SOME EFFECTS OF CADMIUM SALTS AND RELATED COMPOUNDS ON REPRODUCTIVE ORGANS AND GROWTH OF <u>CULEX QUINQUE</u>-<u>FASCIATUS</u> SAY (CULICIDAE)

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By

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Submitted to the faculty of the Graduate College of the Oklahoma State University in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE May, 1969

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Thesis Approved:

DEHowell Thesis Adviser lon 11

the Graduate College Dean of

For the past two years cadmium compounds have been investigated by entomologists at Oklahoma State University. Research in this area has shown intriguing results on insect growth and reproduction. With this in mind, the author, upon recommendation by Dr. D. E. Howell, decided to investigate the effects of cadmium salts and related compounds on a mosquito species to be colonized the the author.

The author expresses sincere appreciation to Dr. D. E. Howell, his major adviser, for actuating and guiding him in this project. Grateful recognition is expressed to other members of his committee, Dr. R. R. Walton, Professor of Entomology; Dr. R. D. Eikenbary, Associate Professor of Entomology; and Dr. E. E. Sturgeon, Professor and Head of Forestry, for their assistance and encouragement through this study.

Acknowledgement is also made of Dr. J. A. Hair, Department of Entomology, for his helpful advice and suggestions.

In addition, the author would like to thank Dr. H. L. Chada, Professor of Entomology, for permitting the use of United States Department of Agriculture equipment and facilities during a portion of this study; Dr. P. N. Saxena, Research Associate, for his time and suggestions on serial sectioning and microscopic photography; and H. G. Raney and M. M. Moyer for their encouragement and help.

Finally, the author expresses appreciation to the United States Army, Medical Service Corps, for allowing time for this study.

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INTRODUCTION

Noxious insects have plagued man for centuries. Researchers have massed many devices, materials, and techniques to eliminate or reduce arthropod intruders. Insecticides, thought to be the panacea for control, were found to have drawbacks, such as harmful effects on man and wildlife and the development of heritable resistance to insecticide by insects. Even so, safer insecticides, better method of application, more safety devices, and increased legislation combined to improve chemical Researchers have found other methods to suppress insect popucontrol. lations. The potentials of chemical sterilization have been clearly expressed by the rapidity with which researchers have published information about different alkylating agents. These have been used to sterilize vertebrates and invertebrates (Kilgore and Doutt, 1967). The entomological "dragnet" has been developed to find sterilizing materials and techniques that are effective, safe, easy to work with, and inexpensive. In the past few years many chemicals have been evaluated for their sterilizing and toxicity effectiveness (LaBrecque and Smith, 1968). An insect chemosterilant is a chemical compound which deprives an insect of its ability to reproduce (Borkovec, 1962). This action might be accomplished by the compound causing (1) the insect to fail to produce ova or sperm, (2) the ova or sperm to die after production, or (3) dominant lethal mutations. The mode of action theories by which the chemosterilants might operate are suggested as cross-linking (Stacey et al., 1958), changes in living DNA other than cross-linking (Anerbach, 1958),

and the blocking of important metabolites (Timmis, 1962). The chemosterilants include radiomimetic compounds, antimetabolites, mitotic poisons, and alkylating agents. Multiple actions are evident in some compounds. Cadmium compounds have shown sterilizing activity in insects (Kunz, 1967; Abdel-Razig, 1966). Cadmium acetate, cadmium iodide, cadmium succinate, and cadmium chloride caused reduction in numbers of eggs laid by Stomoxys calcitrans (L.) (Kunz, 1967, unpublished data). Egg production of Musca domestica (L.) was reduced by feeding adults cadmium chloride, cadmium acetate, and cadmium succinate (Abdel-Razig, 1966, unpublished data). The sterilizing activity of cadmium on mammals and rodents has been indicated by such workers as Parizek and Zahor (1956), Kar (1961), Gunn et al. (1961), and Kar and Pandoj (1963). The chemicals were introduced by subcutaneous injection, scrotal inunction, diet incorporation, intramuscular injection, and intra-testicular injection. The testes were found to be affected more than the ovaries. Deterrents to the use of cadmium as chemosterilants are toxicity to man and animals, damage to tissue, delayed growth, and high mortality to insects. Current literature shows that compounds damage the reticulo-endothelial cells of the lungs, liver, kidneys, and spleen of various mammals. Cadmium has also caused hyperplasia, sarcomata, necrosis, neoplasia, and interstitial cell tumors. The selectivity of cadmium for testes could be due to the sensitivity of the seminiferous epithelium, and blocking of sulphydryl groups (Parizek, 1957). Oestrogen producing organs seem to be the site of cadmium activity (Parizek, 1964). Other workers have found that zinc compounds counteract the effects of cadmium (Powell et al., 1964; Gunn et al., 1961; Kar et al, 1960).

