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INSECTICIDE RESIDUES

Chemical Residues in the Milk of Cows Grazed on Chlordan-Treated Pasture

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Dairy cows were grazed on plots of pasture grass that had been treated with granular chlordan at 0.25, 0.50, or 1.0 pound per acre. Analysis of milk samples from the cows revealed the presence of small amounts of heptachlor epoxide (less than 0.1 p.p.m.) that persisted to the end of the experiment, 8 weeks after treatment. Chlordan was also detected in the milk of cows on the 0.5- and 1.0-pound plots, but levels of chlordan plus heptachlor epoxide were generally below 0.1 p.p.m. The identity of the heptachlor epoxide was confirmed by gas chromatographic and spectrophotometric procedures.

CHLORDAN has been proposed for use on pasture land to control the imported fire ant (*Solenopsis saevissima richteri* Forel) provided such use will not result in undesirable chemical residues in the milk of dairy cows grazing thereon. The experiment reported here was designed to furnish data upon which to base a decision on the feasibility of using chlordan.

A farm was selected near Gulfport, Miss., and four plots of good grassland were laid out for the experiment. The soil was light, sandy loam, well covered with a mixture of bahia grass and lespedeza. The forage was not as succulent as desirable for the best feed, but the cattle foraged readily and ate considerable quantities of the grass. On September 25, 1961, three of the plots were treated with granular formulations at estimated rates of 0.25, 0.5, or 1.0 pound of technical chlordan per acre. Exact calculations based upon plot areas and weights of formulation applied revealed that the plots actually received 0.24, 0.43, or 0.96 pound per acre. The fourth plot was left as an untreated check. Treatment was made under ideal conditions, but rainfall occurred about 30 minutes later.

On September 26, two cows were placed on each treated plot and the untreated check plot. Six additional cows were also placed on the check plot to be used on treated pasture at later dates. On October 2, 1 week after treatment, two additional cows were placed on the 0.5-pound plot, and, on October 9 and 24, 2 and 4 weeks after treatment, two more cows were placed on this plot on each date. The experiment was terminated on November 21, 8 weeks after application of the insecticide.

The cattle were predominantly Jersey, but were mixed with Holstein and beef breeds. They were very thin.

Milk samples were taken from all of the cows before the experiment was begun and analyzed to determine whether or not there was any contamination. The first posttreatment samples were collected from each cow 24 hours after exposure, and daily milk samples were taken for 2 weeks. Samples were then taken on a weekly basis during the remainder of the exposure period. The milk samples were processed to extract the butterfat in the laboratory of the Methods Improvement Operations, Plant Pest Control Division, Gulfport, Miss., and the butterfat was shipped by air to the pesticide chemists of the Entomology Research Division at Beltsville, Md., for analysis.

Samples of grass and soil from the treated plots were collected and analyzed in the Gulfport laboratory.

Analytical Procedure

Milk Samples. Milk samples were first put through a cream separator to separate most of the butterfat in heavy cream. Approximately 150 ml. of the cream was placed in a 1-quart fruit jar equipped with a rubber-sealed lid, granular anhydrous sodium sulfate added, and the contents mixed until the cream was dehydrated. About 300 ml. of *n*-pentane was added, the lid sealed, and the jar shaken vigorously for several minutes. The butterfat and insecticide were extracted by the pentane under these conditions. The pentane solution was then decanted into an Erlenmeyer flask equipped with a three-ball Snyder column and the solvent removed on a steam bath. The last traces of pentane were removed at room temperature, under vacuum. The

Table I. Recovery of Chlordan and Heptachlor Epoxide from 10 Grams of Butterfat

μG. Added	% Recovered
CHLORDAN[a]	
5	80
10	75
20	79
HEPTACHLOR EPOXIDE[b]	
5	90
10	97
20	98

[a] Corrected for check sample reading of 3 μg.
[b] Check sample reading was zero.

butterfat was then placed in polyethylene-capped vials for storage and shipment to the Beltsville laboratories. This extraction procedure was developed by the authors specifically for pesticides that are known to occur entirely in the butterfat in whole milk. The percentage of butterfat in the milk samples was determined by the standard method, which permits the calculation of residue levels in the whole milk from those found in the butterfat and eliminates the necessity for complete extraction of the fat from the milk.

For analysis, 10 grams of butterfat was transferred to a dry, 500-ml., separatory funnel with *n*-pentane, and the volume adjusted to about 300 ml. The solution was then washed twice by shaking with 7% fuming sulfuric acid, with 30 ml. of the acid used for each wash. Emulsions that occurred were broken by cautiously adding water, a few drops at a time. In the first wash, the acid layer turned bright yellow, then darkened as the emulsion broke, until it became dark brown. The second wash was usually yellow in color but became clear when the emulsion broke. The acid washes were discarded and the pentane solution was washed twice with 150-ml. portions of distilled water. The solution was then dried over anhydrous sodium sulfate, transferred to an Erlenmeyer flask fitted with a three-ball Snyder column, and the volume reduced to about 20 ml. on a steam bath.

