Toxicity of *O,O*-Diethyl *O*-(2-Isopropyl-6-methyl-4-pyrimidyl) Phosphorothioate (Diazinon)

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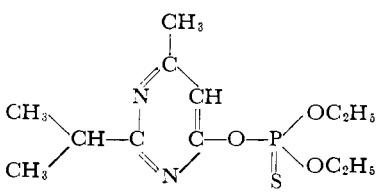
The acute oral LD_{50} of O,O-diethyl O-(2-isopropyl-6-methyl-4-pyrimidyl) phosphorothioate, Diazinon, to rats is 100 to 150 mg. per kg. when administered as technical Diazinon and 264.5 mg. per kg. as a 25% wettable powder. The acute oral LD_{50} to mice is 82 mg. per kg. administered as technical Diazinon. Acute and repeated dermal application to rabbits showed that Diazinon is absorbed through the skin. The chronic feeding of Diazinon 25% wettable powder at levels of 10, 100, and 1000 p.p.m. of active Diazinon in the diets of rats produced no signs of toxicity in 72 weeks. Diazinon produced inhibition of cholinesterase in vivo and in vitro. The feeding of Diazinon 25% wettable powder to dogs for periods up to 43 weeks at levels up to 6.5 mg. per kg. per day of active Diazinon caused no gross signs of toxicity, but inhibited cholinesterase activity. Levels of 9.3 mg. per kg. per day and higher resulted in the usual signs of toxicity associated with cholinesterase inhibition.

AMONG THE NEW ORGANIC INSECTICIDES, Diazinon (G-24480) has shown considerable promise for the control of houseflies and certain other insect pests (*2*, *4*). Diazinon, *0,0*-diethyl *0*-(2-isopropyl-6-methyl-4-pyrimidyl) phosphorothioate, has the following structural formula:



The technical product is a pale brown liquid which is insoluble in water but dissolves readily in most organic solvents.

Diazinon was first synthesized by Gysin (*5*) in 1952. Its toxicological and insecticidal action was reported briefly by Gasser (*3*) in 1953. This investigator found the acute oral LD_{50} of technical Diazinon in mice, rats, guinea pigs, and rabbits to be 96, 235, 320, and 130 cu. mm. per kg., respectively. The oral LD_{50}'s of a 25% wettable powder were reported as 122.5 mg. per kg. for mice and 712.5 mg. per kg. for rats.

The following studies were undertaken to ascertain the action of Diazinon on cholinesterase activity and to obtain additional toxicological data which might be of practical value in connection with its application as an insecticide.

Material and Methods

The samples of Diazinon used consisted of a technical grade containing 85% active compound, and a wettable powder, Diazinon 25W, which contained a nominal concentration of 25% Diazinon. These materials were furnished by The Geigy Chemical Corp., Geigy Agricultural Chemicals Division. Actual analyses made during subacute feeding studies showed a variation from 22.7 to 22.9% active material in Diazinon 25W. Feeding was based on the actual analyses and not on the nominal concentration.

Cholinesterase activity was estimated by Frawley's (*1*) modification of Michel's electrometric method. Results agreed with those found by the manometric method (*6*) used periodically during this study.

Effect of Diazinon on Cholinesterase in Vitro

The effects of technical Diazinon on cholinesterase activity were determined by adding known concentrations of the insecticide to the plasma, red blood cells, and brain of the rat. Solutions of technical Diazinon in 50% ethyl alcohol were added to homogenates of the tissues so that each final dilution contained 6.9% ethyl alcohol, 0.069 ml. of plasma or red cells, and 34 mg. of brain per ml. of solution.

At molar concentrations of 3.79×10^{-4}, 3.79×10^{-6}, and 3.79×10^{-5}, complete inhibition was produced in the plasma, red blood cells, and brain, respectively. The estimated inhibition 50's were 5.4×10^{-5} for the plasma, 4.2×10^{-7} for the red cells, and 2.5×10^{-6} for the brain.

