Determination of Methoxychlor and/or Metabolites in Milk Following Topical Application to Dairy Cows

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Possible excretion of methoxychlor or its chlorine-containing metabolites in milk following topical application of this insecticide has been studied using two independent methods of analysis. Direct dusting at recommended levels of 10 grams of 50% powder per animal produced no detectable residues in milk. Spray applications at normal dosages resulted in traces of methoxychlor shortly after treatment, but the excretion level decreased rapidly with time. Repeated topical applications produced no increase in the excretion levels. Organic chlorine compounds other than methoxychlor were not detected.

Livestock ectoparasites such as biting or bloodsucking flies and lice can, if uncontrolled, cause annual losses of as much as half a billion dollars (*10*). These potential losses would result from damage to hides and meat, loss in weight and milk production, and ineffective utilization of feed. Methoxychlor, 1,1,1-trichloro - 2,2 - bis(*p* - methoxyphenyl)-ethane, applied directly to cattle is effective in combating certain of these pests. It controls hornflies and lice, and, in addition, affords protection against stable flies, horse flies, and mosquitos. Because it is well suited for the treatment of dairy cows, the excretion of methoxychlor in milk, following topical applications, has been the subject of considerable study.

Shortly after the introduction of methoxychlor, Carter *et al.* (*2*) studied the excretion of various chlorinated insecticides in milk following topical applications. Using an analytical procedure which was sensitive to a few tenths of a part per million of organic chlorine, he was unable to detect methoxychlor in milk following topical application of wettable-powder suspensions containing 0.5% methoxychlor. These results, coupled with the low mammalian toxicity of methoxychlor (*8, 11*), the even lower toxicity of its metabolites (*9*), and the low retention of methoxychlor in body tissues (*11*) indicated the safety of this pesticide for use on dairy cattle. Following this early work, somewhat conflicting results have appeared in the literature. Claborn and Wells (*5*) reported the detection of 0.7 p.p.m. of methoxychlor in the milk of a single cow, 1 day after spraying with a 0.5% emulsion of methoxychlor, and up to 0.4 p.p.m. after spraying with the same concentration of a wettable-powder suspension. Helrich *et al.* (7), working with herds of 4 to 6 animals, reported residues of a few tenths of a part per million of methoxychlor in milk shortly after spraying. On the other hand Birk and Dixon (*1*) and Cheng *et al.* (*3*) published data showing that less than 0.1 p.p.m. of methoxychlor is excreted in milk following topical applications.

The studies reported in this paper were conducted to re-examine the question of excretion of methoxychlor or its chlorine-containing metabolites in milk following topical application to cows. Methoxychlor was applied to cows by spraying and hand dusting at normal levels and recommended intervals, using commercial formulations. The effects of applying gross overdosages of methoxychlor were also investigated. To minimize variations caused by differences in individual animal responses, most of these tests were conducted on the same group of Guernsey-Jersey grade dairy cows, and composite milk samples were analyzed.

Two independent analytical methods were used to characterize the milk samples, one based on a selective colorimetric reaction, the other on total organic chlorine. Several sensitive colorimetric reactions have been employed for detecting traces of methoxychlor (*4*). The method used in this study is based on the reaction employed originally by Fairing (*6*). It is sensitive to a few micrograms of methoxychlor; DDT, lindane, toxaphene, and many other pesticidal chemicals do not respond. However, fats and oils must be carefully removed from methoxychlor residues to avoid interferences caused by the charring action of sulfuric acid on such substances.

Organic chlorine was chosen as the general index for metabolites because of its sensitivity, and because of the likelihood that such materials would contain chlorine. The procedure used in this investigation involves solvent extraction of whole milk, a Pregl catalytic combustion of the extract residue (*13*), and, finally, spectrophotometric determination of the resulting chloride (*15*).

Catalytic combustion was used because it requires only small amounts of a few reagents, an advantage for obtaining an over-all low chlorine blank. Using a platinum-silver preburner to purify the tank oxygen, the combustion procedure contributed only 2 γ of chloride to the blank in control milk.

