Residues of Chlorpyrifos, Its Oxygen Analogue, and 3,5,6-Trichloro-2-pyridinol in Milk and Cream from Cows Fed Chlorpyrifos

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Cows were fed a complete ration containing chlorpyrifos at 5 levels from 0.3 to 30 ppm for 2 weeks at each level. Milk and cream samples were collected at predetermined intervals during the feeding of the chemical and for 14 days following withdrawal of the highest feeding level. Residues of chlorpyrifos (*O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate) and its oxygen analogue (*O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphate) were determined by flame photometric gas chromatography and the 3,5,6-trichloro-2-pyridinol (the principal degradate of chlorpyrifos) was determined as the trimethylsilyl derivative by electron capture gas chromatography. The methods were validated to 0.01 ppm for the three compounds in milk and 0.01 ppm for chlorpyrifos and its oxygen analogue and 0.025 ppm for 3,5,6-trichloro-2-pyridinol in cream with overall average recoveries of greater than 80%. The average residues found were 0.01 ppm of chlorpyrifos, <0.01 ppm of chlorpyrifos oxygen analogue, and 0.01 ppm of 3,5,6-trichloro-2-pyridinol in milk and 0.10 ppm of chlorpyrifos, <0.01 ppm of chlorpyrifos oxygen analogue, and <0.025 ppm of 3,5,6-trichloro-2-pyridinol in cream at the highest feeding level. Residues of all chemicals decreased rapidly upon removal of chlorpyrifos from the feed.

Lorsban brand insecticides, a product of The Dow Chemical Co. containing chlorpyrifos (*O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate), are biologically active against many species of plant, animal, and human health insects and other arthropod pests. Recommended agricultural uses include control of corn rootworms, seed-corn maggots, and peach tree borers. Other promising uses are being studied.

The diversity and increasing number of pests and insect control situations for which Lorsban insecticides may be employed have increased the need for information relating use of the products to residues in many substrates. Animals grazing pastures or crop areas recently treated with the insecticide or fed feeds prepared from such sources could potentially ingest residues of chlorpyrifos. The subsequent secretion of chlorpyrifos into milk of dairy cows ingesting residues is of prime importance. Studies (Dishburger et al., 1967) showed no residue of chlorpyrifos in milk of cows confined up to 9 days on a pasture sprayed at the rate of 0.05 lb of chlorpyrifos per acre. Chlorpyrifos administered in 50 lb of feed per day for 4 days produced no detectable residues in milk of a dairy cow (Gutenmann et al., 1968). In subsequent studies (Johnson et al., 1969), cows were fed silage prepared from corn sprayed at the dent stage of maturity with Lorsban insecticides at rates of 0.28, 0.56, and 1.12 kg of chlorpyrifos per hectare. The silage prepared from the corn contained 2.71, 5.87, and 11.59 ppm of chlorpyrifos (dry basis). Milk from the cows fed the silage for 7 weeks was free of chlorpyrifos (<0.002 ppm) and its oxygen analogue (<0.005 ppm).

Reported herein are the results of additional studies to determine the level of residues of chlorpyrifos, its oxygen analogue and 3,5,6-trichloro-2-pyridinol in milk and cream from cows fed known amounts of chlorpyrifos.

EXPERIMENTAL SECTION

The animal care and milking were conducted at the Ag-Organics Department, The Dow Chemical Co., Midland, Mich., in animal care facilities fully accredited by the American Association for Accreditation of Laboratory Animal Care. Six Holstein dairy cows, weighing 953 to 1228 lb each, were stanchioned in separated pens during the entire study. The cows were conditioned for 2 weeks in their quarters on a basal ration. Three animals were continued on the basal ration to serve as controls and three were then fed the basal ration containing chlorpyrifos at 0.3, 1, 3, 10, and 30 ppm for 14 days consecutively at each level. At the end of this time the chlorpyrifos-fortified feed was withdrawn and the animals were fed the basal ration for 14 days.

A total of 36 lb of the basal ration or basal ration plus chlorpyrifos was given each cow daily, half at each milking. Any feed not eaten before the next milking period was removed and weighed. Records of feed consumption and milk production of the animals were maintained and are shown in Table I. All of the feed provided was consumed most of the time.

