Plant Industry, Div. Soil Management and Irrigation, Research Rept. 237 (1952).

- (18) Lindquist, A. W., Roth, A. R., Hoffman, R. A., and Butts, J. S., J. Econ. Entomol., 44, 931 (1951).
- (19) Lindquist, A. W., Roth, A. R., Yates, W. W., Hoffman, R. A., and Butts, J. S., Ibid., 44, 167 (1951)
- (20) March, R. B., and Metcalf, R. L., Soap Sanit. Chemicals, 26, 121, 123, 125, 139 (1950). (21) Metcalf, R. L., "Mode of Action
- of Organic Insecticides," Natl. Research Council, Natl. Acad. Sci., Chem. Biol. Coordination Center, Rev. No. I., 1948.
- (22) Natl. Research Council, Natl. Academy Sci., "Conference on Insecticide Resistance and Insect Physiology," Pub. 219 (1952).
- (23) O'Brien, R. D., and Spencer, E. Y., J. Agr. Food Chem., 1, 946 (1953).
- (24) Pearce, G. W., and Jensen, J. A., *Ibid.*, **1**, 776 (1953).
- (25) Perry, A. S., and Hoskins, W. M., J. Econ. Entomol., 44, 850 (1951).
- (26) Perry, A. S., and Hoskins, W. M., Science, 111, 600 (1950).
- (27) Roan, C. C., Fernando, H. E.,

and Kearns, C. W., J. Econ. Entomol., 43, 319 (1950).

- (28) Roth, A. R., Lindquist, A. W., and Terriere, L. C., Ibid., 46, 127 (1953).
- (29) Schechter, M. S., Soloway, S. B., Hayes, R. A., and Haller, H. L., Ind. Eng. Chem., Anal. Ed., 17, 704 (1945).
- (30) Skipper, H. E., Bryan, C. E., White, L., Jr., and Hutchison, O. S., J. Biol. Chem., 173, 371 (1948).
- (31) Sternburg, J., and Kearns, C. W., Ann. Entomol. Soc. Amer., 43, 444 (1950).
- (32) Sternburg, J., and Kearns, C. W., J. Econ. Entomol., 45, 497 (1952).
- (33) Sternburg, J., and Kearns, C. \overline{W} .,
- Science, 116, 144 (1952).
 (34) Sternburg, J., Kearns, C. W., and Bruce, W. N., J. Econ. Entomol., 43, 214 (1950).
- (35) Sternburg, J., Vinson, E. B., and Kearns, C. W., *Ibid.*, **46**, 513 (1953).
- (36) Tahori, A. S., and Hoskins, W. M., Ibid., 46, 302 (1953).
- (37) Vinson, E. B., and Kearns, C. W., Ibid., 45, 484 (1952).
- (38) White, W. C., and Sweeney, T. R., U. S. Public Health Repts., 60, 66 (1945).

- (39) Williams, R. T., Ann. Rev. Bio-
- (40) Williams, R. T., "Detoxication Mechanisms," Wiley, New York, 1949.
- (41) Winteringham, F. P. W., Science, 116, 452 (1952).
- (42) Winteringham, F. P. W., Harrison, A., and Bridges, R. C., Nature, **166,** 999 (1950).
- (43) Winteringham, F. P. W., Loveday, P. M., and Harrison, A., *Ibid.*, I. IVI., and Harrison, A., Ibid., 167, 106 (1951).
 Woke, P. A., J. Agr. Research, 58, 289 (1939).
 Total Marcinet Content of Co
- (45) Zeid, M. M. I., Dahm, P. A., Hein, R. E., and McFarland, R. H., J. Econ. Entomol., 46, 324 (1953).

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PESTICIDES IN FOODS

Determination of Malathion and Its Influence on Flavor of Milk from Cows Fed Malathion-Sprayed Alfalfa

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Malathion, applied at the rate of 0.4 pound per acre in California, caused nearly 100% mortality among mosquito larvae. But no malathion was detectable in the milk of cows fed on alfalfa sprayed with malathion, and taste tests showed that more persons found off-flavor in milk from untreated than from treated pastures.