This paper will show some effects of cadmium and other compounds on reproductive organs and growth of <u>Culex quinquefasciatus</u> Say.

MATERIALS AND METHODS

The mosquitoes were collected and colonized at Oklahoma State University. Maintenance of the colony followed some of the procedures outlined by DeMeillon and Thomas (1966). Modifications involved a mean temperature of 78 F \pm 3; Purina rat and mouse laboratory chow for a larval food; adult feeding on 3% sugar syrup; and white leghorn cockerels for blood. Test chemicals were reagent grade cadmium chloride, cadmium acetate, cadmium iodide, indium sulfate, antimony potassium tartrate, zinc chloride and technical grade Cadminate (60% cadmium succinate). All chemicals except the antimony compound were stored in a vacuum desiccator.¹

Larvae.--First and late third or early fourth instars were exposed to soluble compound concentrations from 0.1 ppb to 1.0 ppt^2 in 100 or 200 ml of tap water in porcelain dishes to determine effective levels. First instars were placed in serial diluted concentrations within 24 hr after hatching and remained in treated water until pupation. Insoluble Cadminate was mixed in 1:1 - 1:1000 ratios with larval diet. The scum which formed on the surface of the water was removed daily and records of mortality and growth were taken. Evaporated water was replaced with tap water. After pupation, the pupae were placed in 1-oz plastic cups

 1 Silica gel was the desiccant, and the vacuum was approximately 454 g pressure.

²ppt means parts per thousand and ppb means parts per billion.

and kept in 1-qt carton cages for adult emergence. Testes and ovaries were dissected from adults and measured in normal saline at least three days after emergence. Peculiarities in gonads were observed. Some gonads were mounted in CMC series media³ for microscopic observations. Seminal vesicles were broken with pin points to observe spermatozoa under an oil immersion lens. Treated mosquitoes were allowed to feed overnight on immobilized chickens to make possible ovarian development. Concentrations of 10 ppm cadmium chloride (20 ml) and 10 ppm zinc chloride (20 ml) were mixed with tap water (180 ml) to form a combined solution of 1.0 ppm cadmium chloride and 1.0 ppm zinc chloride. This combination is later referred to as $CdCl_2 - ZnCl_2$ concentration of 1.0 ppm. First instar larvae were reared in the combined solution until pupation. Testes and ovaries were dissected from the adults three days after emergence.

First instar larvae were also reared in water containing 1.0 ppm of cadmium chloride for three days until approximately 50% were dead. The survivors were then placed in untreated water and reared to adults. When the adults were three days old, the abdomens of six males and six females were fixed in Carnoy's fluid, embedded in 59 C mp paraffin and serial sections made. After staining with Delafield's haematoxylin and Eosin Y, these preparations were compared with those from mosquitoes reared in the normal manner.

<u>Pupae</u>.--Pupae were placed in concentrations of 10.0 ppt, 1.0 ppt, 0.1 ppt of cadmium chloride, cadmium acetate, and zinc chloride until adult emergence. Three days later gonads were checked.

³General Biological Supply House CMCP-9AF, CMCP-9AB, CMC-9.

<u>Adults</u>.--One-day-old adults from the stock colony were separated according to sex, grouped into lots of 25, and maintained for 4 days in 1-gal cartons with 3% sugar solutions containing 1000, 100, 10, or 0 ppm cadmium chloride in cotton pads. Then mating crosses were made in 1-qt cartons using treated males x untreated females, treated females x untreated males, treated males x treated females, and untreated males x untreated females.⁴ Immobilized chickens were placed on each cage one day later. A similar experiment was conducted with 50 males and 50 females with 10 and 50 ppm cadmium chloride.⁵ The numbers of egg rafts, numbers of eggs, numbers of unembryonated rafts, and per cent hatch of viable rafts were recorded.

⁴Total of 6 males and 6 females/carton ⁵Mating crosses, 10 males and 10 females/carton

RESULTS

Larvae.--The effects of various concentrations of test compounds on survival are shown in Table I. The survival of larvae treated with selected concentrations are given in Table II. Concentrations of 0.1 ppm - 0.1 ppb did not affect either survival or median pupation time.⁶ The relationships of compound concentrations to average median pupation time are shown in Fig. 1. Cadmium chloride, cadmium acetate, and cadmium iodide, each at concentration of 1.0 ppm resulted in the average median pupation times of 13.8, 13.3 and 15.0 days, respectively. Zinc chloride at 0.1 ppt lengthened median pupation time to 17 days. Zinc chloride-cadmium chloride at concentration of 1.0 ppm caused 62% mortality of first instar larvae and caused the surviving larvae to pupate at a median time of 11 days.

