become repellent when its concentration is too high.) However, flies may make direct contact with lures from which the output is weak through loss or a low application rate. Of all the synthetic lures discussed here, trimedlure seems to have the least repellency to medflies at close range. At cool temperatures (50° to 69° F.) trimedlure outperformed a high-grade angelica seed oil, previously the best low-temperature lure; thus in 12 weeks, 434 medflies were caught with angelica seed oil, 368 with medlure, and 1433 with trimedlure. Trimedlure is the most promising medfly lure developed thus far.

Few attempts to relate insect attractancy and chemical structure have been made. Unquestionably, a correlation may be made from the compounds of this study, since they are all structurally similar and many of them are attractive to the medfly, although few are sufficiently attractive to be considered useful. However, there is little that the authors can predict on the basis of these or past studies with siglure. Although the *tert*-butyl ester analog of siglure (*sec*-butyl ester) was much inferior to siglure (olfactometer ratings 8 and 87), trimedlure (*tert*-butyl ester) was much superior to medlure (*sec*-butyl ester). It does seem remarkable, however, that the medfly will exhibit a marked preference for one compound over some very closely related ones, such as homologs and positional isomers.

The ultimate criterion as to the value of a lure is based on field performance. Such variables as temperature, humidity, altitude, competing natural attractants, wind conditions, rainfall, insect population, host plants, and even trap design may affect catches and some of these may have a bearing on which chemical is chosen.

Too high a volatility will cause the lure to lack persistence and possibly to issue in so high a concentration that it becomes repellent to an insect approaching the trap. Too low a volatility that cannot be compensated for by increasing wick surface and application rate is not good either, because not enough of the lure will volatilize to permit the insect to detect it from a distance. Most of the lures discussed herein differ in their most effective application rates, which can be determined only by field tests under a wide range of conditions. The most attractive of the chlorinated lures in this study were the branched-chain esters—specifically the isopropyl, 1,1-dimethylpropyl, *sec*-butyl, and *tert*-butyl esters. Of the brominated lures, only the ethyl ester showed up well; higher homologs probably were not sufficiently volatile.

The best of the new medfly lures may be sufficiently potent (when mixed with a toxicant) to eliminate males and serve as direct control agents, if used in accordance with the methods developed for the oriental fruit fly (*Dacus dorsalis* Hendel) (*10*).

Acknowledgment

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HERBICIDE RESIDUES IN MILK

Form and Magnitude of 2,2-Dichloropropionic Acid (Dalapon) Residues in Milk

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AFTER it had been established that residues may occur in forage crops sprayed with dalapon at dosages necessary for the control of noxious grass (*1*), it became important to know the magnitude of dalapon residues in milk from dairy cattle which ingest such forage. A series of related tests was conducted to accrue this information.

Dalapon could conceivably occur in milk in at least two forms—as the free acid (or more probably a salt) in the aqueous phase and as a glyceride in the fat. The first test, involving a single animal fed 200 p.p.m. of dalapon (as its sodium salt) for 8 weeks, was designed to determine the relative magnitudes of dalapon residues in each of these forms. A second test wherein three cows were fed dalapon at 20, 50, and 100 p.p.m., respectively, for 4 weeks was conducted to establish the relation between residue and quantity of dalapon ingested. In a third test, this information was augmented by milk residue data from six cows, two each fed 100, 200, and 300 p.p.m. of dalapon for an 8-week period.

Test Procedure

The first test was conducted from March 8 to May 24, 1956, using a Hereford cow weighing 900 pounds and producing an average of 5.5 kg. of milk daily. The animal was confined to its stanchion during the entire test period. Control milk samples were collected for a 2-week period prior to feeding dalapon. The chemical was administered twice daily, morning and evening, by mixing with silage contained in a washtub. Each dose consisted of 1.13 grams of dalapon, as the sodium salt, in 50 ml. of H_2O, approximating 200 p.p.m. based on the average dry weight of feed consumed. The balance of the diet consisted of alfalfa hay.

