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INSECTICIDE RESIDUES IN BUTTERFAT

Residues of Heptachlor Epoxide in Butterfat of Dairy Cows Grazing Pastures Treated with Granular Heptachlor

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Six groups of two dairy cows each (a high and low producer) were placed on a pasture at various intervals after application of approximately 0.25 pound of granular heptachlor per acre. The butterfat from these animals showed continuous low level residues (maximum 3 p.p.m.) of heptachlor epoxide when animals were allowed to graze the treated pasture continuously after 1, 8, 15, 29, and 43 days following application. Residue was present in trace (indeterminate) amounts in the butterfat of the animals placed on the pasture 57 days after treatment. Animals continued to excrete residue in their butterfat after removal from the treated pasture (80 days after application) and placed in a dry lot for 40 days.

HEPTACHLOR applied as a spray has been used effectively on pastures and range land to control many insects. Milk and fatty tissues of dairy cattle fed heptachlor-treated hay or grazing heptachlor-treated pastures have been found to contain heptachlor epoxide, the metabolite of heptachlor (*2–5, 8*).

A maximum level of 13.3 p.p.m. of heptachlor epoxide was found in the butterfat of four dairy cows grazing pasture sprayed with 2 ounces of heptachlor in Diesel oil for grasshopper control. This peak was reached after 28 days and, thereafter, consistently and slowly declined with levels reaching 5.9 and 1 p.p.m. after 84 and 165 days, respectively (*3*). In the one other study involving direct grazing of heptachlor-treated pasture (0.5 pound in emulsion per acre) by cattle, Gannon and Decker (*4*) reported a maximum concentration of 0.2 p.p.m. of heptachlor epoxide in the milk, or approximately 5.0 p.p.m. in the butterfat, within 3 to 7 days, which declined thereafter.

These previous studies with heptachlor were based on spray applications. The present recommendation for eradication of the imported fire ant, *Solenopsis saevissima richteri*, is the application of heptachlor granules at the rate of 0.25 pound (12.5 pounds of 2% heptachlor granules) per acre in each of two treatments at 3- to 6-month intervals (*9*). The label issued for use of heptachlor in this manner requires that dairy cattle not be allowed to graze treated pasture for 30 days after application. Because of the physical properties of granular heptachlor, it was assumed that there would be little contamination of forage, and consequently little danger of residue in milk. Since no published reports were found in the literature to verify this assumption, this study was undertaken. In addition, it was desirable to determine how much time should elapse after application of heptachlor before it would be safe to place cattle on treated pasture.

Experimental Procedure

Experimental Plots. FORAGE AND SOIL SAMPLES. Approximately 15 acres of dairy pasture with no previous history of insecticide application was mowed to a height of approximately 4 to 6 inches in March, prior to initiation of the experiment, and 200 pounds per acre of nitrate of soda were applied. It was disked lightly and seeded to Johnson grass, *Sorghum holepense* (L.). The 15 acres were divided, with wire fence, into five plots approximately equal in size. Samples of forage and soil from each plot were collected prior to and after insecticide treatment for determination of heptachlor and heptachlor epoxide. For forage, ten randomly distributed subsamples, approximately 1 foot square, were collected from each plot, placed in a 5-gallon lard can, and stored at 40° F. until ready for processing. Twenty-five randomly distributed soil samples were collected from each plot with a soil auger. Each soil sample was 2 inches in diameter and was trimmed to include only the top inch of soil, as described by Murphy and Barthel (*7*). Soil samples were stored in the same manner as forage samples.

Two days prior to application of insecticide, the plots were clipped to a height of 4 to 6 inches. Similar clipping commensurate with good pasture management was performed during the course of the experiment. After application of the insecticide, forage samples were again collected as described above at 2-, 4-, 6-, and 8-week intervals. Soil samples were also collected 6 and 30 days after application, as previously described.

