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Effects of the Chemical Pesticides DDT and Sevin on Rabbits

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EFFECTS OF THE CHEMICAL PESTICIDES DDT AND SEVIN ON RABBITS

A Thesis

Presented to

the Graduate Faculty

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In Partial Fulfillment

of the Requirements for the Degree

Master of Education

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by

James Allen Alban

July, 1967

APPROVED FOR THE GRADUATE FACULTY

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James A. Alban

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EFFECT OF THE CHEMICAL PESTICIDES DDT AND SEVIN ON RABBITS

Fifty-Word Summary of Thesis

by

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July, 1967

The study involves investigation into the toxicity of the chemical pesticides DDT (p, p' dichlorodiphenyltrichloroethane) and Sevin (1-naphthyl n-methyl 1-carbamate) to domestic rabbits. Evidence for disruption of digestive and reproductive processes are discussed as well as degeneration of organs. Chemical residues of less than 1 ppm to 50 ppm were found in adipose tissue.

"EFFECTS OF THE CHEMICAL PESTICIDES DDT AND SEVIN ON RABBITS"

Examination of available literature led to the conclusion that more study is needed for the evaluation of the effects of chemical pesticides on wildlife. The question of whether or not pesticides are instrumental in the population decline of many organisms is a critical conservation problem. Attempts to destroy or repress harmful and undesirable pests frequently affect the well-being of many desirable forms of wildlife, both aquatic and terrestrial. A number of investigations, concerned with determining pesticide tolerances and the effects of the chemical residues on organisms, have been, and are presently being, conducted.

Exposure to pesticides occurs from ingestion of contaminated foods, water, direct skin absorption and inhalation of air-borne materials. There are approximately sixty-thousand registered pesticide products (FDA Pub. No. 18, 1964) used for the control of insects, weeds, plant diseases and as defoliants. Studies indicate (U.S. Dept. of Ag., 1952) that many of the chemicals cause harmful effects to organisms. Chemical poisons affect the normal functions of specific cells and tissues. The chemical process in the animal is affected so as to bring about changes in its basic metabolic functions.

The purpose of this study is to evaluate the effects of the chemical pesticides DDT (p, p'dichlorodiphenyltrichloroethane) and Sevin (1-naphthyl n-methyl-carbamate) on domestic rabbits.

Toxic effects of DDT and Sevin

DDT solutions may be absorbed through skin. Acute: Tremors of head and neck muscles, tonic and clonic convulsions, depression, respiratory failure and death. Estimated oral fatal dose is 500 Mg/Kg. body weight of the solid material. Solvents such as kerosene increase toxicity-death occurs 2-24 hrs. Chronic: Necrosis and hypertrophy of the liver and degenerative changes in the brain. (Merck Index, 52). Sevin is primarily a contact material, but possesses some stomach and fumigant effects as well. It inhibits cholinesterase. Acute oral LD_{50} of the technical material to rats is 500-700 Mg./Kg. of body weight, which is about one-half as toxic as DDT (Hanna, 1958). Four factors were investigated, using 0.5 and 0.25 per cent solutions. The first factor considered was the effect of the chemicals on the microorganisms of the caecum.

The relationship between the rabbit and its intestinal microorganisms (this study concerned with bacteria only) is considered a mutualistic one. The intestinal organisms produce vitamins, a more nutritive type of protein, volatile fatty acids and smooth out the carbohydrate fermentation process (Levine, 1961). Gutierrez and Davis (1959) indicate that ciliates should not only be considered as involved in conversion of starch materials of plant origin into protozoan reserve polysaccharides, but also in eating associated bacteria. The accumulated evidence on bacterial feeding by the proto-

zoa points to them as agents altering the intestinal microflora, both qualitatively and quantitatively.

Protozoa found in the rabbit caecum are Chilomastix cuniculi, Giardia duodenalis, Monocercomonas cuniculi and Retortamonas cuniculi (Levine, 1961). A study concerned with the bacterial types in the rumen of cattle (Maki and Picard, 1965) revealed that the most consistent types present were Escherichia coli, Streptococcus bovis, Bacillus subtillus and Bacillus pumilis. Also the molds, Mucor and Streptomyces, and the yeasts, Candida krusei and Torolopsis formata, were present. Whether these types are somewhat consistent with those in rabbits are as yet unknown, as there are no related studies concerned with the bacteria of rabbits. The purpose of this phase of the study was to note any variations in normal population, by making bacterial plate counts from fecal pellets.

The second factor investigated was observable morphological and physiological changes, such as weight loss, daily activity, nerve tremors, diarrhea, and to organs of the body. Coburn and Treichler (1946) reported that 2 of 4 cottontail rabbits, given an oral dose of 2,000 (2%) mg/kg of DDT (Mg/Kg of body weight), survived and that 1 of 3 given a 2,500 (2.5%) mg/kg dose also survived. They also reported, that 3 of 4 cottontails on a diet containing 0.4 per cent DDT died within 3 weeks.

