Insecticide Residues in the Milk of Dairy Cows Fed Insecticides in Their Daily Ration

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Aldrin, dieldrin, heptachlor, DDT, and methoxychlor were fed in the daily rations to dairy cows for 16 weeks. For varying periods afterwards, the cows were fed on insecticide-free rations. Milk samples were analyzed through both periods to determine the rate of accumulation and decline. The rates of accumulation for the various insecticides were as follows: aldrin (excreted as dieldrin) > dieldrin > DDT > heptachlor (excreted as heptachlor epoxide) > methoxychlor.

Certain chlorinated hydrocarbon insecticides are excreted in milk at different rates per unit of intake. Many studies have contributed to the determination of their relative safety in actual usage.

There has been a considerable need, however, for an insecticide feeding test on dairy cows selected for uniformity which, through the use of closely controlled conditions and sensitive analytical methods, would more accurately determine the relative rates of excretion, in milk, of the common chlorinated hydrocarbon insecticides. The present work is an attempt to fill this need. In determining relative rates for the various insecticides, it was necessary, especially with methoxychlor, to feed levels much in excess of any which would ever be found as residues on forage as a result of a field application. Lower levels would not have provided data from which rates of excretion could be determined.

Procedure

The Holstein cows selected for testing were all fairly uniform in weight, food consumption, condition, and milk production.

Rations for the cows consisted of number one grade, first cutting alfalfa and a concentrate mixture of $^1/_3$ oats and $^2/_3$ ground corn. Each cow was stanchioned and fed the oats–corn concentrate from the same individual feed box. During hay feeding periods, each cow was tied with a rope halter and fed from the same feed rack throughout the experiment. Before beginning the test, efforts were made to estimate the approximate feed intake for each cow. During the experiment, the amount of feed provided for each cow was slightly less than the average amount the animal had been found to consume. This procedure reduced to a minimum the amount of unconsumed feed, and there were very few occasions when it was necessary to weigh back feed. Immediately prior to each feeding, dieldrin formulated in acetone at various concentrations was pipetted onto the rations so that 1 ml. of the solution was sufficient to add the desired amount of dieldrin, in terms of parts per million, to 1 pound of feed.

Before the cows were put on dieldrin intake, both rations and milk samples were analyzed to ensure that they contained no dieldrin residues.

On days when milk samples were to be collected, the entire production from each cow was placed in a separate, sterile galvanized can. The milk in each container was thoroughly mixed and representative samples were placed in milk cartons lined with Pliofilm bags. Samples were then labeled and placed in a cold room at 0° F. until analyzed.

Because of the uniformity of the cows tested, it was deemed unnecessary to determine the butterfat content of the milk from each cow at each sampling period. In related tests, where animals were more variable, butterfat determinations were made and analytical results were adjusted to 4% butterfat.

Each of the 18 cows in the test was fed a different dosage of insecticide. Rates, in terms of parts per million, for the various insecticides fed were as follows: aldrin: 1, 10, and 40; dieldrin: 10, 50, and 75; heptachlor: 50, 75, 100, and 200; DDT: 10, 25, 100, and 200; and methoxychlor: 800, 1000, 4000, and 7000.

All cows were fed on treated rations for 16 weeks and milk was sampled at intervals throughout. At the end of this period, four consecutive milkings were taken and analyzed separately. The results were averaged to establish a more accurate end point. One cow from each insecticide-feeding group was slaughtered at the end of the 16 weeks of feeding and her fat was analyzed for insecticide content.

To determine the decline in insecticide content in the milk, the remaining cows were fed on insecticide-free diets for up to 7.5 additional weeks and their milk was analyzed at intervals.

Methods of Analysis

Aldrin and Dieldrin. Milk samples, 300 grams, from cows fed either aldrin or dieldrin in their rations were cleaned up and analyzed for dieldrin according to the Shell method series (*10*), which is a modification of the method of O'Donnell, Johnson, and Weiss (*7*).

Heptachlor. The epoxide of heptachlor was determined by first extracting 75-gram milk samples with colorimetric pentane in centrifuge bottles, then following the method of cleanup and analysis used by Fahey (*4*). Final determination of heptachlor epoxide was done by reacting it with a reagent—composed of $0.5M$ ethanolamine and $0.5M$ potassium hydroxide in Butyl Cellosolve—designed originally by Polen and Silverman (*8*) for the determination of heptachlor.

