

EVALUATION OF TOXICITY OF DIAZINON TO
SEVERAL SPECIES OF STORED GRAIN INSECTS

by

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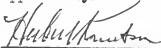
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INTRODUCTION AND OBJECTIVES

Numerous insecticides have been developed since the start of World War II, some of which are undoubtedly of potential use for control of stored grain pests. However, some have not been recognized as such, or insufficient research has been done to obtain approval from the Food and Drug Administration (FDA) for their use on stored grains. Today there are few recommended grain protectants, such as premium grade malathion and pyrethrins, that are considered safe enough to use on stored grains. If these become ineffective because of development of insect resistance, or for other reasons, there would be no approved insecticide to substitute for stored grain insect control.

Stored grain insects must be controlled to prevent contamination and loss of wheat, which is a major source of human food. If wheat is found to contain one percent by weight of weevil damaged kernels, it is considered unfit for human consumption and must be classified as livestock feed (L-30 KSU Oct '61). From the standpoint of economic loss, it is estimated that insects destroy between 5 and 10% of the world's grain production every year (Wilbur 1963).

The primary objective of the present study was to determine through biological evaluation, whether diazinon, O-O-diethyl-O-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate, is effective as a stored grain protectant at low treatment rates. In addition, information on diazinon was sought concerning its effect upon seed germination, its relative toxicity to insects, and the length of its residual life.

When possible, diazinon was compared with naled (dibrom), 1,2-dibromo-2,2-dichloroethyl dimethyl phosphate; malathion, O,O-dimethyl dithiophosphate of diethyl mercaptosuccinate; sevin, 1-naphthyl N-methylcarbamate; and dichlorvos (Vapona [®]), O,O-dimethyl 2,2-dichlorovinyl phosphate.

LITERATURE REVIEW

Strong and Sbur (1961 and 1964), Telford et al. (1964), and Kalkat et al. (1961) have published on the laboratory evaluation of diazinon as an insecticide against stored grain insects. These papers were concerned with the effect of temperature, humidity, and moisture content of the wheat, on the residual life and relative toxicity of diazinon and other chemicals. They found that: (1) as the temperature increased, the chemical's residual life was shortened; (2) as the relative humidity increased, the higher the initial insect mortality due to the increased volatility of the chemical giving a fumigant action; and (3) the higher the moisture content of the wheat, the less effective was the chemical. They also classified diazinon according to its relative toxicity and residual life with other chemicals, primarily malathion. They found diazinon to be as good or better under the laboratory conditions studied.

No field work on diazinon is known.

The Diazinon Handbook (1964) published by the Geigy Agriculture Chemical Company states: "The fact that diazinon controls strains of insects that have become resistant to other insecticides makes it particularly effective." This refers to uses other than on stored

grains. Since malathion, which is now used on wheat as a protectant, has shown some signs of inducing resistance in the German cockroach (Grayson, 1963) within 15 generations, it would be logical to assume that stored grain insects may also build up a resistance, but no published or unpublished cases are known. Diazinon within the same study (Grayson, 1963) did not show signs of inducing resistance in the German cockroach after 15 generations.

Many articles dealing with malathion as a grain protectant have been written, one of which is Strong and Sbur (1960), who determined the influence of grain moisture and storage temperature on the effectiveness of malathion. They found, using wheat at 60°F with a moisture content of up through 16% treated with 10 ppm malathion, that 100% control of all stored grain insects studied was obtained for 12 months. At 90°F, using 10 ppm malathion treated wheat with moisture content of 10%, insect control was maintained up through 9 months; but under the same conditions with a moisture content of 14%, control lasted only through the third month.

Watters (1959) studied the effects of grain moisture content on residual toxicity and repellency of malathion. He found that the higher the moisture content the less control of the insects being studied.

Gunther et al. (1958) found that malathion lost biological effectiveness with time and had a residual half life of 5.6 months.

Naled and dichlorvos have been mentioned in the article of Strong and Sbur (1960), but the chemicals were not desirable as stored grain protectants because of their short residual life.

Sevin, as indicated by unpublished work in this laboratory, requires a very high ppm treatment rate to achieve insect control on wheat. Its residual life is not as good as that of diazinon or malathion under the conditions studied.

EXPERIMENTAL PROCEDURE AND MATERIALS

General Procedure

Four major investigations were conducted: (1) laboratory studies of insecticides in small cartons; (2) simulated field studies involving 4 bushel samples of wheat in 55 gallon drums; (3) small scale field studies involving 400 bushel samples of wheat in Butler bins; and (4) laboratory germination tests of treated grain. Only biological evaluation of the insecticide is included in this thesis.

Laboratory Studies

Conditions controlled were temperature, humidity, age of insect (Table 2), method of handling insects, and the moisture content of the wheat.

Application of the insecticides. Three different application methods were used, depending on the amount of wheat and the type of insecticide formulation used (Table 3). The treated wheat varied in amount from 3 kg to 400 bushels.

Table 1. Test grains used and types of investigations conducted with each grain. All experiments at ambient temperatures. (a)

Common name and scientific name	Laboratory Studies	Germination Study	Simulated Field	Small Scale Field
Wheat (a)				
<u>Triticum aestivum</u>	X	X	X	X
Rye				
<u>Secale cereale</u> L.		X		
Oats				
<u>Avena sativa</u>		X		
Barley				
<u>Hordeum vulgare</u> L.		X		
Corn				
<u>Zea mays</u> L.		X		
Peas				
<u>Pisum sativum</u> L.		X		
Lima beans				
<u>Phaseolus lunatus</u> L.		X		
Sorghum				
<u>Sorghum vulgare</u> Pers.		X		

(a) Laboratory, 70°-80°F, mean 76°F; simulated field, 60°-90°F, mean 82°F; small scale field, 30°-86°F, mean 76°F.

(b) Hard red winter, moisture content 12.05 ± .55% as determined by a Steinlite Model S Moisture detector. Test weight 60 ± 1.5 pounds per bushel.

Table 2. Test insects.

Common name and scientific name	Abbr.	Temp. (°F)	Hum. (%)	Rearing methods		Age of insect		Number of insects		
				Culture media (a)	Quart glass jars	Lab.	Sim. field	Laboratory	Sim. field	Small scale field
Rice weevil <i>Sitophilus oryzae</i> (L.)	(RW)	80±2	70±5	1	Mason jars	7 wk.	all ages	50/half pt. or 100g of wheat	many many	(b)
Confused flour beetle (CFB) <i>Tribolium confusum</i> (Duval)		80±2	70±5	2	Mason jars	9 wk.	all ages	50/half pt. or 100g of wheat	many many	
Lesser grain borer (LGB) <i>Rhyzopertha dominica</i> (F.)		80±2	70±5	1	Mason jars		all ages		many many	
Granary weevil (GW) <i>Sitophilus granaria</i> (L.)	(GW)	80±2	70±5	1	Mason jars		all ages		many many	
Sawtooth grain beetle (STGB) <i>Oryzaephilus surinamensis</i> (L.)		80±2	70±5	3	Mason jars		all ages		many many	
Flat grain beetle (FGB) <i>Cryptolestes pusillus</i> (Schonh.)		80±2	70±5	3	Mason jars		all ages		many many	
Angoumois grain moth (AngM) <i>Sitotroga cerealella</i> (Oliv.)		80±2	70±5	1	Mason jars		all ages		many few	(c)
Indian meal moth (IMM) <i>Plodia interpunctella</i> (Hbn.)	(IMM)	80±2	70±5	4	Mason jars		all ages		many none	

(a) Culture media

"Procedures for rearing stored grain insects"—Department of Entomology, Kansas State University, 1962. Compiled by Professor Donald A. Wilbur.

1—Whole hard red winter wheat, 12.0% + .5% moisture.

2—Wheat shorts (fortified with dry yeast at the rate of 5% by weight).

3—Rolled oats (fortified with dry yeast at the rate of 5% by weight).

4—Finely ground corn meal 4 parts, glycerin 1 part, dog food (ground) 2 parts, dried yeast 1 part, honey 4 parts, wheat germ 1/2 part, wheat shorts 4 parts, oatmeal 1 part, whole wheat 14 1/2 parts.

(c) Few—over 1,000.

Table 3. Insecticides and formulations.

Commercial name	Structural name	Formulation	Lab. studies	Lab. nation	Simu- lated field	Small scale field
Diazinon AG 250	O-O-diethyl O-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate	25% emulsifiable concentrate	X	X	X	X
Naled (dibrom)	1,2-dibromo-2,2 dichloroethyl dimethyl phosphate	Ortho Dibrom 8 emulsive	X			
Malathion	O,O-dimethyl dithiophosphate of diethyl mercaptosuccinate	57% emulsifiable concentrate	X		X	
Sevin	1 naphthyl, N-methylcarbamate	5% dust 80% wettable powder			X	
Dichlorvos (Vapona ^(R))	O,O-dimethyl 2,2-dichlorovinyl phosphate	22% emulsifiable concentrate	X		X	

Table 4. Major equipment used.

Laboratory studies	Germination study	Simulated field	Small scale field
Thayer and Chandler Air brush sprayer Plate I, Fig. 3 right	Laboratory sprayer Plate I, Fig. 3 left	Portable electric cattle sprayer (Hudson)	Laboratory sprayer Plate I, Fig. 3 left
Laboratory sprayer Plate I, Fig. 3 left	15-gallon barrel with vanes Plate I, Fig. 4	55-gallon barrels Plate II, Fig. 3	Plenum chamber Plate III, Fig. 1
2-gallon drum with vanes Plate I, Fig. 1	Barrel roller Plate II, Fig. 1	4-inch auger with electric motor	2 1,000-bushel Butler bins
Steinlite moisture detector Model S	5-gallon cans with nylon covers Plate II, Fig. 2	Non-divisional 3-foot brass probe Plate III, Fig. 2B	6-inch auger, gasoline motor operated
1/2-pint Pres-Lok cartons		Barrel roller Plate II, Fig. 1	Divisional 5 1/2-foot probe Plate III, Fig. 2A
Boerner divider		Steinlite moisture detector Model S	Non-divisional 3-foot brass probe Plate III, Fig. 2B
Laboratory drum roller Plate I, Fig. 2			Steinlite moisture detector Model S

EXPLANATION OF PLATE I

- Fig. 1. Interior of two-gallon drum with vanes, used in laboratory mixing and treating procedures.
- Fig. 2. Laboratory roller with the 2-gallon drum and artist air brush sprayer in their respective positions for spraying.
- Fig. 3. Laboratory sprayer (left) and artist air brush sprayer (right).
- Fig. 4. Interior of the 15-gallon barrel with vanes and cover with a hole in the center for entrance of orifice of the sprayer.

PLATE I



FIG. 1

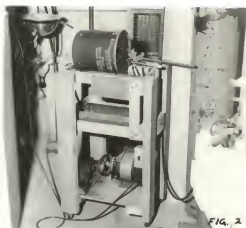


FIG. 2

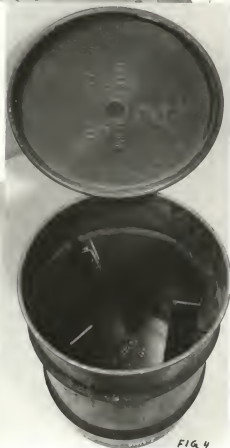


FIG. 4

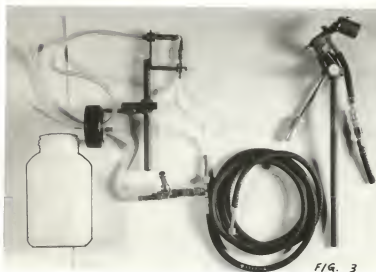


FIG. 3

The 3 kg samples were treated by one of two methods, depending upon whether the insecticide was (1) a dust; or (2) an emulsifiable concentrate.

