Effects of Feeding Dieldrin- and Heptachlor-Treated Alfalfa Hay to Dairy Cows

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Eight dairy cows were fed alfalfa hay treated with either heptachlor or dieldrin and feeding trials were conducted for 112 days, during which milk samples were taken weekly. A small amount of dieldrin was found in the milk and a considerable amount in the butter from cows fed hay treated with 1 and 4 ounces of dieldrin per acre. No heptachlor was excreted in the milk. Heptachlor epoxide was found in milk and butter from cows fed hay treated with 4 ounces of heptachlor per acre. There was no detectable effect on milk production, feed consumption, or health of cows. Histological examination of kidney, liver, muscle, and fat tissue showed no abnormalities associated with insecticide treatment.

Some of the newer insecticides, including dieldrin and heptachlor, are so effective against many of the insects common to forage crops that only a small amount is required for control. This is a definite advantage, for residues remaining on the forage crops after harvest may be harmful to animals consuming it or, indirectly, to man. In the latter respect excretion of insecticides in the milk, and storage in the tissues of animals, have been reported by Bateman and coworkers (*1*) and many others.

Claborn and Wells (*2*) showed that dieldrin was excreted in the milk of dairy cows after the animal was sprayed with this insecticide and Ely and coworkers (*6*) demonstrated that dieldrin was excreted in milk of dairy cows ingesting forage sprayed with dieldrin. Davidow and Radomski (*4*) found that when dogs were fed heptachlor a metabolite of heptachlor, identified as heptachlor epoxide, appeared in the dogs' tissues, while Davidow, Radomski, and Ely (*5*) discovered that this same metabolite was excreted in the milk of dairy cows which had ingested heptachlor. Radomski and Davidow (*11*) reported the toxicity of heptachlor epoxide to mice to be greater than that of the parent compound, heptachlor.

The purpose of the present study was to determine transmission of dieldrin and heptachlor into the milk and tissues of dairy cows which had been fed alfalfa hay sprayed with a sufficient amount of insecticide to control insects.

Materials and Methods

A 40-acre field of first crop alfalfa was divided into 20 plots (1.6 acres each), which were separated from each other by buffer strips 33 feet wide in one direction and 50 feet wide transversely. The five treatments were as follows: (1) no treatment; (2) 1 ounce of dieldrin per acre; (3) 4 ounces of dieldrin per acre; (4) 1 ounce of heptachlor per acre; and (5) 4 ounces of heptachlor per acre. The five treatments were distributed at random within each field replication. The formulations used were emulsifiable concentrates and they were applied with a ground rig sprayer in 12 gallons of spray per acre.

Counts of alfalfa weevil larvae were made prior to application of the insecticide and also 5 and 7 days after spraying. Seven days after treatment the hay was cut. After being windrowed, suncured, and baled, it was stored in a barn mow for about 4 months until the start of the feeding experiment. Only a trace of rain fell between the treatment and storage of the alfalfa.

Ten Holstein cows were divided into two groups and one cow from each group was assigned to each of the five treatments. The treated hay was fed *ad libitum* during the 4-month feeding trial from October 22, 1953, to February 12, 1954. Grain was fed at the rate of approximately 1 pound for each 4 pounds of milk produced. Cows were weighed biweekly, and daily milk weights were recorded.

Milk samples were taken at weekly intervals throughout the period. Hay samples were taken before the hay was cut, at the time it was placed in storage, and also from alternate bales as the hay was fed. At the termination of the experiment butter was churned from a composite sample of the cream from the two cows on each treatment. Two of the cows, one from group 3 and one from group 5, were sacrificed and tissue samples were taken of the kidney, liver, muscle, and fat tissues of each cow.

Dieldrin was determined in the hay, milk, tissue, and butter samples by the spectrophotometric method of O'Donnell, Johnson, and Weiss (*8*); heptachlor by the use of the Polen-Silverman reaction (*10*); and heptachlor epoxide as outlined by Radomski and Davidow (*11*), except that benzene was used as the eluent, as recommended by Feinstein (*7*), rather than a mixture of benzene and hexane. To increase the sensitivity of the determination of heptachlor, 0.2-ml. reaction tubes were used, and the color was read in a Beckman Model DU spectrophotometer using microcuvettes.

The samples were prepared for analysis as follows: Milk was extracted with Skellysolve B according to the method of Schechter, Pogorelskin, and Haller (*12*) up to the point of filtration through cotton just prior to the acid extraction. Shaking with acid for removal of fat was not used. Fat, butter, and hay samples were extracted with Skellysolve B. The remainder of the tissues was ground with a meat grinder, further macerated with water and ethyl alcohol (1 to 1) in a Waring Blendor, and extracted with Skellysolve B in the same manner as milk.

Interfering substances were removed from the hay samples by adsorption chromatography. In the case of heptachlor samples, the extract was run first through a Florisil column and then through a Florex column. When heptachlor was to be determined, a Davidow column (*3*) was used to remove fat and

other interfering substances from all samples, other than hay. All dieldrin samples were treated for removal of interfering substances according to methods outlined by O'Donnell and coworkers (9), except that a mixture of magnesium oxide–Hyflo Super-Cel (2 to 1 by weight) was used as a chromatographic adsorbent in place of the Attasol–Hyflo Super-Cel mixture.