Each day a suitable portion of milk from the morning's production was mixed with an equal amount from the preceding evening and 250-gram aliquots were

Ten dairy cows were fed the herbicide 2,2-dichloropropionic acid (dalapon) for periods up to 8 weeks, at rates of 20 to 300 p.p.m. based on dry feed intake. Approximately 0.3% of the ingested dose appeared in the aqueous phase of the milk. No significant amount was found in the fat. Analytical procedures are given for determining as little as 0.1 p.p.m. of dalapon in milk either in free form or combined as a glyceride.

Table I. Test Animal Data and Dosage Rates

Cow No.	Animal Wt., Lb.	Breeding Date	Av. Daily Milk Production, Kg.	Grams Dalapon Fed Twice Daily	Approx. P.P.M.
T-1	1610	1/20/56	17.9	0.12	20
T-2	1490	3/24/56	14.4	0.30	50
T-3	1490	12/1/55	11.8	0.60	100
T-4	1560	8/20/56	15.3	0.60	100
T-5	1310	2/22/56	12.1	0.60	100
T-6	1470	2/6/56	12.8	1.20	200
T-7	1100	5/6/56	10.6	1.20	200
T-8	1470	...	6.5[a]	1.80	300
T-9	1490	2/22/56	18.0	1.80	300

[a] Milk production from this animal declined rapidly shortly after test began, dropping from average level of nearly 16 kg. per day to less than 2 kg. This cow was selected on the basis of dairyman's recorded breeding date of 2/6/56. This date proved to be erroneous.

Table II. Blank Values for Control Milk

Procedure	No. of Detns.	Grams Milk Represented by Sample Analyzed	Blank[a] (as P.P.M. Dalapon) High	Low	Av.
Distribution					
Free dalapon	6	250	0.07	0.05	0.06
	6	100	0.10	0.06	0.08
Glyceride	9	250	0.04	0.03	0.04
	3	100	0.09	0.08	0.09
Elution	14	250	0.05	0.04	0.05
	11	100	0.12	0.10	0.11

[a] Includes reagent blank which is major portion of total. All absorbance measurements made using water as reference.

Table III. Recovery Data

Dalapon Added, P.P.M. Free acid	Glyceride	Recovery, % Free acid	Glyceride
	Distribution Procedure		
0.15	0.09	...	98
0.15	0.09	97.5	98
0.15	0.09	100	100
0.30	0.18	95	102
0.30	0.18	99	...
0.75	0.27	104.5	98
0.75	0.27	104.5	94.5
0.75	0.90	105.5	101
0.75	0.90	101.5	100
		Av. 101	99
	Elution Procedure		
0.10		108	
0.10		102	
0.30		98.5	
0.30		96	
0.30		94.5	
0.60		103.5	
0.60		95	
0.60		94.5	
0.75		94.5	
0.75		104	
0.75		103	
1.05		99.5	
1.50		95.5	
1.50		98.5	
		Av. 99	

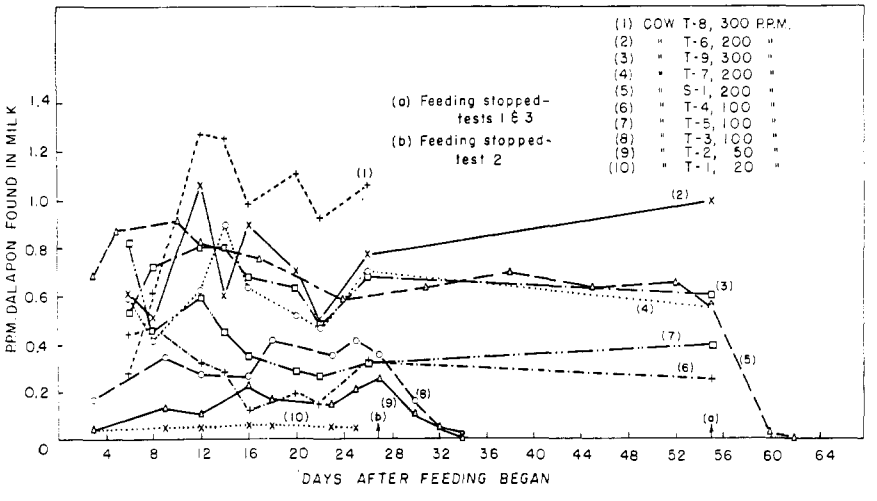

Figure 1. Dalapon residue in milk

taken for analysis. Milk not analyzed immediately was stored in a deep-freeze.

