the conclusions of Lofgren, Adler, and Barthel (*6*).

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Literature Cited

(1) Agr. Research Service, U. S. Dept. Agr., Interim Federal Specification 0-I-00528, Feb. 6, 1958.
(2) Barthel, W. F., Murphy, R. T., Mitchell, W. G., Corley, Calvin, J. Agr. Food Chem. **8,** 445–7 (1960).
(3) Lichtenstein, E. P., *Ibid.*, **7,** 430–3 (1959).
(4) Lichtenstein, E. P., Schulz, K. R., *J. Econ. Entomol.* **52,** 124–31 (1959).
(5) *Ibid.*, **53,** 192–7 (1960).
(6) Lofgren, C. S., Adler, V. E., Barthel, W. F., *Ibid.*, **54,** 45–7 (1961).
(7) Lofgren, C. S., Stringer, C. E., Gulfport, Miss., unpublished data, 1959.
(8) Murphy, R. T., Barthel, W. F., J. Agr. Food Chem. **8,** 442–5 (1960).

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INSECTICIDE RESIDUES IN MEAT AND MILK

Feeding of Malathion to Cattle: Residue Analyses of Milk and Tissue

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The use of malathion for insect control in stored cattle feed and forage requires knowledge of the possible excretion of the insecticide in milk, or of its accumulation in tissue, at higher levels of cattle feeding than have heretofore been investigated. No malathion was found in milk of cows fed up to 800 p.p.m. of the insecticide based upon a 12-pound daily ration of dairy chow. Hay was permitted *ad libitum*. Similarly, no malathion was detected in blood, liver, kidney, heart, muscle, or fat of ruminating calves fed 200 p.p.m. of the insecticide, based on total food intake, for 41 to 44 days. Some malathion was found in the liver of two calves that were sacrificed after 14 days at this level of intake. Statistical analysis permits the conclusion that, at a 95% level of confidence, 95% of such samples will contain less than 1 p.p.m. of malathion. No ready explanation of why malathion should be present after the shorter interval of feeding is apparent.

Malathion, *S*-[1,2-bis(ethoxycarbonyl)ethyl]*O,O*-dimethyl phosphorodithioate, is a broad-spectrum insecticide that has widespread use in plant and animal health areas. Previous investigations to determine the occurrence of malathion residues in milk and meat of cattle have been concerned with either dermal application of the insecticide, or its feeding at relatively low levels in the diet. Smith *et al.* (*7*) found that two cows, fed malathion at the established tolerance level for alfalfa (8 p.p.m.) for three weeks, did not excrete the insecticide in milk, nor was it detectable in blood, brain, liver, kidney, round, or rib-eye of one animal that was slaughtered immediately upon termination of the experiment. Gjullin, Scudder, and Erwin (*4*) pastured cows on alfalfa sprayed with known amounts of malathion, and subsequently failed to detect any in milk. It must be presumed that the animals ingested some of the insecticide, but the amount is unknown. Wells *et al.* (*8*), using 0.5 and 1.0% malathion sprays and 4% dust for controlling flies in dairy barns and on dairy cattle, found that malathion is excreted in milk under these conditions in varying small amounts, the magnitude of which depends upon the type of treatment, and the interval before sampling. However, Goulding and Terriere (*5*), using a 4% dust for horn fly control on dairy cattle, reported that only occasional milk samples collected 12 to 60 hours after treatment contain any detectable amount of malathion. The two results considered positive by these authors were 0.01 p.p.m. from which it would appear that they attribute an unusually high degree of precision to the analytical procedure. Some malathion (0.03 to 0.05 p.p.m.) was found in milk when a 10% dust or a 0.5% aqueous dispersion of a wettable powder was employed. The report of Claborn *et al.* (*2*) on the application of 0.5 and 1% malathion sprays to cattle shows residues of 0.08 to 0.36 p.p.m. in milk 5 hours after spraying. The duration of a detectable residue was 3 days. These authors were unable to find malathion (< 0.5 p.p.m.) in fat of cattle that had received 16 sprays at 1-week intervals, when the fat was sampled 1 week after the last spraying.

Interest in the use of malathion for control of insects in stored cattle feed—e.g., citrus pulp—prompted the present investigation. In such uses, insecticide residues on the feed may attain relatively high levels; therefore, it is necessary to determine the amount of malathion, if any, in milk and edible tissues of cattle under such conditions.

