Table I. Residues of Heptachlor Epoxide in Milk from Cows Fed 0.5 and 1.0 P.P.M. of It for 2 Weeks

Days after First Feeding	Heptachlor Epoxide in Butterfat, P.P.M.[a]	Absorbance	
		Sample	Check
Cow No. 22 fed at the 0.5 p.p.m. level[b]			
−1[c]	0.01		
1[d]	0.13	0.093	0.062
2	0.21	0.124	0.068
3	0.25	0.139	0.068
4	0.36	0.182	0.074
5	0.38	0.191	0.074
7	0.35	0.169	0.068
14	0.29	0.153	0.068
16	0.30	0.188	0.082
18	0.19	0.147	0.082
28	0.24	0.162	0.098
Cow No. 30 fed at the 1.0 p.p.m. level[e]			
1	0.05	0.082	0.062
3	1.34	0.444	0.078
4	1.04	0.303	0.078
7	1.71	0.460	0.057
14	1.94	0.460	0.057
16	1.20	0.431	0.062
21	0.72	0.287	0.103
28	0.52	0.230	0.103

[a] Uncorrected for check and recovery.
[b] $t = 9.25$. $t(0.01) = 3.25$.
[c] 1 day before feeding heptachlor epoxide was begun.
[d] First day of feeding heptachlor epoxide.
[e] $t = 5.12$. $t(0.01) = 3.49$.

tachlor epoxide. The cream was separated from the milk using a mechanical separator. The butter oil was extracted from the cream as in the procedure of Westlake (*5*). About 50 grams of the cream were tumbled with 300 ml. of pentane for 15 minutes. One hundred fifty grams of sodium sulfate were added and the pentane extract was decanted. One hundred milliliters of pentane were added, tumbled for 30 minutes, and the pentane was decanted off. One hundred milliliters more of pentane were added, tumbled for 45 minutes, and the remaining pentane was decanted off. The pentane extracts were combined and the pentane was distilled off through a Snyder column, on a hot water bath. Fifteen grams of butter oil were taken for analyses. Activated Florex XXS was used in the chromatographic step and 6% of diethyl ether was employed to elute heptachlor epoxide from this column. A new standard recovery curve was prepared with each pair of samples analyzed. The curve followed Beer's law. Recovery of heptachlor epoxide added to cream averaged 113.5%. Time did not permit analyses of the milk from all of the cows in the experiment.

Results and Discussion

Table I shows the residues of heptachlor epoxide found in milk. All standard curves prepared were recovery curves; hence, the check values become 0 γ. Because milk from epoxide-fed cows and control cows was taken at every sampling date, it was believed desirable to analyze the milk for heptachlor epoxide together with the check taken on the same day, so the values might be compared as paired values. These values could then be tested by the Student's t test for significance (*4*).

Heptachlor epoxide was present in the milk when either level of epoxide was fed to the dairy cattle (Table I). The amounts present at any sampling data were real and greater than that in the controls at the 1% level.

Prior to the feeding of the heptachlor epoxide, at the middle of the test period, and again at the termination, a trained veterinarian examined each cow for any signs of poisoning by checking its weight, temperature, pulse, respiration, nature of feces, etc. No flavors or odors that could be attributed to the feeding of heptachlor epoxide were found present in the milk. None of the cows exhibited any signs of poisoning or showed any weight loss. All test animals remained normal and healthy during the course of the experiment.

Conclusion

These data suggest that if heptachlor epoxide does appear in the field as residues from normal applications of heptachlor for insect control, the feeding of such residues will result in the secretion of heptachlor epoxide in milk.

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INSECTICIDE RESIDUES IN MILK

The Effects of Feeding High Levels of Sevin on Residue, Flavor, and Odor of the Milk of Dairy Cattle

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SEVIN (1-naphthyl-*N*-methyl carbamate), a new insecticide with relatively low mammalian toxicity and a wide range of effectiveness on many insects, was particularly effective on the gypsy moth caterpillar, and hence was chosen as a possible replacement for DDT for 1959 in the U. S. Department of Agriculture gypsy moth eradication program. Because forage as pasture, hay, grain, and silage would be contaminated by the blanket coverage needed in the eradication program, it was the purpose of this experiment to determine if the feeding of high levels of Sevin would affect the health of dairy cattle, cause residues to appear in the milk, or result in off-flavors or odors.

Earlier tests by the U. S. Department of Agriculture at Kerrville (*2*) had indicated that Sevin was not detected in milk of dairy cows fed levels of Sevin varying from 2.5 to 50 p.p.m. Therefore, it was arbitrarily decided to feed technical Sevin for a period of 2 weeks at levels of 50, 150, and 450 p.p.m. of the average total daily roughage (hay and silage) intake of the dairy cattle.

Five cows of the breeds Brown Swiss, Holstein, Jersey, and Ayrshire were as-

Technical Sevin was fed to dairy cows of the Brown Swiss, Jersey, Holstein, and Ayrshire breeds at 50, 150, and 450 p.p.m. of their average total daily roughage intake (dry weight) for a period of 2 weeks. Samples of milk were taken at regular intervals and the cream was analyzed for Sevin by means of the p-nitrobenzenediazonium fluoborate coupling method (Union Carbide). The concentration of Sevin, if present, was below the sensitivity of the analytical method, 0.01 p.p.m.

signed at random to each of the three insecticide levels fed and to a control. Each treatment had, as far as possible, at least one animal of each breed.