Experimental

Animal Treatment In conducting these tests, detailed precautions were taken to avoid exogenous contamination. The cows were stanchioned in alternate stalls of a conventional dairy barn, and long hair was clipped from their udders and flanks. All applications of methoxychlor were made in a closed pen, remote from the dairy barn. During spraying, the udders were shrouded in cotton sacking and care was taken not to direct methoxychlor onto the shrouded areas. The cows remained tied until the spray had dried, after which the udder shrouds were removed and the cows returned to their stanchions. In the dusting tests, weighed amounts of powder were topically applied, using a shake-can, to the topline and sides of the cows. Working forward from the rump, the hair coat was raised by hand, and the dust was applied, gently rubbed into the hair and onto the skin. The cows were returned to their stanchions immediately after being dusted.

The cows were milked twice daily with a machine milker. Prior to each milking their udders were thoroughly sponged

and rinsed and the milking machines were cleaned with methanol, a good solvent for methoxychlor. After milking, the machine was removed to the laboratory where an appropriate aliquot was withdrawn. Butterfat content of composite milk samples, determined by the detergent method described by Sager and Sandars (*12*), averaged 5.0% during the test period, ranging from 4.6 to 5.4%.

Colorimetric Method for Determining Methoxychlor in Milk

Reagents and Apparatus. Dehydrohalogenation reagent, 4% (w./v.) of reagent grade potassium hydroxide in 95% ethanol, freshly prepared before use.

Colorimetric reagent, 85% sulfuric acid (82.5 to 88.0% is acceptable), containing 10 mg. of ferric chloride per liter.

Chromatographic column, 1-to-1 weight ratio of Celite No. 545 and heavy, adsorptive magnesia, U.S.P. grade. The composition is slurry mixed in redistilled hexane, dried, and dry-packed to a depth of 60 mm. in an all-glass, coarse, fritted-disk filter tube (100 × 20 mm.).

Procedure. One hundred grams of whole milk, 100 ml. of 95% ethanol, and 200 ml. of redistilled, commercial grade *n*-hexane are added to a 1-liter separatory funnel, and the mixture is shaken vigorously for at least 2 minutes. The resulting emulsion is divided into two equal portions, transferred to two 250-ml. centrifuge bottles, and centrifuged for approximately 5 minutes at 1800 to 2000 r.p.m. The upper (hexane) layers are removed, and transferred to a 600-ml. beaker.

An additional 100-ml. portion of hexane is added to each of the centrifuge bottles. The bottles are stoppered with polyethylene plugs and again shaken thoroughly. The contents are centrifuged, and the layers are separated as described above. The hexane layers are added to those previously collected, and the hexane extraction is repeated a third time.

The combined hexane extract is concentrated to approximately 125 ml. at room temperature, using a slow stream of oil-free air to hasten evaporation. The concentrated extract is transferred to a 500-ml. separatory funnel, 50 ml. of redistilled, technical grade nitromethane is added, and the contents are shaken vigorously for at least 2 minutes. When the layers have separated, the nitromethane is transferred to a second 500-ml. separatory funnel, containing about 50 ml. of hexane.

The original hexane is extracted with three additional 50-ml. portions of nitromethane as described above, each time draining the nitromethane layer into the second separatory funnel. The combined nitromethane extract is shaken with the 50 ml. of hexane to remove traces of interfering substances which had partitioned into the nitromethane. After phase separation, the nitromethane layer is drained into a 400-ml. beaker and carefully evaporated on a steam bath to a volume of approximately 25 ml. This residue is transferred quantitatively to a 250-ml. standard taper Erlenmeyer flask, using small portions of nitromethane for washing. The solvent is then carefully removed on a steam bath, using a current of air. To avoid losses of methoxychlor, solvent evaporation should be taken just to dryness.

Fifty milliliters of the alcoholic potassium hydroxide solution is added, the flask is attached to a water-cooled condenser, and the contents are refluxed gently for 30 minutes. The flask is cooled, the condenser is rinsed with 50 ml. of distilled water, and the entire contents of the flask are transferred to a 500-ml. separatory funnel with the aid of 100 ml. of petroleum ether (C.P. grade with boiling range of 35° to 60° C.) which has been passed through a column of chromatographic alumina. The contents of the separatory funnel are shaken thoroughly and allowed to separate, and the petroleum ether layer is removed and retained.