The fortified feeds were prepared by blending 10 or 25% (w/w) concentrates of chlorpyrifos on silica gel with sufficient feed to make rations containing 0.3, 1, 3, 10, and 30 ppm of chlorpyrifos. The concentrates were prepared using a Dow commercial production lot of chlorpyrifos (lot

Table I. Milk Production and Body Weight of Cows Fed Chlorpyrifos

Dates		Chlorpyrifos in diet, ppm	Av daily milk production, lb					
			Control cows			Treated cows		
Started	Stopped		7417	1584	99	96	90	12
6-7	6-20-69	0	43	35	34	49	*a*	43
6-21	7-4-69	0.3	43	34	32	42	41	43
7-5	7-18	1	41	33	31	42	39	42
7-19	8-1	3	41	34	32	41	40	40
8-2	8-15	10	37	33	29	36	40	41
8-16	8-29	30	40	32	25	37	38	41
8-30	9-15	0	36	30	27	35	34	39

	Body wt, lb					
	Control cows			Treated cows		
Dates	7417	1584	99	96	90	12
6-6-70	1080	1182	1099	953	1228	1113
9-5-70	1185	1258	1210	1010	1288	1181

[a] Record sheet lost.

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no. CP523-CD235C) dissolved in acetone and mixed with silica gel.

Milk samples from each cow were obtained at predetermined times by combining 0.5 pt from the evening milking with an equal amount collected the following morning. The evening samples were stored overnight in a refrigerator. The composite samples were stored frozen in 1-pt screw-cap metal cans until analyzed. All milking was done by machine according to the same daily schedule. The control cows were milked with the same machine which was washed after milking each cow. Individual milking machines were used for the animals on treated feeds.

Cream samples were collected from morning milk only, by pooling 1.5 gal of milk from each cow of its respective group. The composited milk was separated on a DeLaval, Model 100, electric farm separator which was adjusted to give medium heavy cream (about 45% butterfat). Samples were collected in 1-pt metal cans with screw tops and immediately frozen. All excess milk and cream were destroyed by incineration.

Analytical. Gas chromatographic methods were employed for the analysis of chlorpyrifos, its oxygen analogue, and 3,5,6-trichloro-2-pyridinol. Residues of chlorpyrifos and its oxygen analogue in milk and cream were determined by the modified methods of Claborn et al. (1968b), described below. Duplicate samples of milk and cream were analyzed for 3,5,6-trichloro-2-pyridinol by Dow method ACR 71.2 (McKellar, 1971).

Gas Chromatography Equipment and Operating Conditions. A Tracor Model 160 instrument equipped with the flame photometric detector (Tracor, Inc., Austin, Tex.) operated in the phosphorus (526-nm filter) mode was used with a 4 ft × 0.25 in. glass column packed with 5% (w/w) DC 200 on Gas-Chrom Q. Flow rates are as follows: nitrogen, 120 ml/min; oxygen, 25 ml/min; and hydrogen, 200 ml/min. Temperatures were: column, 205 °C; injection point, 215 °C; and detector, 225 °C; recorder, 1 mV input; chart speed, 15 in./h.

For the 3,5,6-trichloro-2-pyridinol a Barber-Colman, Model 5000 gas chromatograph equipped with ^{90}Sr electron capture detector was used. The column was a U-shaped borosilicate glass, 3 mm i.d. × 1.83 m packed with 5% DC 200 on Gas-Chrom Z, 80–100 mesh. Carrier gas was prepurified nitrogen at a flow rate of 100 ml/min (40 psi inlet pressure), passed through a 4A molecular sieve. The column temperature was 140 °C, the injector 205 °C, and the detector 215 °C. The recorder had a 5 mV input and sensitivity at 3.3×10^{-10} A full scale; chart speed, 20 in./h.

Reagents. Methylene chloride, hexane, acetonitrile, and acetone were redistilled. Methanol and benzene were glass distilled. The silicic acid was Mallinckrodt's 100-mesh powder, analytical reagent grade (each lot of silicic acid must be calibrated to determine correct volume of eluting solvent). The Woelm acidic alumina was kept at 130 °C in a beaker covered with a watch glass and poured directly from there to the column when ready for use.