Late third and early fourth instar larvae reared in 1.0 ppm cadmium chloride or cadmium acetate solutions pupated approximately the same time as untreated larvae, but 50% of the pupae drowned during emergence. Death followed soon after straightening of the abdomen just before emergence. Lengths of testes and ovaries from adults of exposed larvae are shown in Table III. Cadmium chloride 1.0 ppm affected both ovaries in each of two females and one ovary in each of two females. Cadmium acetate 1.0 ppm affected both ovaries of one female. Cadmium chloride

 $^{6}\mathrm{Median}$ pupation time is defined as the time when approximately 50% of the larvae pupate.

	Concentration in Water											
Compounds	1.0 ppt	0.1 ppt	10.0 ppm	1.0 ppm	0.1 ppm	0.01 ppm	1.0 ppb	0.1 pp				
CdC1 ₂	Х	Х	X	/	S	S	S	S				
Cd(C ₂ H ₃ O ₂) ₂	X	X	Х	/	S	S	S	S				
CdI ₂	Х	X	Х	/	S	S	S	S				
In ₂ (S0 ₄) ₃	-	S	-	S	S	S	S	S				
ZnCl ₂	Х	. 1	-	S	Ś	S	S	S				
к(sb0)с ₄ н ₄ 0 ₆	Х	/	S	- -	- -	· · · · ·	-	-				
ZnC1 ₂ - CdC1 ₂	-	-	Х	/	S		-	-				
		· · · · · · · · · · · · · · · · · · ·		Chemical:Di	et ratio			<u></u>				
	1:1	1:10	1:20	1:40	1:50	1:60	1:100	1:1000				
Cadminate	Х	Х	Х	X	Х	Х	/	S				

DOSAGE-MORTALITY OF FIRST AND LATE-THIRD OR FOURTH INSTAR LARVAE CAUSED BY VARIOUS COMPOUNDS

TABLE I

/ = Partial Mortality

- = No Treatment

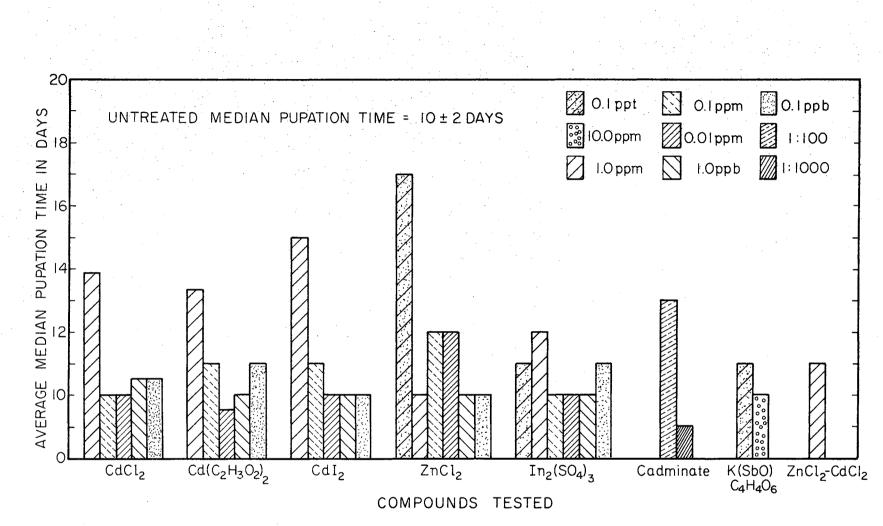
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TABLE	ΙI
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SURVIVAL	0F	LARVAE	REARED	ΙN	SELECTED	CONCENTRATIONS
		· (OF EACH	CON	1POUND	

Compounds	Concen- tration	Stage of instar tested	No. larvae tested	Average % survival
CdC1 ₂	1.0 ppm	1	260	35
	 .	3-4	50	72
Cd(C2H302)2	1.0 ppm	1	90	49
	-	3-4	50	94
CdI2	1.0 ppm	1	20	60
In ₂ (S0 ₄) ₃	0.1 ppt ^a	1	20	85
	1.0 ppm	1	20	85
ZnCl ₂	0.1 ppt	1	20	55
	1.0 ppm	1	20	80
к(sb0)с ₄ н ₄ 0 ₆	0.1 ppt	1	50	72
	10.0 ppm	1	50	84
$ZnCl_2 - CdCl_2$	1.0 ppm	1	50	38
Cadminate	1:100	1	50	36
	1:1000	1	50	84