DDT. Following the extraction of 75-gram milk samples with *n*-hexane, DDT was separated from the butterfat by chromatography on a sulfuric acid–Celite column after the method of Davidow (*3*). Analyses were run by the method of Pontoriero and Ginsburg (*9*).

Methoxychlor. After extraction of 75-gram samples with *n*-hexane, partition distribution with nitromethane *vs.* *n*-hexane was used to separate methoxychlor from butterfat. Final color development was with sodium methylate. Both the cleanup and analytical procedure are described by Claborn and Beckman (*2*).

Table I. Dieldrin and Heptachlor Epoxide Excreted in Milk of Dairy Cows as a Result of Feeding Various Levels of Insecticides

Time, Days	In Milk,[a] P.P.M.											
	Aldrin, P.P.M. on Ration[b]			Time, Days	Dieldrin, P.P.M., on Ration[b]			Time, Days	Heptachlor, P.P.M., on Ration[c]			
	40	10	1		75	50	10		200	100	75	50
After Start of Intake												
1	0.06	...	...		0.07	0.08	...		0.15	0.07	...	...
2	1.49	0.31	...		0.17	0.15	0.09		0.32	0.13	...	...
3	2.82	0.82	0.09		...	2.11	0.31		0.64	0.15	0.07	0.05
7	5.22	1.18	0.12		1.61	2.18	1.10		1.40	0.60	0.32	0.24
14	9.80	1.04	0.18		2.32	3.57	1.22		1.79	0.60	0.36	0.29
28	10.01	2.69	0.32		6.68	3.86	1.27		1.87	0.81	0.44	0.39
42	12.46	2.41	0.27		9.20	8.93	1.66		2.29	1.39	0.53	0.47
49	12.27	2.22	0.28		...	8.94	1.62		2.77	0.93	0.51	0.39
56	14.96	2.39	0.33		12.33	10.32	1.15		3.20	...	0.79	0.41
70	15.45	2.51	0.39		13.02	8.22	1.18		3.87	1.17	0.87	0.69
77	13.66	...	0.33		...	10.08	1.03		...	...	...	...
84	...	2.45	0.33		12.89	9.40	1.19		3.73	1.19	0.92	0.63
91	13.75	2.35	0.35		...	9.47	1.22		4.20	1.41	1.25	0.84
98	14.57	2.09	0.37		13.35	11.10	1.71		4.20	1.71	1.37	1.23
105	13.95	1.94	0.35		...	12.10	1.37		4.27	1.08	0.97	0.91
112[d]	16.10	3.42	0.41		13.36	10.96	1.78		4.14	1.86	1.52	1.13
112-p.p.m. in fat	...	31.58	...		...	123.7	...		...	17.24	...	...
After Removal from Intake												
1	12.00	...	0.39	1	Cow went	...	1.26	1	3.93	...	1.50	1.10
7	9.07	...	0.35	7	dry	...	0.76	3	3.97	...	1.33	1.04
14	5.00	...	0.23	14		...	0.69	5	3.50	...	1.25	0.97
21	0.98	...	0.19	21		...	0.47	7	3.33	...	1.03	0.86
28	0.77	...	0.18	28		...	0.34	9	3.37	...	0.85	0.78
35	0.73	...	0.12	35		...	0.28	11	3.19	...	0.81	0.64
39	...	...	0.08	39		...	0.19	13	3.09	...	0.79	0.60
								15	2.20	...	0.61	0.50
								17	1.81	...	0.44	0.25

[a] Corrected for checks.
[b] Sensitivity 0.01 p.p.m.
[c] Sensitivity 0.04 p.p.m.
[d] Average four consecutive milkings.