APPLICATION. Two per cent heptachlor granules were applied to the five pasture plots between 12 noon and 2 P.M. on May 13, 1960, by a jeep equipped

Table I. Residue in Soil 6 and 30 Days Following Application of Insecticide

Plot	Residue, P.P.M. 6 Days: Heptachlor	6 Days: Heptachlor epoxide	30 Days: Heptachlor	30 Days: Heptachlor epoxide
I	0.12	0.06	0.6	0.10
II	0.62	0.19	1.20	0.05
III	0.39	0.19	0.86	0.29
IV	0.31	0.10	0.84	0.10
V	0.41	0.13	0.69	0.14
Control	0.0	0.0	0.0	0.0

Table II. Analyses of Residue in Treated Forage 1 to 8 Weeks after Treatment

Plot No.	Residue, P.P.M.[a] Heptachlor, 1 wk.	Heptachlor epoxide, 1 wk.	Heptachlor Epoxide 2 wks.	4 wks.	6 wks.	8 wks.
I	0.22	0.20	0.86	0.50	0.16	0.26
II	0.32	0.10	1.40	0.44	0.10	0.22
III	0.20	0.10	0.10	0.42	0.16	0.10
IV	0.22	0.20	0.48	0.28	0.26	1.10
V	0.28	0.16	0.32	0.70	0.42	0.16
Control	0	0	0	0	0	0

[a] All heptachlor determinations after 1 week were less than 0.1 p.p.m. as heptachlor.

with a Buffalo turbine. Swath widths were 30 feet and the machine was calibrated to deliver approximately 12.5 pounds of insecticide per acre. Samples of insecticide were taken from each bag for chemical determination of heptachlor content. Weather conditions were satisfactory for application. Foliage was dry and wind velocity was well below that which would interfere with uniform application; however, terrain of the plots was too rough to permit the most uniform application. Shallow drainage ditches in most of the plots caused some interference.

The amount of insecticide applied to each of the five plots was checked in two ways. The insecticide was weighed prior to being placed in the machine and after each plot was treated and insecticide remaining in the machine was removed and weighed. This gave the amount actually applied. The distribution was determined by placing 10 dishpans, each 1 sq. foot in area, at random over each plot to collect the insecticide as it was applied. Following treatment the insecticide collected in the pans was transferred to shell vials. It was taken to the laboratory, where any foreign matter was removed, and the sample weighed. Amount of insecticide actually collected per pan and calculated as pounds per acre was determined.

Dairy Cows. Twelve lactating Jersey cows, obtained from an area in Louisiana with no known history of insecticide use, and two lactating Holstein cows from the Louisiana State University dairy herd were used in the test. After analyses showed no heptachlor or heptachlor epoxide present in the milk of the cows, the animals were brought to the Louisiana State University dairy farm. They were held in a dry lot and fed silage, hay, and an 18% protein concentrate until time to be placed on test. All feed had been previously tested and found to be free of heptachlor and heptachlor epoxide. After the cows were placed on treated plots, their only source of forage, they received only grain concentrate as supplemental feed at the rate of 1 pound per 4 pounds of milk, so as to limit the amount of grain consumed. All animals were weighed at regular intervals during the course of the experiment.

After application of insecticide to the pasture the experimental animals were divided into groups of two cows each, comprising a high and a low producer. A group was placed on a separate treated plot for continuous grazing at 1, 8, 15, 29, and 43 days following application of the insecticide. At 57 days following treatment of the pasture, gaps were opened in fences separating plots, so that cattle could roam at will over the pasture. Group VI was placed on the treated area at this time. A control group of two lactating Holstein cows (Group VII) was pastured on an untreated plot located about 1/4 mile from the treated plots. After animals had been placed on their respective plots, they were allowed to graze the treated pasture continuously until August 1, 1960, 80 days after application of the insecticide. All animals, including the controls, were then removed from the two pastures and held on a dry lot for 40 days, where they were again fed silage, hay, and concentrate until the termination of the experiment on September 12, 1960. This was done to determine any carry-over effect of the insecticide residue in the butterfat. It was not possible to keep the animals on dry lot for a longer period because of management problems.