^appt = parts per thousand



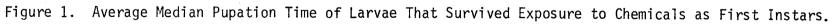


TABLE III

Compound	Con- centra- tion	No. Male	Adults Female	<u>Length</u> Testis	ovary ^b		No. Distor- tions
CdC1 ₂	1.0 ppm	6	17	0.64	0.77	3	6
2	* *	. 1	4	0.71	0.84	-	-
	0.1 ppm 0.01 ppm	5 7	10	0.71	0.90	5 12	- 3
	0.01 ppm 1.0 ppb	-	31 4	0.65	0.83 0.78	2	э -
	0.1 ppb	7	21	0.63	0.85	9	-
Cd(C ₂ H ₃ O ₂) ₂	1.0 ppm	3	9	0.65	0.82	4	2
	* 0.1 ppm	1	5 4	0.59 0.71	0.88 0.72	1 2	· · · ·
	0.1 ppm 0.01 ppm	3 6	11	0.65	1.03	1	-
	1.0 ppb	-	10	-	0.74	~	
.	0.1 ppb	-	4		0.78	3	-
CdI ₂	1.0 ppm 0.1 ppm	1 3 3	4 5	0.67	0.90 1.00	- 2	-
	0.01 ppm		6	0.63	0.84	2 2	-
	1.0 ppb	+C	+ 5	· · · · ·	- · 0 01	- 2	-
7	0.1 ppb	-		-	0.81		· •
ZnC1 ₂	0.1 ppt 1.0 ppm	1 4	4 12	0.61	1.18 0.81	3 2 4 3 2	
· · · ·	0.1 ppm	-	5		0.78	2	- -
	0.01 ppm 1.0 ppb	1	7 5	0.76	0.72	4	_
	1.0 ppb 0.1 ppb	-	4	-	0.83	2	-
In ₂ (S0 ₄) ₃	0.1 ppt	1	6	0.67	-	6	_
2. 4.3	1.0 ppm	-	4	-	0.71	6 3	-
	0.1 ppm 0.01 ppm	+ 2	+ 7	- 0.68	- 0.85	- 3	-
· .	1.0 ppb	1	5	0.67	0.84	3 2 2	-
	0.1 ppb	-		-	0.83	2	· · ·
ZnCl ₂ -CdCl ₂	1.0 ppm	-	3q	-	0.76		-
<(Sb0)C ₄ H ₄ O ₆	0.1 ppt	5 5	9d 7d	0.71	0.91	- 1	-
	10. ppm			0.72	0.90	-	-
Cadminate	1:100 1:1000	1	6	0.69 0.77	0.89 0.97	· -	. <u> </u>
Control		23	67	0.71	0.94	23	·

SIZES AND DISTORTIONS OF ADULT GONADS FROM TREATED LARVAE^a TREATED AT VARIOUS CONCENTRATIONS OF COMPOUNDS

Adult females not blood fed + Lost adults b

С

d Adults not offered blood meal

0.01 ppm affected both ovaries in one adult and one ovary in another.

The reproductive organs of both male and female are shown in Figs. 2 and 3, respectively, while Figs. 4 to 8 show distortions apparently caused by cadmium compounds. The gross ovarian distortions were found to have dense masses of tracheae with or without ovarioles. The different sizes of ovarioles are apparent in Fig. 8. The first egg chamber is either absent or reduced in size and the cellular walls are obliterated. A normal ovariole is shown in Fig. 9. The distorted ovaries were shorter in length and narrower in width than untreated or normal ovaries. Oviposition of an unusual egg raft on the surface of the water was observed from a cadmium treated female (cadmium chloride at 1.0 ppm). There were seven clustered, elongate (approximately 0.9 mm), non-viable, reddish-orange eggs (Fig. 10). Other dissected ovaries and testes showed no apparent differences in length compared with the controls. The average sizes of gonads from adults of treated larvae fell well within the range of lengths in the controls. Normal mature ova are shown in Fig. 11.

Histological sections of the gonads of adults reared from larvae maintained in 1.0 ppm cadmium chloride solution until approximately 50% of the larvae had died and the survivors kept in untreated water until emergence showed no abnormalities.