Chlorpyrifos and Its Oxygen Analogue. *Method of Claborn et al. (1968a,b) and Ivey and Claborn (1968), Modified Extraction.* Silica gel (40 g) was added to a 250-ml beaker containing 40 ml of milk or cream and mixed to a free-flowing powder. This mixture was transferred to a funnel filter containing Whatman No. 2v filter paper over a 500-ml Erlenmeyer flask. The mixture was washed with 500 ml of redistilled methylene chloride, first using some of this volume to rinse the mixing beaker. The rinse was added onto the mixture. The volume of solution in the Erlenmeyer flask was reduced to about 25 ml by means of a hot plate and 3-ball Snyder column. The remaining solvent was removed at reduced pressure (water aspirator) with intermittent, low heating of the flask. Particular care was observed during this step to avoid possible loss of chlorpyrifos or the oxygen analogue. The residue in the flask was transferred to a 500-ml separatory funnel, using 100 ml of redistilled *n*-hexane to make the transfer. Fifty milliliters of redistilled acetonitrile was added to the funnel and then shaken for 2 min. The layers were allowed to separate and the acetonitrile layer was drawn off into a 300-ml Erlenmeyer flask. The process was repeated 3 times, using 50 ml of acetonitrile each time. The collection of four acetonitrile extracts were concentrated to a volume of 10 to 15 ml by distillation with hot plate and Snyder column. The remaining acetonitrile was removed by addition and evaporation of four 20-ml portions of *n*-hexane, retaining a volume of 10–15 ml. The residue was taken to dryness at reduced pressure with intermittent heat, then dissolved in 5 ml of *n*-hexane and held for silicic acid cleanup.

Cleanup. A chromatographic column was prepared by adding, successively, a 1.5-cm layer of anhydrous sodium sulfate, 5 cm (8.5 g) of well-packed silicic acid (as received), and a 1.5-cm layer of anhydrous sodium sulfate and prewashed with 50 ml of a 7.5% (v/v) methylene chloride in *n*-hexane solution. The residue was transferred onto the column using about 20 ml of the solvent, then a total of 85 ml of solvent was added to the column. The eluate was discarded and the chlorpyrifos eluted with 200 ml of methylene chloride–*n*-hexane solvent. The column was washed with 50 ml of methylene chloride and the eluate discarded. Seventy-five milliliters of methylene chloride was added and the eluate was held for analysis of chlorpyrifos oxygen analogue.

The solvents were removed by distillation and evaporation, the last 10–15 ml with low heat and vacuum or N_2 sparge. The residues were dissolved in 2 ml of acetone and 2 μl of the respective dilutions was injected into the gas chromatograph, then diluted if necessary. The concentration of the injected aliquots was determined quantitatively by comparison of the resulting peak heights with the peak heights of standard solutions. The residue was calculated in parts per million based on the weight of the sample.

3,5,6-Trichloro-2-pyridinol. *Extraction from Milk.* A 10-g portion of milk was weighed into a 4-oz bottle. Ten milliliters of methanol, 5 ml of water, 8 g of sodium chloride, and 0.1 ml of 40:60 v/v concentrated hydrochloric acid–water solution were added. The sample was shaken 5 min with 20 ml of benzene and centrifuged. The partition was repeated with a second 20-ml portion and the benzene phases combined.

Extraction from Cream. Ten grams of cream was weighed into a 4-oz bottle. Twenty milliliters of methanol and 4 g of filter aid were added and blended on a Lourdes blender for 3 min. The sample was capped and shaken for 15 min, then filtered on a 0.5-cm pad of filter aid and rinsed to 50 ml total volume. A 20-ml aliquot of the methanolic solution was pipetted into a 4-oz bottle. Twenty milliliters of water, 8 g of sodium chloride, and 0.1 ml of a 40:60 v/v concentrated hydrochloric acid–water solution were added. The sample was shaken for 5 min with 20 ml of benzene and centrifuged. The partition was repeated with a second 20-ml portion and the benzene phases were combined.

Cleanup. A 1 × 3.5 cm column (3.5 g) was prepared by transferring acidic alumina directly from a 130 °C oven

Table II. Recovery of Chlorpyrifos, Its Oxygen Analogue, and 3,5,6-Trichloro-2-pyridinol from Milk and Cream

Chemical	ppm added	Milk			Cream		
		No. of determ.	Recovery, %		No. of determ.	Recovery, %	
			Range	Av		Range	Av
Chlorpyrifos	0.01	3	85-95	90	2	85-90	88
	0.02	2	73-84	78	1	73	
	0.03	2	84-92	88			
	0.04	1	88		1	88	
	0.05	1	98		1	98	
	0.10	1	95		1	95	
Oxygen analogue	0.01	7	85-98	92	6	78-95	87
	0.05	1	92				
3,5,6-Trichloro-2-pyridinol	0.01	6	84-92	88			
	0.025				11	68-92	83
	0.10	2	81-88	85			
	1.0	2	83-85	84			
	5.0	2	68-83	76			

into a 1-cm diameter glass liquid chromatographic column to a height of 3.5 cm and tapped lightly to settle. The benzene phase from the partition was poured onto the alumina column and allowed to run completely through, discarding the eluate. A capsule vial (12 dram) was placed under the column and the compound was eluted with 20 ml of a 40:60 v/v concentrated hydrochloric acid–water solution by applying a slight air pressure (about 2 drops/s). The eluate was shaken with 9 ml of benzene for 5 min. The benzene phase was centrifuged and decanted. The step was repeated with a second 9 ml of benzene and combined. The volume was adjusted to 20 ml. A 5-ml portion of the benzene was pipetted into a 10-ml flask and 80 μl of BSA (*N,O*-bis(trimethylsilyl)acetamide was added. The sample was diluted to 10 ml with benzene.

