	64	15/17	• -	1870	-	1870	1865	-	1865	99.7	-	99.7	110.0		110.0	0.0	-	0.0

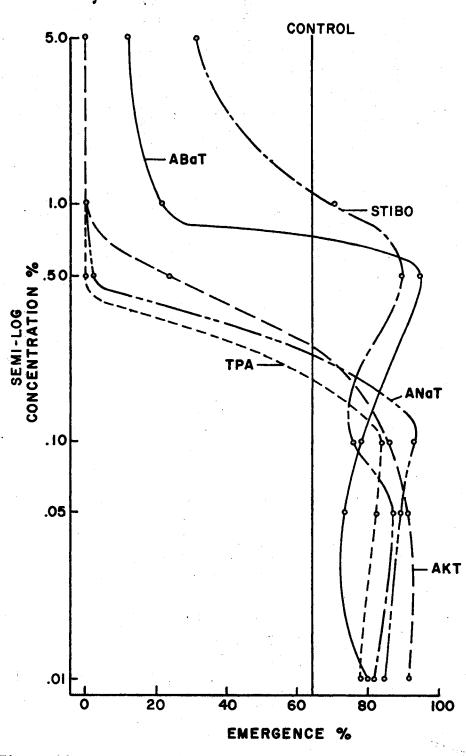


Figure 14. Percent emergence of adults resulting from 2nd instar larvae fed on medium containing antimony barium tartrate (ABaT), antimony potassium tartrate (AKT), antimony sodium tartrate (ANaT), stibophen (STIBO) and triphenylantimony (TPA).

Not only did triphenylantimony produce the lowest mean percentage emergence of the tested compounds but reached the zero emergence level at a lower concentration (0.50%) than did the others (Table 14). Both antimony sodium tartrate (Table 15) and antimony potassium tartrate (Table 16) prohibited any emergence at the 1.0% concentration levels, with the former compound allowing only a mean of 3.0% emergence at the 0.50% concentration level. The zero emergence level was apparent with antimony barium tartrate only at the highest treatment concentration (Table 17).

With all compounds tested the 3 lowest concentrations allowed more emergence than did the controls possibly suggesting that some therapeutic effect was involved. It is possible that at the lowest concentrations (0.01%, 0.05% and 0.10%) enough of the compound was present in the medium to limit microbial growth which could be detrimental to larval development. At the 0.50% concentration and above, however, this beneficial effect was outweighed by the toxic effects of the compounds so that mortality increased significantly. Stibophen, being the least toxic of the compounds would continue to provide a beneficial influence through the 1.0% concentration and possibly beyond only to become detrimental at the 5.0% concentration (Table 18).

Figure 15 illustrates the effects of antimony sodium tartrate and triphenylantimony on the growth of the 2nd instar larvae after being fed on the treated medium for approximately 36 hours. The control larvae fed on uncontaminated medium reached the 3rd instar and were robust and healthy while the increasing concentrations of the antimony compounds showed varying degrees of influence on the growth and development of the larvae. Larvae fed on the medium containing 0.50%

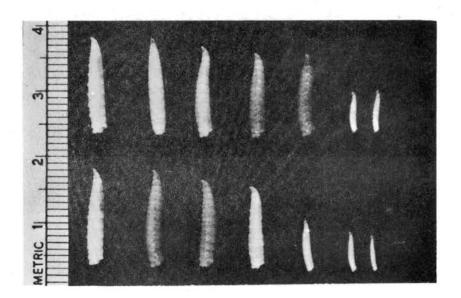


Figure 15. Size variation of <u>C</u>. <u>macellaria</u> larvae of the same age fed antimony sodium tartrate treated medium on the top row and triphenylantimony treated medium on the bottom row with the first larvae on the left being untreated and larvae receiving the highest compound concentration on the right. antimony sodium tartrate were somewhat smaller than the controls but did develop to the 3rd instar and 3.0% of them were successful in reaching adulthood. The larvae fed on medium containing triphenylantimony at the 0.50% concentration also reached the 3rd instar but growth was seriously affected and these individuals died before reaching the adult stage. At the 1.0% and 5.0% concentrations of both of these compounds larval development did not proceed beyond the 2nd instar.

The other tested compounds influenced the growth and development of the larvae but not to the same high degree as encountered with antimony sodium tartrate and triphenylantimony.

Hightower (1972 Personal Communication) concluded that the weights of mature larvae and probable mating capability of male <u>Cochliomyia</u> <u>hominivorax</u> are definitely correlated. It would therefore be feasible to conclude that those larvae which were prohibited from attaining full potential growth by the actions of the antimony compounds would not produce as many offspring as those individuals less affected. The results of these tests tend to agree with this conjectural concept, Although 3.0% of the treated larvae at the 0.50% concentration of antimony sodium tartrate reached adulthood they were unable to produce any offspring. The impaired growth of larvae resulting from treatment at the 0.10% concentration level resulted in the production of a mean of only 53.0 eggs per female as compared to a mean of 110.0 eggs per female for the control. Similarly, 84.0% of the larvae fed triphenylantimony were successful in gaining adulthood but produced a mean of only 15.2 eggs per female.