The effects of reagent grade cadmium chloride, cadmium acetate, and cadmium iodide on survival and median pupation time were compared by rearing first instar larvae to the pupal stage in 1.0 ppm solutions of each. The data were not conclusive (Fig. 12). Survival data suggested that the cadmium ion may have been responsible for the physiological effects on mosquitoes while the delay in time of pupation data suggested

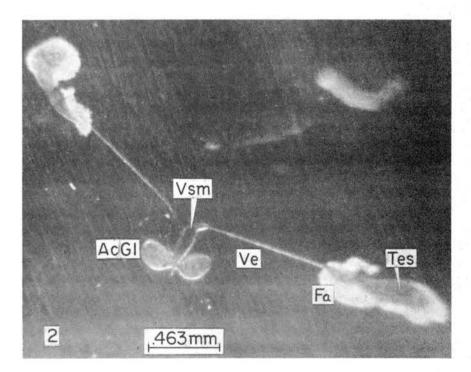


Figure 2. Reproductive Organs of a Male C. <u>quinque-fasciatus</u> Say. AcGl, Accessory Gland; Vsm, Seminal Vesicle; Ve, Vas Efferens; Fa, Fat Bodies; Tes, Testis.

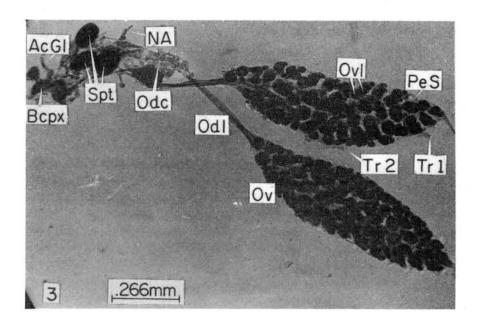


Figure 3. Reproductive Organs of a Female C. <u>quinquefasciatus</u> Say. Bcpx, Bursa Copulatrix; AcGl, Accessory Gland; Spt. Spermathecae; NA, Nerve Attachment; Odc, Common Oviduct; Odl, Lateral Oviduct; Ovl, Ovarioles; PeS, Peritoneal Sheath; Ov, Ovary; Tr1, Anterior Tracheal Trunk; Tr2, Posterior Tracheal Trunk.

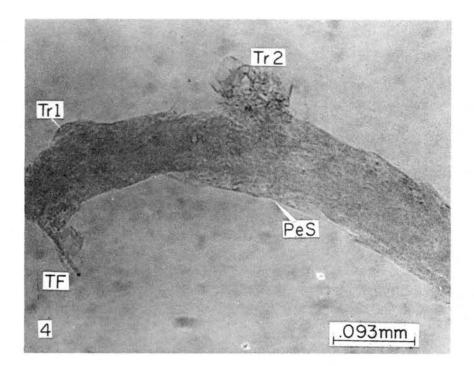


Figure 4. A Distorted Ovary From an Adult of a Larva Reared in 1.0 ppm Concentration Cadmium Chloride Until Pupation. The Ovary is Densely Tracheated, the Tracheal Trunks, Tr1 and Tr2, are Visible, but Tracheae are Filled with Fluid Within the Ovary and not Visible. PeS, Peritoneal Sheath; TF, Terminal Filament.

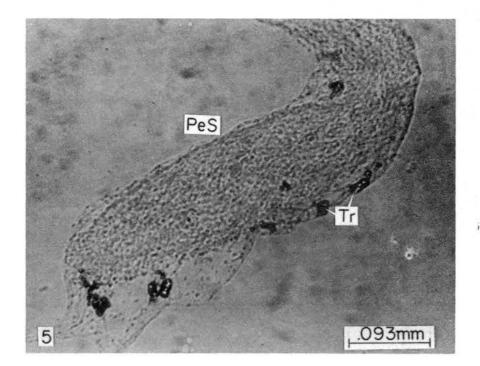


Figure 5. Distortion of an Ovary Treated as in Figure 4. PeS, Peritoneal Sheath; Tr, Air Within Tracheae.

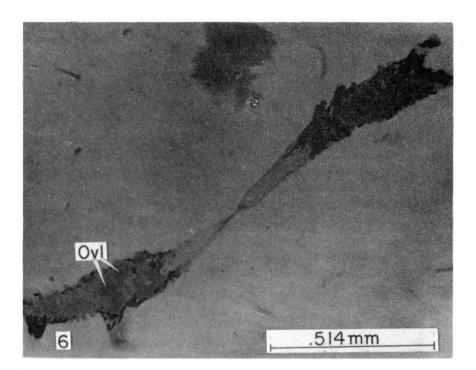


Figure 6. Two Distorted Ovaries from an Adult of a Larva Reared in 0.01 ppm Cadmium Chloride. Tracheal Tubes are Visible When Filled With Air. The Distortion of the Ovarioles (Ovl) Within the Ovaries is Apparent.