Extraction is repeated with 25 ml. of petroleum ether, and the combined extract is backwashed with 20 ml. of an equal-volume mixture of ethanol and water. The washed extract is transferred to a 100-ml. beaker and carefully evaporated to dryness, using a current of air. The residue is redissolved in 50 ml. of hexane and passed through a Celite-magnesia column by gravity flow. After the final incremental addition, the column is washed with an additional 25 ml. of hexane which is collected with the original effluent. This purified hexane solution is evaporated to dryness at room temperature in a 100-ml. beaker, using a current of air.

Twenty milliliters of the sulfuric acid color reagent is added to the residue, and the mixture is allowed to stand 1.5 hours with occasional swirling. The absorbance of the resultant pink solution is then determined in a 1-cm. cell at 550 mμ, using sulfuric acid reagent in the reference cell. The amount of methoxychlor in the sample is determined from a calibration curve prepared from known amounts of pure methoxychlor which have been subjected to the dehydrochlorination and color development steps described above.

Low blanks were obtained on untreated milk with the colorimetric method, averaging 0.02 p.p.m. with an average deviation of 0.01 (Table I). All methoxychlor values reported in this paper are corrected for this 0.02 average blank. In view of the level and variability of the blank, net methoxychlor values less than 0.02 cannot be considered

Table I. Apparent Methoxychlor Level in Pretreatment Milk

Designation[a]	Apparent Methoxychlor, P.P.M.
Delaware herd	
1	0.02
2	0.03
3	0.00
4	0.04
5	0.02
6	0.01
Texas herd A	
1	0.02
2	0.02
Texas herd B	
1	0.01
2	0.03
	Av. 0.02 ± 0.01

[a] Numbers indicate daily composites on different days.

Table II. Recovery of Known Amounts of Methoxychlor Added to 100 Grams of Whole Milk

Methoxychlor, γ		Recovery,	P.P.M.
Added	Found	%	Level
2.5	2.3	92	0.025
5.0	4.7	94	0.05
5.0	5.0	100	0.05
5.0	4.5	90	0.05
10.0	9.5	95	0.10
10.0	9.0	90	0.10
10.0	8.5	85	0.10
15.0	13.5	90	0.15
25.0	24.0	96	0.25
25.0	22.4	89	0.25
		Av. 92	

Table III. Stability of Methoxychlor in Whole Milk

Treatment	Methoxychlor, γ	
	Added	Found
None	20	17.0
Stored 3 days at room temperature	20	18.5
Stored 5 days at room temperature	20	17.5

significant. Satisfactory recoveries were obtained on known amounts of methoxychlor added to untreated milk (Table II). The stability of methoxychlor in milk at microgram levels was demonstrated (Table III); thus, the delays which necessarily occurred between milking and analysis were insignificant.

Method for Determining Total Organic Chlorine in Milk

Reagents and Apparatus. Diethyl ether, *n*-hexane, nitromethane, and methanol (reagent grade solvents) are redistilled through a 9-inch Fenske column or equivalent, discarding a 10% forecut and leaving a 15% bottom.

Deionized water with total electrolyte content preferably less than 0.1 p.p.m. as sodium chloride.

Reducing solution (prepared immedi-

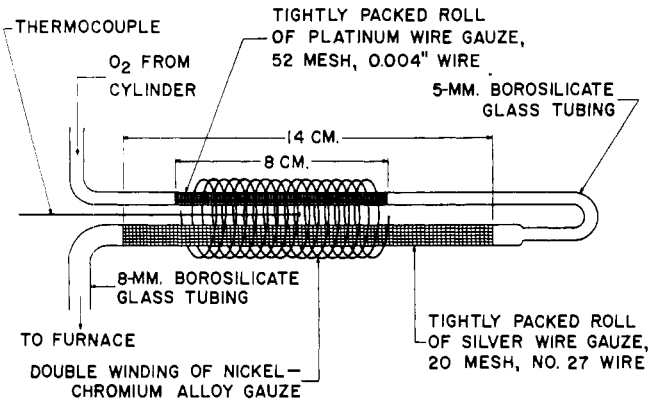

Figure 1. Oxygen preburner

ately before use) contains 5 drops of saturated hydrazine sulfate solution in 5 ml. of a 5% solution of reagent grade sodium carbonate. Deionized water is used throughout.