Acute Oral Toxicity

Solutions of technical Diazinon in corn oil were administered orally by stomach tube to groups of male albino mice and rats (total number of animals 52 and 49, respectively) at graduated dosage levels. Diazinon, 25% wettable powder, was administered to groups of male albino rats (total number 42) as a 5% suspension in methylcellulose. Expressed as active Diazinon, the calculated acute oral LD_{50} of the technical Diazinon for mice is 82 mg. per kg. of body weight and for rats, approximately 100 to 150 mg. per kg. Expressed as active Diazinon, the acute oral LD_{50} of the 25% wettable powder for rats is 264.5 mg. per kg., indicating a significantly reduced toxicity for this formulation. The statistical calculations were by the method of Wilcoxon and Litchfield (*7*). Gross signs of systemic toxicity observed following oral administration of either the technical Diazinon or the wettable powder were characteristic of cholinesterase inhibition and included depression, salivation, lacrimation, rapid respiration, tremors, and diarrhea. Gross pathology observed among the animals that died included hemorrhagic lungs and kidneys, and gastrointestinal irritation. Gross autopsies performed upon the surviving animals after an observation period of one week showed slight gastrointestinal irritation.

Acute and Repeated Dermal Application

A single application of Diazinon 25% wettable powder was applied to the closely clipped abdominal skin of albino rabbits at dosage levels of 0.46, 0.92, 1.84, or 4.00 grams per kilogram of body weight (based on the active ma-

terial). The wettable powder was applied either dry or moistened with sufficient distilled water to form a paste. The material was spread evenly on a nonabsorbent paper backing and secured to the skin by means of a gauze and adhesive tape binder. There appeared to be more complete absorption from the moistened material than from the dry material. The acute dermal LD_{50} is greater than 4.0 grams of active Diazinon per kg. of body weight.

Groups of albino rabbits received repeated daily dermal applications of technical Diazinon at dosage levels of 0.1, 0.3, 0.5, or 1.0 ml. per kg., applied to the closely clipped abdominal skin under rubber damming. The material was absorbed dermally and some deaths occurred at all dosage levels, generally following three to five applications.

Following application of either technical Diazinon or the 25% wettable powder, the dermal irritation observed was mild in nature and was characterized by erythema, atonia, and desquamation. The observed gross signs of systemic toxicity exhibited by the animals that succumbed were characteristic of cholinesterase inhibition. Gross autopsy findings observed among the animals that died included hemorrhagic lungs and irritation of the gastrointestinal tract and peritoneum. Gross and microscopic examination of the organs of the surviving animals revealed no characteristic pathology.

Experiments with Rats

Subacute Feeding Three groups of 10 male albino rats each received 0 (control), 100, or 1000 p.p.m. (0, 0.01, or 0.1%) of technical Diazinon in the diet for 4 weeks. Food consumption for the three groups was comparable. The 1000 p.p.m. Diazinon group exhibited a slight growth retardation. There was no gross evidence of toxicity and no gross pathology at autopsy.

Studies were conducted to determine the effect of Diazinon on the cholinesterase activity of the brain, plasma, and red blood cells of the above rats (Table I). At 100 p.p.m. Diazinon in the diet, only the enzyme activity of the red blood cells was significantly inhibited. Both the red blood cells and brain at 1000 p.p.m. were inhibited. The values obtained for the plasma at 100 and 1000 p.p.m. were statistically evaluated and were not found to be significantly different from the control values.

Table I. Cholinesterase Activities of Tissues from Rats Receiving Diazinon for 4 Weeks

Level, P.P.M.	No. of Rats	% Normal Activity		
		Plasma	Red cells	Brain
Control	6	100	100	100
100	10	90	24	97
1000	10	84	0	29

Chronic Feeding Method Weanling male and female albino rats of the Carworth Farms strain were selected at random and housed individually in wire-mesh cages elevated above the droppings. Water and the appropriate diets were offered *ad libitum*. Weekly records were kept of the individual body weights and food consumption of each rat during the course of the study, and weekly observations were made as to the general appearance, condition, and behavior of each animal. Gross autopsies were performed upon the animals that died and representative tissues were preserved.