The second and third tests were conducted with Holstein cattle at a local dairy farm, the former from May 4 to June 22, 1956, and the latter from July 5 to September 4, 1956. The animal weights, breeding dates, and average daily milk production are listed in Table I. The tests were run in the manner described for test 1, except that the dalapon was mixed with a grain ration instead of silage. Actual amounts fed to approximate the desired levels of ingestion are shown in Table I.

Analytical Methods

Recognizing the possibility that dalapon might occur in milk both as the free acid (or salt) in the aqueous phase and as a glyceride in the fat, an analytical procedure was developed which will separate "free" dalapon from dalapon glycerides and quantitatively determine each separately. As shown in subsequent data (Table IV), no significant quantities of dalapon glycerides were detected in the first test. Since the analytical method used was laborious, an abbreviated method was developed to determine uncombined dalapon only. The latter half of the data from the first test and all data from subsequent tests were obtained using this method. Both methods are outlined below, the former being designated "distribution procedure" and the latter "elution procedure." Only the newly developed isolation techniques are explained here. Details of the chromatographic cleanup technique and spectrophotometric determinative procedure, as well as the extraction apparatus mentioned, are described in detail in an earlier publication (*2*).

Table IV. Residue in Milk from Cow (No. S-1) Fed 200 P.P.M. of Dalapon

Days after Feeding Began	Rep.	Distribution Procedure: Dalapon A[a]	Dalapon μg.	Dalapon P.p.m.	Glyceride A[a]	Glyceride μg.	Glyceride P.p.m.	Elution Procedure: Free Dalapon A[a]	Free Dalapon μg.	Free Dalapon P.p.m.
3	1	0.673	68	0.68	Nil	Nil	Nil			
5	1	0.935	95	0.95						
	2	0.779	79	0.79						
10	1	0.899	91	0.91	0.005	0.5	<0.1			
	2	0.889	90	0.90	0.005	0.5	<0.1			
12	1	0.809	82	0.82						
	2	0.799	81	0.81						
17	1	0.739	75	0.75	0.047	4.5	<0.1	0.743	75	0.75
	2	0.739	75	0.75	0.033	3.5	<0.1	0.753	76	0.76
24	1	0.514	52	0.52	0.035	3.5	<0.1			
	2	0.629	64	0.64	0.027	2.5	<0.1			
31	1	0.625	63	0.63	0.039	4	<0.1			
	2	0.624	63	0.63	0.030	3	<0.1			
38	1							0.673	68	0.68
	2							0.683	69	0.69
45	1							0.623	63	0.63
	2							0.625	63	0.63
52	1							0.628	64	0.64
	2							0.653	66	0.66
55	1							0.535	54	0.54
	2							0.588	60	0.60
		Av. residue uncombined dalapon, 10th through 55th day								0.69
		Feeding Stopped on 55th Day								
60	1							0.061	6	0.02
	2							0.054	6	0.02
62	1							0.011	1	Trace
	2							0.019	2	Trace

[a] Absorbance of final solution corrected for total blank (see Table II). Samples analyzed for glyceride and for free dalapon collected after feeding stopped, were total extracts from 250 grams of milk. Other samples analyzed for free dalapon (by both methods) were aliquots representing 100 grams of milk.

Distribution Procedure. 1. To 250 grams of milk contained in a 16-ounce screw-cap bottle add successively, with thorough mixing, 50 grams of Hy-Flo Super-Cel, 6 ml. of 5*N* H_2SO_4, and 25 ml. of 20% aqueous phosphotungstic acid.

2. Filter the mixture, with suction, through an extra-large borosilicate glass Soxhlet thimble, prepared by depositing a 10-gram pad of Super-Cel on the fritted glass disk.

3. Dry the filter cake in a vacuum oven for 18 hours, at 40° to 45° C. Maintain the pressure in the oven below 30 mm. of mercury while bleeding in a small amount of air. Place in a Soxhlet apparatus and extract with ethyl ether for 4 hours.