The insecticide dust and wheat were weighed, in correct proportions for the desired treatment rate, and poured into a 2-gallon drum with internal vanes (Plate I, Fig. 1). After sealing, the drum was placed on a laboratory roller (Plate I, Fig. 2) which rotated it at 15 rpm for 30 minutes. The treated wheat was removed from the drum and run through a Boerner divider, 7 times, to further assure thorough mixing. The wheat was then placed in containers and stored in brown paper bags in the laboratory, or in aluminum cans in the greenhouse.

After the wheat was placed in a 2-gallon drum with internal vanes (Plate I, Fig. 1), the emulsifiable concentrate and wettable powder were applied to the wheat in the form of a fine spray mist. A hole centered in the lid of the spraying drum allowed the insecticide spray mist to enter the drum. The drum was placed on a laboratory roller (Plate I, Fig. 2) and the calculated amount of insecticide by weight diluted to 15 ml with water was then applied with two water rinses of 5 ml each. The drum was rotated at 15 rpm during treatment and for the 10 minutes following. The propellant used in the sprayers was either carbon dioxide or nitrogen at 20 psi gauge pressure. The wheat was then removed from the drum and run through the Boerner divider 7 times for thorough mixing. It was stored in brown paper bags in the laboratory, or in aluminum cans in the greenhouse.

The third method of application will be explained under the small scale field study portion of this paper.

Sampling. Four procedures for sampling grain were used. The desired subsample from the 3 kg samples was obtained by either a Boerner divider, or by use of a small scoop. Samples of grain were removed from the 400 bushel bins by using one of the two types of probes (Plate III, Fig. 2). A brass non-divided 3-foot probe was used to sample approximately 600-gram aliquots through the sixth week following treatment of the grain. An aluminum 5 1/2-foot divided probe was used for all samples taken from the seventh week until December 30, 1964. A divided probe was used to obtain samples at various depths for biological analysis of the grain. Initially 5 levels were sampled, approximately 600 grams each, but because of the similarity of the results between levels 1 and 2, and between levels 3 and 4, they were combined, respectively, giving 3 levels of grain being sampled: top (1 and 2), middle (3 and 4), and bottom (5). The approximately 600-gram samples of wheat taken from each level were placed into quart Pres-Lok cartons. The insects placed in this grain provided the bioassay data for the small scale field study.

Testing. The insects used were rice weevils, Sitophilus oryzae (L.) and confused flour beetles, Tribolium confusum (Duval). The insects were handled gently with sieves consisting of window screen soldered to quart coffee cans, and a vacuum-operated aspirator with just sufficient vacuum to lift the insects into the aspirator without injury. This procedure was used to place the insects on test and also to count the insects for mortality at 1, 7, 14, 21, and 30 days, when the counts were taken.

Wheat was sampled for laboratory bioassay studies immediately, 30 days after treatment, or at longer periodic intervals as was the case

with the 400-bushel study. The wheat was placed in the laboratory for at least 4 hours before testing, to allow the temperature of the wheat to become uniform at $75^{\circ} \pm 3^{\circ}\text{F}$. Two, three, or four 100-gram samples of wheat were then weighed and placed in 1/2 pint Pres-Lok cartons, infested with 50 rice weevils or 50 confused flour beetles each, and placed in a constant temperature and humidity room at $80^{\circ} \pm 2^{\circ}\text{F}$ and $70\% \pm 5\%$ relative humidity until mortality counts were taken. The insects were kept in their original containers throughout the study with the exception of short periods when the mortality counts were being taken (approximately 3 minutes each). The insects were removed from the wheat at the time of the last mortality count. The wheat was then returned to its original container and placed in the constant temperature and humidity room to await emergence of the new insects (45-60 days).

Germination Study

Treated seeds were sent to the State Seed Testing Laboratory in Topeka, Kansas.

Application. Diazinon AG 250 emulsifiable concentrate was applied to 15-pound samples of seed (Table 1) at two treatment rates, with a laboratory sprayer (Plate I, Fig. 3, left), using 50 ml of water as the carrier and 10 ml of water as the rinse. The controls were sprayed with 60 ml of water. The propellant used was nitrogen at 20 psi gauge pressure. The seeds were treated in a 15-gallon barrel with vanes (Plate I, Fig. 4) and a hole in the center of the lid to allow passage of the spray mist into the barrel. The barrel was put on the barrel roller (Plate II, Fig.1)

and rotated at 15 rpm during treatment and for 15 minutes following. The seeds were placed in five gallon pails with nylon covers (Plate II, Fig. 2) and stored in the laboratory at $70^{\circ} \pm 5^{\circ}\text{F}$ and relative humidity 20-60%.

Sampling. Samples of the seeds were taken with a small scoop immediately, 30, 90, and 180 days after treatment, placed in polyethylene bags, and shipped to the seed testing laboratory for tests.

Simulated Field Studies

a 4-bushel, or simulated field study, was conducted to more nearly reproduce the conditions of farm and commercial storage. Fifty-five-gallon barrels nearly full of wheat were stored in the greenhouse (Plate II, Fig. 4), with a limited amount of temperature control to permit insect activity through the winter months (Note, Table 1). Two separate simulated field studies were conducted. The first of these studies, treated on July 8, 1962, utilized only diazinon. In the second study treated on August 1, 1963, diazinon, malathion, sevin, and dichlorvos were all used in order to compare the usefulness of these insecticides.

Application. The emulsifiable concentrate and wettable powder were applied with a cattle sprayer (Hudson portable) at the desired treatment rate. The desired aliquot of the insecticide for application was brought up to 100 ml with distilled water. Ten ml of water was used to rinse the sprayer. The spray mist was applied to the wheat as it fell

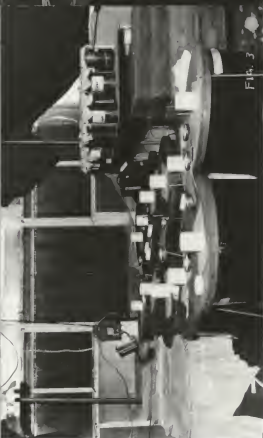
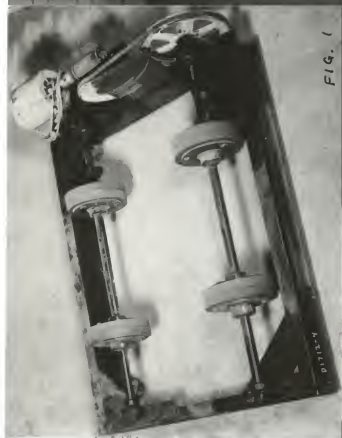
from a 4-inch auger into a 55-gallon barrel. Several trial runs were made for calibration of the sprayer. The barrel, sprayer, and outlet end of the 4-inch auger were covered with a polyethylene canopy to prevent undue loss of the insecticide. After treatment, the vanned barrels were sealed and rotated on the barrel roller (Plate II, Fig. 1) at 15 rpm for 30 minutes, then transported to the greenhouse for infestation.

Infestation. The barrels were arranged in the greenhouse (Plate II, Fig. 3) to allow the insects free access and choice of wheat. Steel grating covered the grain and provided a place to set the insects for infestation (Table 2, and Plate II, Figs. 3 and 4). All adjacent barrels were connected by a sheet of paper and a 1/4-inch nylon cord to provide means for the insects to travel between barrels. Aluminum rods were placed outside and inside the barrels to allow a means of leaving or entering for the insects unable to climb the sides of the barrels. Initially many insects of each of the major stored grain species used (Table 2) were placed atop the barrels in clean cultural media to obtain the infestation.

Sampling and Testing. At regular intervals, samples were removed from the barrels with an undivided probe. Two probings were removed from each barrel, one from the outer edge and one from the center. These probings were placed into quart, Pres-lok cartons, one for each barrel, and returned to the laboratory where they were weighed, the insects removed (handled as previously mentioned) and counted, and the counts converted to the number of insects per kg and recorded. The insects were destroyed and the wheat was returned to the cartons and placed in the constant temperature humidity room previously mentioned for 45-60 days. Then emergence counts were taken and recorded.

EXPLANATION OF PLATE II

- Fig. 1. Barrel roller used for rotating the 15 and 55 gallon barrels.
- Fig. 2. Five-gallon cans with nylon covers in the storage position, for laboratory germination study.
- Fig. 3. Arrangement of 55-gallon barrels used in simulated field studies. Aluminum cans on upper right hand corner shelf used for storing laboratory bioassay wheat prior to infesting.
- Fig. 4. Steel grating which covered 55-gallon barrels and the containers which originally held the insects and clean wheat used for infestation.



EXPLANATION OF PLATE III

Fig. 1. Plenum chamber with hole in the side to allow the sprayer orifice to enter.

Fig. 2A. The 5 1/2 foot divided probe used for sampling wheat.

Fig. 2B. Three foot non-divided probe used for sampling wheat.

PLATE III



FIG. 1
D1712-1



Fig. B

Fig. A

FIG. 2
D1712-2

Small Scale Field Study

In this 400-bushel small scale field study, two 1000-bushel Butler bins with a partition through the center of each were used for the storage facilities. This study is still in progress at the time of writing but ample data have been collected. The wheat used as the control part of the study was donated by the Stored Grain Insect Laboratory, USDA, Manhattan, Kansas, and the remainder of the wheat was purchased from Garvey Elevator, Wichita, Kansas.

Application. Diazinon AG 250 emulsifiable concentrate at the desired treatment rate was applied with a laboratory sprayer (Plate I, Fig. 3, left) as the wheat fell through a plenum chamber (Plate III, Fig. 1) from an auger. The desired aliquot brought up to 1514 ml was applied as a spray mist through a hole at the upper end of the plenum chamber. The control wheat was treated with the AG 250 formulation without diazinon. The propellant used to operate the sprayer was nitrogen at 30 psi gauge pressure.

Sampling. The method was identical to the 400-bushel sampling procedure used in the laboratory study.

Testing. Initially the grain was heavily infested with six species of stored grain insects by placing the insects in quart cartons with clean, untreated wheat. The cartons were submerged until the open top was even with the surface of the grain mass. Four of these cartons were evenly distributed on the surface of each 400-bushel grain mass. The insects had free choice of all the grain within the individual bin. The calculated treatment rates within each bin were Bin #1, control and 8 ppm; Bin #2,

2 ppm and 4 ppm. The wheat was sampled at regular intervals and the insects removed and handled as mentioned above. Live and dead insects present were recorded. The wheat was then used for the laboratory bioassay studies mentioned earlier. Emergence data were taken after the wheat was removed from the laboratory bioassay studies.

RESULTS AND DISCUSSION

General

Dosage levels in parts per million, as indicated by the plates and tables, are calculated dosages. In unpublished work by Dr. C. C. Roan and Dr. B. P. Srivastava of this University, it was found that by using the previously described laboratory procedures for application of the insecticides, approximately 85% of the calculated dosage actually was identified on the wheat. The remaining 15% was lost. This loss occurs when spraying into a closed container. A certain amount of the spray material is forced back out through the lid opening because of a build-up of pressure within the treatment chamber. Some spray materials also adhere to the sides of the treatment chamber. The residue analysis procedures used to determine this percentage loss were gas liquid chromatography and the sulfide method using the spectrophotometer. It was expected and assumed that a comparable percentage of application deposit would be attained with the application procedures used in the simulated field and small scale field studies.