The following groups of rats were initiated, the Diazinon 25% wettable powder being added to each diet on a weight basis:

Level	No. of Rats	
	Male	Female
Control	20	20
0.001% Diazinon, 25% wettable powder (10 p.p.m.)	20	20
0.01% Diazinon, 25% wettable powder (100 p.p.m.)	20	20
0.1% Diazinon, 25% wettable powder (1000 p.p.m.)	20	..

As previous data indicated that a dietary level of 1000 p.p.m. caused initial growth retardation in young rats, the high level group was started at a level of 100 p.p.m. and over a 10-week period the dietary concentration was gradually adjusted upward until the level of 1000 p.p.m. was reached.

The first 72 weeks of the study are included in this publication. The study will continue for 2 years, at which time gross and microscopic examinations of the organs of the surviving animals will be made.

Results. Table II presents a summary of body weights, food and compound consumption, and survival data for the rats. The "per cent survival" is based not only on the actual number of survivors, but also on the length of time the animals survived. Figure 1 presents graphically the growth curves of the control and experimental groups. From inspection of Table II and Figure 1, it is apparent that there are no significant differences between the experimental and control groups with regard to average body weight gains, food consumption, or percentage of survival during 72 weeks of feeding.

The majority of the animals remained in good physiological condition during the study. Several rats in each group, including controls, exhibited evidence of respiratory involvement manifested by wheezing, nasal discharge, or rapid and labored respiration. Other conditions observed among some animals in both control and experimental groups include rough coats, alopecia, and blood-like crusts around the nose and eyes. Body sores have been noted in a few animals in all groups except the 1000 p.p.m. group.

The cause of death in the majority of animals that have succumbed is attributed to pneumonitis, as at autopsy the most consistent finding has been congested and/or abscessed lungs. Accurate autopsies could not be performed upon several mortalities because of advanced autolytic changes.

Table II. Food Consumption and Survival of Rats Receiving Diazinon for 72 Weeks

Level	Sex	Time Interval, Weeks	No. of Rats		Av. Body Weight, G.		Consumed, Av./Rat/Day, G.		Compound Consumed, Av./Rat/Day, Mg.	% of Survival
			Start	Finish	Initial	Final	Total diet	Food		
Control	M	0–72	20	13	73	431	16.4	...	...	93.5
	F	0–72	20	17	70	295	14.6	...	...	97.4
0.001	M	0–72	20	18	72	433	16.6	16.6	0.166	98.7
	F	0–72	20	19	68	301	14.3	14.3	0.143	99.9
0.01	M	0–72	20	17	72	442	16.0	16.0	1.60	96.9
	F	0–72	20	20	68	317	14.7	14.7	1.47	100
0.1	M	0–72[a]	20	17	74	424	15.9	15.9	15.9	95.1

[a] During first 10 weeks dietary levels were gradually adjusted upward from 0.01% to 0.1% level. Total diet and compound consumed refer to 11th through 72nd week.