Column chromatography was employed to complete the cleanup and to separate chlordan from heptachlor epoxide. The column described by Murphy and Barthel (*3*) was used since it had previously been found suitable for butterfat cleanup by Rusoff *et al.* (*5*). The polarity of the eluting solvent was changed to compensate for variations in the adsorptive properties of the Florisil. After the prescribed washing of the column, chlordan was eluted with 200 ml. of pentane and the heptachlor epoxide with 150 ml. of 3% ether in pentane. The eluates were evaporated on a steam bath, to a volume of about 3 ml., in Erlenmeyer flasks fitted with three-ball Snyder columns. The solutions were then transferred to 10-ml. graduated centrifuge tubes, and the volume was reduced to 0.2 ml. in a 40° C. water bath with a gentle stream of air.

Chlordan was determined by the method of Davidow (*2*), heating for 15 minutes at 100° C., with 0.3 ml. of reagent. The absorbance was read at 550 mμ on a spectrophotometer. Heptachlor epoxide was determined by heating at 100° C. for 15 minutes with 0.5 ml. of Polen-Silverman reagent (*4*). The absorbance was determined at 390, 410, and 440 mμ, and the baseline technique described by Wright (*6*) was used to determine the presence of heptachlor epoxide.

Recoveries of the insecticides were determined by adding known amounts to samples of whole milk and carrying them through the entire procedure. It was found that identical results could be obtained by adding the standards to butterfat samples. Recoveries at three different levels are shown in Table I.

Technical Chlordan. Heptachlor was separated from chlordan by chromatographing on an alumina column. The column, containing 20 grams of adsorbent, was wetted with 50 ml. of pentane, and a sample of the technical chlordan used for treating the experimental plots was then introduced and the column eluted with pentane. The first 50 ml. of eluate was discarded, and the next 50 ml. containing the heptachlor was analyzed to determine the amount present. Under the conditions described, the chlordan remained on the column. The value found for heptachlor is considered to be a maximum since small amounts of related compounds that would give the same color test could have been present.

Soil and Grass. Soil samples were collected and processed as described by Murphy and Barthel (*3*). Grass samples were cut with a power-driven rotary mower, diagonally across the plot. About 1000 grams were collected, ground, and subsampled for analysis. Analyses were made for chlordan and

Table II. Heptachlor Epoxide in the Milk of Cows Grazed on Pasture Treated with 0.25 Pound of Chlordan per Acre on September 25, 1961

Date of Sample	P.P.M. for Animal Numbers[a] 1	14
9/6	0	0
9/14	0	0
9/29[b]	0	0
10/2	0	0
10/4	0	0
10/7	0.02	...
10/9	0.03	0.02
10/18	0.01	0.01
10/25	0	0.02
11/8	0	0
11/21	0	0

[a] Check sample readings were zero. [b] Cows exposed on September 26.

Table III. Chlordan (C) and Heptachlor Epoxide (H) in the Milk of Cows Grazed on Pasture Treated with 0.5 Pound of Chlordan per Acre on September 25, 1961

Date of Sample	P.P.M. for Animal Numbers[a] 5[b] C	H	10[b] C	H	4[c] C	H	11[c] C	H	6[d] C	H	12[d] C	H	2[e] C	H	3[e] C	H
9/14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9/29	0.02	0	0.07	0	...	...	...	...	...	...	...	...	...	...	...	...
10/2	0.02	0	...	...	...	...	...	...	...	...	...	...	...	...	...	...
10/3	...	...	0.02	0.02	...	...	...	...	...	...	...	...	...	...	...	...
10/4	<0.02	0	...	...	0	0	0	0	...	...	...	...	...	...	...	...
10/7	<0.02	0	...	...	0.04	0.02	<0.02	0.03	...	...	...	...	...	...	...	...
10/9	0.05	0.02	0.05	0.05	0	0.02	0.05	0.02	...	...	...	...	...	...	...	...
10/11	...	...	...	...	0.03	0.01	0.03	0.01	0.04	0	0	0	...	...	...	...
10/13	...	...	...	...	<0.02	0.04	...	...	0.04	0	...	...	...	...	...	...
10/15	...	...	...	...	...	...	<0.02	0.02	<0.02	0	0	0.02	...	...	...	...
10/18	0.04	0.03	<0.02	0.03	0	0.03	0.04	0.02	<0.02	0	0.04	0.04	...	...	...	...
10/20	...	...	...	...	...	...	...	...	0	0.02	<0.02	0.03	...	...	...	...
10/25	...	0.06	0.07	0.04	0	0.06	0.03	0.02	0	0.03	0.04	0.03	0.03	0	0.02	0
10/28	...	...	...	...	...	...	...	...	0.03	0.03	...	0.06	0.03	0	0.03	0
10/31	...	...	...	...	...	...	...	...	<0.02	0.05	0	0.05	...	0.04	0.02	0.03
11/1	...	...	0.05	0.04	0	0.05	0.04	0.03	0.06	0.06	0.05	0.05	0.04	0.02	0.02	0.03
11/4	...	...	...	...	0.04	0.03	...	...	...	...	...	...	...	...	...	...
11/8	0	0.04	0.07	0.04	0	0.03	0.04	0.03	...	...	0.05	0.04	0.05	0	0	0.03
11/21	0	0.04	0.07	0	0	0.04	0.04	0.02	0	0.03	0	0	0.08	0.03	0.04	0.03

[a] Check sample readings were zero for heptachlor epoxide. Correction made for chlordan average check value of 0.01 p.p.m. (Range: 0 to 0.02 p.p.m.). [b] Exposed on September 26. [c] Exposed on October 2. [d] Exposed on October 9. [e] Exposed on October 23.