Results and Discussion

Both dieldrin and heptachlor were effective against alfalfa weevil, as all the insecticide treatments were between 90 and 100% effective.

The dieldrin and heptachlor residues on the samples taken from alternate bales as the hay was fed are shown in Table I. In every case the dieldrin residues are somewhat higher than are the corresponding values for heptachlor. The hay samples taken when the hay was stored were analyzed but the results did not differ significantly from those in Table I and are not given here. More dieldrin (approximately twice as much) was found on the samples taken prior to the cutting of the alfalfa. This was to be expected, inasmuch as these samples had not been subjected to sunlight and other weather conditions for as long a time as the hay. Such was not the case with heptachlor, as the green alfalfa samples, calculated on an air-dry basis, contained approximately the same amount of heptachlor as did the hay samples taken after harvest. This was probably due to the fact that the residue was already low. Dieldrin hay samples were also analyzed by the Shell Development Co., Denver, Colo., and the results were in agreement with the analyses reported here.

The daily grain and hay consumption and daily milk and butterfat production of the ten cows on experiment are shown in Table II. Milk and butterfat production and feed consumption were normal for all the cows. Similarly, the general health of all the cows was normal throughout and at the completion of the experiment. Table II also lists the amount of insecticide found on the hay each cow received and the corresponding amount of insecticide excreted in the milk. Insecticide excretion in the milk was lower than the amount of insecticide found on the hay.

No heptachlor was found in any of the milk samples. No heptachlor epoxide was found in the milk of the cows which were fed hay from the plots treated with 1 ounce of heptachlor per acre (Table III). Cows fed hay treated with 4 ounces of heptachlor per acre consistently showed a small amount of heptachlor epoxide in their milk from the beginning of the experiment. The amounts shown in Table III are low and are near the limit of sensitivity of the method. Dieldrin was found in the first sample of milk taken (on the fifth day of the experiment) after the feeding of the hay was started. The amount excreted increased slightly for about 60 days, after which it remained about constant.

The amount of dieldrin and heptachlor in the tissues of the two slaughtered animals is shown in Table IV. These figures and those in Table V attest to the similarity of these insecticides to other chlorinated - hydrocarbon insecticides which are stored in the fat. This is particularly apparent in the case of dieldrin. No heptachlor was found in any of the tissues or in the butter. Dieldrin residue in butter was higher than that in milk and indicated that the dieldrin was associated with the fat portion of milk.

Table III. Insecticide Excreted in Milk of Cows Fed Hay Treated with Dieldrin or Heptachlor

Day[a] Milk Sampled	Control Cows, 0 Oz./Acre, Cow No.		Heptachlor[b] 1 Oz./Acre, Cow No.		Heptachlor[b] 4 Oz./Acre, Cow No.		Dieldrin 1 Oz./Acre, Cow No.		Dieldrin 4 Oz./Acre, Cow No.	
	Hu253	W255	Hu224	E237	Hu228	W256	E240	W258	W257	Hu251
	Insecticide Found, P.P.M.									
0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0.2	0.1	0	0	0.5	0.5
9	0	0	0	0	0.1	0.1	0.1	0.2	0.5	0.5
11	0	0	0	0	0.04	0.06	0	0	0.5	0.4
16	0	0	0	0	0.1	0.1	0.1	0.1	0.3	0.3
23	0	0	0	0	0.1	0.1	0.2	0.2	1.2	1.2
33	0	0	0	0	0.0	0.0	0.4	0.5	1.3	1.3
37	0	0	0	0	0.1	0.09	0.3	0.3	1.1	1.2
44	0	0	0	0	0.1	0.2	0.3	0.4	1.3	1.5
51	0	0	0	0	0.2	0.2	0.3	0.4	1.5	1.4
59	0	0	0	0	0.2	0.1	0.4	0.4	1.6	1.0
66	0	0	0	0	0.1	0.1	0.4	0.6	2.0	2.2
73	0	0	0	0	0.2	0.2	0.4	0.5	1.6	1.9
80	0	0	0	0	0.1	0.2	0.5	0.5	1.3	1.5
87	0	0	0	0	0.3	0.4	0.4	0.4	1.8	1.7
93	0	0	0	0	0.2	0.06	0.5	0.4	1.8	1.8
102	0	0	0	0	0.3	0	0.4	0.4	1.8	1.7
106	0	0	..	0	0.1	..	0.5	0.5	1.8	1.8
112	0	0	0	0	0.1	0.1	0.4	0.3	1.4	1.3
Average	0	0	0	0	0.1	0.1	0.3	0.3	1.3	1.3

[a] Days number from beginning of experiment.
[b] Excreted in milk as heptachlor epoxide. No heptachlor found in any milk.