Urea solution (10%) is prepared in deionized water, using recrystallized urea. Recrystallization is accomplished by dissolving 60 grams of reagent grade urea in 50 ml. of deionized water at 30° C., filtering this solution through a fritted-glass funnel, and cooling to 5° C. The crystals are filtered off on a fritted-glass filter and dried overnight in a vacuum oven at 60° C.

Ferric perchlorate solution is prepared by extracting 20 to 30 grams of colorless ferric perchlorate (G. Frederick Smith Chemical Co.) with small volumes of 72% perchloric acid in a glass-stoppered flask. The acid is decanted and the extraction repeated until the acid layer no longer appears yellow. The final extraction mixture is filtered through a coarse fritted-glass funnel, and the crystals are pressed with a glass rod to remove as much of the acid as possible. Nine grams of purified ferric perchlorate is dissolved in 20 ml. of deionized water and 1 liter of 72% perchloric acid is added. The reagent is stored in a Machlett-type closed-system, all-glass buret. No lubricant is used on the stopcock and the drying tubes are filled with indicating anhydrous aluminum oxide.

Combustion Assembly. An oxygen cylinder, pressure reducing regulator, glass wool filter, flowrater, and preburner (Figure 1) are connected in that order by means of pure gum rubber tubing. The preburner is mounted horizontally and heated directly under the nickel-chromium alloy gauze with a Tirrell burner equipped with a fish-tail flame spreader. The flame is adjusted so that the temperature is between 680° and 700° C., as measured by the thermocouple. Neither end of the silver wire gauze should be covered with the nickel-chromium alloy gauze, nor should it receive any direct heat from the flame. The oxygen cylinder pressure is reduced to 20 pounds, and a flow rate of 4 to 5 ml. per minute through the flowrater is maintained by means of a needle valve. (The flowrater is calibrated with oxygen by means of a wet-test meter.) Oxygen must not be permitted to flow through the preburner unless the latter is heated to temperature. The free end of the preburner is connected by means of a section of gum rubber tubing to a small glass tube inserted in a one-holed cork stopper that fits the open end of the combustion tube.

The combustion tube is held vertically in a suitable clamp with the spiral end downward. The tip is immersed in 5 ml. of reducing solution contained in a shortened 15-cm. test tube. Gentle suction is applied to the open end of the combustion tube, so that the reducing solution is drawn up to cover only half the length of the spiral. The reducing solution is allowed to drain back into the test tube. The operation is repeated, again wetting one-half the length of the spiral and allowing the reducing solution to drain. The solution is poured from the test tube, leaving the latter moist. The procedure just described for wetting the spiral is repeated precisely from one analysis to another, in order to maintain a constant blank.

The combustion tube is then placed in the furnace rack, the entire spiral portion extending beyond the long burner, so that it will receive no heat during the combustion. The test tube, still moist with reducing solution, is placed over the tip of the combustion tube. Two clean platinum contact stars are placed in the section which is surrounded by the long burner. The contact stars are placed so that the wire loop of each is about 2 cm. inside the ends of the burner.

Procedure. A 100-gram sample of whole milk and 100 ml. of absolute ethanol are added to a 500-ml. separatory funnel (no lubrication on ground-glass joints). Five milliliters of 3.6*N* sulfuric acid in deionized water is added, and the contents of the funnel are mixed well. One hundred milliliters of 3-to-1 redistilled ether–redistilled hexane mixture is added and the separatory funnel is shaken gently for 3 minutes. The contents of the separatory funnel are poured into a 500-ml. borosilicate glass centrifuge bottle and the mouth of the bottle is covered with dental dam, held in place with an elastic band. The mixture is centrifuged for 5 minutes at about 1800 r.p.m. (explosion-proof centrifuge preferred).

The upper phase is transferred to a 250-ml. beaker with the aid of a 50-ml. pipet and suction bulb. Contamination with the lower phase is carefully avoided. The ether-hexane extraction is repeated three more times in the centrifuge bottles, using 50-ml. volumes of the solvent mixture, shaking vigorously for 3 minutes, and centrifuging as before. During these extractions, a rubber stopper covered with aluminum foil is used to seal the centrifuge bottle. The combined extract is evaporated to 150 to 175 ml. Contamination by dust, etc., is carefully avoided throughout the procedure.