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Experimental

Analytical Method. The colorimetric method of Norris *et al.* (*6*) was used for the determination of malathion in milk, muscle, fat, and liver. Kidney was assayed by the same procedure as described for liver, with the exception that the sodium sulfate–hydrochloric acid wash was omitted. Heart was treated by the procedure described for fat-free meat. Blood (heparinized) was assayed by extracting 100 ml. with two successive 200-ml. portions of carbon tetrachloride, combining the extracts, and proceeding from that point as with fat-free meat.

Pretreatment milk samples from each of the cows used in this study, approximately 6 gallons in all, were pooled in order to obtain samples for establishing a blank value and determining the efficiency of malathion recovery. The composite sample was separated into 500-gram portions; two 500-gram samples were required for one assay. Twenty samples were freeze-dried for blank determinations, while the remaining samples were so treated after the addition of known amounts of malathion. Results of the blank and recovery determination are presented in Table I.

In the case of blood and meat, blank and recovery values were determined using tissues obtained from ruminating calves of approximately the same ages and breeds as those used in the feeding

Table I. Recovery of Malathion Added to 1 Kg. of Whole Milk

Malathion P.P.M., Added	Found	Recovery, %
0	0.013[a]	...
0.020	0.015	75
	0.015	75
	0.018	90
	0.011	55
	0.018	90
0.050	0.043	86
	0.053	106
	0.043	86
0.075	0.058	77
	0.060	80
0.100	0.083	83
	0.077	77
	0.076	76
	0.090	90
	0.084	84

[a] Mean of 10 replicates with a standard deviation of 0.011. All recoveries are corrected for this value.

Table II. Recovery of Malathion Added to Blood and Meat

Sample Wt., Grams	Malathion, P.P.M. Added	Found	Recovery, %
	BLOOD		
100	0	0.043 ± 0.012[a]	...
	0.56	0.42	75
	0.56	0.41	73
	0.94	0.66	70
	0.94	0.69	73
	1.75	1.47	84
	1.75	1.47	84
	MUSCLE (Fat-Free)		
300	0	0.041 ± 0.015[a]	...
	0.06	0.06	100
	0.11	0.12	109
	0.11	0.14	127
	0.20	0.16	80
	0.20	0.25	125
	1.02	0.70	69
	1.14	1.25	110
	HEART		
600	0	0.074 ± 0.028[a]	...
	0.12	0.13	108
	1.01	0.79	78
	1.17	0.85	73
	1.32	1.12	85
	1.55	1.21	78
	1.63	1.19	73
	KIDNEY		
50	0	0.113 ± 0.043[a]	...
	0.39	0.21	54
	0.77	0.49	64
	1.24	0.82	66
	1.24	0.96	77
	1.94	1.33	69
	1.94	1.30	67
	LIVER		
50	0	0.184 ± 0.042[a]	...
	0.40	0.31	78
	0.81	0.63	78
	0.83	0.71	86
	1.21	0.95	79
	1.66	1.36	82
	2.02	1.61	80
	FAT		
100	0	0.052 ± 0.007[a]	...
	0.78	0.53	68
	0.78	0.51	65
	0.94	0.61	65
	1.09	0.66	61
	1.09	0.81	74
	1.56	1.23	79
	1.56	1.24	79

[a] Mean and standard deviation of six replicates. All recoveries are corrected for this value.

study. Muscle tissue, however, was an exception—it was obtained as veal roast at a local market. Results of these determinations are presented in Table II. The fat used for the blank and recovery determinations was trimmed from around the heart, whereas in the case of the test animals, the malathion assays were performed on perirenal fat.

Feed for Lactating Cows. Ten lactating Guernsey dairy cows, 5 to 7 years of age and weighing 770 to 1020 pounds, were used in this phase of the study. Morning and afternoon milk samples were taken from all cows during a 3-day pretreatment period, starting with the afternoon milking on the first day and ending with the morning milking on the fourth day. One quart (including milk from all four quarters) from each cow at each milking was set aside for analysis. The two 1-quart samples for any given day were pooled for malathion determination.

For treatment purposes, the cows were placed in two groups. Group I (cows 1 to 8) was given malathion, 95% technical, in a No. 12 gelatin veterinary capsule immediately after the morning and afternoon milkings. The amount of the insecticide was calculated in terms of parts per million of the 6 pounds of dairy chow eaten at each feeding (12 pounds per day total). Untreated hay was permitted *ad libitum*. Group II (cows 9 and 10) served as controls—i.e., no malathion was administered.

During the treatment period of April 11 to May 16, 1960, each of the cows in Group I received an equivalent of 50 p.p.m. of malathion, as defined above, the first week, and 100, 200, 400, and 800 p.p.m., respectively, each week during the successive weeks. The milk sampling schedule for the treatment period was the same as that for the pretreatment period.