Investigations with cottontails (Pillmore, et al. 1961-62) indicate that one cottontail given an oral dose of 2,000 mg/kg p,p' DDT

showed a loss of weight and appetite, but no other symptoms were observed during the 8 days after treatment. They later placed 4 cottontails on a 0.4 per cent diet. Three of 4 died, a male after 4 days, a female after 10 days and a male after 18 days. Four other cottontails fed on a diet containing .02 per cent p,p' DDT over a period of 70 days, showed no symptoms during the test period.

Stickel et al. (1965), investigating responses to pesticides in woodcocks (Philohela minor) discussed five factors involved in testing: 1. Underweight birds die quicker; 2. Adjustment to capture-excitement and inability to settle down; 3. Stress-Migration time and size of cage; 4. Size of dose (small continued doses are more lethal than one large dose); 5. Different chemicals affect different individuals. In oral dosage studies, response of rats has been delayed or diminished by protecting the animal from environmental disturbance, and effects have been increased by such disturbance (Deichmann et al. 1950).

In nature, animals commonly obtain pesticides through the food chain in small but frequent amounts, which is ideal for assimilation. Therefore, long feeding trials are an important aspect of testing.

The effect on reproductive capacity is the third factor studied. Bernard and Gaertner (1964) investigated the effects of DDT on reproduction in mice. Adult laboratory mice (Mus musculus) were fed diets containing 100 to 300 ppm (parts per million) of DDT. The mice were able to survive for extended periods. However, breeding

tests showed that the fertility of the mice declined following exposure to increased concentrations of DDT. Groups of mice exposed to levels of 200 and 300 ppm of DDT in the diet produced the same number of young per litter as the controls, but the number of females that produced no young was significantly greater in the group fed 300 ppm in the diet. Indications are that reproductive failures can occur following exposure to sublethal quantities of DDT.

Pillmore et al. (1961-62) reported in their investigation, using 0.4 per cent DDT on cottontails, that the mother killed both newborn young. Tissue residue tests of the young indicated 67-380 ppm. Significant observations on the residue data indicate that the surviving mother rabbit contained the least amount of residue in the brain and liver. Also, considerable amounts of DDT residues were found in the newborn, indicating that transferral to the fetus is a means of eliminating the pesticide.

The final and fourth factor examined was the concept of tolerance. The definition of tolerance is usually one that denotes the limits of an environmental condition (minimum or maximum) that causes death if surpassed. It does not set a limit for other adverse effects to the organism, such as the effect on reproductive capacity or other physiological reactions not readily observed.

Bernard and Gaertner (1964) reported that sublethal quantities of DDT produced reproductive failures in mice. In the report referred to previously by Pillmore et al. (1961-62), a residue of 30 ppm in

the brain of one rabbit after 3 weeks on a 0.1 per cent diet of DDT was larger than found in 2 rabbits on a diet containing 0.4 per cent DDT, which were 21 and 27 ppm respectively.

Radeleff and Bushland (1960) state that chemical analyses may confuse rather than aid in diagnosis of insecticide poisoning and cite the fact that higher residues may be found in animals surviving than in those which succumb to the effect of chlorinated hydrocarbons.

The foregoing is but a small assemblage of the growing evidence against the use of pesticides. Needless to say, the American public is still oblivious to the facts. Everyday, by spraying and eating food contaminated with chemicals, we subject not only our wildlife but ourselves to the effects of the chemicals.

This study was conducted at Central Washington State College, Ellensburg, Washington. The building used for the experiment is enclosed and free from abnormal disturbance. The investigation took place during spring and summer (April-August) 1966.

The general hypotheses stated for the investigation are:

1. The consistent intake of the chemicals may cause physiological changes such as bloat, diarrhea and organ damage, resulting in death to the organism if the diet is maintained.
2. The ingestion of toxic chemicals may destroy the infusoria of the caecum, which are helpful in digestion. This may result in digestive problems, weight loss and eventual death.
3. The continued intake and assimilation of the chemicals, may

cause abortive factors or death to the fetus due to damage of the reproductive process or to the fetus per se.

4. Tolerance levels are not valid indicators of damage, due to the definition of the concept, and also because of variations in lethal toxicity and other limiting factors such as stress, control of diet, etc.

It is hoped that information from this study will help in assessing harmful effects of pesticide applications on wildlife, even when no direct or immediate effects may be observed.

EXPERIMENTAL

The experimental data has been collected and separated into four distinct phases, in order to facilitate a clearer understanding of the study.

Phase #1

Twelve rabbits were maintained in separate pens on a controlled diet (unlimited food and water) consisting of W.F.A. (Western Farmer Association) rabbit ration* and alfalfa. The pens were located within a locked building so that environmental conditions could be controlled as much as possible.

*Analysis of Rabbit Ration

Min. cr. protein	20.0%
Min. cr. fat	2.0%
Max. cr. fibre	15.0%
Ingredients:	Ground barley, ground oats, wheat mixed feed, soybean oil meal, alfalfa meal, linseed oil meal, salt, vit. B-12 supplement.