Table II. DDT and Methoxychlor Excreted in Milk of Dairy Cows as a Result of Feeding at Various Levels of Intake

Time, Days	In Milk,[a] P.P.M.								
	DDT, P.P.M. on Ration[b]				Time, Days	Methoxychlor, P.P.M. on Ration[b]			
	200	100	25	10		7000	4000	1000	800
After Start of Intake									
1	0.65	0.52	...	...		0	0	...	...
2	2.80	2.07	...	...		0	0.10	...	...
3	2.97	2.04	...	...		0.60	0.15	0.07	0.08
7	3.67	1.93	0.58	0.28		0.83	0.43	0.21	0.17
14	3.19	3.28	0.73	0.33		0.85	0.34	0.16	0.06
28	3.24	2.60	1.01	0.33		1.08	0.36	0.11	0.06
42	4.62	3.27	1.25	0.47		0.65	0.38	0.13	0.07
49	3.64	3.65	1.74	0.57		0.55	0.44	0.13	0.15
56	5.91	4.69	2.18	0.48		1.56	0.38	0.12	0.13
63	5.66	4.31	1.56	0.52		1.24	0.32	0.05	0.07
70	4.53	4.58	2.16	0.61		1.85	0.80	0.04	0.08
77	5.32	3.81	2.33	0.44		1.33	0.87	0.12	0.09
84	6.07	4.60	2.64	0.60		2.01	0.56	0.08	0.06
91	4.58	3.95	2.11	0.66		2.25	0.43	0.16	0.13
98	5.39	3.86	2.72	0.64		2.35	0.50	0.17	0.06
105	4.51	3.35	2.21	0.59		0.86	0.29	0.17	0.18
112[c]	6.00	4.06	2.29	0.63		2.14	0.51	0.19	0.13
112-p.p.m. in fat	...	65.4	...	...		...	4.93	...	...
After Removal from Intake									
1	4.60	...	2.51	0.73	1	0.40	...	0.11	0.10
4	2.13	...	1.12	0.49	3	0.11	...	0.08	0.03
7	1.61	...	0.88	0.36	5	0.09	...	0.03	0.07
10	1.05	...	0.60	0.05	7	0.07	...	...	0.01
13	0.83	...	0.39	...	9	0.07	...	0	0.07
16	0.66	...	0.16	...	11	0.06	...	0.03	0.01
					13	0.04	...	0.02	0.04
					15	0.05	...	...	0.02

[a] Corrected for checks.
[b] Sensitivity 0.04 p.p.m.
[c] Average four consecutive milkings.

Results and Discussion

The data on the amounts of dieldrin and heptachlor epoxide excreted in milk as a result of feeding aldrin, dieldrin, and heptachlor respectively are presented in Table I. Similar data for DDT and methoxychlor are shown in Table II. These tables demonstrate the concentration build-up for the various dosages of the insecticides over a 16-week period and the decline when the feeding of contaminated rations was discontinued. Tables I, II, and III show that of the materials tested, aldrin has the greatest propensity for storage—as dieldrin—in fat and excretion in milk. At comparable dosages, more dieldrin was stored in fat and excreted in milk when aldrin was fed than when dieldrin itself was fed.

DDT was next in value and appeared in milk at higher levels than did heptachlor epoxide—as a result of feeding heptachlor at comparable dosages. Methoxychlor showed very little tendency to be stored in fat or to be excreted in milk regardless of the large dosages fed. Claborn and his associates (*1*) found that this same order prevailed as a result of spraying cows with the various materials rather than feeding them.

The relative rates of excretion in milk as presented in Table III indicate that

Table III. Propensity of Various Insecticides for Storage in Fat and Excretion in Milk of Dairy Cows Fed Insecticides in Their Rations

Compound	*P.P.M. in Diet*	*P.P.M. in Milk at 16 Weeks*	*P.P.M. Excreted at 16 Weeks per P.P.M. in Diet*	*Rate of Excretion Relative to Methoxychlor as 1*	*P.P.M. in Fat at 16 Weeks*	*P.P.M. Stored in Fat at 16 Weeks per P.P.M. in Diet*	*Rate of Excretion Relative to Methoxychlor as 1*	*Ratio, P.P.M. in Fat to P.P.M. in Milk*
Aldrin[a]	40	16.10	0.40					
	10	3.42	0.34		31.58	3.16	2633	9.23
	1	0.41	0.41		...			
Av.	17.0	6.64	0.391	1685				
Dieldrin	75	13.36	0.18					
	50	10.96	0.22		123.7	2.47	2058	11.29
	10	1.78	0.18		...			
Av.	45.0	8.70	0.193	832				
DDT	200	6.00	0.0300					
	100	4.06	0.0406		65.4	0.65	542	16.11
	25	2.29	0.0916		...			
	10	0.63	0.0630		...			
Av.	83.75	32.45	0.0387	167				
Heptachlor[b]	200	4.14	0.0207		...			
	100	1.86	0.0186		17.24	0.17	142	9.27
	75	1.52	0.0203		...			
	50	1.13	0.0226		...			
Av.	106.25	2.16	0.0203	88				
Methoxychlor	7000	2.14	0.00032		...			
	4000	0.51	0.00013		4.93	0.0012	1	9.85
	1000	0.19	0.00019		...			
	800	0.13	0.00016		...			
Av.	3200.00	0.74	0.00023	1				