Feed for Ruminating Calves. Nine calves of dairy breeds, 6 to 8 months of age and weighing 250 to 300 pounds each, were fed 12 pounds of diet per calf per day for 2 weeks prior to administration of malathion. The purpose of this was to acclimate each animal to this level of intake. The feed was formulated to accepted standard specifications, and contained no antibiotics or medication. Following this, calf 1 received no malathion. Calves 2 to 9 were given malathion, 95% technical, in a No. 12 gelatin veterinary capsule at feeding time, which was 2:00 P.M. each day. The amount of malathion administered was equivalent to 200 p.p.m. of the 12-pound total daily feed intake.

After 14 daily doses of malathion, calves 2 and 3, together with calf 1, were sacrificed for determination of malathion in tissues. The remaining six calves continued to receive daily doses of malathion until the results from the first three animals were known. Calves 4 and 5 were sacrificed after 41 days, calves 6 and 7 after 42 days, and calves 8 and 9 after 44 days. All test animals were sacrificed the morning following the day of the last dose.

Results and Discussion

Milk Analyses. Statistical analysis (*7*) was carried out on the raw data from the assays for malathion in milk, Table III. These results are uncorrected for any blank value or factor for incomplete recovery. The two components of variance (between-cow and within-cow) were evaluated from the two-way classifications for each dosage of malathion, and one-tailed tests for the significance of differences between treatment and control means were carried out. In no case was the t test significant at the 5% level. The least difference that would have been significant was, on the average, 0.01 p.p.m.

Results in Table III show a somewhat progressive increase in apparent malathion over the course of the study. The fact that this increase runs parallel in both treatment and control samples indicates that it is time-dependent rather than treatment-dependent. No explanation of this can be offered.

Milk production ranged from 20 to 30 pounds per cow per day with little variation in individual cases. Toward the end of the study, cow 8 became inactive and dropped in production. The following day she returned to normal behavior and milk output, and continued in good condition for the remaining 10 days of the study. Otherwise, no untoward signs were observed among the animals at any time. The administration of 800 p.p.m. of malathion was continued for 2 weeks, but no milk samples were analyzed for the second week.

Tissue Analyses. Results of the determination of malathion in tissues are given in Table IV. These values are likewise uncorrected. The two-way classifications for each tissue and length of treatment were analyzed, and differences between treatment and control

Table III. Apparent Malathion (P.P.M.) in Milk before and during Feeding of the Insecticide to Cattle

Day	P.P.M. Fed[a]	Cows on Treatment 1	2	3	4	5	6	7	8	Controls 9	10
					PRETREATMENT						
1	0	0.000	0.003	0.003	0.004	0.000	0.000	0.000	0.000	0.000	0.000
2	0	0.002	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000
3	0	0.010	0.003	0.002	0.000	0.000	0.025	0.026	0.024	0.024	0.011
					TREATMENT						
1	50	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
3	50	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
7	50	0.000	0.000	0.000	0.000	0.000	0.000	0.007	0.007	0.000	0.000
8	100	0.007	0.013	0.000	0.000	0.007	0.000	0.000	0.000	0.000	0.011
10	100	0.013	0.000	0.008	0.000	0.019	0.018	0.021	0.000	0.011	0.011
14	100	0.008	0.000	0.015	0.010	0.007	0.000	0.000	0.007	0.007	0.007
15	200	0.016	0.018	0.018	0.024	0.034	0.018	0.019	0.018	0.016	0.011
17	200	0.015	0.007	0.019	0.028	0.028	0.014	0.023	0.023	0.014	0.018
21	200	0.007	0.007	0.000	0.021	0.011	0.004	0.010	0.003	0.000	0.000
22	400	0.007	0.028	0.019	0.044	0.033	0.030	0.032	0.028	0.030	0.025
24	400	0.040	0.034	0.035	0.011	0.000	0.000	0.000	0.000	0.000	0.000
28	400	0.067	0.093	0.007	0.007	0.042	0.038	0.045	0.043	0.030	0.032
29	800	0.038	0.044	0.042	0.044	0.038	0.041	0.044	0.044	0.044	0.044
31	800	0.018	0.018	0.023	0.045	0.030	0.030	0.045	0.044	0.038	0.037
35	800	0.034	0.013	0.025	0.025	0.013	0.021	0.025	0.023	0.023	0.054

[a] Based on 12 pounds of dairy chow fed per day. Hay was permitted *ad libitum.*

