not consider the codistillation of DDT from test systems.

The percentage distribution of the sizes of particles of DDT in the 15-p.p.b. preparation is shown below:

Diameter, A.	Per Cent
>50,000	50.7
1100–50,000	30.7
83–1100	6.6
59–83	2.7
41–59	1.3
7–41	8.0

Most of the particles were larger than 1100 A. in diameter. Only about 10% were in the range of 41 to 1100 A., but this quantity, if it were not removed from the filtrate, is sufficient to cause serious error in the solubility value.

Figure 1 shows a model of the DDT molecule—constructed from Fisher-Hirshfelder-Taylor atomic models—as compared with a sphere 7.1 A. in diameter that has a volume equivalent to that of the DDT molecule, and with the smallest particle, 41 A. in diameter, that could have been removed from the filtrate in the centrifugation experiments. The shape of the molecule is close enough to a sphere that Stokes' law may be used for the determination of particle size without appreciable correction. A centrifugation time in the No. 40 rotor of about 17.5 days at 39,400 r.p.m. is required to remove undissolved amicron particles of DDT larger than molecular size from the top half of the filtrate. Fortunately, stability to ultracentrifugal force was approached within 6 hours and it was not necessary to centrifuge for such an impractical period.

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INSECTICIDE RESIDUES IN MILK

Effects of Feeding Low Levels of Heptachlor Epoxide to Dairy Cows on Residues and Off-Flavors in Milk

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Heptachlor epoxide was fed to dairy cows at 0.5 and 1.0 p.p.m. of roughage intake. Analysis of the butter fat indicated residues reached a level of 0.38 and 1.94 p.p.m. for the two levels, respectively. These feeding rates simulate the residue of heptachlor epoxide shown to be present on alfalfa after practical application of heptachlor.

HEPTACHLOR is an effective insecticide for controlling many insects. It is recommended, particularly for the control of the meadow spittlebug and alfalfa weevil in the Northeast, at dosages ranging from 0.25 to 1 pound per acre, (Gyrisco *et al.*, *2*). Gannon and Decker (*1*) reported that heptachlor was converted to its epoxide on alfalfa and resulted in a residue of 2.42 p.p.m. when as little as 1 pound per acre of heptachlor was applied; 0.17 p.p.m. of the epoxide was still present after 21 days.

Heptachlor epoxide is more persistent and toxic than heptachlor. An experiment was conducted, therefore, to determine if heptachlor epoxide is excreted in the milk of cows fed levels of it, duplicating what comes from forage as a result of practical applications of heptachlor for insect control. Technical heptachlor epoxide was fed to dairy cows at levels of 0.5 and 1.0 p.p.m. of their average total daily roughage for a period of 2 weeks.

Materials and Methods

Three Holstein cows were assigned to each level of technical heptachlor epoxide and five cows were to serve as controls. The cattle were housed at random, in stables so constructed that no animal could steal food from her neighbor. Each cow was fed 40 pounds of hay, 50 pounds of silage, and grain of a good general herd mix at a rate roughly approximating 1 pound of grain for each 4 pounds of milk produced. All feed was carefully weighed out and portions not eaten were weighed and recorded. Thus, an accurate record of food intake was available for each animal over the 2-week standardization or pretest period and the following 2-week test period.

Each week the actual dosage of technical heptachlor epoxide fed to each cow was based on the dry weight of the previous week's intake of roughage. The amount of technical epoxide to be fed each cow to obtain the desired 0.5 or 1.0 p.p.m. dosage was weighed out carefully on a microbalance for each day, divided in half, and added to 1-pound lots of grain. Just prior to grain feeding for the herd each morning and evening, the pound of treated grain was fed in metal containers to each cow. Untreated grain was fed in a similar manner to the five control cows. Samples of milk consisting of 1 quart of fresh, well-mixed, raw milk were taken at the regular morning and evening milkings at −1, 1, 2, 3, 4, 5, 7, and 14 days, while the heptachlor epoxide was being fed, and again at 16, 18, 21, and 28 days after the initiation of the experiment.

Prior to feeding of the heptachlor epoxide and once a week thereafter, additional samples of milk were taken for butterfat, flavor, and odor tests.

The analytical procedure of Meyer, Malina, and Polen (*3*) was used, with several modifications, to determine hep-

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-