Absorption and Toxicity of Dieldrin. (19336)

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(Introduced by Robert MacVicar.)

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Dieldrin,* a relatively new insecticide, is very satisfactory for many purposes. It is compatible with most agricultural fertilizers, herbicides, fungicides, and insecticides. It shows promise for the control of many insects, particularly household pests, animal parasites, cotton insects, and soil inhabiting larvae. Its usefulness is enhanced by its high toxicity to insects, stability in acids and bases, and failure to impart flavors when used on fruits and vegetables.

Dieldrin is known to be toxic to warm blooded animals; hence it was deemed desirable to determine (a) its toxicity when applied at various concentrations, (b) its chronic toxicity when fed orally, (c) the amount of insecticide accumulated in the organs of the laboratory animals, and (d) whether sprayed cows eliminated dieldrin in their milk.

Experimental animals. The rats and rabbits used as experimental animals in determining the toxicity of Dieldrin were kept in wire cages and observed for abnormal actions and other symptoms of toxicity. Jersey cows under normal farm conditions were used to determine the amount of Dieldrin excreted in the milk. At death or at the termination of an experiment, an autopsy was performed on all small animals. Liver and kidney samples from the individual rats of each group were pooled, placed in sealed jars, quickly frozen, and stored until analyzed. The milk samples were treated immediately with formaldehyde and refrigerated. The Dieldrin was determined as organic chlorides according to the method adapted by Carter(1), the liberated chlorine being determined by the McLean and Van Slyke(2) method.

Toxicity of external applications of Dieldrin. Rabbits were dipped in a dilute emulsion of xylene, Triton x-155, Dieldrin, and water. The weight of the animal, mg/kilo Dieldrin used, survival period, and p.p.m. Dieldrin in the liver and kidney are given in Table I. These data show that all animals absorbed Dieldrin and retained it in their livers and kidneys. Refusal to eat and drink and listlessness were constant symptoms. When lethal doses were given, 14 to 100 p.p.m. Dieldrin was found in the livers and 21 to 165 p.p.m. in the kidneys. Mature rabbits receiving 70 mg/kilo or more at weekly intervals died; 50 mg/kilo or more caused death in immature rabbits. Symptoms noted were salivation, grinding of teeth, and spasms. Animals receiving less than 30 mg/kilo showed no symptoms during the 70 days of the experiment. Lower concentrations produced no outward symptoms. Three lots of immature rats were dipped respectively in .125%, .187%, and .250% Dieldrin emulsions once a week. Resulting data are listed in Table II. The average weight of animals increased gradually. None of the animals dipped in the .125% emulsion died. One rat dipped in the .187% emulsion died after 3 treatments, and one in the .250% after 2 treatments; indicating that individual animals have different tolerances. The Dieldrin found in the liver ranged from 25 to 35 p.p.m. and in the kidney from 42 to 52 p.p.m. The concentrations of insecticide applied to these experimental animals were many times the amount normally recommended for practical insect control.

Oral toxicity of Dieldrin. The oral toxicity to rabbits was determined by force feeding Dieldrin in capsules once a week for 25 weeks or as long as the animal survived. Results are given in Table III. Rabbits numbered 3, 4, and 18, receiving the highest dosages, had convulsions and ground their teeth excessively. Opisthotonos was followed by calmness, impaired vision, and bleeding of the

* Dieldrin is the name for the insecticidal product containing not less than 85% of 1, 2, 3, 4, 10, 10-hexachloro-6, 7-epoxy-1, 4, 4a, 5, 6, 7, 8, 8a-octahydro-1, 4, 5, 8-dimethanonaphthalene.

TABLE I. Nine Rabbit Dipping Experiments. (Each animal was treated once a week).

Wt (g)	mg Dieldrin per kilo body wt	Application period (wk)	Days of survival	P. P. M. Dieldrin	
				Liver	Kidney
3675	250	1	4	82	165
3500	250	1	6	48	21
3550	250	1	8	—	111
3575	250	1	2	14	38
3450	250	2	11	73	57
3630	200	2	9	92	148
2235	80	3	15	100	135
1475	70	2	10	68	162
3560	30	10	70*	12	12

* Animal was sacrificed.

TABLE II. Rat Dipping Experiments. Average Weight in g. (Dipped weekly).

Sol.	Weeks										P.P.M. Dieldrin*	
	1	2	3	4	5	6	7	8	9	10	Liver	Kidney
.125%	285	276	279	309	325	321	374	379	392	†	25	42
.187%	290	268	262	310	345	355	370	†			30	51
.250%	262	277	325	316	340	350	353	†			35	52

* Pooled samples. † Rats sacrificed.

TABLE III. Eight Rabbit Feeding Experiments.