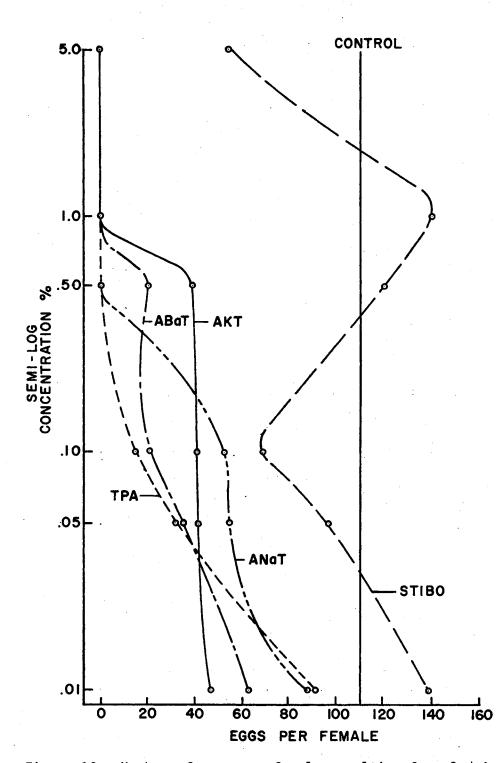
House (1967) found the larvae of <u>Agria [= Pseudosarcophaga]</u> <u>affinis</u> could distinguish nutritional differences among similar synthetic foodstuffs and would select the food most suitable for optimum growth and development. In a later work this investigator (House 1970) reported evidence that suggested rates of growth and development and dietary choice were both relative to the level of amino acids and glucose. Larvae on foods with suitable amino acid levels would probably make no distinction nor would growth and development rates differ, though the nutrient composition of the foods varied (House 1971).

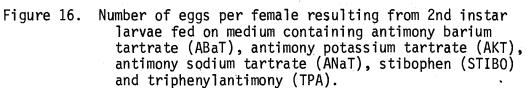
It cannot be ruled out that the introduction of the antimony compounds altered the amino acid content of the media but this is probably not the cause for growth and development cessation.

Figure 16 illustrates the eggs produced by those adults that had received the antimony compounds as larvae. Subjection of these data to the F test (Snedecor 1968) showed there were significant differences at the 1% level between all treatments of all compounds except for stibophen which was the least effective in inhibiting reproduction. Further subjection of the data to Duncan's multiple-range test (Steel and Torrie 1960) showed significant differences at the 1% level between all 0.50%, 1.0%, and 5.0% concentrations and the controls except for stibophen and antimony potassium tartrate.

Antimony sodium tartrate and triphenylantimony reached the zero eggs per female level at the 0.50% concentration while total reproductive inhibition was gained at the 1.0% concentration for antimony potassium tartrate and antimony barium tartrate (Figure 16).

It is unknown whether the size of the larvae and subsequent adult size was the most influential on inhibition of reproduction or if





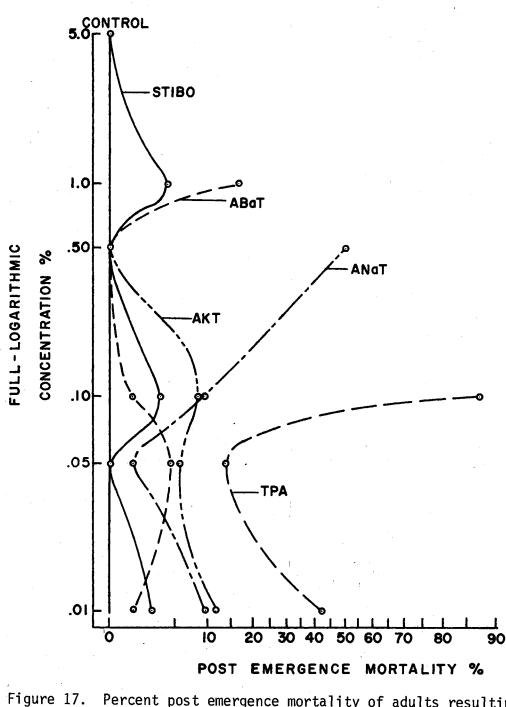
action of the antimony compounds on the gonads proved to be more detrimental. Gross dissections of adults showed some decrease in size of the ovaries at the higher concentrations but no effects on the size of testes could be observed. The earliest stage in which Lowne (1893) was able to identify the gonads of <u>Calliphora erythrocephala</u> with certainty was the first day of the pupal stage. In histological sections of <u>C. macellaria</u> larvae, no cellular structure was found that could be identified as being gonads.