Milking. Cows were removed from their plots only during milking. They were placed in the same stanchion at each milking and milked at 6 A.M. and 5 P.M. daily. Separate Surge milking machines and milk buckets were used for each group of cows to prevent contamination. The milk was weighed daily and recorded. After each milking the machines and buckets were washed thoroughly and cleaned with a standard dairy cleaner (G.L.X., Wyandotte Chemical Co.). A careful check was made after each milking to see that the animals were returned to their respective plots.

Sampling of Milk and Fat. Milk from each animal was transferred into an individual St. John milk jug. After evening milking, these containers were held at 40° F. until the following morning, when they were combined with the morning milk for separation. After combining, individual sterile milk thiefs were used to collect a sample to prepare a 7-day composite for Babcock butterfat tests.

A Model 100AE DeLaval separator was used to separate the cream at an approximate test of 40% butterfat. Milk from the control animals and those that had not been placed on treated plots was separated first. Milk from each pair of test animals was separated after the controls. The separator was rinsed in warm water after milk from the first animal in the pair had been separated. After separating the milk from the second animal the separator was completely disassembled, thoroughly washed with the standard dairy cleaner, and rinsed in warm water before milk from subsequent pairs of animals was separated. After the first 20 to 25 ml. of cream from the separator had been discarded, the cream from each day's milk from each animal was collected in thoroughly clean Mason fruit jars, using a new lid insert for each sample. Cream was stored in new plastic bags or new Mason jars, labeled with cow number and date, then frozen until analyzed.

Analyses for Residue. Forage and Soil. The method of Murphy and Barthel (7) was used for determining residues in soil and forage.

Butterfat. All heptachlor epoxide in butterfat was determined by the technique of Meyer, Malina, and Polen (6) as modified by Murphy and Barthel (7). The modification involved the use of 7% fuming sulfuric acid for removing the butterfat from the pentane solution and a three-part chromatographic column, consisting of Florisil, Florisil–carbon black, and Florisil in three successive layers for the separation of the heptachlor epoxide from the interfering substances. Determinations of split samples of butterfat from individual cows were made at the U.S.D.A. Plant Pest Control Division Laboratories and the Louisiana State University Dairy Nutrition Laboratory. Absorbance readings of samples were made with a Bausch and Lomb Spectronic 20 spectrophotometer at the former laboratory