Table IV. Chlordan (C) and Heptachlor Epoxide (H) in the Milk of Cows Grazed on Pasture Treated with 1.0 Pound of Chlordan per Acre on September 25, 1961

Date of Sample	P.P.M. for Animal Numbers[a] 7 C	7 H	8 C	8 H
9/14	0	0	0	0
9/29[b]	0.05	0	0.07	0
10/2	...	...	0.07	0.01
10/3	0.02	0.02	0.07	0.01
10/4	0.03	0.03	...	...
10/7	<0.02	0.01	0.04	0.04
10/9	0.03	0.03	0.05	0.03
10/18	<0.02	0.02	<0.02	0.06
10/25	0.04	0.05	...	...
10/27	...	...	0.03	0.05
11/8	0	0.04	0.06	0.05
11/21	0.02	0.02	0.05	0.03

[a] Check sample values were zero for heptachlor epoxide. Chlordan results corrected for average check value of 0.01 p.p.m.
[b] Cows exposed on September 26.

Table V. Chlordan Residues in Grass and Soil, in P.P.M., 1961[a]

Treatment, Pounds/Acre	Date Sampled 9/29	10/5	10/25	11/20
	GRASS			
0.25	0.2	0.3	0	0
0.5	0.7	0.8	0.2	0.1
1.0	1.0	1.5	1.2	0.6
	SOIL			
0.25	0.3	...	...	0.1
0.5	0.4	...	...	0.3
1.0	1.6	...	...	1.3

[a] Corrected for check samples.

heptachlor epoxide in the manner described by these authors.

Analytical Results

Milk. The data from the analyses of representative milk samples taken throughout the experiment are shown in Tables II, III, and IV. The values given were calculated from the amounts found in the butterfat based on the actual butterfat content of the individual samples.

No chlordan was detected in the milk of the cows on the 0.25-pound treatment. Heptachlor epoxide appeared 11 days after treatment and persisted for about 3 weeks. The highest level reached was 0.03 p.p.m.

Chlordan appeared in the milk of cows on the 0.5-pound plot within 3 days after exposure and persisted at low levels throughout the experiment. Heptachlor epoxide first appeared in the milk about 10 days after exposure and also persisted for the duration of the experiment. Delaying exposure of cows until 1, 2, or 4 weeks after treatment had very little effect on insecticide levels in the milk.

The milk from cows placed on the 1-pound plot showed the same pattern of residues as those on the 0.5-pound plot.

Carter *et al.* (*1*) fed alfalfa hay containing chlordan to dairy cattle for 150 days. At a daily intake of 0.36 to 0.42 mg. per kg. of body weight, the milk contained 0.1 to 0.2 p.p.m. These results are similar to those reported here although the type of feed, time of feeding, method of exposure, and analytical methods are quite different.

Technical Chlordan. The heptachlor content of the technical chlordan used in making the granular formulation was 6.4%. Heptachlor content may possibly vary in different lots of technical chlordan, and the value given cannot be assumed as correct for any other lot.

Soil and Grass. No heptachlor epoxide was found in any of the soil or grass samples. Chlordan was found in amounts consistent with the treatments. Chlordan residues are given in Table V.

Discussion

The levels reported in milk, generally less than 0.1 p.p.m. of chlordan and heptachlor epoxide combined (from about 1.5 to 3.3 p.p.m. in the butterfat), were so low that the significance of the data was a matter for concern. The differences between samples for the control cows and those on the treated plots were consistent, and the data appeared valid, even at these marginal levels. Positive confirmation of the identity of the compound recorded as heptachlor epoxide was made by other means, however, to dispel any doubt.

The absorption curve for the colored solution obtained in the colorimetric analysis for heptachlor epoxide was run on a recording spectrophotometer, and a positive peak was observed at 410 mμ. A sample was then analyzed in a microcoulometric gas chromatograph, and the contaminant was positively identified as heptachlor epoxide. For further substantiation, samples were run in a conventional gas chromatograph, and pure heptachlor epoxide, alone or added to control samples, and the contaminant in the samples from treated cows had identical retention times. Furthermore, the response of both types of gas chromatographs showed good quantitative agreement with the colorimetric analyses.

It must be concluded that the technical chlordan used in this study contained enough heptachlor to cause detectable amounts of heptachlor epoxide to appear in the milk of cows grazed on treated pastures. Chlordan was also found in the milk of cows on the two higher levels of treatment. The combined chlordan and heptachlor epoxide content of all samples analyzed was so small that the practical significance of the residues may be questionable.

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