All post-emergence mortality followed increasing concentration levels (Figure 17). Antimony sodium tartrate and triphenylantimony reached complete mortality at lower levels, in the former case at the 0.50% concentration and with the latter at the 0.10% concentration.

## Larval Dipping

Results of dipping 2nd instar larvae of <u>C</u>. <u>macellaria</u> in aqueous solutions of three antimony compounds are presented in Tables 19-21. Antimony sodium tartrate was the most toxic to the larvae with little variation existing between it and antimony potassium tartrate. Stibophen had little effect upon the 2nd instar larvae. Toxicity to the 2nd instar larvae was independent of submergence time with all compounds.

Toxicities at all submergence times for antimony sodium tartrate and antimony potassium tartrate paralleled each other, with mean percentages reaching the 3rd instar of 50.8% and 51.9% respectively (Tables 19, 20). Of the treated control larvae 96.1% reached the 3rd instar and 96.0% of the untreated check reached the 3rd larval instar.



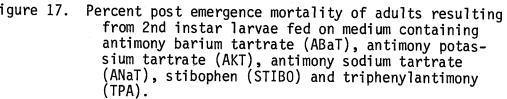


Table 19. Effects on molting capabilities, emergence, post-emergence mortality, total offspring production and eggs per female resulting from 2nd instar larvae dipped in aqueous solutions of antimony sodium tartrate.

· · · · · · · · · · · · · · · · · · ·					R	eachin	ig 3rd Ii	nstar			Rea	ching	Pupal S	tage <sup>a</sup>	
	No	. Trea	ted		<u>Total</u>		Percent			•	Total			Percent	
Time (Min.)	5	15	30	5	15	30	5	15	30	5	15	30	5	15	30
Conc. %															
.05	100	100	100	99	100	97	99.0	100	97.0	95	100	97	95.9	100	100
.10	100	100	102	96	91	96	96.0	91.0	94.1	96	91	96	100	100	100
1.0	100	100	100	66	42	53	66.0	42.0	53.0	64	40	51	96.9	95.2	96.2
5.0	100	100	100	9	7	5	9.0	7.0	5.0	7	6	5	77.7	85.7	100
10.0	100	100	100	2	0	1	2.0	0.0	1.0	2	0	1	100	0.0	100
Control <sup>d</sup>	115	100	100	112	99	92	97.4	99.0	99.0	112	99	91	100	100	98.9
Check <sup>e</sup>	100	-	-	96	-	-	96.0	-	-	96	-	-	100	-	-

<sup>a</sup>Percent column represents the percentages of those individuals successfully reaching the preceding stage. <sup>b</sup>Percent treated larvae successfully reaching adulthood.

<sup>C</sup>Post-emergence mortality.

<sup>d</sup>Dipped in distilled water only.

eNot dipped.

Table	19.	(Continued)
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	Reaching Adulthood <sup>a</sup>					Percen Adulth			D	ercent		Eggs Produced						
T	otal		P	ercent		Treate				Mortal			Tota]		Per Female			
5	15	30	5	15	30	5	15	30	5	15	30	5	15	30	5	15	30	
67	85	61	70.5	85.0	62.9	67.0	85.0	61.0	7.5	5.9	18.0	2699	3226	3655	103.8	80.6	182.7	
79	50	86	82.3	54.9	89.6	79.0	50.0	84.3	2.5	32.0	2.3	3710	3063	3676	112.4	153.1	147.0	
47	19	21	73.4	47.5	41.2	47.0	19.0	21.0	10.7	15.8	52.4	1638	1647	1549	81.9	137.5	387.3	
7	5	5	100	83.3	100	7.0	5.0	5.0	14.3	0.0	100	703	418	0	140.6	139.3	0.0	
2	0	1	100	0.0	100	2.0	0.0	1.0	100	-	100	0	0	0	0.0	0.0	0.0	
92	90	89	82.1	90.9	97.8	80.0	90.0	89.0	5.4	6.7	1.1	2073	2175	1788	162.2	157.0	184.8	
90	0	-	93.8	-	-	90.0	-	-	13.3	-	<b>-</b> 1	1998	-	-	90.9	-	-	

Table 20. Effects on molting capabilities, emergence, post-emergence mortality, total offspring production and eggs per female resulting from 2nd instar larvae dipped in aqueous solutions of antimony potassium tartrate.