[a] Excreted in milk and stored in fat as dieldrin.
[b] Excreted in milk and stored in fat as heptachlor epoxide.

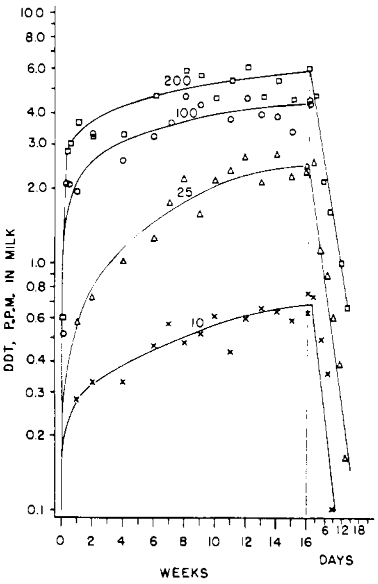

Figure 1. DDT in milk at indicated intervals when:

Cows were fed 10, 25, 100, and 200 p.p.m. of DDT in the daily ration for 16 weeks and 1–16 days after chemical intake was discontinued (semi-log relationship)

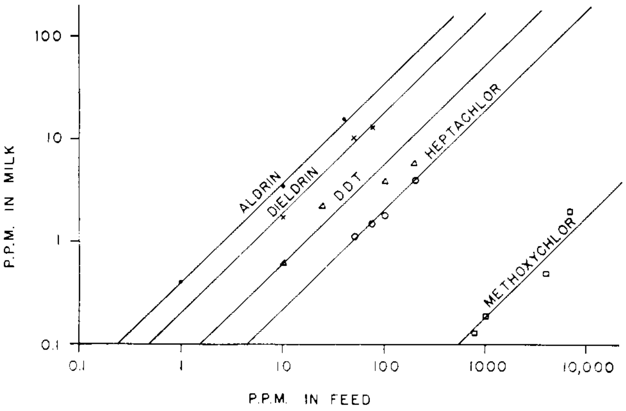

Figure 2. Relative amounts of various insecticides found in milk when fed at the rates indicated in the daily diet for 16 weeks (log-log relationship)

aldrin is excreted—as dieldrin—roughly twice as readily as dieldrin itself, 10 times as readily as DDT, 20 times as readily as heptachlor—as heptachlor epoxide—and 1600 times as readily as methoxychlor. Propensity for storage in fat follows the same order with variations in magnitude. Methoxychlor was lost most rapidly. While DDT was lost less rapidly than methoxychlor, it was lost considerably faster than the cyclodiene insecticides.

As pointed out by the authors in a related paper (*5*) using dieldrin and by Kiigemagi, Sprowls, and Terriere (*6*), using endrin, the common belief that fat should contain about 25 times as much insecticide as milk does not hold true. Ratios of parts per million in fat to parts per million in milk for the various insecticides tested here ranged between 9 and 16 to 1 rather than 25 to 1.

When the insecticides were removed from the diet and the animals were supplied only uncontaminated feed for a period of time, with the exception of aldrin and heptachlor, the insecticides tested tended to disappear from milk samples in the inverse order of their propensity for storage. Methoxychlor was lost most rapidly. While DDT was lost less rapidly than methoxychlor, it was lost considerably faster than the cyclodiene insecticides. In the case of aldrin, which was stored as dieldrin, the rate of loss was characteristic of dieldrin, which offset its greater propensity for storage. On the other hand, in the case of heptachlor, which was stored as heptachlor epoxide, the rate of loss was comparable to that of dieldrin and thus heptachlor lost some of the advantage of its low propensity for storage.