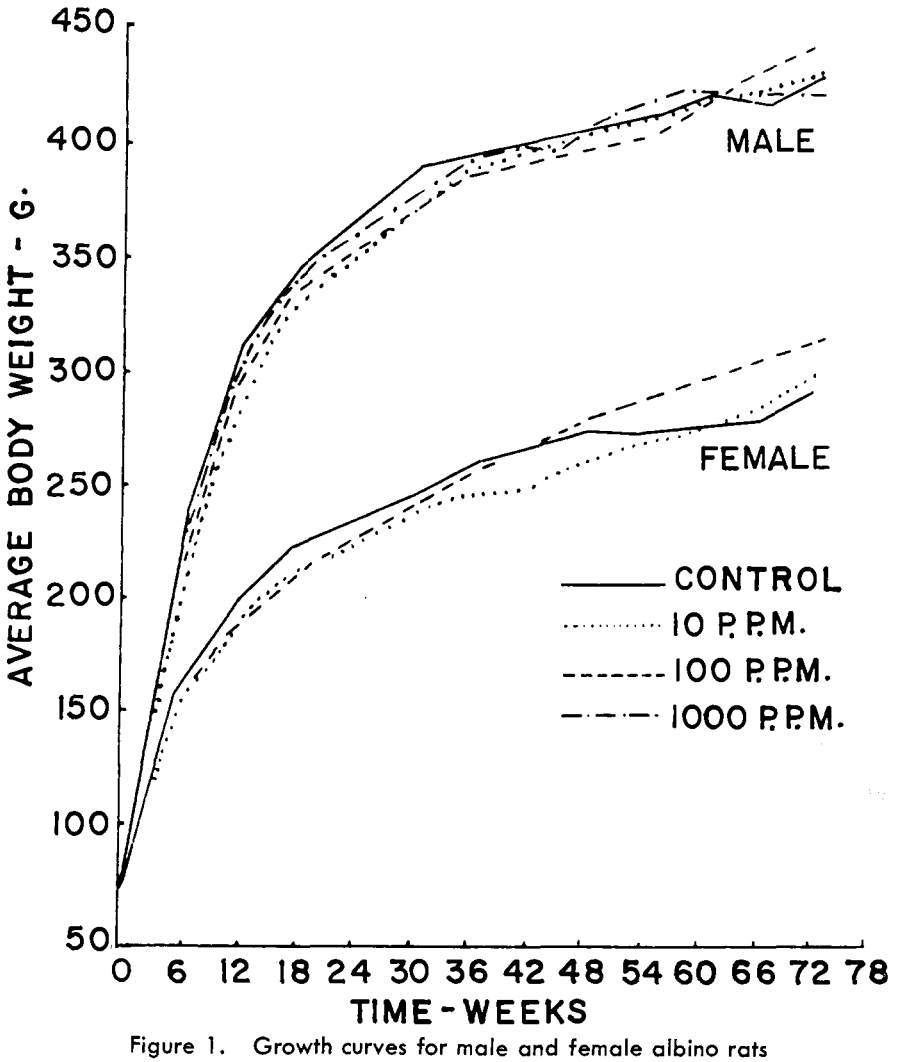


Figure 1. Growth curves for male and female albino rats

Experiments with Dogs

Chronic Feeding Method Eight male and female mongrel dogs were used in these studies. Preexperimentally, the dogs were immunized against rabies, given distemper-hepatitis serum, and allowed to adapt to laboratory conditions and diet. The animals were initiated on the study, receiving orally by capsule the Diazinon wettable powder, 6 days per week. Initial dosage levels and subsequent changes in dosage levels are shown in Table III.

Each animal was observed daily for signs of systemic toxicity; body weights and compound consumption were recorded at weekly intervals; food was offered once a day, and water was available at all times. The dogs were housed individually.

Complete blood counts, sedimentation rates, hematocrits, blood urea nitrogen determinations, bromosulfalein liver function tests, and urine analyses were conducted on each dog initially, at 90-day intervals, and at sacrifice.

Blood samples were taken initially and at various intervals during the study for estimation of cholinesterase activity of the plasma and red blood cells. The blood for the enzyme assay was taken from the jugular vein by venipuncture and placed in small beakers containing heparin as an anticoagulant. Initial measurements of cholinesterase activity were made before the feeding studies were started, each dog serving as his own control for calculation of inhibition.