					R	eachin	ig 3rd I	nstar			Rea	ching	Pupal S	tage <sup>a</sup>	
	No	. Trea	ted		Total	<u> </u>	Percent				Total		Percent		
Time (Min.)	5	15	30	5	15	30	5	15	30	5	15	30	5	15	30
Conc. %									, , <b>, , , , , , , , , , , , , , , , , </b>						
.05	105	100	100	94	94	85	89.5	94.0	85.0	92	94	84	97.9	100	98.8
.10	76	100	88	74	94	76	97.4	94.0	86.4	71	93	74	95.9	98.9	97.4
1.0	74	100	78	67	77	52	90.5	77.0	66.7	66	76	52	98.5	98.7	100
5.0	100	100	73	10	7	7	10.0	7.0	9.6	10	7	7	100	100	100
10.0	100	100	82	2	2	4	2.0	2.0	4.9	2	2	3	100	100	75.0
Control <sup>d</sup>	115	100	100	112	99	92	97.4	99.0	92.0	112	99	91	100	100	98.9
Check <sup>e</sup>	100	-		96	-	-	96.0	-	-	96	-	-	100	-	-

<sup>a</sup>Percent column represents the percentages of those individuals successfully reaching the preceding stage. <sup>b</sup>Percent treated larvae successfully reaching adulthood.

<sup>C</sup>Post-emergence mortality.

<sup>d</sup>Dipped in distilled water only.

eNot dipped.

Table 20.	(Continued)	

	Reaching Adulthood <sup>a</sup>				l		nt Rea hood <sup>b</sup>		p	ercent				Eggs	Produced			
T	otal		P	ercent			ed Lar			Mortal			Total		P	er Fema	le	
5	15	30	5	15	30	5	15	30	5	15	30	5	15	30	5	15	30	
30	68	71	32.6	72.3	84.5	28.6	68.0	71.0	6.6	8.8	14.0	2802	2166	454	90.4	75.5	10 <b>.6</b>	
53	74	55	74.6	79.6	74.3	69.7	74.0	62.5	13.2	18.9	14.5	1050	850	1797	45.7	26.6	94.6	
46	55	38	69.7	72.4	73.1	62.2	55.0	48.7	6.5	10.9	13.4	789	920	694	31.6	34.1	43.4	
7	6	7	70.0	85.7	100	7.0	6.0	9.6	14.2	0.0	12.5	3	51	69	1.0	8.5	17.3	
1	1	3	50.0	50.0	100	1.0	1.0	3.7	100	0.0	50.0	0	O	0	0	0	0	
92	90	8 <b>9</b>	82.1	90.9	97.8	80.0	90.0	89.0	5.4	6.7	1.1	2073	2175	1788	162.2	157.0	184.8	
90	<i>u</i>	-	93.8	-	-	90.0	-	-	13.3	-	1	1998		-	90.9	-	_ 1	

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<u></u>					R	eachin	ng 3rd In	nstar			Read	ching	Pupal S <sup>.</sup>	tage <sup>a</sup>	
	No	. Trea	ted		Total		Percent				[ota]		Percent		
Time (Min.)	5	15	30	5	15	30	5	15	30	5	15	30	5	15	30
Conc. %												-			
.05	100	100	63	94	96	59	94.0	96.0	93.7	94	93	55	100	96,9	93.2
.10	100	100	76	93	98	76	93.0	98.0	100	93	98	73	100	100	96.1
1.0	100	100	100	97	98	93	97.0	98.0	93.0	97	98	92	100	100	98.9
5.0	100	100	78	96	95	76	96.0	95.0	97.4	93	93	75	96.9	97.9	98.7
10.0	100	100	100	98	94	90	98.0	94.0	90.0	94	92	87	95.9	97.9	96.7
Control <sup>d</sup>	115	100	100	112	99	92	97.4	99.0	92.0	112	99	91	100	100	98.9
Check <sup>e</sup>	100	-		96	-	-	96.0	-	-	96	-	-	100	-	<b>-</b> ·

Table 21. Effects on molting capabilities, emergence, post-emergence mortality, total offspring production and eggs per female resulting from 2nd instar larvae dipped in aqueous solutions of stibophen.

<sup>a</sup>Percent column represents the percentages of those individuals successfully reaching the preceding stage. <sup>b</sup>Percent treated larvae successfully reaching adulthood.

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<sup>C</sup>Post-emergence mortality.

<sup>d</sup>Dipped in distilled water only.

eNot dipped.

	Reaching Adulthood <sup>a</sup>				l		nt Rea hood <sup>D</sup>		F	Percent			Eggs Produced						
T	otal	<u></u>	P	ercent	<u>,                                     </u>		ed Lar			<u>Mortal</u>			Total_		Pe	r Femal	e		
5	15	30	5	15	30	5	15	30	5	15	30	5	15	30	5	15	30		
91	88	50	96.8	94.6	90.9	91.0	88.0	79.4	21.9	7.9	18.0	1364	1733	1208	42.6	38.2	46.5		
87	84	69	93.5	85.7	94.5	87.0	84.0	90.8	4.7	19.7	10.1	1562	1692	1280	37.2	44.5	41.3		
95	86	71	97.9	87.8	77.2	95.0	86.0	71.0	16.8	12.0	16.9	2993	1888	1550	71.3	56.4	55.3		
87	87	57	93.5	93.5	76.0	87.0	87.0	73.1	16.1	7.4	7.3	1826	2234	1099	49.4	69.8	33.3		
89	65	82	94.7	70.7	94.3	89.0	65.0	82.0	8.0	33.8	6.1	1297	909	895	30.1	39.5	38.9		
92	90	89	82.1	90.9	97.8	80.0	90.0	89.0	5.4	6.7	1.1	2073	2175	1788	162.2	157.0	184.8		
90	-	-	93.8	-	-	90.0	-		13.3	-	-	1998	-	-	90.9	-	-		