The concentrated ether-hexane solution is transferred to a 500-ml. separatory funnel, and 100 ml. of 1-to-1 absolute ethanol–deionized water solution, adjusted to pH 2 to 3 with sulfuric acid, is added. The mixture is shaken for 3 minutes and the layers are allowed to separate. The lower aqueous layer is drained into a second separatory funnel and the ether-hexane layer is returned to the original beaker. The aqueous layer in the second separatory funnel is further extracted with three 50-ml. portions of 3-to-1 ether-hexane. Before each of these extractions, the first separatory funnel is washed with the solvent mixture. Following each extraction, the upper layer is transferred to the original beaker. The combined ether-hexane extract is evaporated to approximately 25 ml. and transferred to a 500-ml. separatory funnel with a total volume of 200 ml. of redistilled *n*-hexane. This solution is extracted successively with three 50-ml. volumes of redistilled nitromethane. The combined nitromethane extract is back-extracted with 50 ml. of redistilled hexane, transferred into a 250-ml. beaker, and evaporated on a steam bath to an oily residue. This residue, which is usually about 0.2 ml. in volume, is transferred to a 10-ml. beaker, using several small volumes of methanol, and evaporated again. Using a 0.1-ml. measuring pipet and small volumes of methanol, the oily residue is quantitatively transferred to an small platinum microcombustion boat. The boat is heated gently on a steam bath to evaporate the methanol continuously during the transfer.

The boat containing the sample is placed in the combustion tube about 5 cm. from the end of the long burner (*13*). The long burner is fixed to surround the combustion tube and heated to produce a temperature between 680° and 700° C. within the combustion tube. When this

Table IV. Chlorine Level in Pretreatment Milk

(100-gram samples analyzed)

Designation[a]	Chloride Found, P.P.M.
1a	0.17
1b	0.10
1c	0.10
1d	0.13
2a	0.10
2b	0.08
2c	0.08
Av.	0.11 ± 0.03

[a] Samples 1 and 2, milk from two different herds of cows; samples a, b, etc., composites of the daily productions of each herd on different days.

Table V. Recoveries of Methoxychlor and 1,1,1-Trichloro-2,2-bis-(p-hydroxyphenyl)ethane

(100-gram milk samples analyzed)

Chlorine Added, P.P.M.	Chloride Found[a], P.P.M.
As methoxychlor	
0.16	0.17
	0.12
	0.07
	0.09
	0.19
Av.	0.13
As 1,1,1-trichloro-2-bis-(*p*-hydroxyphenyl)ethane	
0.17	0.16
	0.12
	0.14
	0.19
Av.	0.15

[a] Corrected for Cl level in control milk (Table IV).

Table VI. Methoxychlor Excreted in Milk Following Topical Application[a]

Post-treatment Period	Amount of Methoxychlor Found in Raw, Whole Milk, P.P.M.							
	Hand Dusting			Spraying				
	Test 1[b]	Test 2[b]	Test 3	Test 4	Test 5	Test A	Test B	Test 6
6 hours	<0.02	...	...	0.02	...	...	...	...
1 day	<0.02	<0.02	0.03	0.06	0.10	0.08	0.05	0.23
2 days	<0.02	...	...	...	...	0.08	0.04	...
3 days	<0.02	<0.02	0.05	0.06	0.07	0.04	0.04	0.19
4 days	<0.02	...	...	...	...	0.02	<0.02	...
5 days	...	<0.02	...	0.04	0.03	0.02	<0.02	0.17
7 days	<0.02	...	0.04	0.02	0.02	<0.02	<0.02	0.13
14 days	...	...	...	...	...	<0.02	<0.02	...
21 days	<0.02	...	...	...	...	<0.02	<0.02	...

[a] Tests 1–6 conducted in Del. with 4 Guernsey-Jersey grade cows. Tests 1 and 2, 10 grams of Marlate 50; 3, 40 grams of Marlate 50; 4, 3 qt. of suspension containing 8 lb. of Marlate 50 per 100 gal. of water; 5, 3 qt. of emulsion containing 8 qt. of Marlate 2-MR per 100 gal. of water; 6, 3 qt. of suspension containing 32 lb. of Marlate 50 per 100 gal. of water.