Results. Blood and urine hematological and biochemical values were consistent and within normal limits for the control and test dogs throughout the study. Gross autopsies of the eight dogs revealed no pathological findings. Microscopic examination of the livers, kidneys, bone marrow, small and large intestines, adrenals, urinary bladder, and testes or ovaries revealed no significant histopathology in any of the animals.

GROUP I. Dogs C-272 and C-273, female and male, respectively, served as controls. The female dog was sacrificed during the 27th week and the male dog was sacrificed at termination of the study, during the 46th week. Both dogs exhibited normal behavior, maintained good appetites, and gained weight during the period of the study.

The results of cholinesterase determinations on blood samples taken from the dogs at weekly intervals during the study are shown graphically in Figures 2 (plasma) and 3 (red blood cells). Each point on the curves is the average of values obtained for the two dogs from each group.

Average values obtained for the plasma of the two control dogs over a 45-week period varied from 79 to 105% of normal activity, with a standard deviation of ±6.7%, while the normal red blood cell activity ranged from 95 to 113% with a standard deviation of ±4.2%.

GROUP II. Dogs C-274 and C-275,

Table III. Dosage Levels Received by Dogs during Feeding Study

Group No.	Dog No.	Sex	Week No.	Dosage of Active Diazinon, Mg./Kg./Day
1	C-272	F	1–27	Control
	C-273	M	1–46	Control
2	C-274	F	1–12	4.6
			13–46	4.3
	C-275	M	1–12	4.6
			13–46	4.3
3	C-276	F	1–5	9.3
			5–11	0
			12–27	4.3
	C-277	F	1–5	9.3
			5–11	0
			12–25	4.3
			25–26	25.0
			26–27	4.3
4	C-278	F	1–2	23.1
			3–11	0
			12–27	6.5
	C-279	M	1–2	23.1
			3–11	0
			12–25	6.5
			25–26	25.0
			26–27	6.5