The twelve rabbits were six males and six females. Five of each sex were three to four months old and weighed 3 1/4 to 3 1/2 lbs apiece on April 5. The older male (2 yrs) and female (3 yrs) weighed 6 3/8 and 6 1/2 lbs respectively on the same date. The project was initiated on this date. The rabbits were maintained on this diet for a month in order to stabilize the intestinal types of organisms. This precaution was taken due to the fact that, during a study with cattle,

it was learned a change in diet caused a variation in number and types of bacteria present (Maki and Picard, 1965).

In developing dilutions for reliable plate counts and a medium for optimum growth, a number of test dilutions (1, 1 X 10^2; 1, 1 X 10^4; 1, 5 X 10^4; 1, 1 X 10^5; 1, 1 X 10^6) were run. E.M.B. (Eosin Methylene Blue) and Trypticase Soy Agar were tested as a medium. Aerobic and anaerobic conditions were tested with both media. Another medium was considered, I.C.G.S.A. (Intestinal fluid-cellobiose-glucose-starch agar), but due to the difficulty in making the medium and maintaining the necessary conditions, it was abandoned. This medium was considered, due to the excellent results along with E.M.B. and Eugon that were observed in studies of rumen flora of cattle (Maki and Picard, 1965). The final choice resulted in the use of trypticase Soy Agar** (autoclaved at 121°C for 15 min.) using a 1, 5 X 10^4 dilution (adjusted as necessary) of the fecal pellets. The plates were maintained in an incubation chamber with aerobic conditions at 37°C for eighteen hours (Optimum period for counting colonies).

**Trypticase Soy Agar

Trypticase Soy Agar	15 gms./liter	(B-B-L pancreatic digest of casein)
Phytone	5 gms./liter	(B-B-L Soy peptone)
Sodium Chloride	5 gms./liter	
Agar	15 gms./liter	

Two pour plates containing 16 ml. of Trypticase Soy Agar and 1 ml. of the 1, 5 X 10^4 dilution were incubated. Test results indicated 66 colonies in one plate and 72 in the other. The average, 69, was multiplied by the dilution factor, resulting in the number of bacteria per ml. of the sample. (No. of colonies X dilution factor = no. of organisms/ml.)

The following table (2-A) illustrates the results of six samples (date, June 6). The average of two samples was used plus a group average was determined. Individual differences are noted, as well as variations between male and female and the large difference found in the older male.

2-A Bacteria Count/Test Sample

Dilution sample 1, 5 X 10^4 Ave. of duplicate plates		Formula-No. of colonies X dilution factor = No. of bacteria per ml. of original sample
No.	Sex	
1	M	112 X 50,000 = 5,600,000/ml
2	M	100 X " = 5,000,000 "
3	F	89 X " = 4,450,000 "
4	M	95 X " = 4,750,000 "
5	F	65 X " = 3,250,000 "
6	F	69 X " = 3,450,000 "

The group average for the six young is 4,416,666. The older male because of the radical change was left out of the group. His count showed 32 X 50,000 = 1,600,000. It is also noted that the females

have a lower count than the males.

By June 6, the rabbits were in good condition and had gained weight steadily, averaging approximately two pounds in two months. The bacteria count varied from 3.2×10^6 to 5.6×10^6 and averaged 4.4×10^6.

Bacteria counts and weights for the duration of the study, including those after the diet had been treated with insecticides, are tabulated in charts 2-B and 2-C.

The W.F.A. ration and alfalfa were sprayed with .25 and .5 per cent of the insecticides, DDT and Sevin. The variously treated feeds were then stored in separate and closed containers. The treated diet began on July 10 and was maintained until Aug. 5, a period of 25 days. One of each sex was maintained on the individually treated diets.

The following chart (2-B) indicates the weight of the rabbits and the gain in weight over the observed period.

Chart 2-C lists dates, bacteria counts and per cent of chemicals used for the duration of the study.

Phase #2

This section of the study was concerned with observations of physiological and morphological changes in the animals before and after the chemically treated diet began.

Refer to charts 2-B, 2-C for variations in weights of rabbits. Some diarrhea was noted the first two weeks on the chemicals. The test animals along with the controls gained regularly with some varia-