Experimental Design and Methods

The cows were housed in a completely randomized order in stalls so constructed that no animal could steal from her neighbor.

Jerseys were fed 25 pounds of hay and 30 pounds of good corn silage daily, while Ayrshires received 30 pounds of hay and 40 pounds of silage. Brown Swiss and Holsteins each were given 40 pounds of hay and 50 pounds of silage. Grain of a good general herd mix was fed on a formula which took into account the amount and quality of roughage fed, milk and butterfat produced, etc., but which in general amounted to about 1 pound of grain for each 4 pounds of milk produced. All feed and roughage for each cow were weighed carefully into the mangers at each feeding and weighbacks of portions not eaten were recorded. Thus, an accurate record of food intake was available for each animal over the test period and a 2-week standardization, or pretest period.

The moisture content of each roughage was determined and computed as a mean of several samples, and the dosage of Sevin fed was then based on the mean dry weight of the daily roughage intake averaged over a week's feeding period. Each week the dosage of insecticide fed to each cow was based on the previous week's intake of roughage and was adjusted accordingly for each cow. The level of technical Sevin to be fed each cow daily was weighed out separately on a Mettler H-5 balance, divided in half and added to two 1-pound lots of grain. Just prior to grain feeding each morning and afternoon, the pound of treated grain was fed to each cow in metal baskets. At the same time, nontreated 1-pound lots were fed to the 5 control cows. The treated grain was quickly consumed as the cows soon learned that more grain was forthcoming, once the treated grain was consumed. None of the treated grain was ever rejected by any of the cows even at the 450 p.p.m. level of Sevin.

The experiment was initiated on January 5, and on January 18, the first Sevin-treated grain was fed. Feeding of treated grain continued until February 1. Samples of milk for residue analysis were taken at −1, 0, 1, 2, 3, 4, 5, 7, and 14 days, while the Sevin was being fed, and again at 16, 18, 21, and 28 days in the third week after the insecticide had been stopped.

Milk samples were taken twice daily at the regular morning and afternoon milking times and consisted of 1 quart of raw, well-mixed milk from each cow.

Just prior to the start of the feeding of Sevin, and once weekly thereafter, samples of milk were taken for butterfat, flavor, and odor tests.

Prior to the feeding of Sevin, at the middle of the experimental period, and again at its conclusion, an experienced veterinarian checked the weight, temperature, pulse, respiration, and type of feces of each animal, observing them for any symptoms of insecticide poisoning.

All feed (hay, silage, and grain) was of excellent quality and was from a single source in sufficient quantity to last for the entire experimental period. Analysis of all cattle feed was made to ascertain that no contaminants were present.

Methods of Chemical Analysis

Mechanically separated cream was extracted with hexane-ether and partition extracted with hexane-acetonitrile according to the unpublished method of Westlake (*3*) as follows:

The cream was extracted by mechanical separation from 400 grams of milk, three times in a separatory funnel with 150-ml. portions of a mixture of equal volumes of *n*-hexane and ether. The extracts were combined, filtered through dry cotton, and evaporated to a 25-ml. volume. The evaporated solvent mixture was transferred to a separatory funnel and extracted three times with 15-ml. portions of acetonitrile. The acetonitrile solutions were combined in a separatory funnel, 200 ml. of water were added and the mixture was extracted three times with 50-ml. portions of diethyl ether. The combined ether extracts were filtered through dry cotton and the filtrate was evaporated to dryness. Spectrophotometric determination of Sevin then followed using the *p*-nitrobenzenediazonium fluoborate coupling method (*1*). Recovery of Sevin added to cream was usually about 84%. The sensitivity of the analytical method was about 0.01 p.p.m.

Discussion and Results

Seventy-eight samples of milk including those of the checks were analyzed for Sevin. The net residues in the milk ranged from (−)0.0062 to 0.0087 p.p.m. Therefore, the concentration of Sevin, if present in the milk, was below the sensitivity of the analytical method. All of the milk samples were not analyzed for residues because milk from the highest levels of Sevin fed failed to disclose measurable Sevin residues. However, some samples of milk from cows fed lower levels of Sevin were selected at random and analyzed. The readings from these samples did not differ from the milk of cows fed 450 p.p.m. of Sevin.

No difference in the reaction of cattle to Sevin was noted among the four breeds.

Unsuccessful attempts were made to recover 1-naphthol, a hydrolysis product of Sevin, added to cream. It was thought that 1-naphthol or esters of it which are known to be formed in other animals might exist in cow's milk. Saponification of milk, followed by analysis for 1-naphthol, was unsatisfactory; hence this work was not continued.

No off-flavors or odors that could be attributed to Sevin were found in any of the milk samples.

None of the cows showed any abnormal changes in weight or exhibited any symptoms of poisoning. Food intake and excretion were normal.

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