The controls used in the various test generally produced no mortality, but when mortality exceeded five percent, Abbott's formula (1925) was

used. When the percent mortality exceeded ten percent the results were discarded.

The insecticides were evaluated only by biological methods. When viewing the plates or tables to determine the dissipation of the insecticide, it should be noted that the data as presented show only the effects of the insecticide that was available to the insects. Immediately after treatment the insecticide has several ways of killing: (1) contact poison; (2) stomach poison; (3) fumigation; or (4) a combination of the above. On each successive day, more of the insecticide is absorbed into the kernel and dissipated into the air, thus reducing the contact and fumigant effect of the insecticide. Hence, after a time, the insect must eat the inner part of the kernel if the chemical is to be effective, even in the case of the more persistent insecticide residues. This helps to explain why confused flour beetles are not as easily killed, since they scalp only the germ as long as the food supply is plentiful. Another possible reason is that they do not absorb as much of the contact poison, because they do not have pulville lobes on their tarsi as do the rice weevils.

Laboratory Studies

Data presented are composites of at least 3 replicas involving a minimum of 150 insects for each point or figure.

Diazinon. From Plate IV it is apparent that, for the LD_{50} and LD_{90} , a higher dosage of diazinon, by a factor of approximately 10, was required to kill confused flour beetles as compared to rice weevils. For rice weevils, the 7, 14, and 30 day mortality counts fall approximately on the same line.

Plate V shows insects placed on test 30 days after treatment. Compared with Plate IV of the same day, mortality counts show the amount of diazinon that has become unavailable to the insects within the first 30 days after treatment. Using rice weevil and the 7 day mortality counts, the LD₅₀ of rice weevils put on test immediately after treatment was .53 ppm while the LD₅₀ of rice weevils put on test 30 days after treatment was 2.25 ppm. This indicates that 1.72 ppm of diazinon was lost as far as the control of rice weevils is concerned. Plate VI indicates an additional .95 ppm of diazinon lost to rice weevil control between 30 and 64 days. Between 64 and 219 days, the loss of diazinon was somewhat slower, losing only 1.40 ppm for this entire period. The treated wheat used in determining diazinon dissipation for portions of the 30 through 235 day percent mortality counts (Plate VI) was stored in 1000 bushel Butler bins prior to sampling and infesting.

The data in Table 5 were collected from the laboratory studies where 50 each of rice weevils and confused flour beetles were placed on test with emergence counts recorded 45 days after the last mortality counts. It is apparent that a calculated treatment dosage level of approximately 10 ppm is needed to prevent major rice weevil emergence and 6 ppm to prevent major confused flour beetle emergence.

Naled. From Plate VII it is apparent, from the LD₅₀ for confused flour beetles, that with naled they were 3 to 4 times more difficult to kill than rice weevils, and, that at the higher percent mortalities, considerably more naled was required to kill confused flour beetles than rice weevils. This is indicated by the relative slant of the mortality curves. It was found that naled in the laboratory has a relatively short residual

Table 5. Emergence counts of rice weevil (RW) and confused flour beetles (CFB) placed on test immediately, and 30 days, after treatment with diazinon. Figures indicate No. of insects per kg of wheat.

Dosage level (ppm)	Immediately				30 days			
	RW		CFB		RW		CFB	
	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
Control	TNC ^(a)		110	0	TNC		130	0
.2	TNC				TNC			
.4	TNC				TNC			
.6	TNC				TNC			
.8	TNC				TNC			
1.0	2,720	60			TNC			
2.0	610	20	260	10	TNC		150	0
4.0	160	0	10	0	4,766	510	50	0
6.0	20	0	0	0			20	0
8.0	10	0			345	395	10	10
10.0	0	0					0	30
16.0	0	0					0	0

(a) Too numerous to count.

life (substantiated by Strong and Sbur, 1960), compared with diazinon. Tests with insects placed in wheat treated with naled 30 days earlier gave no appreciable mortality. As indicated in Table 6, naled did not reduce the emergence rate of either species substantially, even at the higher dosage levels.

Table 6. Emergence counts of rice weevils (RW) and confused flour beetles (CFB) placed on test immediately after treatment with naled, Figures indicate No. of insects per kg of wheat.

Dosage level (ppm)	RW Alive	CFB	
		Alive	Dead
Control	TNC ^(a)	4,710	0
1.2	TNC	820	0
2.0	TNC	820	30
4.0	TNC	680	10
6.0	TNC	670	10
8.0	TNC	540	0
10.0	TNC	730	10
16.0	TNC	570	0
20.8	TNC	630	0
25.6	TNC	530	0
32.0	TNC	590	0
40.0	TNC	1,080	0

(a) Too numerous to count.

Malathion. From Plate VIII it is evident that about 8 times as much malathion is required to obtain an LD₅₀ for confused flour beetles as for rice weevils. Malathion's effect on rice weevils at one day is insignificant since only a small percent mortality was obtained at the highest dosage level. The broken line in Plate VIII is a hypothetical line, drawn from one point parallel to the 7 and 30 day mortality curves. In Plate IX, there is also a

hypothetical line for the 7 day rice weevil mortality counts. This indicates that under the previously described conditions malathion rapidly became unavailable as a toxicant to rice weevils. This substantiates work by Gunther et al. (1958) where malathion was ineffective against rice weevils within 2 months at similar conditions used in this study. Work by Lindgren (1954) showed malathion to be questionable for control against rice weevils at 2 ppm at 1 month. As indicated by Table 7 when compared to Table 5, the emergence is approximately the same for malathion as for diazinon.

Table 7. Emergence counts of rice weevils (RW) and confused flour beetles (CFB) placed on test immediately, and 30 days, after treatment with malathion. Figures indicate No. of insects per kg of wheat.

Dosage level (ppm)	Immediately				30 days			
	RW		CFB		RW		CFB	
	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
Control	TNC ^(a)		310	0	TNC		200	0
.2	TNC				TNC			
.4	TNC				TNC			
.6	TNC				TNC			
.8	TNC				TNC			
1.0	2,460	50			TNC			
2.0	530	60	30	10	TNC		30	0
4.0			10	0			10	0
6.0			0	0			0	0
8.0							10	10
10.0							10	40
16.0							0	0

(a) Too numerous to count.

It should be noted that the data presented under the 30 day column were results from wheat treated at least 3 months prior to emergence counts. Examination of Plate IX indicates why no control of the emergence at 3 months was obtained, since very little control at 30 days of the original insects was obtained.

Sevin. As indicated by the mortality curves on Plate X, very high dosage levels were required to obtain control of rice weevils and confused flour beetles. The LD_{50} for sevin was between 12.5 and 25 ppm for rice weevils and between 25 and 50 ppm for confused flour beetles, using the 30 day mortality counts. The mortality counts were corrected by Abbott's formula (1925) since the controls often had a mortality of between 5 and 10%. Unpublished work by Grabbe and Ahmed of this university found similar results, using sevin as a grain protectant.

Table 8 indicates the effect of sevin on the emergence of rice weevils and confused flour beetles.

Table 8. Emergence counts of rice weevils (RW) and confused flour beetles (CFB) placed on test immediately after treatment with sevin. Figures indicate No. of insects per kg of wheat.

Dosage level (ppm)	RW		CFB	
	Alive	Dead	Alive	Dead
Control	17.170 g/kg ^(a)		60	0
12.5	24.520 g/kg		20	0
25.0	430	60	10	0

(a) Weighed insects (approximately 20 RW/.10 gram).

Dichlorvos. Plate XI shows that the relative toxicity of dichlorvos to rice weevils and confused flour beetles did not increase after the 7 day mortality counts. This was probably because of its rapid dissipation. Strong and Sbur (1960) showed dichlorvos to be completely dissipated within one month after treatment. The insects placed on test 30 days after treatment showed no mortality. The emergence of rice weevils and confused flour beetles was not affected by the treatment of dichlorvos as indicated by all treatment levels, having approximately the same number of emerged insects as did the control.

Comparison of diazinon with naled, malathion, sevin, and dichlorvos.

The LD₅₀'s and LD₉₀'s of the above insecticides are listed in Table 9.

Table 9. LD₅₀'s and LD₉₀'s of diazinon, naled, malathion, sevin and dichlorvos taken from 7 day mortality counts of rice weevils (RW) and confused flour beetles (CFB), put on test immediately and 30 days after treatment. The LD₅₀'s and LD₉₀'s are approximate readings taken from Plates IV through IX. All figures are parts per million (ppm).

Insecticide	Immediately				30 days			
	LD ₅₀		LD ₉₀		LD ₅₀		LD ₉₀	
	RW	CFB	RW	CFB	RW	CFB	RW	CFB
Diazinon	.53	5.90	.82	13.50	2.25	22.50	3.70	37.00
Naled	3.20	9.20	5.00	20.00				
Malathion	.62	4.50	.85	8.10	3.70	12.00	7.50	27.00
Sevin	18.00	85.00	27.00					
Dichlorvos	.72	3.50	1.00	7.00				

For rice weevils put on test immediately, diazinon is the most toxic, with malathion second. For confused flour beetles put on test immediately, dichlorvos is the most toxic with malathion again second.

Since rice weevils cause more serious damage to wheat, this would classify diazinon as the better grain protectant when compared with other insecticides used in this study.

GERMINATION STUDY

The germination test (Table 10) indicated that there was no detrimental effects on any of the seeds when treated with 8 or 16 ppm diazinon. Sixteen ppm far exceeds the amount of diazinon that would be required to offer insect protection.

Table 10. Effects of diazinon on seed germination at 8 and 16 ppm.

Figures are percent viable seeds.

Seed	Control				8 ppm				16 ppm			
	<u>days after treatment</u>				<u>days after treatment</u>				<u>days after treatment</u>			
	0	30	90	180	0	30	90	180	0	30	90	180
Sorghum	77	75	81	71	83	76	76	77	79	77	67	69
Barley	93	94	92	96	95	95	94	96	94	94	93	96
Oats	94	95	94	84	93	93	95	92	96	90	93	87
Corn	95	95	93	94	94	94	93	93	95	93	94	94
Lima beans	88	70	77	84	83	75	81	78	87	79	71	77
Peas	92	94	95	93	96	94	94	92	94	95	95	94
Rye	94	91	90	94	(a)	93	91	91	92	95	89	90
Wheat	52	49	49	48	59	52	54	59	54	56	47	52

(a) Results not received.

SIMULATED FIELD STUDIES

Plates XII through XV included data taken from the first of 2 simulated field studies. There were 2 barrels of wheat for each calculated treatment level of diazinon at 1/4 lb. (4 ppm), 1/2 lb. (8 ppm), and 2 lbs. (33 ppm) per 1,000 bushels and 2 barrels for controls. The protection provided the wheat at the 4 ppm level (Plate XIII) lasted approximately 19 weeks. Afterwards, wheat destruction occurred, as indicated by the emergence from the wheat sampled and counted at 11 and 19 weeks. The 8 ppm treatment (Plate XIV) appeared to be losing some of its toxicity, but the emergence counts 45 days later of the 19 week sampling indicated that 98.4% of the emerged insects were killed. From this information it is evident that 8 ppm diazinon will give sufficient protection to wheat of 12.5% moisture, 60 lbs. test weight per bushel, for a period of at least 7 months, and possibly longer. The 33 ppm (Plate XV) showed complete kill through the 19 week original and emergence counts. As indicated in Plates XII through XV, the primary insect pest which developed was the rice weevils. This was also true throughout these studies. These same plates indicate that if rice weevils are controlled, the remaining species of stored grain insects studied will not present a major problem.