Table 21. (Continued)

Of those larvae successful in reaching the 3rd instar, 85.6% of those treated with antimony sodium tartrate pupated; 94.5% receiving stibophen pupated and 96.0% of those receiving antimony potassium tartrate pupated successfully. Of those 3rd instar larvae that reached the pupal stage 61.3% of them reached the adult stage after treatment with antimony sodium tartrate; 86.1% reached the adult stage after treatment with stibophen (Table 21) and 72.6% reached the adult stage after being treated with antimony potassium tartrate (Table 20).

Of those receiving antimony sodium tartrate as 2nd instar larvae a mean total of 35.5% were successful in reaching the adult stage (Table 19). The mean total reaching the adult stage from 2nd instar larvae treated with antimony potassium tartrate was 38.7% and with stibophen a mean total of 83.7% successfully attained adulthood.

With all three compounds the greatest losses occurred during the pupal stage with the highest mortality occurring in those insects receiving antimony sodium tartrate.

The effects of concentration of both antimony sodium tartrate and antimony potassium tartrate were greatest on the 2nd instar larvae beginning with the 1.0% concentration and increasing in severity through the 5.0% concentration to almost total mortality at the 10.0% concentration. Stibophen's effect was minimal throughout development and changed little with increasing concentrations.

Little effect was observed on post-emergence mortality with all compounds and at all concentrations and time. Some concentrations produced a high post-emergence mortality percentage but there were few individuals therein that attained the adult stage so a low number of deaths would necessarily produce a high mortality percentage figure.

The offspring production of the females resulting from 2nd instar larvae treated with the antimony compounds follows generally the same trends as toxicity in the earlier stages. The untreated check and the control in all instances had greater offspring production than all concentrations of antimony potassium tartrate and stibophen; and with antimony sodium tartrate in all but the 1.0% concentration at 30 minute treatment time where the eggs per female jumped to 387.3. This could be attributed to experimental error.

#### Egg Dipping

The effects on hatching, post-emergence mortality and offspring capabilities of <u>C</u>. <u>macellaria</u> eggs dipped in aqueous solutions of antimony potassium tartrate are compiled in Table 22.

Rogers and Howell (1971) performed a similar test with <u>Argas</u> <u>persicus</u> eggs dipped in physiological saline--antimony potassium tartrate and found a higher percentage hatch occurred in these solutions than in saline only. They also found that submergence of <u>A</u>. <u>persicus</u> ova in physiological saline for 1 minute significantly reduced the percentage hatch.

The data in Table 22 show a similar effect on <u>C</u>. <u>macellaria</u> eggs dipped in distilled water--antimony potassium tartrate solutions. A mean percentage hatch of 53.5% was obtained when the eggs were submerged for 1 minute while the control which was dipped in distilled water only for the same period of time resulted in a mean percentage hatch of 39.9%, a reduction of 13.6%. With the exception of the 0.5% concentration, all concentrations provided a higher percentage hatch than the control at the 1 minute submergence time. It should be noted

										ŀ	latch	·			
Time		No.	Trea	ted		<u> </u>		No.	·	<u></u>			Percer	nt	
(Min.)	1	5	10	15	25	1	5	10	15	25	1	5	10	15	25
<u>Conc. %</u>															
.05	180	200	200	200	200	114	76	34	9	12	63.3	38.0	17.0	4.5	6.0
0.1	197	200	200	200-	200	133	70	13	32	27	67.5	35.0	6.5	16.0	13.5
0.2	200	200	200	200	200	87	62	13	5	14	43.5	31.0	6.5	2.5	7.0
0.3	188	200	200	200	200	124	87	16	16	27	66.0	43.5	8.0	8.0	13.5
0.4	200	190	200	200	200	123	47	13	15	13	61.5	24.7	6,5	7.5	6.5
0.5	200	147	200	200	200	42	27	11	8	13	21.0	18.4	5.5	4.0	6.5
Control <sup>b</sup>	153	200	200	200	200	61	77	17	7	27	39.9	38.5	8.5	3.5	13.5
Check <sup>C</sup>	200	200	-	-	-	10	11	-	-	-	5.0	5.5	-	- 1	-
$\bar{\mathbf{x}}^{d}$	194.2	2 189.5	5 200	200	200	103.8	61.5	16.7	14.2	17.7	53.5	32.5	8.3	7.1	8.8

Table 22. Effects on hatching, post eclosion mortality and offspring capabilities of <u>C</u>. <u>macellaria</u> eggs dipped in aqueous solutions of antimony potassium tartrate.