HE FAILURE OF CHLORINATED HYDRO-L CARBON INSECTICIDES to control mosquito larvae in several areas in California during 1950 and 1951 made it necessary to search for new effective larvicides. Malathion, one of the materials tested, was found to cause nearly 100% mortality when used at the rate of 0.4 pound per acre. However, before this insecticide could be generally used in pastures where milk cows graze, it was necessary to determine the amount that might be retained in the milk and whether it caused any change in taste.

Methods

Three cows were pastured on 1/2 acre of alfalfa that was sprayed with malathion emulsion once a week for 4 weeks. The animals were removed at the time of spraying but were turned back into the pasture as soon as the spray had dried. Another portion of the field served as a check pasture. At the end of 4 weeks, because of overgrazing in the sprayed area, it was necessary to move the cattle to a new sprayed area of the field. The animals were kept in this second pasture for 4 more weeks, during the first 3 weeks of which it was sprayed with malathion weekly. The alfalfa ranged from about 6 to 16 inches in height during the tests and was irrigated about once a week.

The first application of malathion was at the rate of 0.4 pound and the six subsequent applications were at 0.5

pound per acre. The emulsion concentrate was diluted with water to a 1.5%strength and applied either with a jeep boom spray or with a 3-gallon handoperated sprayer.

Two of the test cows were of the Holstein breed and one was a Guernsey. Milk for comparison was obtained from the same number of cows of each breed that were pastured on adjacent nontreated alfalfa. These animals obtained from one third to one half of their food from the green alfalfa and the remainder from dry feed. This is common practice in the San Joaquin Valley, although some dairies use dry feed exclusively.

Milk samples from the cows grazing on the malathion-treated and the nontreated alfalfa were collected by the dairy department of Fresno State College. cooled to 40° to 50° F., and shipped in vacuum bottles by air to Berkeley for analysis. The analysis was made on the morning after collection. The following procedure was used for these analyses.

Four hundred milliliters of milk and 400 ml. of carbon tetrachloride were mixed in a Waring Blendor for 5 minutes.

The mixture was allowed to separate in a separatory funnel, and the carbon tetrachloride was drained off and measured. The recovered carbon tetrachloride was taken as an aliquot of the original 400 ml.

Malathion was determined in the extract by the copper-complex method developed by the American Cyanamid Co. (1), and the color readings were made on a Beckman DU spectrophotometer.

The accuracy of this method is indicated by the recovery of 92% of the malathion in samples of milk to which 1 p.p.m. of malathion had been added. This method is sensitive to a minimum sample quantity of 100 γ of malathion, which shows 88% transmittance on the spectrophotometer, but this may be taken as a conservative figure. As all milk samples tested were 200 mg. or larger, the lower sensitivity limit for each sample would be 0.5 p.p.m.

Samples of the milk were taste-tested each week by a panel of 6 to 12 people. Samples of milk from cows fed on the malathion-treated or untreated pasture and of the untreated milk to which 10 and 20 p.p.m. of technical malathion had been added were rated as having faint, medium, strong, or no off-flavor.

Chemical Analyses

No malathion was detectable in any of the samples collected from the cows

grazing in the malathion-treated pasture. The Beckman readings on these samples and the check samples are comparable, 98 to 100% in all samples (Table I). The 92% recovery in the samples to which malathion was added indicates that if any malathion was present in the samples from cows on the treated pasture, it was much less than the test's sensitivity (0.5 p.p.m.). Actually no malathion was indicated by any of the readings with the spectrophotometer.

Table I. Spectrophotometer Readings on Milk from Cows Fed Alfalfa Sprayed with Malathion Emulsion, 1952

(0.4 pound per acre applied July 8 and 0.5 pound thereafter)

Date of Appli- cation			Beckman Reading, % Transmittance						
		Date Samples Analyzed	Treated	Untreated check					
July	8	July 8	98	98					
,		´13	99	100					
	15	16	97	98					
		18	100	100					
	22	25	100	99					
			99ª						
	29	31	98						
Aug.	12	Aug. 21	99						
0	19	28	99						
aç	Sam	ple taken July	7 23.						