CHART 2-B

Weight Gain and Loss 6/6/66-8/10/66

Rabbit No.	Sex	Age	6-6-66 Wt. lbs	Gain or Loss		7-21-66 Wt. lbs	Gain or Loss	7-30-66 Wt. lbs	Gain or Loss	8-10-66 Wt. lbs	Gain or Loss	Chemical Diet
1	M	5-6 mo.	6 1/4	G 2 3/4	Chemically treated diet began 7-10-66.	7 1/4	G 1	6 7/8	L 3/8	7	G 1/8	.5 Sev.
2	M	"	6	G 2 1/2		6 1/2	G 1/2	6 7/8	G 3/8	6 1/2	L 3/8	.25 Sev.
3	F	"	6 3/8	G 2 7/8		6 3/4	G 3/8	6 3/4	0	6 3/8	L 3/8	.25 DDT
4	F	"	5 1/2	G 2		7	G 1 1/2	7 1/4	G 1/4	7 5/8	G 3/8	.25 Sev.
5	F	"	6 1/2	G 3		6 1/2	0	6 3/4	G 1/4	6 1/4	L 1/2	.5 DDT
6	F	"	5 1/4	G 1 3/4		7 3/4	G 1 1/2	8 1/8	G 3/8	7 3/4	L 1/4	Cont.
7	M	"	7 1/8	G 3 5/8		8 3/8	G 1 1/4	8 3/8	0	8 5/8	G 1/4	Cont.
8	M	"	6 1/4	G 2 3/4		6 7/8	G 5/8	7	G 1/8	7 5/8	G 5/8	.5 DDT

CHART 2-B (Cont.)

Weight Gain and Loss 6/6/66-8/10/66

Rabbit No.	Sex	Age	6-6-66 Wt. lbs	Gain or Loss		7-21-66 Wt. lbs	Gain or Loss	7-30-66 Wt. lbs	Gain or Loss	8-10-66 Wt. lbs	Gain or Loss	Chemical Diet
9	M	5-6 mo.	5 7/8	G 2 3/8	Chemically treated diet began 7-10-66.	6 1/8	G 1/4	6 1/8	0	6 3/8	G 1/4	.25 DDT
10	F	"	6	G 2 1/2		7	G 1	7 1/8	G 1/8	6 5/8	L 1/2	.5 Sev.
11	F	3 yrs.	8 1/2	G 2		9 3/8	G 7/8	9 1/2	G 1/8	9 3/4	G 1/4	Old Cont.
12	M	2 yrs.	7 1/4	G 1 1/8		7 1/2	G 1/4	7 3/4	G 1/4	7 1/2	L 1/4	Old Cont.

CHART 2-C

Bacterial Count, Dates, and Dilution Factors

Rabbit No.	Sex	Age	% of Chemical used	7-6-66 cont. count 1/40,000* Plates A	B	Ave	7-15-66 5 days 1/40,000 Plates A	B	Ave	7-22-66 11 days 1/30,000* Plates A	B	Ave	7-29-66 18 days 1/20,000* Plates A	B	Ave	8-5-66 25 days 1/40,000 Plates A	B	Ave
1	M	5-6+ mo.	.5 Sev.	78	66	72	20	24	22	25	36	30	191	206	198	162	173	168
2	M	"	.25 Sev.	88	85	86	15	25	20	37	44	41	256	239	248	214	184	199
3	F	"	.25 DDT	206	177	192	18	24	21	27	26	27	262	259	260	119	125	122
4	F	"	.25 Sev.	144	142	143	21	24	23	27	20	24	212	215	213	121	139	130
5	F	"	.5 DDT	59	70	65	15	15	15	38	28	33	194	185	190	204	210	207
6	F	"	Cont.	64	87	76	61	65	63	165	161	163	119	122	120	162	183	172
7	M	"	Cont.	197	171	184	58	68	63	149	168	158	99	103	100	126	162	144
8	M	"	.5 DDT	33 *1/60,000	31	32	9	23	16	49	46	48	176	187	182	214	210	212

Chemical diet began 7-10-66.

*1/40,000

*1/40,000

*1/40,000

* Deviation from dilution

CHART 2-C (Cont.)

Bacterial Count, Dates, and Dilution Factors

Rabbit No.	Sex	Age	% of Chemical used	A	B	Ave	A	B	Ave	A	B	Ave	A	B	Ave	A	B	Ave
9	M	5-6+ mo.	.25 DDT	233	198	215	17	12	15	43	39	41	132	125	129	125	120	123
10	F	"	.5 Sev.	164	178	171	13	16	15	21	18	20	180	227	203	122	120	121
11	F	"	Old cont.	141	111	126	173	215	183	176	164	170	300+	300+	300+	152	150	151
										*1/40,000			*1/40,000					
12	M	"	Old cont.	35	32	33	53	41	47	110	102	106	153	300+	230	62	80	71
				*1/60,000						*1/40,000								

Chemical diet began 7-10-66.

CHART 2-D

Physiological and Morphological Irregularities

Rabbit No.	Sex	Age	Chemical diet	Muscle tissue	Kidneys	Liver	Fat	Mammary glands	Other	
1	M	5-6+ mo.	.5 Sev.	LP*	pitted	clear	heavy*	no		
2	M	"	.25 Sev.	LP	pitted	clear	heavy	no		
3	F	"	.25 DDT	LG	pitted	clear	light	not developed	Large amount of water in tissue	Lungs white
4	F	"	.25 Sev.	LP	pitted	clear	heavy	not developed	Contains 6 embryos	
5	F	"	.5 DDT	LG	pitted	necrosis	heavy	not developed		
6	F	"	cont.	LP	clear	clear	med.	well dev.		
7	M	"	cont.	LP	clear	clear	med.	no		
8	M	"	.5 DDT	LG	clear	clear	heavy	no		

*LP-Light pink
LG-Light green tinge
med., light, heavy-fat

CHART 2-D (Cont.)