<sup>a</sup>Mortality of the various stages following successful eclosion from treated eggs.

<sup>b</sup>Dipped in distilled water only.

<sup>C</sup>Was not dipped.

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<sup>d</sup>Mean of all concentrations at various times excluding control and check.

Dr	nst Fold	nsion M	ortality	,a					Offsprin	ig Produ	ced	·	=	- <u></u>
		Percent		·			Total				P	er Femal	e	
1	5	10	15	25	1	5	10	15	25	1	5	10	15	25
85.9	92.1	35.3	22.2	16.7	869	692	904	667	679	144.8	230.7	69.5	222.3	169.8
85.7	80.0	46.2	50.0	22.2	696	797	923	692	1128	63.3	99.6	230.8	69.2	102.5
87.4	90.3	7.7	20.0	14.3	625	684	422	547	686	89.3	171.0	70.3	182.3	98.0
78.2	90.8	37.5	6.3	48.1	976	246	798	880	916	81.3	41.0	133.0	97.8	101.8
86.2	82.9	46.2	26.7	38.5	570	582	369	1138	102	114.0	97.0	369.0	162.6	51.0
59.5	37.0	45.5	25.0	30.8	776	459	257	712	559	86.2	57.4	42.8	178.0	139.6
68.9	83.1	58.8	57.1	59.3	173	928	703	265	245	21.6	132.6	234.3	265.0	61.3
50.0	54.5	-	-	-	122	0	-	-	-	40.7	0	-	-	-
82.8	84.0	34.0	30.6	30.2	752	576.7	612.2	772.7	678.3	90.2	98.9	102.0	128.8	110.0

Table 22. (Continued)

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that the two checks against the distilled water showed only a mean percentage hatch of 10.5% indicating that the distilled water alone had an effect on the hatching capabilities of the eggs.

Submergence time had an obvious effect on hatching but hatching was largely independent of antimony potassium tartrate concentration.

Post-eclosion mortality was greatest at the 1 and 5 minute submergence time and became less with increasing submergence times. No explanation for this occurrence can be made nor can one be advanced to explain the high percent post-eclosion mortality for the untreated checks (82.8% and 84.0%) at these times. The control post-eclosion mortality would necessarily be high because of the low number of individuals that had hatched from the treated eggs.

As shown in Table 22 the antimony potassium tartrate had no effect upon the number of offspring produced by the adults resulting from the treated eggs. A beneficial effect would appear to be the rule but no valid comparisons can be made due to the adverse effects of the distilled water alone on the control group and the high post-eclosion mortality experienced by the check group. It would not be expected that an effect on fecundity would be experienced since a large amount of the antimony potassium tartrate would be absorbed by the egg shell and would be lost at the time of hatching. It is not probable that the individual which did absorb some of the compound would retain enough of it, without its being toxic, to carry over into the pupal stage at which time the gonads are developing.

# SUMMARY AND CONCLUSIONS

The effects of five antimony compounds on reproduction, growth and development of the secondary screw-worm fly <u>Cochliomyia macellaria</u> (Fabr.) were investigated in the laboratory. The eight tests conducted included adult feeding, injection and dusting; pupal dipping and inunction; larval feeding and dipping; and egg dipping.

In the adult feeding experiments antimony potassium tartrate proved to be the most toxic of the test compounds (resulting in a mortality mean of 22.2%), followed in order by stibophen, antimony sodium tartrate, triphenylantimony and antimony barium tartrate.

Total mortality caused by antimony potassium tartrate showed a linear relationship with concentration levels. No significant differences in toxicity of antimony sodium tartrate were evident between the lower concentrations (0.01, 0.05, 0.10, 2.0, 5.0%). At the 5.0% concentration the effects were marked with a significant increase in total mortality from 16.0% to 59.3%. Toxicity in response to triphenylantimony was similar to that of antimony sodium tartrate but was not as profound at the higher levels. There were no obvious differences in toxicity between all concentrations of stibophen above the 0.10% level and none between all concentrations of antimony barium tartrate.

Antimony sodium tartrate was the only tested compound that had an effect on the hatch of eggs resulting from treated females. The compound's effects were not significant until reaching the 1.0% concentration level. All compounds showed a reduction in the percentage

hatch over the controls but in no case were these reductions significant.