Beginning wt (g)	Final wt (g)	Sex	mg Dieldrin fed per wk	Feeding period (wk)	P.P.M. Dieldrin	
					Liver	Kidney
3150	3400	♀	0	15	0	0
3575	4600	♀	30	25	13	6
3500	3170	♀	60	13	49	77
3575	2825	♀	110	11	76	181
975	3363	♀	0	14	0	0
950	3275	♀	10-20*	14	—	—
1015	3300	♂	20-40*	14	23	48
875	3075	♂	35-70*	13	88	201

* Amount of Dieldrin fed doubled from 9 to 14th week.

gums. Weight gains were not appreciably affected until the rabbits received 60 mg/kilo or more. Dieldrin found in the liver ranged from 13 to 88 p.p.m. and in kidneys from 6 to 201 p.p.m. Rabbit No. 2, which received 30 mg per week for 25 weeks, did not die. Rats were weighed and force fed weighed quantities of Dieldrin once a week as long as they survived, or until the termination of the experiment. Lot I served as a control. Lot 2, receiving 15 mg Dieldrin per animal per week, made gains comparable to the controls. Lot 3 received 30 mg per rat per week, equivalent to 150 mg/kilo body weight. One rat died the second week, one the fourth, one the fifth, and the last one the eleventh week. The average growth rate was comparable until the time of death.

Excretion of Dieldrin in milk of dairy cattle. In determining the amount of Dieldrin eliminated in the milk of cows sprayed for parasite control, a herd of dairy animals under normal farm conditions was used. Each animal was sprayed with one gallon of 0.1% spray mixture at approximately 3 week intervals for tick control. Pooled milk samples were taken during milking. Following spraying, the concentration of Dieldrin in the milk gradually increased to a maximum of 5.9 p.p.m. during the first 3 weeks and then decreased steadily despite continued applications of Dieldrin.

Observations. Toxic symptoms in rabbits, such as excessive salivation, grinding of the teeth, rolling of the eyes, muscular twitching, and convulsions were observed. Later the animal became listless, did not drink, and appeared to be blind. Except for the death of 2 of the rats, which were dipped, no abnormalities were noticed. Sprayed cattle showed no symptoms.

Mature rabbits given 60 mg/kilo body weight or more at weekly intervals died; an immature rabbit died after repeated weekly treatments of approximately 50 mg/kilo body weight. Younger animals generally were more susceptible to insecticide poisoning than older ones. Rats given approximately 75 mg/kilo body weight were not affected after repeated weekly treatments. Those given 150 mg/kilo body weight died after repeated weekly treatments. This would seem to indicate that the lowest toxicity for rats falls between these 2 concentrations, considerably above the amount causing death in rabbits. The accumulation of Dieldrin in the organs of the animals treated indicates that repeated treatments may be potentially dangerous.

Conclusions. 1. Dieldrin produced chronic toxicity in rabbits when it was applied once a week at 70 mg/kilo body weight. Less than 30 mg/kilo produced no symptoms. 2. Only 2 of 11 rats dipped in .125, .187, and .250% Dieldrin spray solutions died. 3. Dieldrin produced chronic toxicity in mature rabbits when fed orally approximately 60 mg/kilo body weight. Similar feeding of approximately 150 mg/kilo body weight was fatal to rats. 4. Liver and kidneys of all animals treated and analyzed were found to contain Dieldrin. The amount recovered in these organs was generally in proportion to the amount fed per week, not the duration of feeding. 5. Analyses of milk from cows sprayed with Dieldrin for external parasite control indicated a maximum of 5.9 p.p.m., which was gradually decreased regardless of continued application.

1. Carter, R. H., *Anal. Chem.*, 1947, v19, 54.
2. McLean, Franklin C., and Van Slyke, Donald D., *J. Am. Chem. Soc.*, 1915, v37, 1128.

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Calcification VI. Adenosinetriphosphate Content of Preosseous Cartilage.* (19337)

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Enzymes acting on organic phosphate(1,2), particularly those involved in phosphorylative glycogenolysis(2,3), have received serious attention among the "local factors" proposed as being responsible for initiating the process of mineralization. While the existence of glycogen(2-4) and presumably some of the enzymes in the cycle has been indicated(2) the presence of adenosinetriphosphate (ATP), required in such a cycle has not been demonstrated. Cartier(5,6), however, has indicated that if ATP were present it might considerably enhance the degree of mineralization. He reported that added ATP greatly enhances *in vitro* calcification of embryonic sheep bone cartilage.

The purpose of the present investigations was to establish the existence of ATP in bone cartilage. As will be shown in this paper, ATP does exist in significant amounts in the bone cartilage of the rat; furthermore, there appears to be a correlation between the preservation of the calcifying mechanism by calcium and the ATP level.

Procedure. Lutwak-Mann(7) was not able to detect either acid hydrolyzable phosphorus nor significant quantities of other forms of organic phosphorus in trichloracetic acid extracts of articular cartilage. This must be related to the fact that the inorganic phosphorus content of cartilage is very high as compared to the organic phosphorus. It is apparent that if organic phosphorus is to be detected, methods other than those heretofore employed must be used. We decided to use the enzymatic procedure developed by one of

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