Physiological and Morphological Irregularities

Rabbit No.	Sex	Age	Chemical diet	Muscle tissue	Kidneys	Liver	Fat	Mammary glands	Other
9	M	5-6+ mo.	.25 DDT	LG	pitted	clear	heavy	no	
10	F	"	.5 Sev.	LP	pitted	clear	med.	well developed plenty of milk	
11	Not tested		old cont.	-	-	-	-	-	
12	Not tested		old cont.	-	-	-	-	-	

CHART 2-E

Breeding Test

Rabbit No.	Sex	Age	Mate	Chemical diet	Number of young	Lived	Died	Other
1	M	5-6 mo.	10	.5 Sev.	X			
2	M	"	4	.25 Sev.	X			
3	F	"	9	.25 DDT	3	0	3	Did not prepare nest.
4	F	"	2	.25 Sev.	6 embryos			Not born when killed.
5	F	"	8	.5 DDT	5	0	5	Prepared after birth.
6	F	"	7	cont.	13	8	5	Prepared nest.
7	M	"	6	cont.	X			
8	M	"	5	.5 DDT	X			
9	M	"	3	.25 DDT	X			
10	F	"	1	.5 Sev.	5	3	2	Of the 3 which lived, 1 died at 3 weeks, 1 at 5 weeks and the other at 3 months.
11	F	3 yrs.	12	old cont.	4	0	4	Killed, as had done previously.
12	M	2 yrs.	11	old cont.	X			

tions until end of test (Aug. 5).

The animals were then sacrificed, dissected, and any anatomical changes within the organism were noted. The observed variation from the control organism was change in color of flesh of DDT fed animals from normal pink to a greenish tinge. Pitting of the kidneys and necrosis of the liver was observed in some of the animals on the treated diet. Other irregularities noticed were lack of development in the mammary glands of some test animals and large deposits of fat around the intestinal organs and in the caudal region.

The preceding chart (2-D) illustrates normal and abnormal conditions observed within each animal.

Phase #3 is concerned with irregularities in reproductive capacity. All females were bred between July 1 and July 10. The gestation period for rabbits is 30 to 35 days. All gave birth to young except number 4 which was pregnant at time of sacrifice. None of the animals prepared a nest or fed the young except number 10 and number 6. All young died, except 3 of a litter of five from number 10 (diet-.5 Sevin) and eight of 13 from number 6 (control). The old female killed her young as she had done previously. The preceding chart (2-E) illustrates the results of Phase #3.

Phase #4 is a residual test for the determination of DDT and Sevin in the adipose tissue of test rabbits.

Sampling (8-10-66)

Adipose tissue samples were taken from the caudal region of the rabbits. The samples were wrapped in plastic bags, labeled, and kept frozen until analyzed.

Extraction and analytical procedure

Samples were removed from the freezer, cut into small pieces, and a representative portion of one gram was sliced paper thin and used for analysis. The sample was placed in an evaporative condenser (250 ml. cap.) with 100 ml. of N-hexane (for DDT) or acetone (for Sevin) and allowed to filter through the sample for 12 hours. The filtrate was allowed to cool and then was washed to dryness with anhydrous sodium sulfate. Enough of the solvent was added to make exactly 100 ml. of filtrate. The fats were then frozen out of the N-hexane-DDT solution and three samples were analyzed for DDT in the Beckman (DB) Spectrophotometer at 236 mu. (quartz cells were used in ultraviolet spectum). No freezing was necessary for the Sevin and the material was analyzed at 590 mu. (glass cells were used in the visible spectrum).

The following chart and graphs (2-F) indicate the results for recovery of the chemicals from the adipose tissue. The per cent transmission was recorded and compared to a previously recorded standard for the two chemicals. From the comparison of the standard and sample, parts/million of the chemicals found in the tissue, were recorded for each sample. Due to the difficulty of detecting small amounts of 100

CHART 2-F 1 (Part 1)

% Deviation for Recovery of DDT and Sevin

Dilution No:			1		2		3		4		5	
Rabbit No.	Sex	Age		% Dev.*		% Dev.		% Dev.		% Dev.		% Dev.
1	M	5-6 mo.	97.5	2.5								
2	M	"	98.0	2.0								
3	F	"	2.0	1.0	12.5	1.5	33.5	.5	55.0	1.0	72.5	.5
4	F	"	97.0	3.0								
5	F	"	0	?	0	?	0	?	3.0	53.0	13.5	59.0
6	F	"	3.0 100		14.5		33.0		56.0		72.5	
7	M	"	3.0 100		14.0		33.0		56.0		72.0	
8	M	"	0	?	3.5	11.0	15.5	17.5	35.5	20.5	57.5	14.5
9	M	"	3.5	+.5	17.5	3.5	39.5	6.5	60.0	4.0	76.0	4.0
10	F	"	97.5	2.5								