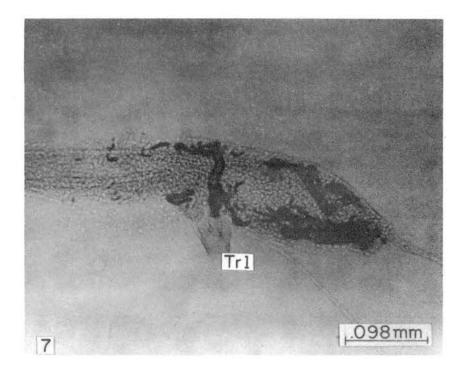


Figure 7. A Distorted Ovary From an Adult of a Larva Reared in 1.0 ppm Cadmium Acetate. Tr1, Anterior Tracheal Trunk.

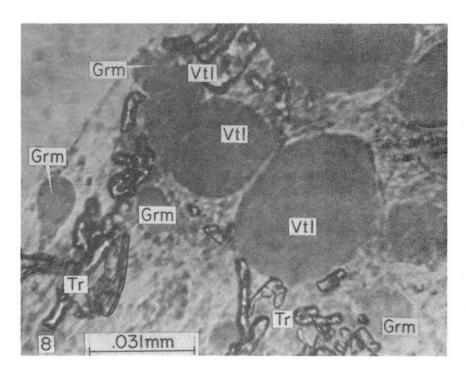


Figure 8. Part of an Ovary From an Adult of a Larva Reared in 0.01 ppm Cadmium Chloride. Grm, Germarium; Vtl, Vitellarium; Tr, Tracheae.

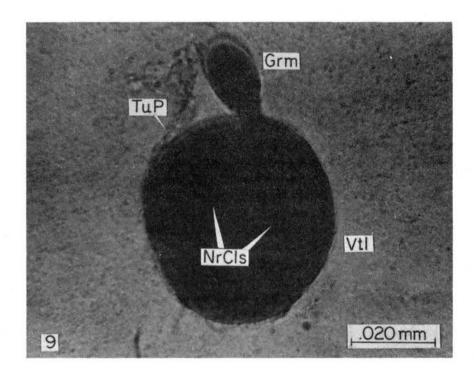


Figure 9. Ovariole From the Ovary of an Untreated Adult. Grm, Germarium; Vtl, Vitellarium; NrCls, trophocytes; TuP, Tunica Propria.

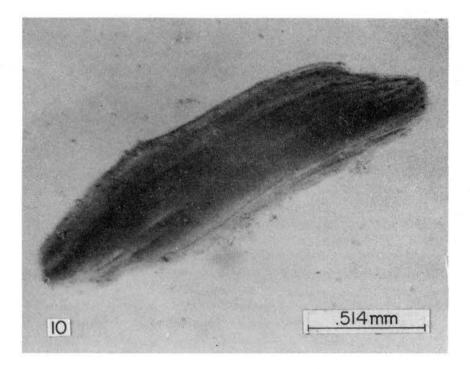


Figure 10. Distorted Egg Raft Oviposited by the Adult of a Larva Reared in 1.0 ppm Concentration of Cadmium Chloride. The Eggs are Clearly Distorted, and the Shells are not Well Formed.

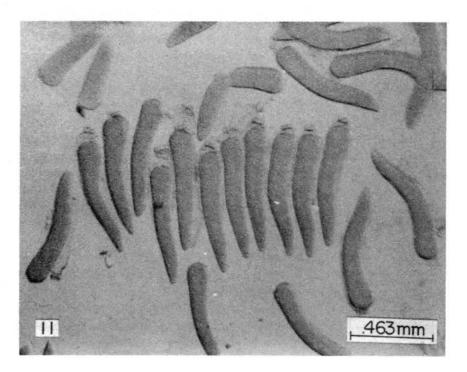


Figure 11. Mature Ova Dissected From an Untreated Adult.

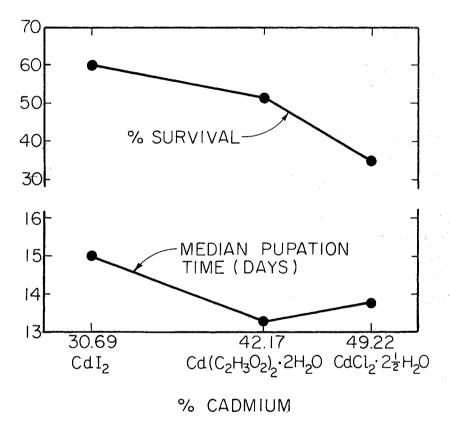


Figure 12. The Effects of Per Cent Cadmium in 1.0 ppm Cadmium Compounds on Time of Pupation and Survival.

that the compounds rather than the cadmium ions determined the effects.