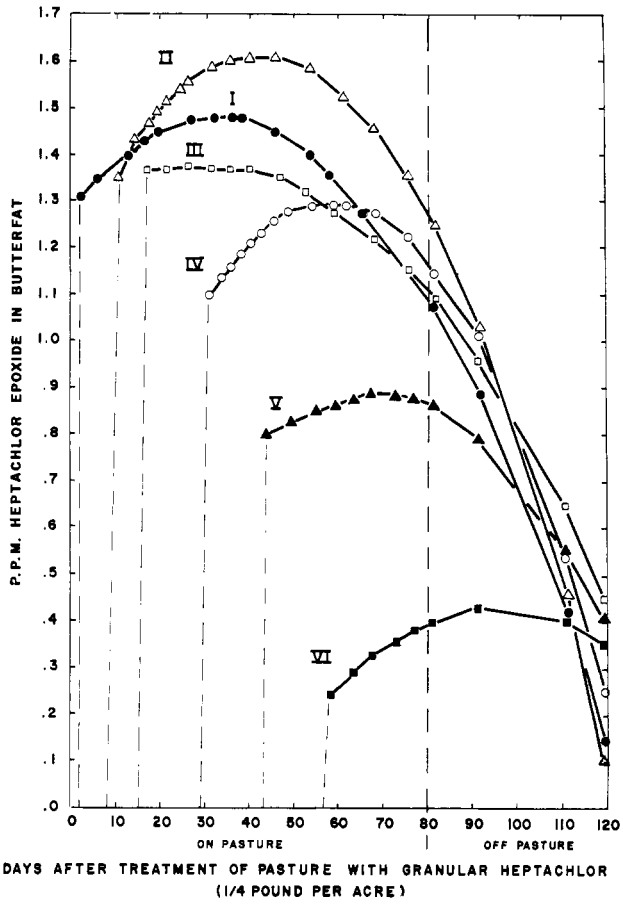

Figure 1. Residues of heptachlor epoxide in butterfat of dairy cows

and with a Beckman DU spectrophotometer in the latter. Blank determinations were run on the control group throughout the experiment to detect any changes in blank due to feed, weather, or other unknown factors. The average pretreatment blank value for each animal was determined from at least three pretreatment samples of butterfat. Whenever the difference in absorbance between a treated sample and pretreatment blank for a particular animal was less than one half of the absorbance, the value was considered indeterminate. A standard blank was run with every three samples analyzed, to give a systematic check on method and instrument.

Results and Discussion

Application. As expected, there was considerable variation in the amount of heptachlor applied within and between plots. The over-all application, as estimated from the amount collected in the dishpans, was at the rate of 13.97 pounds per acre instead of 12.5 pounds.

Residues in Soil. The results of soil analyses for insecticide residue, presented in Table I, are in good agreement with work reported by Barthel *et al.* (*1*).

Residues on Forage. Table II shows the results of residue determinations made on the forage. At the end of the first week most of the residue found was heptachlor, although a significant amount of epoxide was already present. After the second week heptachlor was not present in detectable amounts and only heptachlor epoxide was recovered. The epoxide on the forage varied within wide limits and fluctuated up and down, probably reflecting the characteristics of the granular application, in which contamination seems dependent on the number of granules mechanically caught by the grass. Even the best sampling procedure does not overcome this erratic pattern. Rainfall which occurred during this period possibly may have splashed soil containing insecticide residue on the forage.

Heptachlor Epoxide in Butterfat. No heptachlor was found in any of the butterfat samples, so that the cow either metabolized the insecticide to its epoxide or consumed grass or soil containing the epoxide. The values of heptachlor epoxide obtained from split samples of butterfat from individual cows by both laboratories were in good agreement. Variations in the amount of residue excreted in the butterfat of each cow from day to day were present, as would be expected because of the different metabolic pattern between animals, but the over-all trend for the cows in each group was comparable.

Figure 1 presents the butterfat analyses for heptachlor epoxide for each group of animals; the best fitting curve was used as calculated from the multiple-regression technique using the linear and quadratic effects of the independent variables from both laboratories. The vertical line at 80 days indicates the day at which all cattle were taken from treated pasture and placed on dry lot for feeding. Zero day at origin indicates the day of treatment of the pasture. The day on which each group was placed on the pasture is indicated by the dotted line to the point on the abscissa in the figure in which the curve originates. All animals were showing some heptachlor epoxide in the butterfat by the second day after being placed on the treated pasture. The ordinate is scaled in parts per million of heptachlor epoxide in the butterfat.

The most striking observation in this study was the rapidity in which heptachlor epoxide showed up in the butterfat of dairy cows (Groups I through V) when they were placed on the treated pasture. The epoxide found in the butterfat of any one cow never exceeded 3 p.p.m. and for groups I to IV the over-all excretion rate was remarkably uniform while the animals were on pasture, averaging 1.1 to 1.6 p.p.m., with means for the group ranging from 1.22 to 1.37 p.p.m. even though group I was placed on a plot one day after treatment and group IV, 30 days after treatment. Cows in groups V and VI showed evidence of epoxide in the butterfat even when they were placed on the treated pasture 43 and 57 days after treatment, the values averaging between 0.8 and 0.9 p.p.m. with an average mean of 0.77 p.p.m. for group V, and between 0.25 and 0.4 p.p.m. with an average mean of 0.35 p.p.m. for group VI. Residue values less than 0.5 p.p.m. were too far down the standard curve to be read accurately, so that any value in this category was used to indicate a probable positive value, too low to be accurately defined. This is true of the values for the animals in group VI. The R^2 values for groups I and II amounted to 25% each and decreased to 17, 11, 8, and 5% for the remaining groups, respectively. Since the butterfat analyses for the insecticide between laboratories were comparable, these low R^2 values indicate a great deal of variation between and/or within cows.