4. Add 10 ml. of concentrated HCl to the filtrate from step 2 and extract with ether for 8 hours, using a continuous liquid-liquid extractor.

5. Combine the ether solutions from steps 3 and 4 in a separatory funnel and extract successively with 10 and 5 ml. of 8% aqueous sodium bicarbonate.

6. Determine the amount of "free" dalapon in the extract (or an appropriate aliquot) by the procedure previously described (*2*). In essence, the procedure involves adding excess 2,4-dinitrophenylhydrazine to the acidified extract and passing it through a siliconized Super-Cel column, following with water saturated with benzyl alcohol. Any dalapon appearing in the eluate is hydrolyzed to pyruvic acid, which is converted to its 2,4-dinitrophenylhydrazone. The color produced in alkaline solution is measured spectrophotometrically.

7. Transfer the ether solution from step 5 to a 250-ml. Erlenmeyer flask and evaporate the ether on a steam bath. While still hot, add 25 ml. of isopropyl alcohol and 7 ml. of 50% aqueous KOH. Swirl for 5 minutes, add 5 ml. of H_2O, and let stand one hour at room temperature to complete saponification.

8. Warm the mixture to about 40° C. and pass it through a 50-ml. column of 20- to 50-mesh Dowex 50 (H^+ form) previously flushed with warm isopropyl alcohol. Wash the column with 25 ml. of warm isopropyl alcohol, followed by 100 ml. of water. Admit the effluent to a 1-liter separatory funnel containing 500 ml. of H_2O, through a capillary tube immersed nearly to the vortex of the funnel. Fatty acids ascend to the surface, while any dalapon present remains in the aqueous phase.

9. Draw off the aqueous phase into the flask to be used for extraction and wash the fatty acids three times with 50 ml. of H_2O; add washings to flask.

10. Acidify the sample with 10 ml. of concentrated HCl and extract with ether for 8 hours, using a continuous liquid-liquid extractor.

11. Extract the ether solution as in step 5.

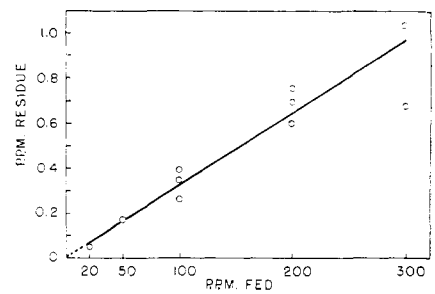

Figure 2. Dalapon residue in milk from cows fed various amounts

12. Determine the "combined" dalapon by following the procedure outlined in step 6.

Elution Procedure. Follow steps 1 and 2 of the distribution procedure. While filtering, do not allow the liquid level to recede below the surface of the filter cake.

Rinse down the walls of the Soxhlet thimble three times, drawing the water nearly into the filter cake each time. Then complete the elution with 600 ml. of H_2O fed continuously.

Transfer the filtrate and washings to a suitable vessel, add 10 ml. of concentrated HCl, and extract with ether for 8 hours, using a continuous liquid-liquid extractor.

Table V. Dalapon Residue in Milk, Test 2

Days after Feeding Begun	Residue, P.P.M.								
	Cow T–1			Cow T–2			Cow T–3		
	Rep. 1	Rep. 2	Av.	Rep. 1	Rep. 2	Av.	Rep. 1	Rep. 2	Av.
3	0.04	..	..	0.04	..	..	0.17	..	..
9	0.05	0.05	0.05	0.13	..	..	0.34	..	..
12	0.04	0.05	0.05	0.10	0.09	0.10	0.28	0.25	0.27
16	0.06	..	..	0.21	0.22	0.22	0.28	0.24	0.26
18	0.06	0.06	0.06	0.17	0.16	0.17	0.42	0.39	0.41
23	0.05	0.05	0.05	0.14	0.13	0.14	0.36	0.34	0.35
25	0.04	0.05	0.05	0.23	0.16	0.20	0.41	0.41	0.41
27	..	..	..	0.22	0.28	0.25	0.35	0.35	0.35
Feeding Stopped on 27th Day									
30	..	..	..	0.10	..	..	0.16	..	..
32	..	..	..	0.05	0.04	0.05	0.05	0.05	0.05
34	..	..	..	Nil	Nil	Nil	0.02	0.02	0.02