<u>Pupae</u>.--Pupae placed in concentrations of 10.0 ppt cadmium chloride, cadmium acetate, and zinc chloride had more mortality than controls. Adult gonads of those that survived appeared unaffected by the treatments. These data are shown in Table IV.

<u>Adults</u>.--When adults were exposed to 3.0% sugar water containing 1.0 ppt cadmium chloride, complete mortality occurred in three days. When females which had been exposed to 0.1 ppt cadmium chloride in 3% sugar water or 3% sugar water only were offered blood meals, 4 of 19 and 14 of 18 fed, respectively. All treated adults died on the seventh day. Females treated at 10.0 ppm were not deterred from taking blood meals; 22 of 24 fed. Dissected ovaries revealed no peculiarities.

The results of mating crosses which were made using untreated adults and those treated at concentrations of 10 and 50 ppm are given in Table V. Even though the data suggest some effect on per cent hatch and numbers of eggs deposited, the variability of the results indicate little credence can be given these indications. Dissections of the adults were made 13 days after the crossings and no abnormalities were noted.

Compounds	Concentra- tion - ppt	No. Pupae	No. Dead	Effects on Ovaries
CdC1 ₂	10.0	10	4	Na
	1.0	15	1	N
	0.1	15	1	N
Cd(C ₂ H ₃ 0 ₂) ₂	10.0	10	4	N
	1.0	15	1	N
	0.1	15	2	Ň
ZnC1 ₂	10.0	5	5	-
	1.0	15	_	N
	0.1	15	2	N
Untreated	۵۰ ۱۹۹۰ - ۲۰۰۰ ۱۹۹۰ - ۲۰۰۰ - ۲۰۰۰ - ۲۰۰۰	15	1	1 - 1 - 1 - 1 - 1 - 1 - 1

ADULT OVARIES AND MORTALITY OF PUPAE EXPOSED TO VARIOUS COMPOUND CONCENTRATIONS

TABLE IV

^a No effects on ovaries

Crosse Male	s ^b Female	Concen- tration ppm	Number engorged	Number eggs/raft	Average number eggs/raft	No. unem- bryonated rafts	Per Cent Hatch
Т	С	10	10	5	224.4	2	88.1
С	T	10	10	4	237.7	2	78.0
С	T	50	10	8	181.0	3	95.1
Т	C	50	8	2	203.0	1	90.4
T	T	10	9	4	184.5	1	81.2
Т	T	50	9	2	183.0	han an an tha an tha Tha an tha an	87.5
C	С	-	10	3	157.7	1	97.8
Colony Sa	nple	-	-	177	239.6	24.1	93.1

MATING CROSSES OF UNTREATED AND TREATED^a ONE-DAY-OLD ADULTS

TABLE V

^a Cadmium chloride was exposed to adults in 3% sugar solution for four days

^b 10 males and 10 females/carton

C = Untreated

T = Treated

DISCUSSION

Parizek and Zahor (1956), Kar (1961), and others have shown that cadmium compounds cause more adverse effects to mammalian testes than to ovaries. In contrast Abdel-Razig (1966, unpublished data) and Kunz (1967, unpublished data) found that cadmium compounds may affect insect ovaries far more than testes. Abdel-Razig (1966) observed that certain cadmium compounds delay and reduce oviposition of <u>Musca domestica</u> L. and <u>Periplaneta americana</u> L. Kunz (1967) found numerous ovarian distortions in <u>Stomoxys calcitrans</u> (L.), caused by cadmium compounds. The effects of cadmium compounds on <u>Culex quinquefasciatus</u> Say ovaries were also shown to be more affected than the testes. No abnormalities in testes have been found.

Larvae.--Kunz (1967) incorporated various concentrations of cadmium acetate, cadmium chloride, and Cadminate in the larval diet of <u>Stomoxys</u> <u>calcitrans</u> (L); at concentrations greater than 80 ppm each compound caused high mortality or stunted growth in surviving larvae. Both cadmium chloride and Cadminate used at 80 ppm caused a reduction in numbers of eggs laid, while no influence on reproduction was observed at concentrations below 80 ppm. Abdel-Razig (1966) exposed late instar <u>Drosophila</u> larvae to 100, 10, and 1 ppm concentrations of various cadmium compounds. Rate of growth and pupation in 10 ppm solutions approached that of the check, but high mortality, sluggishness, and smaller size were apparent. Similarly, first instar larvae of <u>Culex</u> <u>quinquefasciatus</u> Say were reared in various concentrations of cadmium

compounds and abberations of the ovaries occurred. High mortality and increased pupation time were apparent.