Tests A and B. Separate herds of 30 cows sprayed in Tex. with 2 qt. of suspension containing 8 lb. of Marlate 50 per 100 gal. of water.

[b] Recommended treatment.

temperature is reached, the cork stopper which connects the oxygen supply is inserted into the open end of the combustion tube. The sample burner is then fixed to surround the combustion tube so that the end toward the long burner is in line with the trailing edge of the sample boat, and heated so that it produces a temperature between 680 and 700° C. in the combustion tube. Purified oxygen is allowed to flow through at the rate of 4 to 5 ml. per minute. The sample burner is moved slowly across the sample and up against the long burner, leaving it there for 10 minutes. (Caution: Heat the preburner to temperature before starting the oxygen flow.) A total of 45 minutes should be taken to complete the combustion, to ensure that a minimum of unburned material passes through to the spiral.

The burners are removed from the combustion tube and the latter is allowed to cool while oxygen is swept through. When cool, the platinum boat and contact stars are removed. The combustion tube is mounted vertically and the spiral is washed by drawing up twice, by means of mild suction, 5 ml. of deionized water contained in a 10-ml. platinum crucible. Three 1-ml. washes of deionized water are added from the top of the tube and collected in the platinum crucible. The test tube is washed with 2 or 3 small volumes of deionized water, and these washings are also added to the platinum crucible.

The sample is evaporated to dryness on a steam bath. The platinum crucible, with cover slightly displaced to allow gases to escape, is placed in a muffle furnace at 500° C. for 30 minutes; then it is removed and cooled. Deionized water (0.5 ml.) and 0.50 ml. of 10% urea solution are added to the crucible.

Ferric perchlorate reagent (7.4 ml.) is added to a 10-ml. volumetric flask, painted black nearly up to the mark. The 1-ml., urea-treated sample is transferred to the flask with the aid of a polyethylene dropping pipet, using three 0.5-ml. washes with deionized water to complete the transfer. After diluting to the mark, the flask is shaken to mix the solution, removing the stopper frequently to release pressure. The neck of the volumetric flask is covered with a small bag of opaque paper, and the solution is allowed to cool for 15 minutes. The absorbance of the sample solution is measured at 353 mμ in a 1-cm. cell against a blank which contains the same volume of hydrazine reducing solution, urea, ferric perchlorate, and deionized water. The micrograms of chloride present in the sample is determined from an appropriate calibration curve.

Calibration is carried out by adding 10, 30, 50, 70, and 100 γ of chloride (appropriate aliquots of a standard sodium chloride solution prepared to contain 100 γ of Cl^- per ml.) to a series of painted 10-ml. volumetric flasks, each containing 7.4 ml. of ferric perchlorate reagent. After dilution to the mark with deionized water, the flasks are shaken to mix the solutions, covered with opaque bags, and allowed to cool for 15 minutes. The absorbance of each solution is determined at 353 mμ in a 1-cm. cell *vs.* a blank containing only 7.4 ml. of the ferric perchlorate reagent and deionized water. Absorbance is plotted *vs.* micrograms of chloride. A new curve must be established each time a fresh ferric perchlorate reagent is prepared. If the reagent is kept over an extended period of time, occasional checks on the working curve should be made.

The average level of chlorine found in control milk was 0.11 p.p.m. with an average deviation of 0.03 p.p.m. (Table IV). This estimated precision of ±0.03 p.p.m. of organic chlorine includes both the variability of the method and possible variations in the actual chlorine content of milk from the same herd on different days and milk from different herds. Recoveries of known microgram amounts of organic chlorine as methoxychlor were excellent through the Pregl combustion procedure and the extra heating at 500° C. The recoveries obtained on milk samples "spiked" with known amounts of methoxychlor and 1,1,1-trichloro-2,2-bis(*p*-hydroxyphenyl)ethane are shown in Table V. The latter compound was included in the recovery work, because Weikel found that its polarity is similar to that of unidentified methoxychlor metabolites in rats (*14*).