Plates XVI through XXIV included data from the second simulated field study. There were 2 barrels of wheat treated with each insecticide: diazinon, malathion, sevin, and dichlorvos with 2 different calculated dosage levels for each insecticide. Dosage levels were:

diazinon, 2 and 4 ppm; malathion, 4 and 8 ppm; sevin, 25 and 50 ppm; and dichlorvos, 2 and 4 ppm. Plate XXV shows photos taken on January 2, 1964 (5 months after treatment) of quart samples of wheat sampled on September 19, 1963, then placed in Pres-Lok cartons. These cartons were held at laboratory conditions of approximately $80^{\circ} \pm 2^{\circ}\text{F}$ and 20-60% relative humidity until the 5-months-after-treatment photos were taken. The wheat showed no damage to the 4 ppm diazinon and 8 ppm malathion treatment levels, with varying amounts of damage found throughout the other treatment levels and total devastation of the control samples.

SMALL SCALE FIELD STUDY

Plates XXVI through XXIX are data collected from wheat stored in the two 1,000 bushel partitioned Butler bins. The reason for the substantial mortality in the control (Plate XXVI) is not fully understood. However, since the 8 ppm treated wheat was in the same bin it is believed that the insects reared in the control section migrated to the 8 ppm section (Table 11). Then, after they had obtained a toxic dosage of diazinon, they migrated back into the control wheat to die. Wheat damage was severe in the control and little or no damage occurred in the 8 ppm treatment. Also, sampling based upon dividing the 8 ppm section of the bin into 3 equal areas with the dividing lines running parallel with the partition (Table 11), showed that migration was occurring.

As is indicated by Plate XXVII, the calculated 2 ppm treatment level provided protection up through 15 weeks. At 22 weeks, the protection was questionable. The 4 ppm treatment level (Plate XXVIII) was still effective

Table 11. Number of living insects collected in the 8 ppm treatment, located adjacent to the untreated control.

Insects	A ^(a)	B ^(b)	C ^(c)
Rice weevil	28 ^(d)	4	2
Confused flour beetle	2	0	0
Sawtooth grain beetle	212	74	22

(a) Located next to the partition dividing treated from untreated grain.

(b) Middle area of treated grain.

(c) Outside area farthest from the partition.

(d) No. insects per kg, sampled by 5 1/2 foot probe, August 21, 1964.

at 22 weeks against the bin infestation. However, Plate VI indicates that when samples of wheat were removed from the bin, the original insects removed, and the wheat reinfested with rice weevils in the laboratory, a 76% mortality was obtained from wheat at 64 days after treatment, using the 7 day mortality counts. At 219 days after treatment, protection offered the wheat at the 4 ppm treatment level was questionable with only a 30% rice weevil mortality from the 7 day mortality counts. The 8 ppm treatment level (Plate XXIX) gave excellent control at 22 weeks. However, Plate VI again indicates that, at 235 days after treatment 87% mortality of rice weevils was still obtained using the 7 day mortality counts. Table 12, 8 ppm column, 22 week data, shows that there was virtually no emergence at 31 weeks after treatment. This indicates that the 8 ppm treatment may be expected to be effective through 10 months, and possibly beyond a year, for bin protection.

Of the numerous (ten or more) 45-60 day emergence counts from the bin samples, only data for the 15 and 22 week counts (Table 12) are given. Counts prior to this period were zero.

Table 12. Adult emergence from the 15 and 22 week, original wheat samples, taken from the Butler bins. Figures are No. insects per kg of wheat.

Insects	Dosage level							
	Control		2 ppm		4 ppm		8 ppm	
	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
	15 weeks							
Confused flour beetle	10	0	0	0	0	0	0	0
Lesser grain borer	100	0	20	0	10	0	0	0
Rice weevil	110	0	310	10	180	0	0	0
	22 weeks							
Confused flour beetle	0	0	0	0	10	0	0	0
Lesser grain borer	290	0	610	0	230	0	0	0
Rice weevil	2,770	0	90	30	10	10	10	0
Sawtooth grain beetle	20	0	0	0	0	0	0	0
Flat grain beetle	10	0	30	0	0	0	0	0

SUMMARY AND CONCLUSIONS

The purpose of these studies was to evaluate diazinon as a grain protectant. Evaluations included determining its effect upon seed germination, its relative toxicity to insects using comparisons with other protectants when possible, and the length of its residue effectiveness.

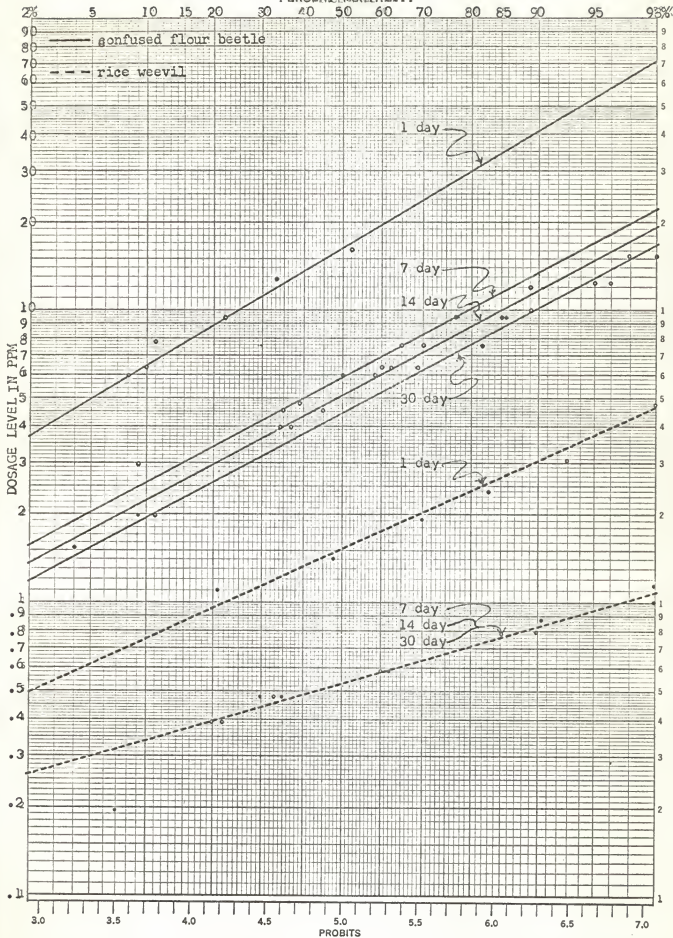
Diazinon had no effect on seed germination to at least 6 months after treatment. Its relative toxicity to rice weevil in the laboratory is superior to malathion. Rice weevils were the most destructive of the stored wheat insects studied. The effectiveness of diazinon on wheat treated with 8 ppm is beyond 8 months, when used on wheat of 12.57% moisture content, stored at a mean temperature of 76^oF.

A comparison of malathion with diazinon was not made in the large bins simulating field conditions. However, since the results with diazinon were approximately the same for the large bins as they were in the laboratory and simulated field studies, it appears that diazinon is as good as, or superior to, malathion under field conditions.

EXPLANATION OF PLATE IV

Diazinon. Mortality of rice weevils and confused flour beetles put on test immediately after treatment. Testing period between June 14 and July 17, 1962.

PERCENT MORTALITY



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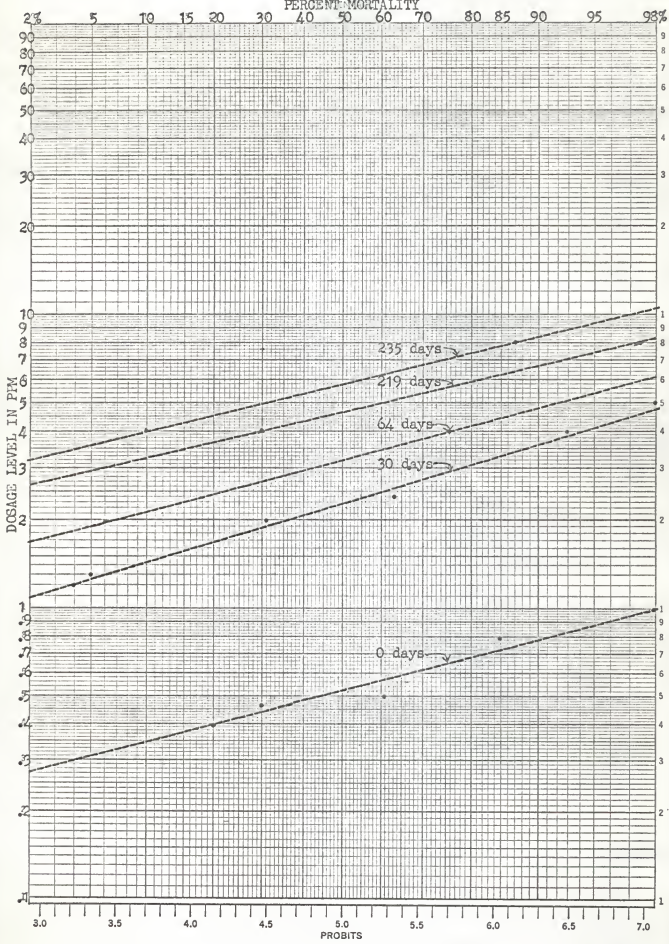
EXPLANATION OF PLATE V

Diazinon. Mortality of rice weevils and confused
flour beetles put on test 30 days after treatment.
Testing period between July 14 and August 16, 1962.

EXPLANATION OF PLATE VI

Diazinon. Seven day mortality counts of rice weevils put on test at immediately, 30, 64, 219, and 235 days after treatment. Testing periods June 14 through August 16, 1962, and June 8, 1964, through February 5, 1965.

PERCENT MORTALITY

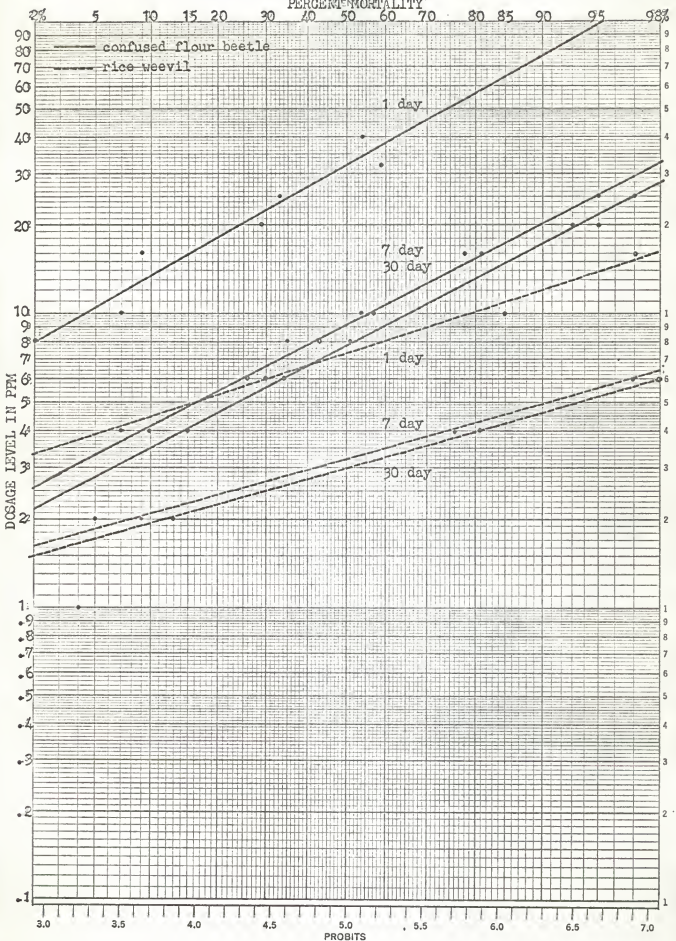


MADE IN U.S.A.
 KUPFFEL B. FENNER CO.