In work reported by the Entomology Research Division, U.S. Department of Agriculture (*3*), with dairy cows foraging treated pastures sprayed with 2 ounces of heptachlor in Diesel oil, approximately 6 times the maximum level of residue reported in this study was reached, but only after a much longer grazing period. Gannon and Decker

(4), using 0.5 pound of heptachlor emulsion per acre, reported higher residues in milk of dairy cows grazing treated pastures as compared to those found in this study, but their experiment used a higher treatment level and was of short duration. The granular form of heptachlor resulted in lower levels of residue in butterfat as compared to the above reports where heptachlor in emulsion or Diesel oil was used.

Inspection of the curves after the animals were taken off the treated pasture (80 to 120 days after treatment) shows that heptachlor epoxide was still being excreted in the butterfat. This is probably due to the accumulation of the residue in the fatty tissues while animals were grazing the treated pasture, since the butterfat percentages for each cow were very comparable. The level of heptachlor epoxide excreted in the butterfat appears to be dependent upon the amount of insecticide or metabolite ingested and stored in the body fat and not the amount of butterfat produced by the dairy cow. A high intake of residue results in a high level excreted in the butterfat.

While on the treated pasture all animals were in good health and gained weight. The cows also showed good persistency in milk and butterfat production during the experiment.

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INSECTICIDE RESIDUES

Analytical Method for Determining 1,1-Dichloro-2,2-bis (*p*-ethylphenyl)ethane in Rat Fat and Cow's Milk

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Perthane, 1,1-dichloro-2,2-bis(p-ethylphenyl)ethane, was administered to rats under a 2-year feeding study for toxicological evaluation. A method was completed for analyzing the fatty tissue from rats and the analytical results have evinced well-defined patterns of absorption and storage. A procedure has also been developed for residual Perthane in cow's milk. These methods involve a general reaction based on dehydrohalogenation of Perthane, followed by reaction with strong sulfuric acid to produce the carbonium ion, which gives a color with maximum absorbance at 495 mμ. The complexities of handling milk and rat fat necessitated a modification of these methods to minimize background interference from extractives.

PERTHANE, which structurally is diethyldiphenyldichloroethane, has found wide acceptance for use on fruits and vegetables and in space sprays. Registration under the Miller Bill required a 2-year feeding study on rats for chronic toxicological evaluation. The analytical method developed involves a procedure reported by Miles and others (*4, 7, 8*) for determination of chlorinated 1,1-diphenylethane–type compound residues on agricultural crops. However, the complexities of handling rat fat necessitated a modification of these methods, to minimize background interference from extractives and allow accurate analysis at the sensitivity desired.

Procedure for Rat Fatty Tissue

The method, in general, involves the dehydrohalogenation of Perthane, followed by reaction with strong sulfuric acid to produce the colored carbonium ion complex with a maximum absorption at 495 mμ. In the procedure for rat fat, the tissues are macerated using sand, followed by extraction with diethyl ether added directly to the mortar. The ether extract is filtered and then evaporated off. The resulting fatty extractables plus any Perthane residue are picked up in petroleum ether and the Perthane is partitioned into acetonitrile and treated with adsorbent to remove substrate interferences. After removal of the solvent, dehydrohalogenation is carried out, followed by washing and evaporation of solvent and determination with 95% sulfuric acid.

Reagents. Perthane. Purified, technical material, recrystallized from methanol twice. Melting point 59–60 ° C.

Petroleum ether.

n-Hexane. Purified, technical grade, 95 mole % minimum passed through activated alumina. With a column