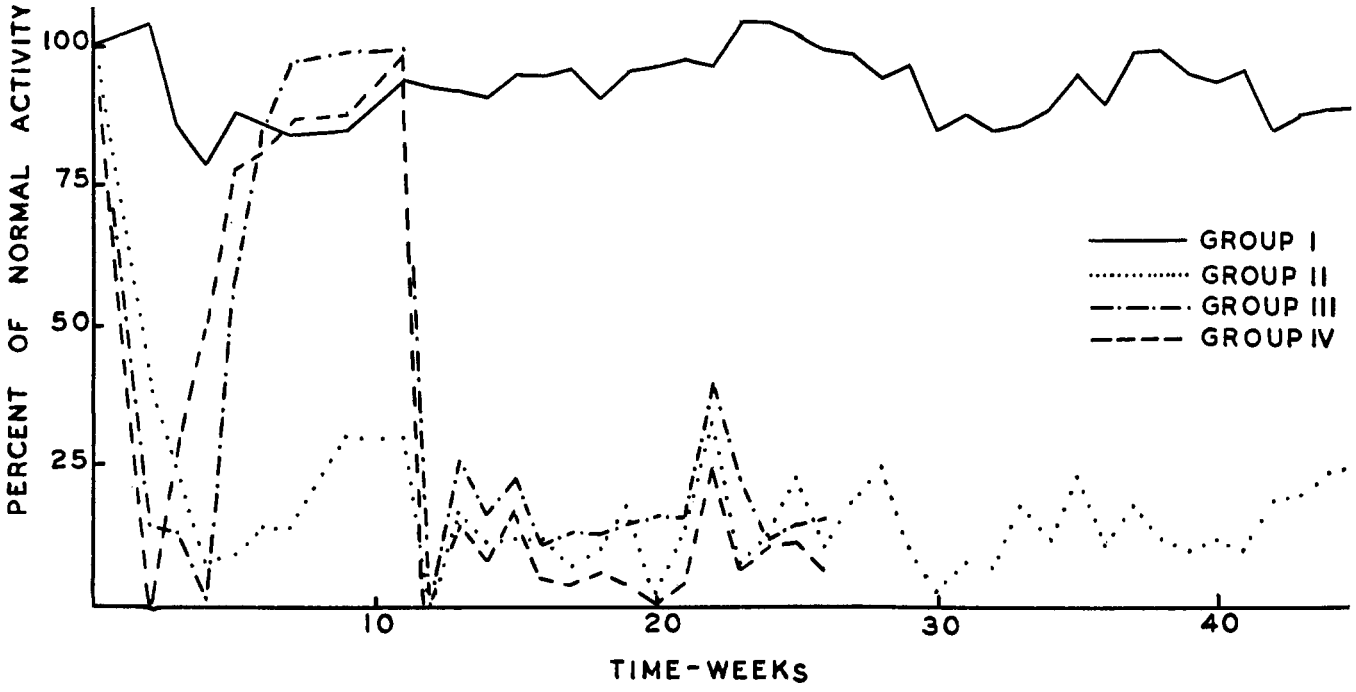


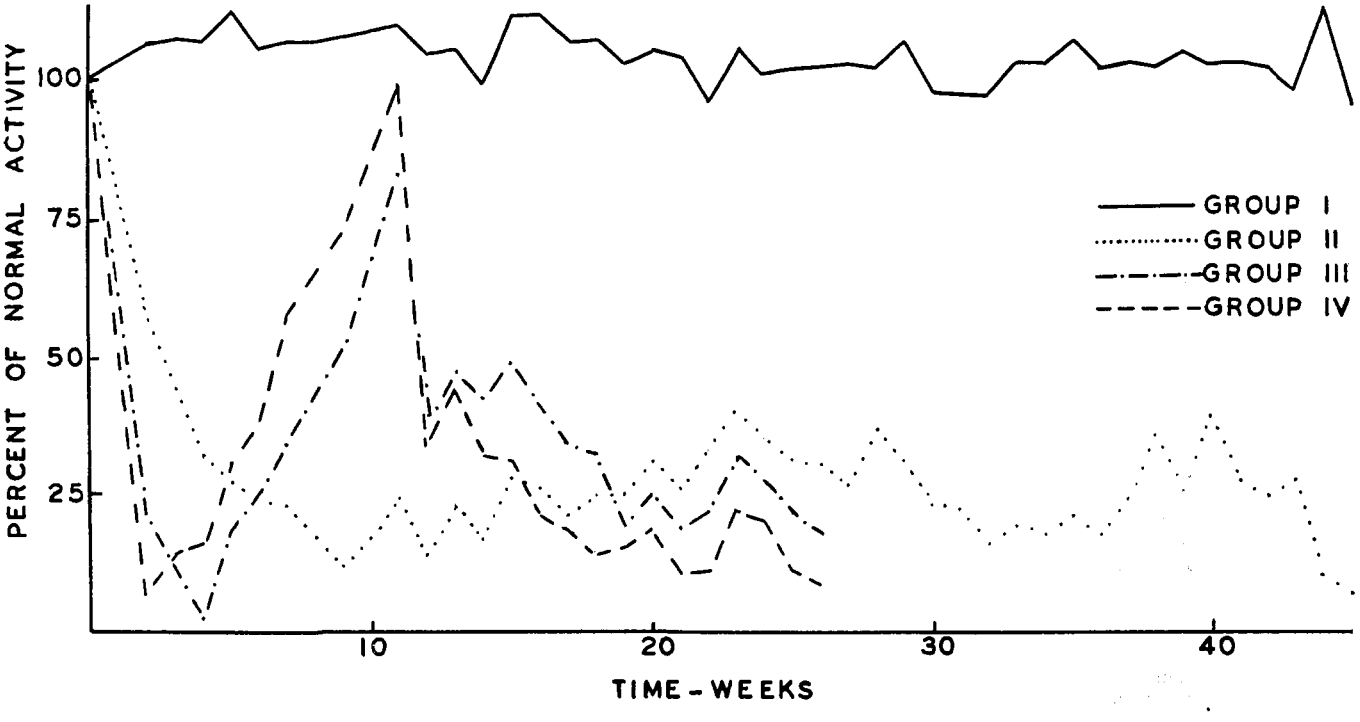
Figure 2. Plasma cholinesterase activity in dogs