Abundant references in the literature state, and a casual inspection of the data presented may lead to the conclusion, that the concentration of an insecticide in milk will rise rapidly for a matter of a few hours or days following initiation of the experiment and then level off at a plateau characteristic for each concentration of chemical in the diet. A closer inspection of the data suggests the possibility that the concentration of insecticide may continue to show slight increase over a period of several months. In considering DDT as a typical example, Figure 1 illustrates the trends developed during the periods of storage and dissipation.

Figure 2 is presented to show graphically the relative propensities for storage and/or excretion of the insecticides tested at the end of the 16-week feeding period.

Weight gains and milk production of treated cows were comparable to those of untreated animals. Throughout the feeding period, and at slaughter, in spite of some of the high levels of insecticide fed, an examination of organs and tissues failed to show any evidence of pathology.

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HERBICIDE STRUCTURE AND ACTIVITY

The Action of Alkyl *N*-Phenylcarbamates on the Photolytic Activity of Isolated Chloroplasts

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Inhibition of the photolytic activity of isolated turnip green (*Brassica* spp.) chloroplasts by various alkyl *N*-phenylcarbamates was investigated. All highly active carbamates possessed a free imino hydrogen atom. Substitution of this hydrogen by an alkyl or aryl group resulted in a loss in, or decrease of, inhibitory power. Possible roles played by the various substituents of the carbamate molecule in inhibiting the photolytic reaction are discussed. Responses obtained were correlated with the herbicidal activity of the same compounds reported by other workers from greenhouse studies. In both the laboratory and greenhouse studies maximum activity was obtained with the propyl and butyl esters and when the ring was halogenated in the meta position.

The action of esters of carbamic acid and *N*-substituted carbamic acids on animals and plants has held the interest of investigators for many years. Initially the compounds most intensively studied were ethyl carbamate (urethan, EC) and ethyl *N*-phenylcarbamate (phenylurethan, EPC). Research on this group of compounds was given new impetus when the herbicidal properties of isopropyl *N*-phenylcarbamate (IPC) and isopropyl *N*-(3-chlorophenyl)carbamate (3-ClPC) were discovered. The last two compounds are currently being used to control weeds in certain crops.

Carbamates such as EPC and IPC have a profound effect on cell division and mitosis, and are most active as herbicides when applied to the soil and subsequently absorbed by roots. Monocotyledonous plants are more susceptible to their action than dicotyledonous plants. These effects, together with their history and development, have been concisely and adequately reviewed in the literature.

The exact mechanisms through which the carbamates (urethans) exert their phytotoxic effects are not known. EC and EPC are reported to inhibit photosynthesis of microorganisms (*25–28*). More specifically, they inhibit the photolytic cleavage of water in photosynthesis. In this photolytic (Hill) reaction (*12*) oxygen is evolved by isolated chloroplasts or chloroplast fragments under the influence of light in the presence of a suitable hydrogen acceptor. This reaction can be considered as "photosynthesis with a substitute oxidant" or photosynthesis without carbon dioxide fixation (*2*). As chloroplasts (*8, 13, 16, 29*) and chloroplast fragments (*3, 4*) have been obtained from different sources and different techniques have been used to follow the course of the photolytic reaction, the amount of inhibition obtained by different investigators with a particular concentration of an inhibitor has shown some variation.

The herbicidal properties, together with the selective action of many of the *N*-phenylcarbamates, have been studied and reported by Shaw and Swanson (*20*) and George *et al.* (*10, 11*). The compounds were applied by Shaw and Swanson as pre-emergent sprays. George *et al.* reported results based on studies with both pre- and postemergent applications.

The specific objectives of this study were to investigate relationships between structural configurations of alkyl *N*-phenylcarbamates and their ability to inhibit the photolytic activity of isolated chloroplasts, and to correlate these responses with the herbicidal properties of the same compounds which have been evaluated by other workers under field and greenhouse conditions.

Materials and Methods

Chloroplasts were isolated from turnip greens (*Brassica* spp.), obtained through commercial sources, by the procedure of Spikes *et al.* (*21*). After the initial isolation, the chloroplasts were washed three times with $0.5M$ sucrose.

The photochemical activity of the isolated chloroplasts was measured in 50-ml. beakers. A 10-ml. reaction mixture which had the following composition was used: $0.0002M$ potassium ferricyanide, $0.01M$ potassium chloride,