EXPLANATION OF PLATE VII

Naled. Mortality of rice weevils and confused
flour beetles put on test immediately after treatment.
Testing period between July 20 and August 31, 1962.

PERCENT MORTALITY

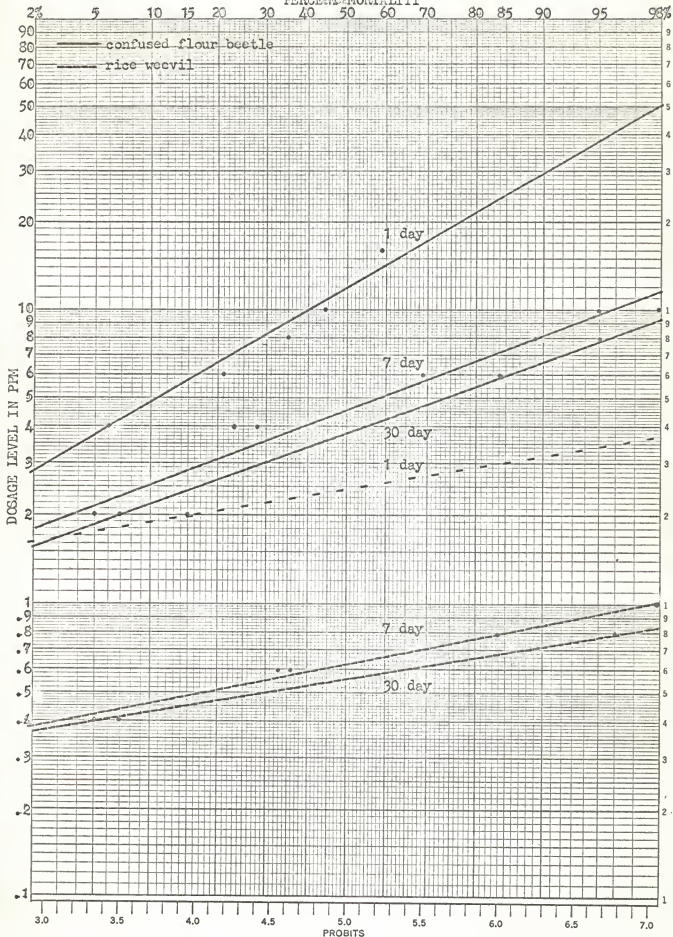


MADE IN U.S.A.
KEUFFEL & ESSER CO.

EXPLANATION OF PLATE VIII

Malathion. Mortality of rice weevils and confused flour beetles put on test immediately. Testing period between July 18 and August 17, 1962.

PERCENT-MORTALITY

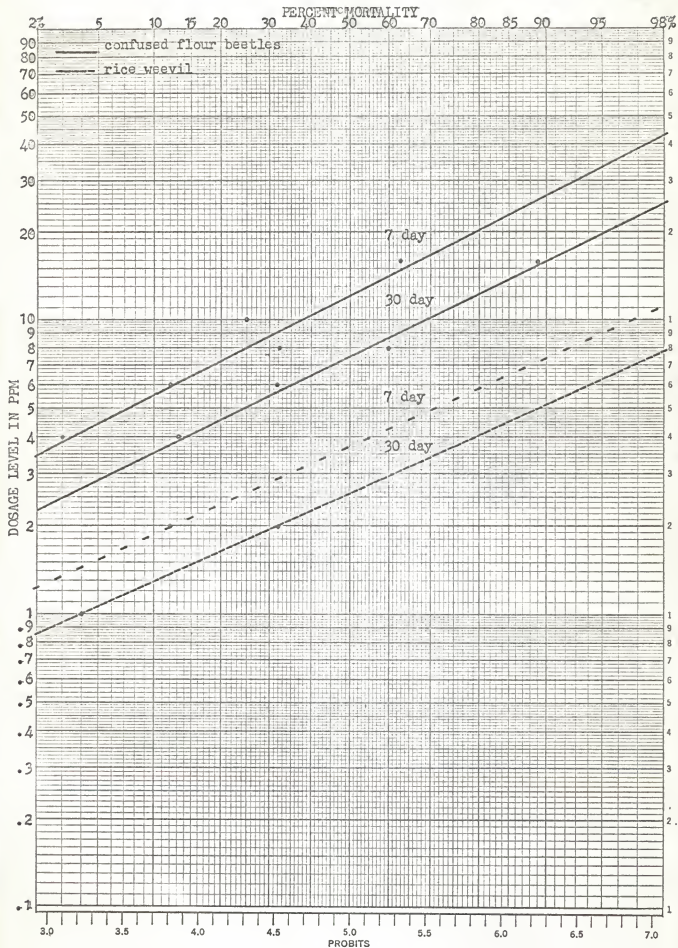


MADE IN U.S.A.

KEUFFEL & FENNER CO.

EXPLANATION OF PLATE IX

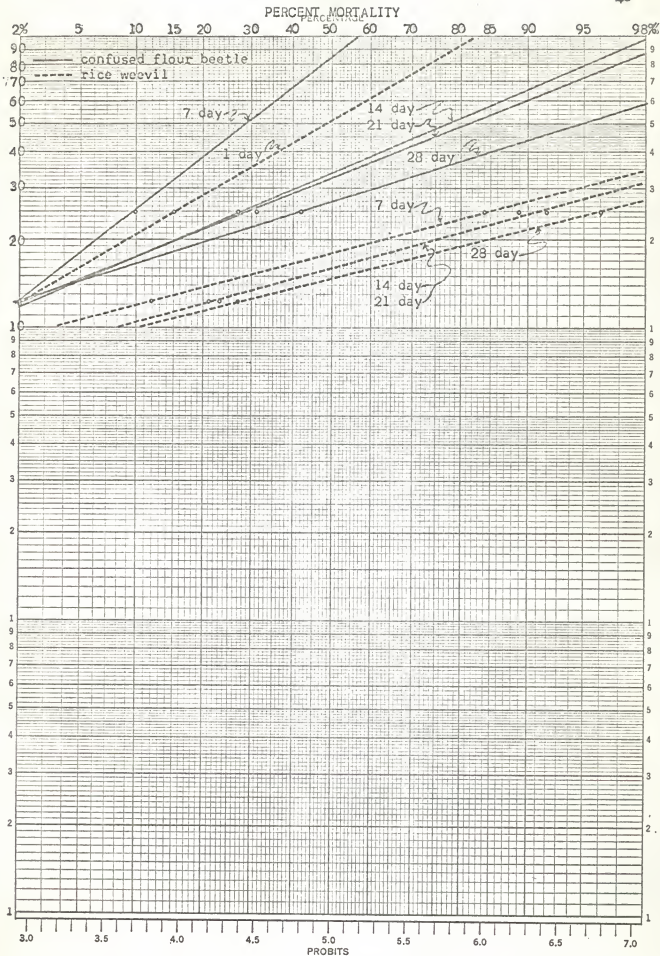
Malathion. Mortality of rice weevils and confused flour beetles put on test 30 days after treatment. Testing period between August 17 and September 17, 1962.



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EXPLANATION OF PLATE X

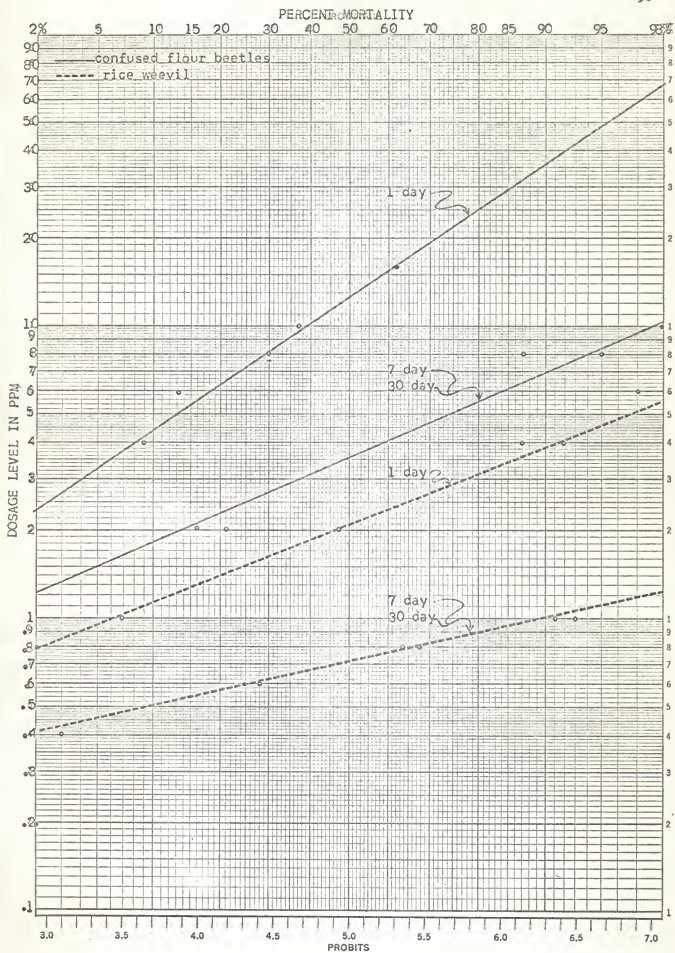
Sevin. Mortality of rice weevils and confused flour beetles put on test immediately after treatment. Testing period between March 23 and August 16, 1964.



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KEMPFER & FRYER CO.

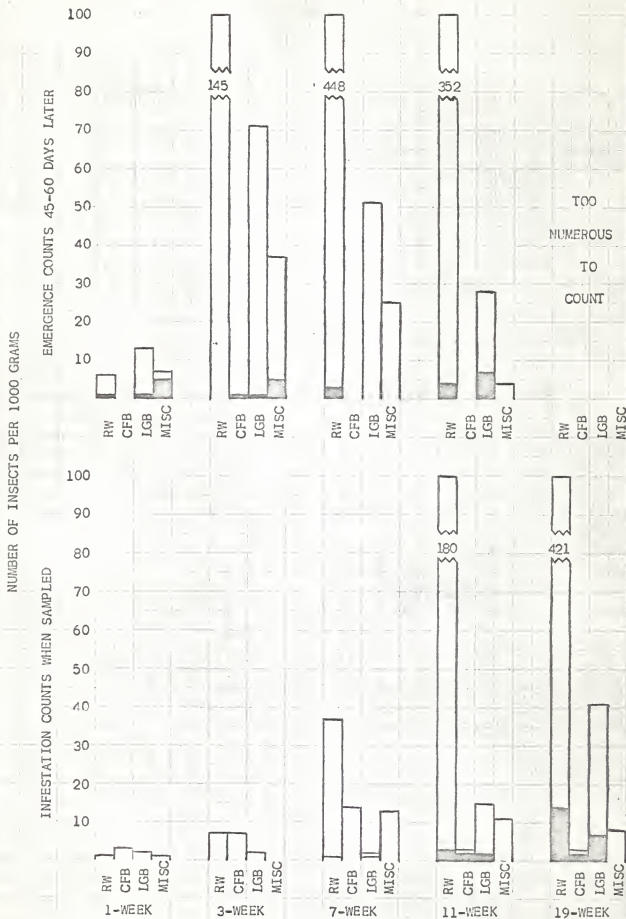
EXPLANATION OF PLATE XI

Dichlorvos. Mortality of rice weevils and confused
flour beetles put on test immediately after treatment.
Testing period between March 23 and August 16, 1964.