* % Deviation from control

Analysis for % Transmission of DDT and Sevin from Fat

CHART 2-F 1 (Part 2)

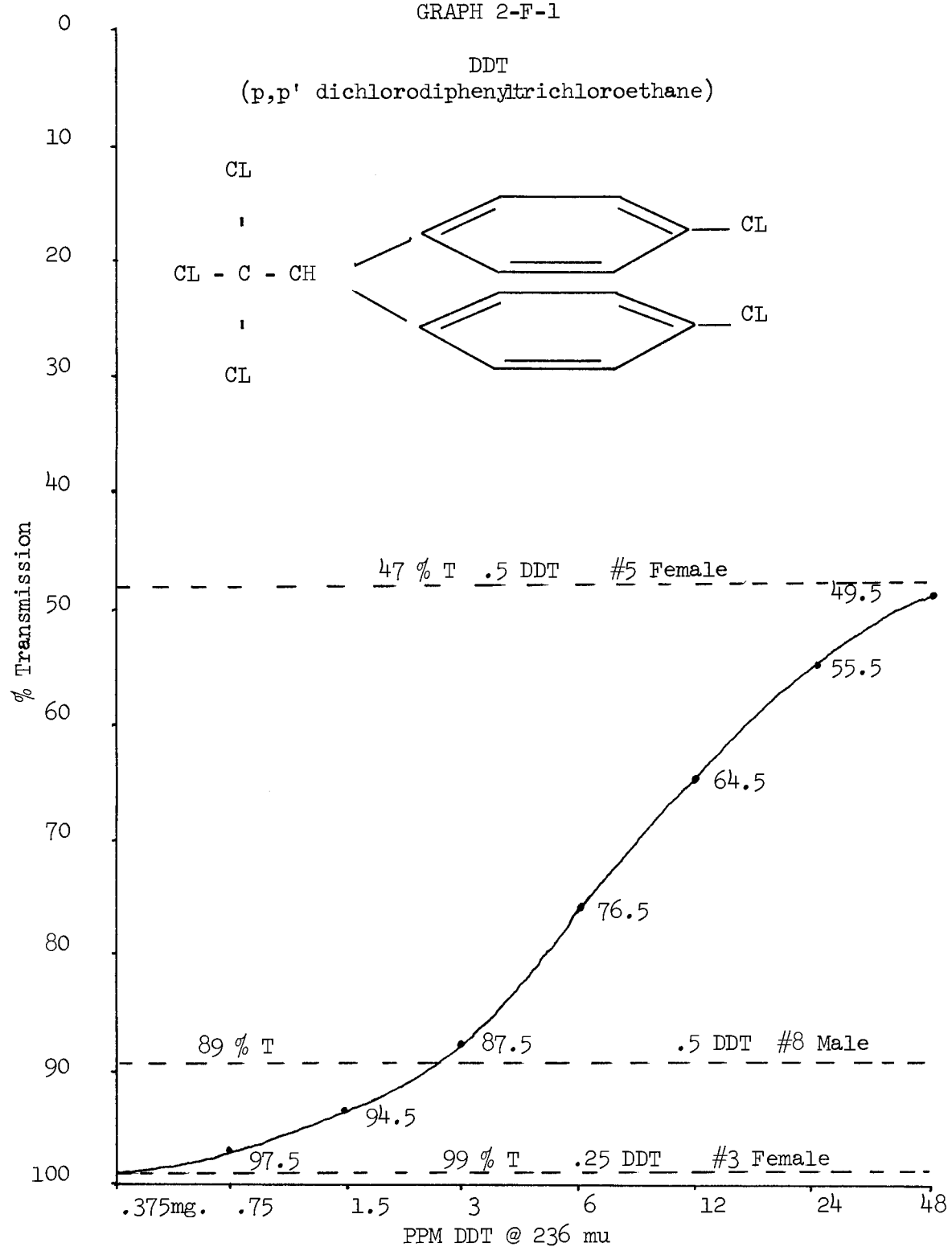
% Deviation for Recovery of DDT and Sevin

Rabbit No.	6	% Dev.	7	% Dev.	8	% Dev.	9	% Dev.	10	% Dev.	Chemical Diet
1											.5 Sev.
2											.25 Sev.
3	84.0	1.0	90.5	.5	95.0	2.0	96.5	1.0	97.0	1.0	.25 DDT
4											.25 Sev.
5	34.0	49.0	56.0	34.0	71.5	21.5	83.0	12.0	89.0	7.0	.5 DDT
6	83.0		89.5		93.0		95.5		96.0		cont. DDT Sevin
7	83.0		90.0		93.0		95.0		96.0		cont. DDT Sevin
8	74.0	9.0	85.0	5.0	90.5	2.5	94.0	1.0	96.0		.5 DDT
9	86.0	3.0	90.5	.5	95.0	2.0	96.0	1.0	98.0	2.0	.25 DDT
10											.5 Sev.