The LD_{50} acute oral dosage of 90% malathion in vegetable oil for rats has been shown by Hazleton (3) to be about 480 mg. per kg. of body weight, and chronic toxicity tests showed no mortality or abnormality of rats even at 1000 p.p.m. in all food given over a period of 2 years. The acute oral LD_{50}

dosage of 99% malathion for rats has been shown by Du Bois and others (2) to be 1500 mg. per kg. The present commercial product contains from 95 to 99% of malathion. It is safe to use larger dosages than would be applied to pastures in mosquito control, as less than 2 to 4 p.p.m. of malathion exists on the foliage after spraying. This amount is not injurious to cattle. The danger that toxic amounts will be present in the milk of cows grazing on treated pastures seems slight indeed.

Taste Tests

The results of the taste tests (Table II) showed that more persons of the panel found off-flavor in milk from the untreated pasture than in that from the malathion-treated pastures; there was no indication of malathion in the milk from the malathion-treated pasture. Moreover, only a limited number of people could detect malathion in milk at a concentration of 10 p.p.m.

Acknowledament

Malathion in samples was determined by W. R. Erwin.

Literature Cited

- (1) American Cyanamid Co., unpublished method.
- Du Bois, K. P., Doulle, John, Deroin, Jere, and Cummings, O. K., Arch. Ind. Hyg. and Occupational Med., 8, 350-8 (1953).
 Hazleton, L. W., Ibid., 8, 399-405
- (1953).

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Table II. Weekly Ratings by Taste Panels of Four Types of Milk Samples

(Off-flavor ratings. 1, none; 2, faint; 3, medium; 4, strong)

Individuals										Malathion Added							
	Check Pasture			Treated Pasture			10 P.P.M.			20 P.P.M.							
	1 st	2nd	3rd	4th	7 st	2nd	3rd	4th	l st	2nd	3rd	4th	1 st	2nd	3rd	41h	
Desson	2	1	1	1	1	1	1	3 .	1	2	2	2	2	2	3	2	
Waterman	1	1	1	4	1	1	2	3	3	3	4	1	3	3	3	3	
LaRoux	1	1	1	2	1	2	1	1	1	1	1	1	2	2	1	1	
Diel		2	1	2		1	1	1		1	1	1		2	2	1	
Maisler	1	1	1	2	1	1	1	1	2	2	2	1	2	2	3	2	
Henderson	2	1	2	3	2	1	1	1	4	2	1	3	4	1	1	1	
Wiseman	2	2	2		1	1	3		2	2	3		3	4	4		
Shaw	_	_	1		-		2				2				4		
Pacheco	• •	3	-	• •	••	2		• •		4		•••		4			
Meeks		1	• •		• •	1		•••		2				2			
Eno	1	1	3	• •	1	1	1	••	2	1	2		1	4	3		
Scudder	•	•	5	2	•	•	•	1	-	•	-	3	-	•	5	4	
Myers	• •	• •	•••	1		• •		1		• •	• •	ž				3	
Peters	• •	• •		1	• •	• •	• •	1			•••	1	• •	••		ĭ	
Hushands	3			1	1	• •	• •	1	4	• •		3	2			2	
Holten	5	• •		1	•	•••		2		•••	• •	1	-		• •	2	
Walker	• •	• •		1	• •		• •	2			••	1		• •	• •	ĩ	
Mevers	1	••	• •	-	1	• •	• •	-	2	• •	••	-	3	• •	• •	•	
Rosav	2	•••	• •	••	1	• •	••	• •	2	• •	••		ă	••		• •	
Schramm	ž		• •	• •	1		• •	• •	2		• •	••	4	••		•••	
Davis	1		• •	••	1	• •	• •	•••	3	• •	• •		2			•••	
	•			•••	•			• •	5				-	•••		•••	
Total		67					56				87				104		