EXPLANATION OF PLATE XII

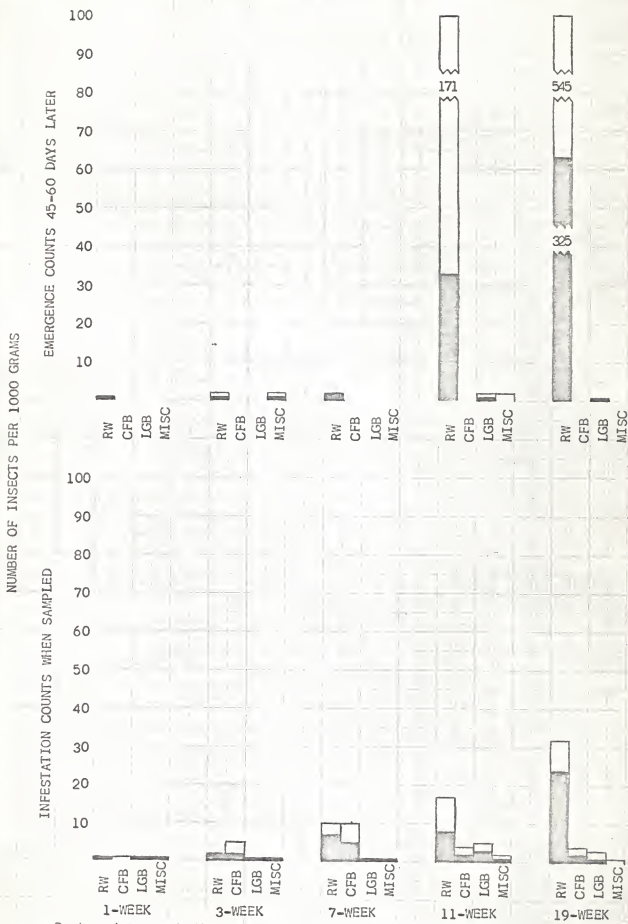
Diazinon bioassay mortality data from wheat of the control barrels of the simulated field study treated on July 8, 1962. Testing period between July 8 and December 30, 1962.



Darkened areas indicate the number of dead insects

EXPLANATION OF PLATE XIII

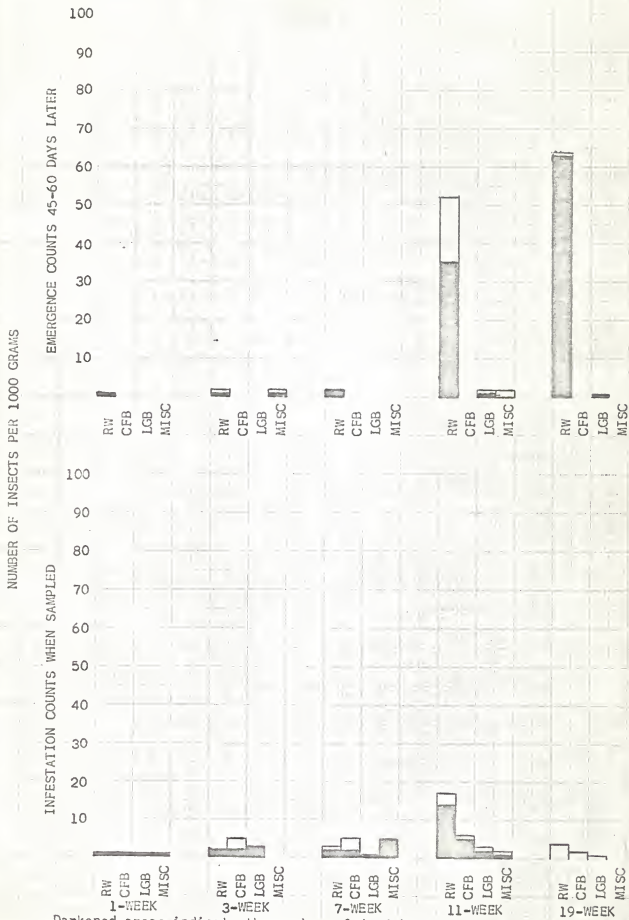
Diazinon bioassay mortality data taken from the wheat of the 4 ppm treated barrels of the simulated field study treated on July 8, 1962. Testing period between July 8 and December 30, 1962.



Darkened areas indicate the number of dead insects

EXPLANATION OF PLATE XIV

Diazinon bioassay mortality data taken from the wheat of the 8 ppm treated barrels of the simulated field study treated on July 8, 1962. Testing period between July 8 and December 30, 1962.



Darkened areas indicate the number of dead insects.

EXPLANATION OF PLATE XV

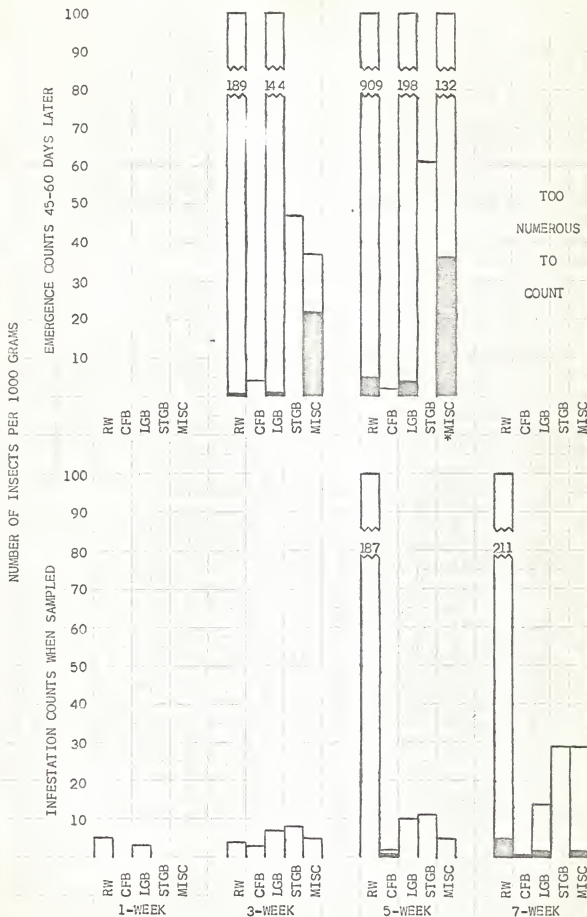
Diazinon bioassay mortality data taken from the wheat of the 33 ppm treated barrels of the simulated field study treated on July 8, 1962. Testing period between July 8 and December 30, 1962.



Darkened areas indicate the number of dead insects.

EXPLANATION OF PLATE XVI

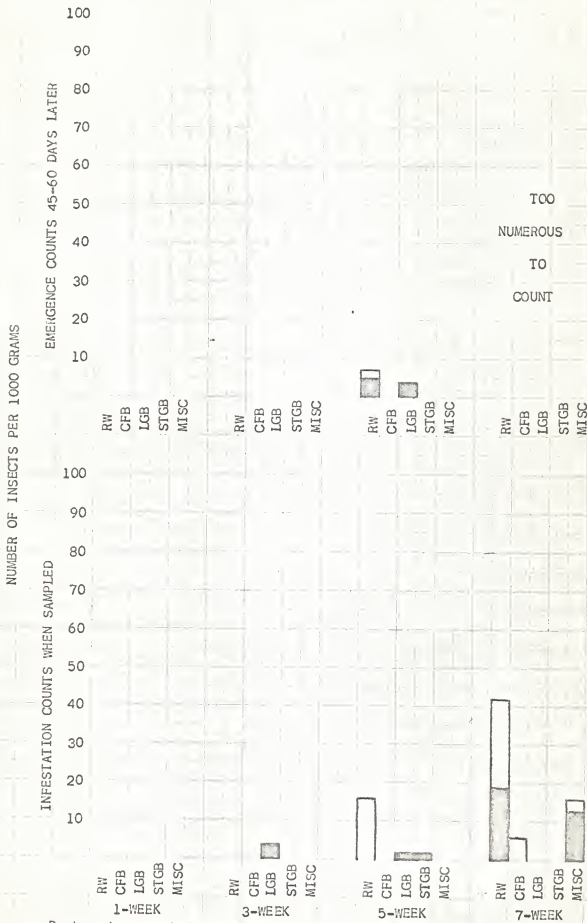
Simulated field bioassay mortality data taken from the wheat of control barrels put on test August 1, 1963. Testing period between August 1 and November 1, 1963.



Darkened areas indicate the number of dead insects.
 *This darkened area indicates the number of live insects.

EXPLANATION OF PLATE XVII

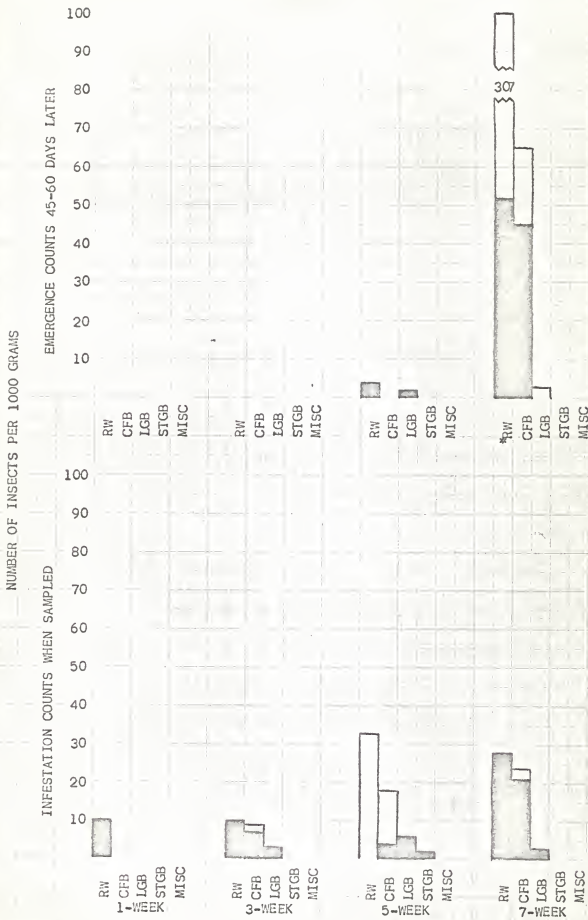
Simulated field bioassay mortality data taken
from wheat treated with 2 ppm diazinon on August 1, 1963.
Testing period between August 1 and November 1, 1963.



Darkened areas indicate the number of dead insects.

EXPLANATION OF PLATE XVIII

Simulated field bioassay mortality data taken from wheat treated with 4 ppm diazinon on August 1, 1963. Testing period between August 1 and November 1, 1963.