Table VI. Dalapon Residue in Milk, Test 3

Days after Feeding Begun	Residue, P.P.M.					
	Cow T–4	Cow T–5	Cow T–6	Cow T–7	Cow T–8	Cow T–9
6	0.44	0.82	0.61	0.59	0.28	0.53
8	0.47	0.45	0.51	0.41	0.61	0.72
12	0.32	0.59	1.06	0.62	1.27	0.80
14	0.28	0.45	0.60	0.89	1.25	0.80
16	0.12	0.35	0.89	0.63	0.98	0.68
20	0.19	0.28	0.70	0.51	1.11	0.63
22	0.15	0.26	0.50	0.46	0.92	0.47
26	0.33	0.32	0.77	0.70	1.06	0.68
55	0.25	0.39	0.99	0.55	..	0.60

Extract the ether solution with bicarbonate as in step 5 of the distribution procedure and determine dalapon in the extract as in step 6.

Blanks and Recovery Data. Samples of control milk, obtained from the various cows before feeding dalapon, were analyzed by the procedures just described to establish "blank" values (Table II). No variation in blanks among the various animals was found.

To determine the efficiency of the distribution procedure in recovering both combined and uncombined dalapon from milk, synthetic knowns were prepared by adding various amounts of dalapon and its glycerol tris ester, in various ratios, to control milk samples. These fortified samples were then subjected to the analytical scheme to determine per cent recovery. The results, summarized in Table III, show that recovery is complete.

Table III also summarizes the results obtained by applying the elution procedure to milk samples fortified with various amounts of dalapon.

Analytical Results

Test 1 (Cow S–1). Five milk samples were analyzed in duplicate for both combined and free dalapon. The data, presented in Table IV, show the absence of any significant quantity of combined dalapon. After this fact had been established, the remaining results were obtained by using the shorter elution procedure. Milk collected 17 days after feeding of dalapon had begun was analyzed by both methods and excellent agreement was obtained.

The data relating to uncombined dalapon are plotted in Figure 1. The average residue (10th through 55th day) of 0.69 p.p.m. is plotted in Figure 2.

Test 2 (Cows T–1 to T–3). Sufficient analyses, using the elution procedure, were run in duplicate to establish the residue levels in milk from cows fed 20, 50, and 100 p.p.m. of dalapon, respectively. These data are presented in Table V and plotted in Figure 1.

Test 3 (Cows T–4 to T–9). Analysis of milk samples from cows T–4 and T–5, fed 100 p.p.m. of dalapon, cows T–6 and T–7, fed 200 p.p.m., and cows T–8 and T–9, fed 300 p.p.m., yielded the data shown in Table VI and plotted in Figure 1. Single determinations were made, since good agreement was obtained between duplicate samples in the two previous tests.

Discussion and Conclusions

The data presented show clearly that a residue to dalapon does appear in milk from cows fed the herbicide. Since no significant quantity of dalapon glycerides could be detected, the residue probably exists only as a simple salt. The magnitude of the residue appears to be directly proportional to the quantity ingested by the animal over the range studied. This is shown in Figure 2, where average residue levels are plotted against corresponding ingestion levels. The slope of the curve indicates that the level of residue to be expected in milk is approximately 0.3% of the level ingested.

The reason for the abnormally low residue found in milk from cow T–9 is not apparent. It is true that milk production by this animal was somewhat higher than for others in test 3 (see Table I). However, there appears to be no relation between residue level and milk production. The data from cow S–1 show good agreement of residues with those from other cows fed at the same level, despite the cow's low milk production rate.

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Correction

Preparation of Labeled 2-Ethylthioethanol, a Demeton Intermediate

In this article by Kermit Groves and Roger Haugwitz [J. Agr. Food Chem. **9**, 262 (1961)], the name of the second author was misspelled. The correct spelling is Haugwitz.