female and male, respectively, were initiated on active Diazinon in the form of a wettable powder. Both dogs received 4.6 mg. per kg. per day for the first 12 weeks. For the remaining period of administration, the dogs received 4.3 mg. per kg. per day. Both dogs exhibited normal behavior, maintained good appetites, and either gained or maintained their body weight during the period of the study. Neither dog exhibited uptoward gross manifestations of systemic toxicity from the oral administration of the Diazinon wettable powder. The Group II animals were sacrificed on the fourth day of the 46th week.

The administration of 4.6 mg. per kg. per day of the Diazinon wettable powder produced significant inhibition of enzyme activity in both the plasma and red blood cells. After 2 weeks of feeding, the plasma activity had dropped to 36% and the red blood cells to 59% of normal activity. The enzyme activity of both tissues reached a minimum value during the 12th week, at which time complete inhibition was found. The values obtained for the plasma varied from 0 to 33% after the initial 2 weeks' decrease. The red blood cells activity reached a minimum value during the ninth week. The values varied from 12 to 40% of normal after the second feeding week.

GROUP III. Female dogs C-276 and C-277 were initiated on a dose of 9.3 mg. per kg. per day of active Diazinon in the form of wettable powder. Both dogs received 25 daily doses of the material during the first 30-day interval. The compound was withdrawn from

Figure 3. Red blood cell cholinesterase activity in dogs



both dogs during the fifth week, when it was found that inhibition of cholinesterase activity was complete. Both dogs were losing weight and exhibited decreased appetites, and the feces were soft. During the interval that Diazinon was withdrawn the dogs exhibited normal behavior, had good appetites, and gained weight.

Both dogs again were placed on the Diazinon wettable powder during the 12th week at a reduced level of 4.3 mg. per kg. per day. Dog C-276 received this dosage through the second day of the 27th week before being sacrificed. During this interval the dog exhibited normal behavior, ate well, and gained weight. Dog C-277 received the 4.3 mg. per kg. per day dosage from the 12th week through the first day of the 25th week. On the second day of the 25th week the level was increased to 25 mg. per kg. per day of active Diazinon. At this level the dog exhibited poor appetite, lost a slight amount of body weight, appeared excited, and developed tremors. After receiving six daily doses at the 25 mg. per kg. per day level, the dosage was again reduced to 4.3 mg. per kg. per day and the animal received this level for 7 days before being sacrificed on the third day of the 27th week. When the level was reduced from 25 mg. per kg. per day to 4.3 mg. per kg. per day of active Diazinon, the dog exhibited no gross signs of systemic toxicity.

The enzyme activity of the plasma and red cells of the dogs receiving 9.3 mg. per kg. per day was decreased to 8 and 21% of normal, respectively, at the end of 2 weeks. After 4 weeks on the compound, the cholinesterase activity had dropped to 1% in the plasma and 2% in the red cells.

From the fifth to the 11th week, the blood cholinesterase regeneration rates were followed at weekly intervals. As is characteristic, the plasma enzyme activity regenerated rapidly, reaching the normal limits after 2 weeks' withdrawal of the insecticide. The regeneration rate of the red cells, however, was a much slower process, normal limits of activity being reached approximately 6 weeks after compound withdrawal.