Powell et al. (1964), among others, have found that zinc compounds counteract the effects of cadmium in mammals. Abdel-Razig (1966) suggested that the physio-chemical relationship of cadmium and zinc could counteract the effects of cadmium on <u>Musca domestica</u> L. by mixing equal parts of zinc. The life span of cadmium acetate exposed adult M. <u>domestica</u> L. when compared with the controls was appreciably shorter and approximately normal in the cadmium acetate-zinc chloride exposed flies. In like manner, zinc chloride-cadmium chloride at 1.0 ppm reduced pupation time and increased survival of first instar mosquito larvae as compared with first instar larvae reared in cadmium chloride (1.0 ppm) only.

<u>Pupae</u>.--Cadmium solutions caused high mortality to <u>Drosophila</u> pupae, while there was little effect on the fecundity or fertility of surviving adults (Abdel-Razig, 1966). Kunz (1967) dipped <u>Stomoxys calcitrans</u> (L.) pupae in concentrations of cadmium compounds and found no influence on reproduction of subsequent adults. Mosquito reproductive organs were not affected, but pupal mortality increased as the concentrations tested increased.

<u>Adults</u>.--When Kunz (1967) exposed <u>Stomoxys calcitrans</u> (L.) to blood pads containing various cadmium concentrations, many ovarian atrophies were noted. Abdel-Razig (1966) found that by mixing various concentrations of cadmium compounds in solid food of <u>Musca domestica</u> L. and <u>Periplaneta americana</u> L. reproduction inhibition was obtained. Cadmium compounds in semisolid and liquid diets were highly repellent. Kunz (1967) reported no deterrence to <u>Stomoxys calcitrans</u> (L.) feeding on the

concentrations described by Abdel-Razig (1966). Adults of <u>Culex</u> <u>quinquefasciatus</u> Say fed readily on all concentrations of cadmium chloride in 3% sugar solutions, but most adult females exposed to 0.1 ppt of cadmium chloride for four days did not feed on the immobilized chickens offered the next day. Dissections of exposed adults revealed no abnormalities.

The differences in the permanence of cadmium effects in mammals are indicated by the permanent sterility of male rats (Gunn et al., 1961) and the temporary sterility of steers and hogs (Abdel-Razig, 1966). The permanence in insect sterility caused by cadmium compounds has not been determined. Abdel-Razig (1966) suggested the temporary sterility was evoked when adult <u>Musca domestica</u> L. were exposed to cadmium concentrations for a short period of time. However, mosquito larvae were reared in various cadmium solutions throughout ovarian imaginal bud development and caused what appears to be permanent sterility (Figs. 4 and 5).

There is very little published literature on the effects of cadmium compounds on insect reproductive systems. The sterilizing effects on testes of mammals and the sterilizing effects on the ovaries of insects are still not clear. However, it is intriguing to note that there is a similarity between the physio-chemical antagonism of zinc to the effects of cadmium on the testes of mammals and to the ovaries of insects. Continued research on the effects of cadmium and the counteractions of zinc are needed.

SUMMARY

Cadmium acetate, cadmium chloride, and cadmium iodide each at concentrations of 1.0 ppm caused high mortalities in first instar larvae of 51, 65, and 40%, respectively, and increased median pupation time to approximately 13, 14, and 15 days, respectively. Cadminate at 1:100 in larval diet caused 64% mortality to first instar larvae and increased pupation time to 13 days. Median pupation time in untreated larvae was about 10 days. No effects on testes were found. Most gross distortions in ovaries were found in ovaries of adults which had been treated as first instar larvae in 1.0 ppm of cadmium chloride. Pupae exposed to 10.0 ppt concentrations of cadmium chloride, cadmium acetate, and zinc chloride either died in that stage or during emergence. A concentration of 1.0 ppm did not affect pupal survival or their gonads. Treated adults were killed by cadmium chloride concentrations of 1.0 ppt and 0.1 ppt but were not killed at 50.0 or 10.0 ppm. No effects on reproduction were observed after crossing treated males and females with untreated individuals.

Indium sulfate at 0.1 ppt neither affected pupation time nor reproductive organs. Zinc chloride at 0.1 ppt did increase median pupation time to 17 days. Zinc chloride and antimony potassium tartrate did not affect reproductive organs. Zinc chloride and cadmium chloride at 1.0 ppm each in the rearing water showed slight reduction in mortality and median pupation time as compared with cadmium chloride at 1.0 ppm alone.

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