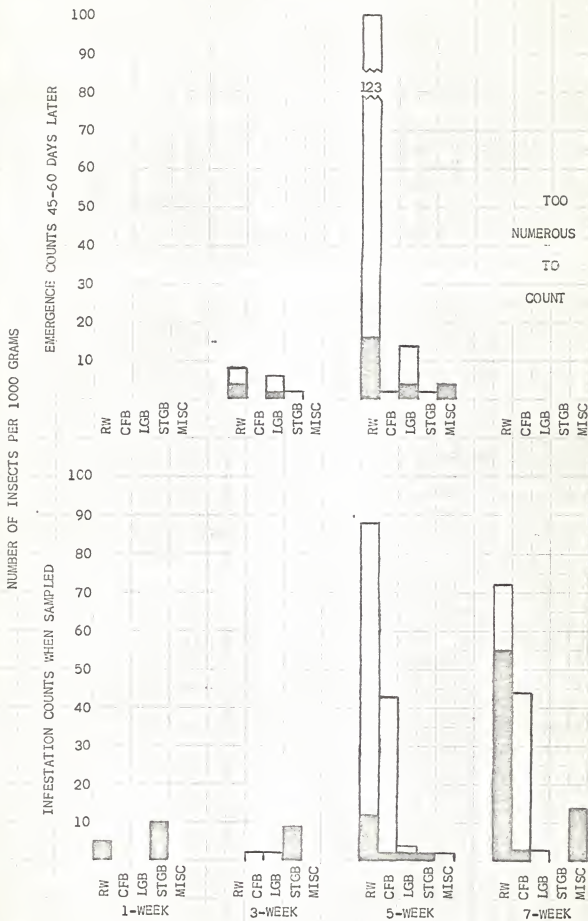


Darkened areas indicate the number of dead insects.

*This darkened area indicates the number of live insects.

EXPLANATION OF PLATE XIX

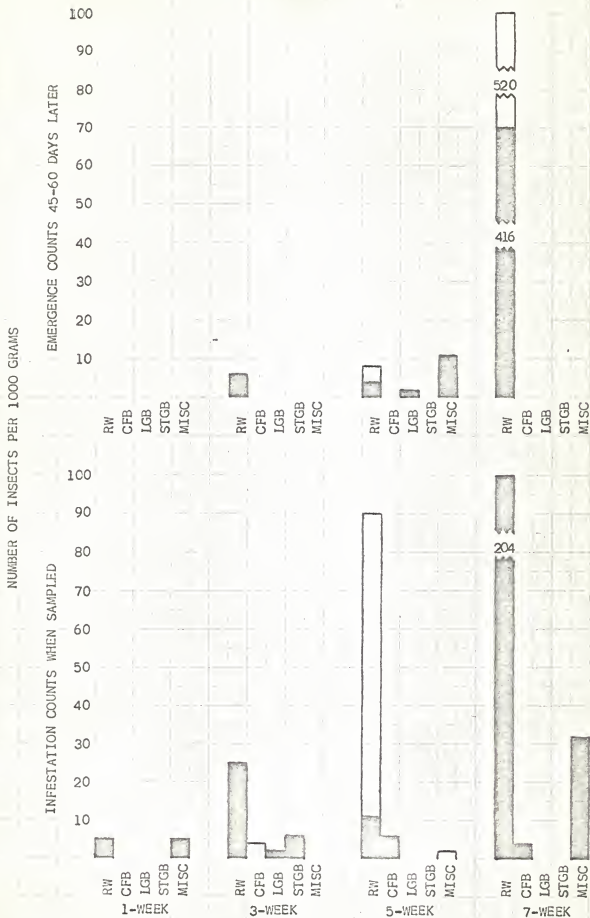
Simulated field bioassay mortality data taken from
wheat treated with 4 ppm malathion on August 1, 1963.
Testing period between August 1 and November 1, 1963.



Darkened areas indicate the number of dead insects.

EXPLANATION OF PLATE XX

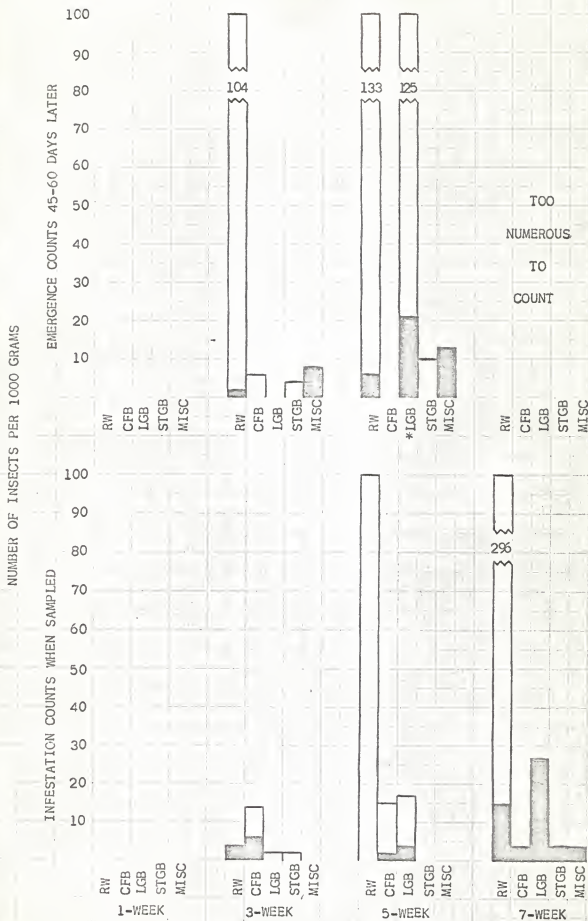
Simulated field bioassay mortality data taken from wheat treated with 8 ppm malathion on August 1, 1963. Testing period between August 1 and November 1, 1963.



Darkened areas indicate the number of dead insects.

EXPLANATION OF PLATE XXI

Simulated field bioassay mortality data taken from wheat treated with 25 ppm sevin on August 1, 1963. Testing period between August 1 and November 1, 1963.

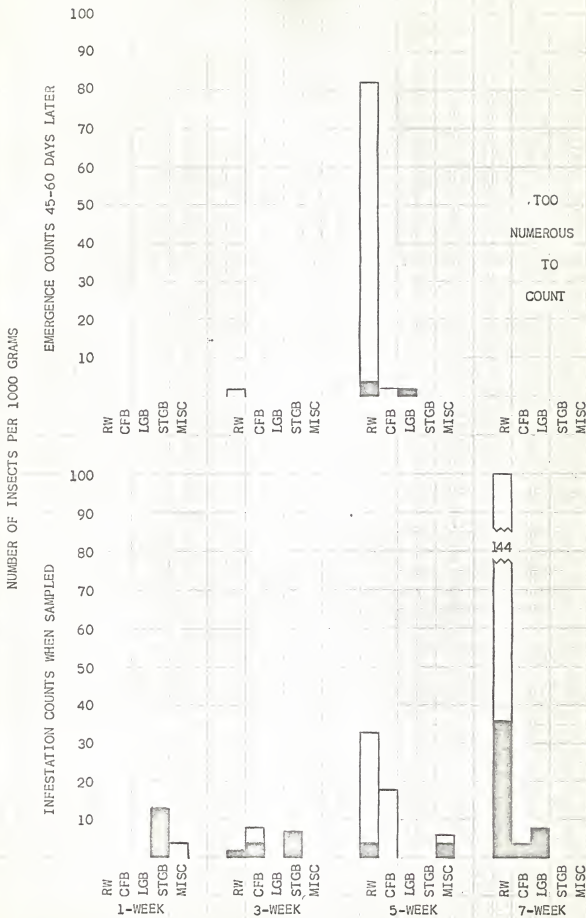


Darkened areas indicate the number of dead insects.

*This darkened area indicates the number of live insects.

EXPLANATION OF PLATE XXII

Simulated field bioassay mortality data taken from wheat treated with 50 ppm sevin on August 1, 1963. Testing period between August 1 and November 1, 1963.

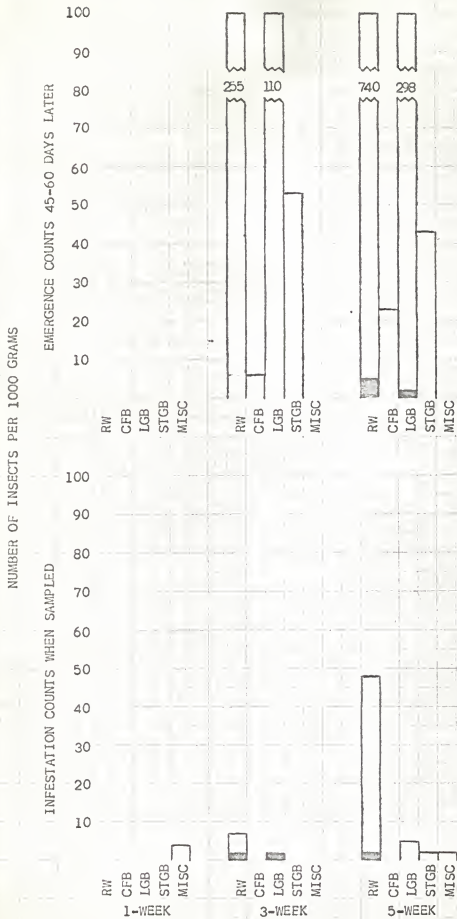


Darkened areas indicate the number of dead insects.

TOO
NUMEROUS
TO
COUNT

EXPLANATION OF PLATE XXIII

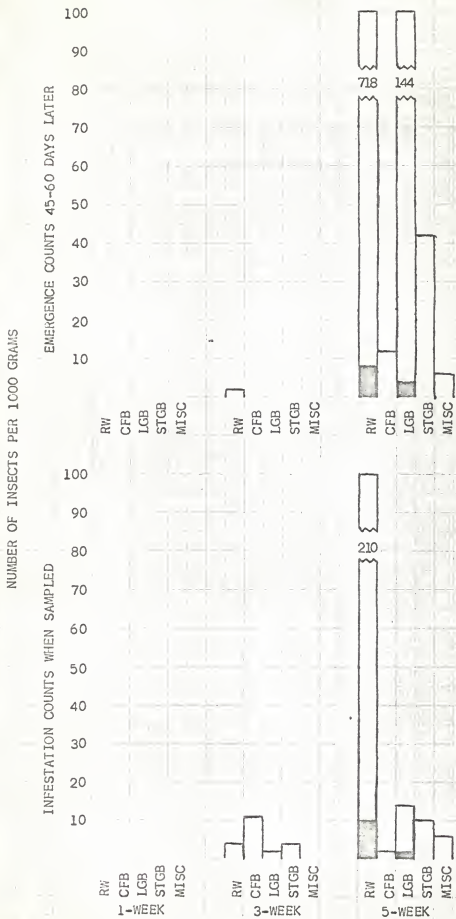
Simulated field bioassay mortality data taken from
wheat treated with 2 ppm dichlorvos on August 1, 1963.
Testing period between August 1 and November 1, 1963.



Darkened areas indicate the number of dead insects.

EXPLANATION OF PLATE XXIV

Simulated field bioassay mortality data taken from
wheat treated with 4 ppm dichlorvos on August 1, 1963.
Testing period between August 1 and November 1, 1963.



Darkened areas indicate the number of dead insects.