When the dogs were replaced on Diazinon, during the 12th week at a level of 4.6 mg. per kg., the enzyme activity of the plasma and red cells was again significantly decreased. During the remaining period of the study, prior to sacrificing during the 27th week, weekly plasma and red blood cell determinations showed constant and significant inhibition of enzyme activity. Increasing the dose from 4.3 to 25 mg. per kg. per day for dog C-277 during the 24th and 25th weeks caused a slight decrease in cholinesterase activity in both plasma and red blood cells. Final enzyme determinations made prior to sacrificing the dogs showed the average values of cholinesterase for the two dogs to be 16 and 18% of normal for the plasma and red cells, respectively.

GROUP IV. Dogs C-278 and C-279, female and male, respectively, were initiated on a dose of 23.1 mg. per kg. per day of active Diazinon in the form of Diazinon wettable powder. Each dog received a total of 12 daily doses during the first 14 days. During this time both dogs exhibited decreased appetite, weight loss, and depression. Blood cholinesterase determinations for both dogs during this interval revealed that plasma and red blood cell cholinesterase was almost completely inhibited. Because of the development of toxicity and complete cholinesterase inhibition, the Diazinon was withdrawn from both dogs from the third through the 11th weeks, during which time the dogs were observed grossly and regeneration of cholinesterase activity of the blood was followed. Following withdrawal, both dogs showed marked improvement in their physical condition, with return of appetite and increase in body weight.

Beginning with the 12th week, both dogs were replaced on Diazinon wettable powder, at a reduced level to provide 6.5 mg. per kg. per day of active Diazinon. Dog C-278 received this level from the 12th week to the time of sacrifice on the second day of the 27th week. Dog C-279 received this level from the 12th through the first day of the 25th week. On the second day of the 25th week, the level was increased to 25 mg. per kg. per day and this dog was given six daily doses at this level. On the second day of the 26th week the level of active Diazinon was again changed back to 6.5 mg. per kg. per day, which the dog received until sacrifice on the second day of the 27th week. From the 12th week to the time of sacrifice, both dogs exhibited normal behavior, maintained good appetites, and gained weight. There were no gross manifestations of systemic toxicity during this interval.

The high level dogs which received 23.1 mg. per kg. per day were taken off the compound after a total of 12 daily doses during the first 14 days. Blood cholinesterase determinations for both dogs during this interval revealed that the enzyme was almost completely inhibited. The enzyme activities of the two tissues returned to normal values at a rate comparable to the regeneration observed in the middle level dogs.

When the dogs were replaced on Diazinon, during the 12th week, at a reduced level of 6.5 mg. per kg. per day, the enzyme activities of both the plasma and red blood cells were again significantly decreased. During the remaining period of the study, prior to sacrificing during the 27th week, weekly plasma and red cell determinations showed constant and significant inhibition of enzyme activity. Increasing the dose from 6.5 to 25 mg. per kg. per day for dog C-279 during the 25th week caused a slight decrease in the enzyme activity of both the plasma and red cells. Final enzyme assays made prior to sacrifice showed the average values of cholinesterase for the two dogs to be 7 and 9% of normal for the plasma and red cells, respectively.

Conclusions

The effects of Diazinon in the animal body appear to be typical of organic phosphates and thiophosphates. The administration of large doses of Diazinon produces the usual symptoms associated with cholinesterase inhibition.

The low cholinesterase activities produced in the dogs over long periods of time without the production of gross signs of toxicity tend to confirm the above conclusions. Plasma cholinesterase was reduced to levels of less than 30% (average 15%) of normal values for a period of 43 weeks with no appearance of gross abnormalities in the Group II dogs. The corresponding red blood cell activity during this period did not rise above 40% of normal. while the average activity was 25% of normal.

The regeneration rate of cholinesterase activity for the dogs in Groups III and IV appears to be consistent with those found by other workers. No gross signs of toxicity appeared in these dogs unless the cholinesterase activity was reduced to less than 10% of normal. Withdrawal of the material from the diet resulted in the rapid disappearance of signs of toxicity and a return to normal of cholinesterase activity.

If the usual precautionary measures as regards the use of organic phosphate insecticides are followed, there should be no hazard from the use of Diazinon.

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