Table I. Insecticide Residues on Alfalfa Hay Fed to Dairy Cows

Insecticide, Oz./Acre	Heptachlor Hay lot No.	Heptachlor Cow No.	Heptachlor[a] residue, p.p.m.	Dieldrin Hay lot No.	Dieldrin Cow No.	Dieldrin[a] residue, p.p.m.
0	5A,5B,5C,5D	W255	0	5A,5B,5C,5D	Hu253	0
1	3A,3B,3C,3D	Hu224	0.05 ± 0.007	1A,1B,1C,1D	E240	1.64 ± 0.89
1	3A,3B,3C,3D	E237	0.07 ± 0.036	1A,1B,1C,1D	W258	1.71 ± 0.58
4	4A,4B,4C,4D	Hu228	0.24 ± 0.05	2A,2B,2C,2D	W257	3.74 ± 0.72
4	4A,4B,4C,4D	W256	0.20 ± 0.08	2A,2B,2C,2D	Hu251	4.24 ± 0.63

[a] Average of determinations for four periods with standard deviation for each mean.

Table II. Feed Consumption and Milk and Butterfat Production During the 112-Day Feedng Period

	Field Treatment, Oz./Acre	Cow No.	Hay Consumed, Av. Lb./Day	Grain Consumed, Av. Lb./Day	Milk Produced, Av. Lb./Day	Butterfat Produced, Av. Lb./Day	Insecticide on Hay, P.P.M.	Insecticide[a] in Milk, P.P.M.
Heptachlor	0	W255	32.0	7.61	29.8	1.25	0	0
	1	Hu224	31.8	5.63	19.7	0.85	0.05	0
	1	E237	34.2	8.52	33.7	1.28	0.06	0
	4	Hu228	32.1	5.34	18.8	0.71	0.25	0.2
	4	W256	31.5	7.44	27.7	1.08	0.18	0.1
Dieldrin	0	Hu253	29.3	7.35	28.0	1.09	0	0
	1	E240	30.3	6.75	25.0	1.00	1.60	0.4
	1	W258	29.7	6.93	25.8	1.07	1.62	0.4
	4	W257	26.6	7.03	26.7	1.01	3.25	1.6
	4	Hu251	24.4	6.57	25.1	0.97	3.65	1.7

[a] In the case of heptachlor residue given is heptachlor epoxide. Residues in the milk are averages of last 7 weeks.

Table IV. Insecticide in Tissues of Cows Fed Hay Treated with 4 Ounces of Dieldrin or Heptachlor per Acre

Insecticide	Cow No.	Insecticide Content, P.P.M. Liver	Muscle	Kidney	Fat
Dieldrin	Hu251	0	0	0.2	2.9
Heptachlor	W256	0	0	0	0.12[a]

[a] Heptachlor epoxide. No heptachlor found in tissues.

Table V. Insecticide in Butter Churned from Milk of Cows Fed Dieldrin or Heptachlor

Insecticide	Oz./Acre Applied	Cow No.	Insecticide Content, P.P.M.
Dieldrin	0	Hu253-W255	0
	1	E240-W258	9.5
	4	W257-Hu251	39.3
Heptachlor	0	Hu253-W255	0
	1	Hu224-E231	0
	4	Hu228-W256	0.2[a]

[a] No heptachlor found in butter. This value is heptachlor epoxide.

The liver, muscle, kidney, and fat tissues of the two slaughtered animals were examined histologically and no abnormalities were observed.

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PESTICIDE TOXICITY

Serum Alkaline Phosphatase Levels, Weight Changes, and Mortality Rates of Rats Fed Endrin

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This work is a part of a larger research project on the toxicity of newer insecticides to animals and the accumulation of such insecticides in products consumed by man. When various levels of endrin (0, 1, 5, 25, 50, and 100 p.p.m.) were added to a basal diet, the serum alkaline phosphatase values were higher among rats consuming endrin than in the control group. All rats receiving 100 p.p.m. of endrin died within the first 2-week period. Mortality rates indicated that male rats were significantly more susceptible to the toxic effects of endrin at the lower levels (1 and 5 p.p.m.) than were females. There was a loss of weight in all rats ingesting endrin. The greatest weight loss occurred in rats consuming the two highest levels of endrin. The total average feed consumption of endrin-fed rats was less than that of the control group. All rats consuming endrin demonstrated hypersensitivity to various stimuli.

THE AMOUNT AND DISTRIBUTION of chlorinated insecticides in the tissues and organs of the animal body have been studied in an attempt to determine their harmful effects. The majority of these studies have been concerned with histological observations and accumulation rates (*1, 3, 6, 11, 12*). The reports indicate that other ways of studying the effects of the insecticides on animal tissues might supplement histological and accumulation data. Various workers (*8, 10, 12, 16*) have indicated that one of the changes occurring after the ingestion of chlorinated hydrocarbons is hepatic cellular alteration and degeneration, with hypertrophy of the organ. It is generally accepted that the liver plays an important role as a source of alkaline phosphatase (*4, 14, 17*). Elevated serum alkaline phosphatase levels have been associated with func-

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