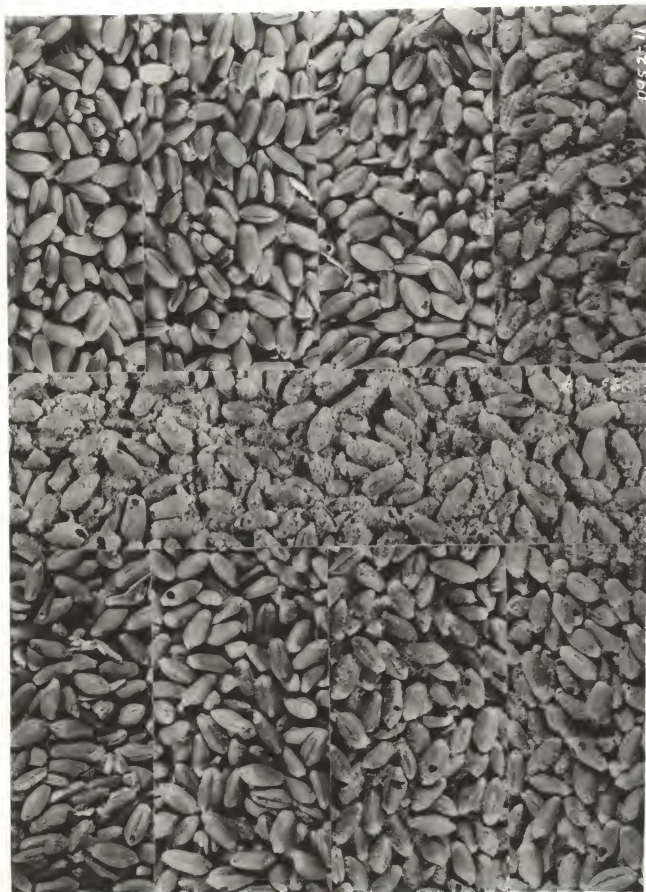
EXPLANATION OF PLATE XXV

Wheat treated with several insecticides at 2 dosage levels each. Following is a positioning diagram showing what insecticide and at what dosage level used.

Diazinon 4 ppm	Malathion 8 ppm	Sevin 50 ppm	Dichlorvos 4 ppm
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Control

Diazinon 2 ppm	Malathion 4 ppm	Sevin 25 ppm	Dichlorvos 2 ppm
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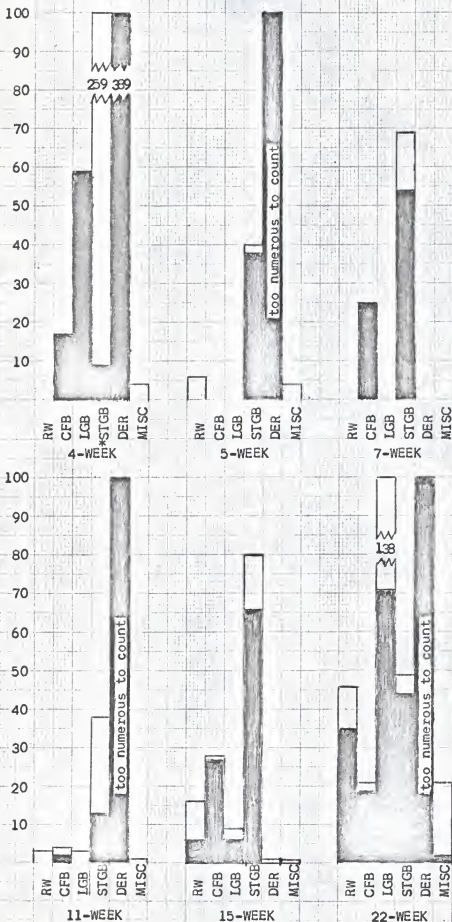


EXPLANATION OF PLATE XXVI

Bin bioassay mortality data from wheat located in the control section of the Butler bins. Wheat had a natural infestation of dermestids. Testing period between June 8 and November 30, 1964.

INFESTATION COUNTS WHEN SAMPLED

NUMBER OF INSECTS PER 1000 GRAMS

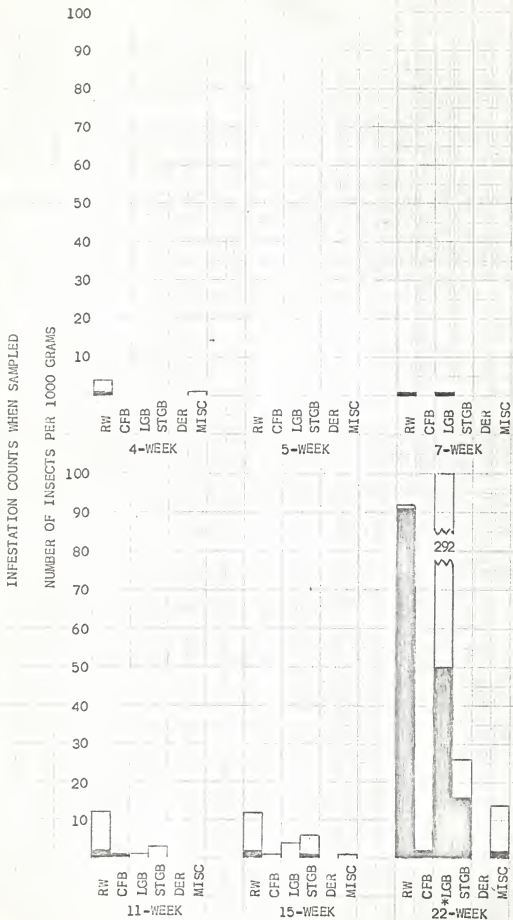


Darkened areas indicate the number of dead insects.

*This darkened area indicates the number of live insects.

EXPLANATION OF PLATE XXVII

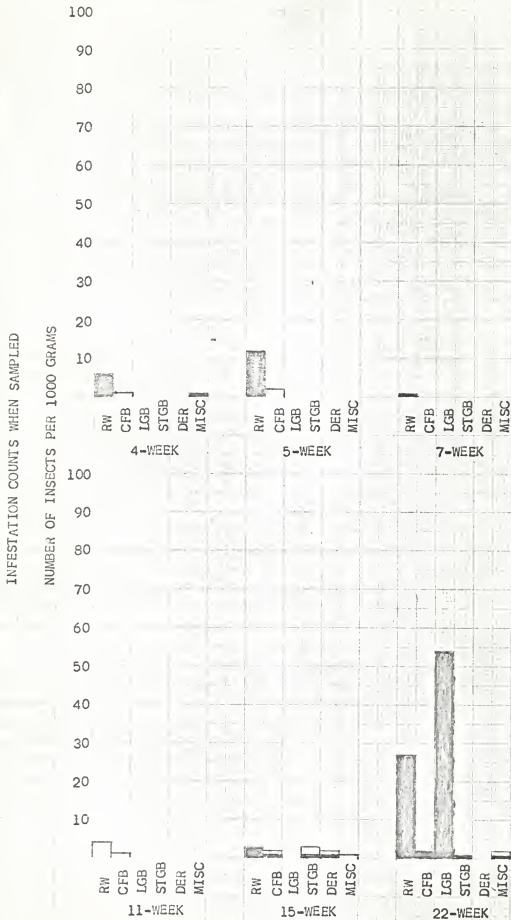
Bin bioassay mortality data collected from wheat
located in the 2 ppm section of the Butler bins.
Testing period between June 8 and November 30, 1964.



Darkened areas indicate the number of dead insects.
 *This darkened area indicates the number of live insects.

EXPLANATION OF PLATE XXVIII

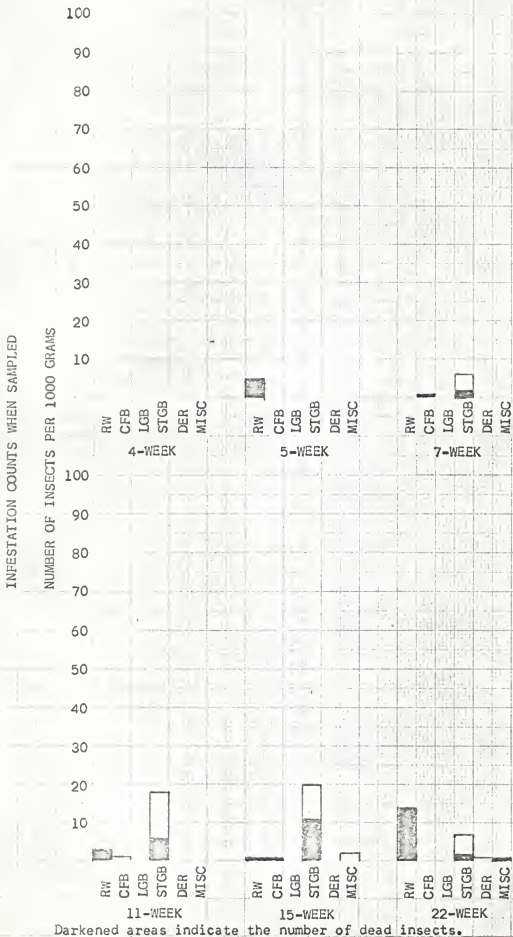
Bin bioassay mortality data collected from wheat
located in the 4 ppm section of the Butler bins.
Testing period between June 8 and November 30, 1964.



Darkened areas indicate the number of dead insects.

EXPLANATION OF PLATE XXIX

Bin bioassay mortality data collected from wheat
located in the 8 ppm section of the Butler bins.
Testing period between June 8 and November 30, 1964.



Darkened areas indicate the number of dead insects.

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EVALUATION OF TOXICITY OF DIAZINON TO
SEVERAL SPECIES OF STORED GRAIN INSECTS

by

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Studies were conducted to evaluate the capabilities of diazinon as a grain protectant. Limited comparisons were made with naled, malathion, sevin, and dichlorvos. Bioassays were conducted in small paper cartons in the laboratory, in 55-gallon drums (4 bushels) to simulate field conditions to some extent, and in 1000 bushel bins to evaluate under field conditions.

The laboratory studies consisted primarily of the treatment of 3 kg samples of wheat at different dosage levels, using various insecticides. Two, three, or four 100 gram aliquots were removed and placed in 1/2-pint Pres-Lok paper cartons, and infested with 50 rice weevils per carton or 50 confused flour beetles per carton, respectively. Then the infested cartons of wheat were placed in a rearing room at $80^{\circ} \pm 2^{\circ}\text{F}$ and $70\% \pm 5\%$ relative humidity. Mortality counts were made at one or more of the following times after introduction of the insects into the treated samples: 1, 7, 14, 21, and 30 days. The insects were then removed from the wheat at the final mortality count and the cartons of wheat returned to the rearing room for 45-60 days, after which emergence counts were taken.

Confused flour beetles were more difficult to kill than rice weevils. Compared with other insects put on test immediately after treatment, diazinon was the most effective against rice weevils and dichlorvos best for use against confused flour beetles. Ten ppm of diazinon was required to eliminate rice weevil emergence and 6 ppm to eliminate confused beetle emergence. Emergence from malathion-treated wheat was similar to that of diazinon treated wheat. The other insecticides tested had little or no effect upon the insect emergence.

Seed germination tests were conducted with diazinon on wheat, rye, oats, barley, corn, peas, lima beans and sorghum, at dosages of 8 and 16 ppm. There was no effect upon germination.

The first simulated field study, using barrels, consisted of diazinon at 4, 8, and 33 ppm. The second study consisted of 4 insecticides, at 2 dosage levels: diazinon, 2 and 4 ppm; malathion, 4 and 8 ppm; sevin, 25 and 50 ppm; and dichlorvos, 2 and 4 ppm. The samples were stored in 55-gallon oil drums in a greenhouse with limited temperature control to allow insect activity during winter months. Samples were infested with 8 major stored grain insect species. Rice weevils were the most prolific and did the most damage. When rice weevils were controlled, no major wheat damage by other species occurred. In the second simulated field study no damage occurred in the 4 ppm diazinon treated wheat and 8 ppm malathion treated wheat, 5 months after treatment. Varying degrees of damage occurred in the other samples. There was complete destruction of the untreated wheat.

The field bin study consisted of four, 400 bushel samples of wheat in 2 partitioned, 1000 bushel Butler bins. The untreated and 8 ppm treatments were in one bin; and the 2 and 4 ppm in another bin. Although the study is still in progress, it was found that at 8 months the 8 ppm treatment was still providing complete protection to the wheat, at a moisture content of 12.57% and temperature between 30° and 86°F.