* % Deviation from control

Analysis for % Transmission of DDT and Sevin from Fat

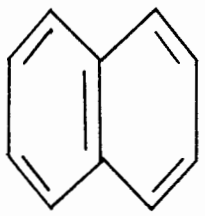
GRAPH 2-F-1


DDT
(p,p' dichlorodiphenyltrichloroethane)
CL
CL - C - CH
CL
CL
CL
0
10
20
30
40
50
60
70
80
90
100
% Transmission
47 % T .5 DDT #5 Female
49.5
55.5
64.5
76.5
87.5
89 % T
.5 DDT #8 Male
94.5
97.5
99 % T .25 DDT #3 Female
.375mg.
.75
1.5
3
6
12
24
48
PPM DDT @ 236 mu

GRAPH 2-F-2

SEVIN

(1-Naphthyl-n-methyl carbamate)



#1 Male .5 Sevin 97.5
#2 Male .25 Sevin 98.0
#4 Female .25 Sevin 97.0
#10 Female .5 Sevin 97.5

CHART 2-F-2 (Part 1)

Dilution Table for Fat-Hexane and Acetone

Dilutions

1.		1 gm in 100			=	1 part/100
2.	5 ml =	.05 gm	+	5 ml	=	.05 gm in 10
					=	.5 parts/100
3.	5 ml =	.025 gm	+	5 ml	=	.025 gm in 10
					=	.25 parts/100
4.	5 ml =	.0125 gm	+	5 ml	=	.0125 gm in 10
					=	0.125 parts/100
5.	5 ml =	.00675 gm	+	5 ml	=	.00675 gm in 10
					=	.0675 gm/100
6.	5 ml =	.003375 gm	+	5	=	.003375 gm in 10
					=	.03375 gm/100
7.	5 ml =	.0016875 gm	+	5	=	.0016875 gm in 10
					=	16.875 mgm/100
8.	5 ml =	.84375 mgm	+	5	=	0.84375 mgm in 10
					=	8.4375 mgm/100
9.	5 ml =	.421875 mgm	+	5	=	.421875 mgm in 10
					=	4.21875 mgm/100
10.	5 ml =	.2109375 mgm	+	5	=	0.2109375 mgm in 10
					=	2.11 mgm/100

cont.

CHART 2-F-2 (Part 2)

Dilution Table for Fat-Hexane and Acetone

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21 mgm/1000 = 21,000 mgm

21 gm/10^6

21 mgm/6.60 gm hexane = 3.182 mgm

= .031820 mgm/gm

= 31850 mgm/10^6 = 31.82 gm/10^6 gm hexane

21 mgm/7.92 acetone = 2.525 mgm

= .025250 mgm/gm

25250 mgm/10^6 = 25.25 gm/10^6 gm acetone

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ml of solvent and use quantitative analysis to record the data. (Refer to chart 2-f-2).

DISCUSSION AND CONCLUSION

A variety of problems occurred at the onset of the study. It was necessary to separate the pens in such a manner as to prevent the rabbits from coming in contact with one another and with each other's food supply. It was also necessary to spray the food and collect feces without contaminating the separate materials.

As much time as possible was spent with the rabbits, thus allowing them to become accustomed to the investigator. At the same time they were sheltered from all other environmental factors that may disrupt their normal functions.

Phase #1

All of the test animals gained regularly until the second and third weeks of the chemically treated diet. At that time noticeable variations in loss and gain of weight were observed (Chart 2-B). These variations may be attributed to three factors. The first factor could be the effect of the chemicals on the digestive system. Secondly, the animals may be reaching a plateau in their age, where variation in eating habits is prevalent, such as is noted in the older rabbits. The third factor, found only in the females is change caused by pregnancy.

A number of dilutions were tested for the fecal pellets. A dilution of $1,5 \times 10^{6}$ gave the best results for variation of bacterial

count. It was found that fresh pellets were necessary for a more standard count and approximately six o'clock in the evening was the best time for collecting. Upon collection, the pellets were weighed to 1 gm, deposited in the dilution bottles of distilled water and mixed with the Soy agar. After 18 hours of incubation, counts were taken from the samples and the average calculated. (Chart 2-C)

The results show a number of individual variations among the test animals before the chemical diets began. It also indicated a very definite change after the diet began. The animals on both types of chemicals had a much lower bacterial count than the controls. This situation lasted until the third and fourth weeks at which time the bacterial count of the animals on the chemicals became greater than the controls. In accounting for this change, two possibilities seem feasible. First, the bacteria may have mutated and the mutations were able to combat the change. The second possibility is that certain types of bacteria died out allowing a more resistant type to take its place. The second reason seems more logical due to the fact that the plates showed a number of colonies of the same species of which there had been only a few colonies previously.