Four-microliter aliquots of this benzene solution were chromatographed. The heights of the peaks obtained were measured for the 3,5,6-trichloro-2-pyridinol trimethylsilyl derivative (see preparation below) in terms of percent full-scale deflection and the weight of residue found (in nanograms) was determined by reference to a standard curve derived on the same day.

Standard Curves. Standard solutions of chlorpyrifos and its oxygen analogue were prepared in acetone. The 3,5,6-trichloro-2-pyridinol silyl derivative was prepared in benzene and used to determine response curves.

The trimethylsilyl derivative of 3,5,6-trichloro-2-pyridinol was prepared as follows. Cut a bulb from an eye dropper in half across the width. Snap the prescored top off of a 1-ml ampule of BSA. Place the upper half of the cut bulb over the opening of the ampule. This serves as an airtight septum. Pipet 1 ml of a 0.025 μg/ml 3,5,6-trichloro-2-pyridinol benzene standard into a 10-ml volumetric flask. With a 100-μl syringe transfer 80 μl of the BSA to the 10-ml flask. Mix well and dilute to 10 ml with benzene. The solution is ready for injection into the gas chromatograph. Repeat the procedure with appropriate solutions to prepare other concentrations.

The efficiency of the methods for chlorpyrifos, its oxygen analogue, and 3,5,6-trichloro-2-pyridinol was determined by fortifying control samples with the appropriate chemical over a concentration range of 0.01 to 5.0 ppm and analyzing them as described above. The results are shown in Table II.

RESULTS AND DISCUSSION

Cows fed a complete dairy ration containing graduated levels of chlorpyrifos from 0.3 to 30 ppm for 2 weeks at each level showed no gross ill effects. Chlorpyrifos in the feed did not depress feed consumption, body weight, or milk production. Production of milk from all cows fed the control dairy ration or dairy rations containing chlorpyrifos decreased with time over the length of the test, but no difference was noted between the two groups.

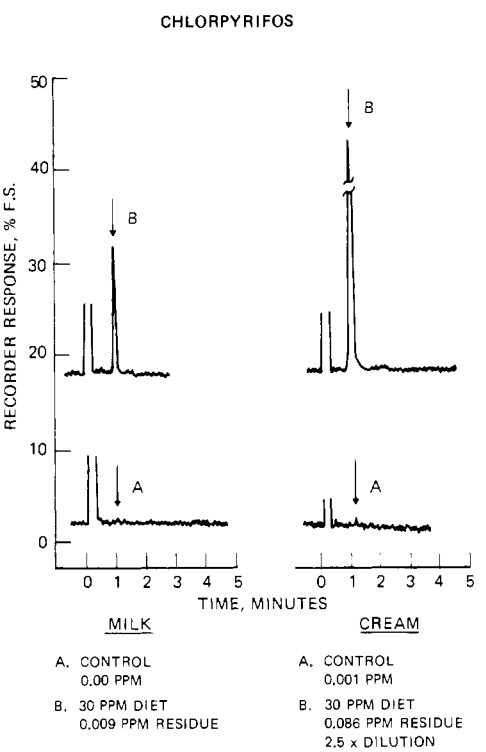

Figure 1. Typical chromatograms from the determination of chlorpyrifos in milk and cream.

The results of analyses for chlorpyrifos, its oxygen analogue, and 3,5,6-trichloro-2-pyridinol in milk and cream are summarized in Table III. Typical chromatograms are shown in Figures 1, 2, and 3.

Levels of chlorpyrifos found in milk ranged from <0.01 at 10 ppm or lower in the diet to 0.01 ppm at 30 ppm. Residues of chlorpyrifos in the cream were roughly ten times higher than in milk as might be expected since chlorpyrifos has been shown to be deposited predominantly in fat tissues (Claborn et al., 1968a). The residues of chlorpyrifos increased in milk and cream with increasing concentration of chlorpyrifos in the diet. Generally, a threefold increase in dietary level, 10 to 30 ppm, caused a threefold increase of residues in cream. Residues of chlorpyrifos in milk were too low (0.01 ppm at the highest feeding level of 30 ppm) to determine if a similar relationship exists. The pattern for 3,5,6-trichloro-2-pyridinol residues was similar to that found for chlorpyrifos in milk.