Antimony sodium tartrate was the most effective of the treated compounds in decreasing fecundity with the production of a mean of 35.8 eggs per female compared with a mean of 76.4 eggs per female for the control, a total reduction of 53.1%. Stibophen was next in decreasing fecundity (-50.1%) and as with toxicity, there were no significant differences in number of eggs per female between concentrations above the 0.01% concentration level. Antimony potassium tartrate showed a total reduction in eggs per female from the control of 33.5% with no significant differences between concentrations 0.01%, 0.05% and 0.10% nor between the 1.0% and 2.0% concentrations. However, significant differences were evident between the lower 3 concentrations and the higher two concentrations (1.0% and 2.0%) and the highest concentration (5.0%).

The number of eggs per female treated with antimony barium tartrate showed a great deal of variation with concentration with significant differences between various levels and the control and no significant differences at other concentrations.

Percent egg reduction from the control with triphenylantimony showed little variability between concentrations with no significance existing between all levels.

Antimony potassium tartrate was the most toxic of the three compounds tested by injection experiments with a mortality mean of 67.4% as compared with 21.5% for the controls which received injections of distilled water and 0.0% for the untreated check. Total mean mortality was only slightly lower for stibophen (52.7%) and as with

antimony potassium tartrate was significantly higher than that of the control or check. Antimony sodium tartrate was the least toxic of the tested compounds.

The toxic effects displayed by both antimony potassium tartrate and antimony sodium tartrate were striking as indicated by day one post-treatment mortalities of 47.8% and 38.4% respectively. Stibophen toxicity was more gradual starting with a mean mortality percentage of 4.0% on day one post-treatment.

Toxicity to antimony sodium tartrate was independent of concentration throughout the 4 lowest concentrations (0.01, 0.10, 0.50, and 1.0%) but mortality was total at the 5.0% concentration. Stibophen and antimony potassium tartrate were similar, both reaching 93.3% at the 5.0% concentration level.

In the injection experiments female mortality exceeded male mortality. This was thought to be in response to increased body temperature of the female over the male due to her increased activity. Results of previous tests show that antimony toxicity increases with temperature. Another possible explanation was that the female elaborated more  $Na^+$  and  $K^+$  ions and in so doing effectively increased the concentration of antimony in the system.

The only injection treatment to significantly reduce fertility was with antimony sodium tartrate at the 1.0% concentration. All other concentrations in all three compounds had no effect upon fertility when compared to the controls.

Fecundity in the injection tests was significantly reduced in all the tested compounds with the greatest reduction resulting from injections of antimony potassium tartrate. With this compound complete

sterilization was obtained at the 5.0% concentration. Stibophen produced total sterilization at both 1.0% and 5.0% concentrations and the total sterilization that was obtained with antimony sodium tartrate at the 5.0% concentration came as a result of total mortality at this level.

Effects of adult dusting proved to be confusing in that all adults exposed to the dust for 5 and 15 minutes resulted in a lower mortality rate than did the controls. Initial indications were that there was a therapeutic value derived from the treatment but the high mortality experienced by the untreated control group would invalidate the results.

Although a decrease in emergence from the controls was obtained by dipping puparia containing pupae in the antimony compounds the differences were not substantial. Antimony sodium tartrate was the most effective in retarding development with stibophen and antimony potassium tartrate producing essentially identical results. In all compounds deleterious effects upon emergence capabilities were independent of dipping time.

Observable malformations of the adults emerging from treated puparia were most pronounced in the antimony sodium tartrate treated group and included twisted wings and soft bodies which apparently could not be hardened properly. The differences in penetration ability of the compounds could have been due to their differences in molecular weights. All females resulting from treated puparia at all concentrations and at all treatment times produced fertile eggs.

The only appreciable influence on development time was obtained

with antimony sodium tartrate and was independent of treatment time but followed increased concentrations.

Pupal inunction tests with lanolin-triphenylantimony mixtures determined that lanolin alone was not toxic to the developing pupae when applied in the same manner as other lanolin-triphenylantimony mixtures. However, it was toxic to the developing pupae when puparia were completely inuncted. This oily covering undoubtedly stopped the transport of oxygen into the developing pupae and death came as a result of asphyxiation.

Post-emergence mortality figures indicated that the antimony compounds were successful in reaching the developing pupae. At all concentrations mortality was higher than the lanolin-only inunction or the controls. Similarly, increased triphenylantimony concentration caused a reduction in the number of offspring produced per female. Considering that the size and mating capability relationship suggested by Spates and Hightower (1970) existed in <u>C. macellaria</u> then triphenylantimony was probably responsible for the decreased egg production.

The effects on emergence capabilities of pupae that had been fed the various antimony compounds as 2nd instar larvae were most pronounced with triphenylantimony at a mean emergence percentage of 40.7%, compared to the control of 64.0%. Antimony sodium tartrate was next in influencing emergence with a mean of 41.8%. Antimony potassium tartrate and antimony barium tartrate were close in their effects upon emergence with means of 48.5% and 46.8% respectively. There was no effect upon emergence capabilities of those larvae receiving stibophen.