Backwashing the original hexane extract with 1-to-1 ethanol–deionized water reduces the chloride blanks considerably. Without this step, control milk analyzes from 0.2 to 0.4 p.p.m. of chloride. A second cleanup of the ether-hexane was not used because fairly stable emulsions were formed. Small amounts of unidentified materials distilled through the catalytic combustion tube and interfered with the spectrophotometric determination of chloride. This interference is eliminated by heating the final chloride residue obtained from the final reducing solution for 30 minutes at 500° C. Treatment of the final perchloric acid

Table VII. Organic Chlorine Found in Milk Following Topical Applications of Methoxychlor

Treatment	Test 1, Spray with Wettable Suspension	Test 3, Hand Dusting with Overdosage	Emulsion Spray with Overdosage[a]
Formulation applied	8 lb. Marlate 50 per 100 gal. water (0.5% methoxychlor)	Marlate 50 powder	8 qt. of Marlate 2-MR per 100 gals. water (0.5% methoxychlor)
Dosage per animal	Single spray with 3 qt.	Single dusting with 40 g. powder (4 times recommended level)	18 1-qt. sprayings at about 3-day intervals
Animals used in test	4 Guernsey-Jersey grade cows	4 Guernsey-Jersey grade cows	4 Guernsey cows
	Total Amount of Organic Chlorine Found in Milk, P.P.M.		
Pretreatment (chloride blank)	0.11 ± 0.03	0.11 ± 0.03	0.11 ± 0.03
After treatment			
6 hrs.	0.14	...	...
12 hrs.	...	...	0.12
1 day	0.13	0.13	0.21
2 days	0.13	...	0.15
3 days	0.10	0.14	...
7 days	0.15	0.10	...

[a] Treatments and sample collection conducted by Helrich *et al.* (7).

solutions with urea is required to eliminate interferences from nitrite, which was identified as one of the combustion products.

Results and Discussion

Duplicate tests were run in which 10 grams of 50% methoxychlor powder was applied to each animal by direct dusting. The data under Tests 1 and 2 in Table VI show that no detectable residues of methoxychlor were present in any of the posttreatment samples analyzed, even those taken immediately following application. Direct dusting at 3-week intervals is the only method of application recommended by the manufacturer, and it is the only method of applying methoxychlor to dairy cows that is registered by the U. S. Department of Agriculture and sanctioned by the Food and Drug Administration.

Slight, but detectable, residues were present in milk shortly after spraying with aqueous suspensions or emulsions (Tests 4 and 5, Table VI). The maximum level encountered (0.10 p.p.m.) occurred 1 day after treatment, and detectable levels persisted for only 1 week. Similar results were obtained on samples of milk from two separate herds of cows sprayed by the U. S. Department of Agriculture in Kerrville, Tex. (Tests A and B, Table VI). This work showed a maximum level of 0.08 p.p.m. of methoxychlor the first day following treatment. It is interesting to note that even following spraying with an oil emulsion, the average level of methoxychlor present during the 3-week interval between treatments would amount to only a few hundredths of a part per million.

Application of four times the recommended quantity of methoxychlor by direct dusting resulted in minute, but detectable, amounts of methoxychlor in milk, attaining a maximum level of 0.05 p.p.m. (Test 3, Table VI). Wettable powder spraying at four times the normal dosage increased the level of methoxychlor in milk (Test 6, Table VI). However, even under these grossly exaggerated treatments, the level of methoxychlor in milk never exceeded 0.23 p.p.m.

The six numbered tests summarized in Table VI were all conducted on the same group of animals, over an 18-week period. At the beginning of each test, the pretreatment samples showed no methoxychlor, thus demonstrating that methoxychlor does not build up in milk following repeated treatments.

To investigate the presence of possible metabolites of methoxychlor which would not respond to the selective colorimetric method, total organic chlorine was determined on aliquots of selected samples. The samples examined were those which contained traces of methoxychlor, because these would be most likely to contain metabolites. The analytical data summarized in Table VII show only slight, if any, increase in the organic chlorine level in milk, following spraying or direct dusting with methoxychlor, even at exaggerated levels. In view of the precision and sensitivity of the organic chlorine method employed in this study, only one sample reported in Table VII contains sufficient chlorine to be of probable significance; however, the positive trend of all the net chlorine results suggests the presence of very small amounts of chlorine contaminants. These are, of course, accounted for by the known amounts of methoxychlor detected colorimetrically, thus establishing that milk from treated animals is not grossly contaminated with unidentified chlorine-containing metabolites.

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