No other variations were noted, except the great deviations in the older rabbits (#11 and 12). These changes appear to be due to their eating habits, which were highly irregular.

Phase #2

This section encompasses mostly observable morphological variations, but some physiological changes are noted.

A few of the animals showed signs of diarrhea at the beginning of the change in diet, although all appeared normal after a few days. Some weight change was noticed (Chart 2-B). No other symptoms or irregularities were noted except nervousness which could be attributed to the initial functioning of reproductive organs.

The major changes observed were found when the animal was sacrificed and dissected (Chart 2-D). Especially interesting was the bleaching of the tissue of the DDT fed animals from normal to a white or greenish tinge. Other irregularities observed were pitting of the kidneys, some necrosis of the liver and heavy deposits of fat.

The diarrhea and loss of weight can be attributed to any substance which upsets the normal digestive process. No answer is available for the bleaching of the muscle tissue except the possibility that the DDT upsets the water balance of the tissue. This suggestion is put forth due to the finding of rabbit number 3 (.25 DDT) having large deposits of water in her tissue.

The pitting of the kidneys and necrosis of the liver is probably due to the degenerative process caused by a number of the chemical pesticides, such as DDT, which have been found in other studies. (Merck Index, 52).

The heavy deposits of fat are possibly a means by which the animal is able to ward off the effects of the chemical. This has been found in other studies, Stickel, et al., (1965), in which body condition has been an important factor in the response to pesticides. Indications

are that any stress, which would cause assimilation of the fatty tissue, would eventually be fatal to the organism.

Phase #3

All the females went through the normal gestation period. No abortive factor was found at this time and all the young appeared healthy. Of interest is the fact that the does did not build nests, nor would they feed their litter. Number 3 failed to pull hair, and number 5 did after the young were born. They did appear highly nervous at this time and were left alone as much as possible. After dissection it was found that the mammary glands had not developed in #3 or #5. Number 10, although on a .5 Sevin diet, built a nest and fed her young as did the control #6. Of the three which lived from #10, all died within three months. None of the young were analyzed for recovery of the chemicals. Chart 2-E lists the results.

Phase #4

A number of methods were tried and rejected in developing a means for analyzing the fat tissue. Eventually, by condensing the materials and using quantitative analysis, an examination for per cent of DDT and Sevin in the adipose tissue indicated a small but positive test for the residues. Charts and graphs (2-F) indicate the results of this particular test.

Best recoveries were found in the DDT fed animals, particularly with those on a .5 DDT diet. Larger deposits were found in the females than the males. This may be due to the pregnant females in-

gesting more food. The highest recovery was found in #5 female (approx. 50 ppm). No recovery was detectable in #9 male (.25 DDT).

Recovery of Sevin was in very small quantities, but approximately the same amounts, a 2 to 3% deviation from the control, was found for all animals on the Sevin diet.

Indications are that DDT is retained in greater quantities than Sevin, and that females are more susceptible to pesticide poisoning than are males.

A number of conclusions may be drawn from a study which encompasses such a diversity of material, but they may be directed to one end: What affect do the chemicals have on the organism when all of the malfunctions are considered?

Evidence from this investigation and other biological research, as previously discussed, indicates morphological changes and disruption of normal physiological functions when pesticides are ingested and eventually assimilated. Disruption of the digestive and reproductive processes as well as degeneration of vital organs, such as found in this study, are prime examples of the effect pesticides have on the organism.

Findings of this study indicate that tissue residues lower than 1 ppm may cause harm to the organism. Methods involving laboratory feeding must always take into account that animals in their natural environment will need greater quantities of food because their increased activity will involve a higher metabolic rate than is true

when they are confined in a laboratory cage or enclosure.

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CENTRAL WASHINGTON STATE COLLEGE

Graduate Division

Final Examination of

James A. Alban

B.A. in Ed., Central Washington State College

1963

for the degree of

Master of Education

Committee in Charge

Dr. Donald H. Baepler

Dr. Virginia P. Harden Dr. Alexander H. Howard Jr.

Samuelson Union Building

Room 211

Friday, July 28, 1967

1:00 p.m.

Courses Included in Graduate Study

Required Courses

Education	507	Introduction to Graduate Study
Education	570	Educational Foundations
Psychology	552	Human Growth and Development

Courses in Field of Specialization

Chemistry	360	Organic chemistry
Zoology	361	Advanced Invertebrate Zoology
Biology	370	Microbiology
Botany	375	Plant Geography
Botany	460	Plant Pathology
Biology	470	Ecology
Biology	597	Individual Study
Biology	598	Seminar
Biology	599	Seminar
Biology	600	Thesis

Elective Courses

Physical Ed.	348	Athletic Training

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CERTIFICATION:

Standard General Certificate

TITLE OF THESIS:

Effects of the Chemical Pesticides DDT and Sevin on Rabbits

FIELD OF SPECIALIZATION:

Biology