Treatment with triphenylantimony produced zero emergence at the lowest concentration level of any of the tested compounds (0.50%).

Both antimony sodium tartrate and antimony potassium tartrate prohibited any emergence at the 1.0% concentration with the former compound allowing only 3.0% emergence at the 0.5% concentration level. The zero emergence level was attained by treatment with antimony barium tartrate only at the 5.0% concentration.

The percentage emergence for all compounds at the 3 lowest concentrations was greater than that of the controls. A therapeutic effect was indicated which could have been due to the compound's effects upon the microbial growth in the medium. At the 0.50% concentration this beneficial influence was probably outweighed by the toxic effects of the compounds, and higher mortality (decreased emergence) resulted. Stibophen, the least toxic of the compounds, probably continued to prove beneficial through the 1.0% concentration and became detrimental at the 5.0% concentration.

Larvae fed on the medium containing 0.50% antimony sodium tartrate were smaller than the controls but reached the 3rd instar and 3.0% of them reached the adult stage. Larvae fed on medium containing 0.50% triphenylantimony also reached the 3rd instar but growth was seriously affected and those individuals died before reaching the adult stage. At the 1.0% and 5.0% concentrations of both antimony sodium tartrate and triphenylantimony larval development did not proceed beyond the 2nd instar.

All the tested compounds showed an influence on growth and development of the treated larvae but none were as pronounced as those treated with antimony sodium tartrate and triphenylantimony.

Recent research has indicated that a definite relationship exists between weights of mature larvae and probable mating capabilities of male <u>C</u>. <u>hominivorax</u>. If this same relationship holds true for <u>C</u>. <u>macellaria</u> it could be concluded that larvae prohibited from attaining full growth potential by actions of the antimony compounds would not produce as many offspring as those individuals less affected. The results of these tests tend to agree with this conjectural concept. Three percent of larvae treated with 0.50% antimony sodium tartrate reached the adult stage but were unable to produce any offspring. Due to the impaired growth of larvae treated at the 0.01% concentration level the resultant adult females could produce only a mean of 53.0 eggs per female. Of those larvae fed 0.10% triphenylantimony, 84.0% were successful in reaching the adult stage but because of impaired growth produced a mean of only 15.2 eggs per female.

It cannot be ruled out that the introduction of the antimony compounds altered the amino acid content of the medium but this was probably not the cause for cessation of growth and development.

It was unknown whether the size of the larvae and subsequent adult size was more influential on reproductive inhibition or if action of the antimony compounds solely on the gonads proved to be more detrimental. Gross dissections of the adults showed a reduction in the size of the ovaries at the higher concentrations but no effects upon the size of the testes were observed.

Antimony sodium tartrate was the most toxic of the tested compounds to 2nd instar larvae receiving dipping treatment followed closely by antimony potassium tartrate. Stibophen showed little toxic effects. Toxicity to the 2nd instar larvae was independent of submergence time with all compounds.

Of those treated 2nd instar larvae successful in reaching the 3rd instar, 85.6% of those treated with antimony sodium tartrate pupated; 94.5% receiving stibophen pupated and 96.0% of those receiving antimony potassium tartrate successfully pupated.

Of those successfully pupating 61.3% reached the adult stage following treatment as 2nd instar larvae with antimony sodium tartrate; 81.6% reached the adult stage after treatment with stibophen and 72.6% of those receiving antimony potassium tartrate were successful in reaching the adult stage.

With all 3 compounds the highest mortality occurred during the pupal stage with toxicity of antimony sodium tartrate being the greatest.

Effects of concentration of both antimony sodium tartrate and antimony potassium tartrate were greatest on the 2nd instar larvae beginning with the 1.0% concentration and increasing in severity through the 5.0% concentration to near total mortality at the 10.0% concentration. Stibophen's effect was minimal throughout development and changed little with increasing concentrations.

Little effect on post-emergence mortality with all compounds and at all concentrations and time was observed.

Untreated check and the control had greater offspring production than all concentrations of antimony potassium tartrate and stibophen. Experimental error would account for the production of 387.3 eggs per female at the 1.0% concentration and 30 minutes treatment time with antimony sodium tartrate.

A mean percentage hatch of 53.5% was obtained with eggs submerged for 1 minute in aqueous solutions of antimony potassium tartrate. The control submerged in distilled water for the same period of time resulted in a mean percentage hatch of 39.9%. All concentrations, with the exception of the 0.5% level provided a higher percentage hatch than the controls at the 1 minute submergence time.

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#### VITA

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## Thesis: ALTERATIONS IN REPRODUCTION, GROWTH AND DEVELOPMENT OF <u>COCHLIOMYIA MACELLARIA</u> (FABR.) IN RESPONSE TO SELECTED ANTIMONY COMPOUNDS

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