Table III. Residues of Chlorpyrifos, Its Oxygen Analogue, and 3,5,6-Trichloro-2-pyridinol in Milk and Cream from Cows Fed Chlorpyrifos

		Av residues found, ppm[a]					
		Milk			Cream (composite of 3 cows)		
Chlorpyrifos in diet, ppm	Days level fed	Chlorpyrifos	O analogue	3,5,6-Trichloro-2-pyridinol	Chlorpyrifos	O analogue	3,5,6-Trichloro-2-pyridinol
3	10				<0.01		
	11				<0.01		
	12				<0.01		
	13				<0.01		
10	3	<0.01	<0.01	<0.01			
	6	<0.01	<0.01				
	10	<0.01	<0.01		0.03		
	11	<0.01	<0.01	<0.01	0.03		
	12	<0.01	<0.01		0.03		
	13	<0.01	<0.01	<0.01	0.03		
30	3	0.01	<0.01	0.01			
	6	0.01	<0.01	0.01			
	10	0.01	<0.01	0.01			<0.025
	11	0.01	<0.01	0.01	0.10	<0.01	<0.025
	12	0.01	<0.01	0.01	0.10	<0.01	<0.025
	13	0.01	<0.01	0.01	0.09	<0.01	<0.025
		Withdrawal – 30					
0	1	<0.01					
0	3	<0.01		<0.01	<0.01		<0.025
0	4	<0.01	<0.01	<0.01	<0.01	<0.01	<0.025
0	5	<0.01	<0.01		<0.01	<0.01	

[a] Mean of samples from three cows per treatment.

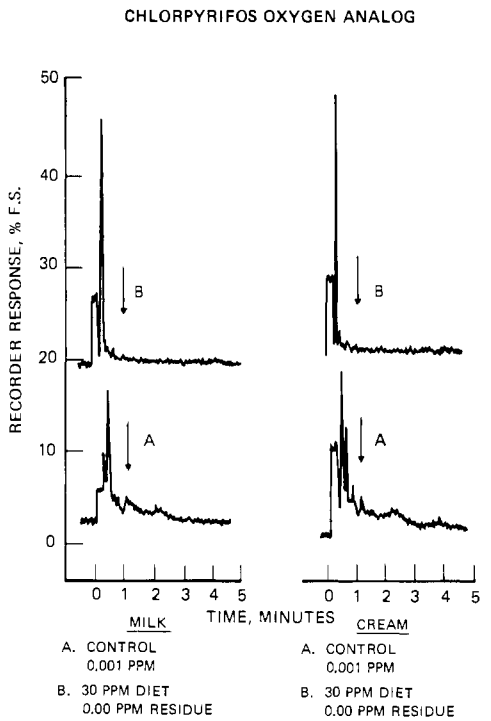

Figure 2. Typical chromatograms from the determination of chlorpyrifos oxygen analogue in milk and cream.

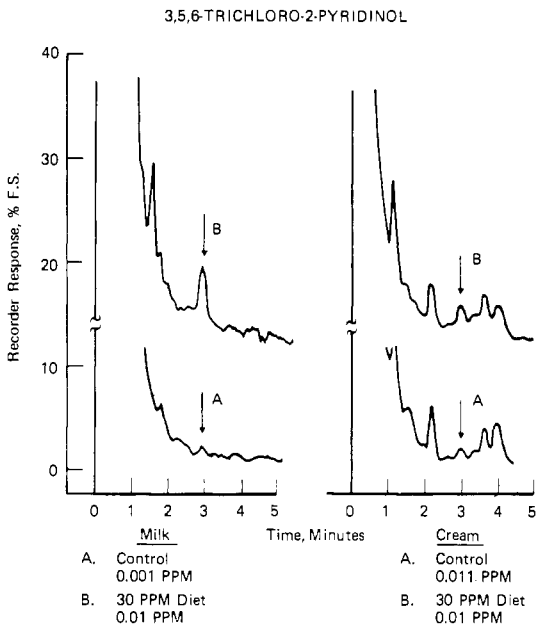

Figure 3. Typical chromatograms from the determination of 3,5,6-trichloro-2-pyridinol in milk and cream.

No residue of the pyridinol (<0.025 ppm) was found in the cream. The oxygen analogue was not detected in any sample of milk or cream at any dietary level of chlorpyrifos. Residues of chlorpyrifos and 3,5,6-trichloro-2-pyridinol disappeared rapidly from the respective substrates when chlorpyrifos was withdrawn from the diet.

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