Table IV. Apparent Malathion (P.P.M.) in Tissues of Ruminating Calves Fed 200 P.P.M. of Insecticide Based on Total Diet

Calf No.	Daily Doses	Liver	Kidney	Heart	Blood	Neck Muscle	Leg Muscle	Loin Muscle	Fat
1[a]	0	0.060	0.040	0.060	0.025	0.022	0.050	0.120	0.200
		0.060	0.040	0.080	0.020	0.035	0.035	0.032	0.230
2	14	0.650	0.190	0.040	0.020	0.056	0.037	0.132	0.132
		0.630	0.236	0.130	0.035	0.076	0.026	0.132	0.130
3	14	0.300	0.300	0.090	0.074	0.084	0.080	0.150	0.150
		0.290	0.260	0.090	0.070	0.084	0.097	0.115	0.140
4	41	0.270	0.200	0.150	0.000	0.105	0.038	0.000	0.197
		0.360	0.302	0.120	0.000	0.085	0.050	0.000	0.124
5	41	0.150	0.234	0.155	0.000	0.085	0.000	0.023	0.072
		0.170	0.106	0.134	0.000	0.100	0.000	0.035	0.085
6	42	0.240	0.200	0.070	0.035	0.150	0.102	0.116	0.085
		0.224	0.202	0.086	0.000	0.000	0.080	0.085	0.075
7	42	0.170	0.234	0.105	0.000	0.134	0.102	0.124	0.075
		0.172	0.140	0.080	0.000	0.134	0.116	0.150	0.070
8	44	0.204	0.170	0.023	0.025	0.023	0.000	0.000	0.132
		0.156	0.172	0.037	0.025	0.000	0.070	0.044	0.102
9	44	0.170	0.141	0.000	0.038	0.000	0.000	0.000	0.805
		0.140	0.150	0.000	0.000	0.000	0.000	0.038	0.120

[a] This animal served as a control, and was sacrificed together with calves 2 and 3. These results were combined with the blanks for the respective tissues in Table II to secure the best over-all estimate of a mean blank. Duplicate analyses represent separate portions of the same tissue.

means subjected to one-tailed t tests. The within-calf error was obtained from the variance of the duplicate determinations in Table IV; the between-calf error was obtained from the variance within the 14-day and 41- to 44-day groups. For treated and control animals, the least difference between means that would have been significant varies with the identity of the tissue and the length of the period of feeding (14 days *vs.* 41 to 44 days). It is least, 0.03 p.p.m., in the case of blood at either interval, and greatest, 0.23 p.p.m., in the case of liver at 14 days.

Only in the case of liver at the 14-day interval was the t test significant at the 5% level. The lower confidence limit indicates the presence of at least 0.1 p.p.m. of malathion. If the underlying distribution of observations is assumed to be normal, a tolerance statement can be made to the effect that 95% of the time, 95% of such samples will contain less than 1 p.p.m. of malathion.

Failure to find malathion in milk and tissue after insecticide feeding is undoubtedly largely a reflection of the circumstance reported by Cook (*3*), that the compound may be destroyed to a considerable extent in the rumen fluid of cattle.

Conclusion

The present work affords a basis for the establishment of additional tolerances for malathion in or on cattle feed and forage.

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Literature Cited

(1) Anderson, R. L., Bancroft, T. A., "Statistical Theory in Research," Chap. XXII, McGraw-Hill, New York, 1952.

(2) Claborn, H. V., Bushland, R. C., Mann, H. D., Ivey, M. C., Radeleff, R. D., J. AGR. FOOD CHEM. **8**, 439–42 (1960).

(3) Cook, J. W., *Ibid.*, **5**, 859–63 (1957).

(4) Gjullin, C. M., Scudder, H. I., Erwin, W. R., *Ibid.*, **3**, 508–9 (1955).

(5) Goulding, R. L., Terriere, L. C., *J. Econ. Entomol.* **52**, 341 (1959).

(6) Norris, M. V., Easter, E. W., Fuller, L. T., Kuchar, E. J., J. AGR. FOOD CHEM. **6**, 111–4 (1958).

(7) Smith, C. T., Shaw, F., Lavigne, R., Archibald, J., Fenner, H., Stern, D., *J. Econ. Entomol.* **53**, 495–6 (1960).

(8) Wells, A. L., Stelmach, Z., Guyer, G. E., Benne, E. J., *Mich. State Univ. Agr. Expt. Sta. Quart. Bull.* **40**, 786–95 (1958).

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