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Determination of Residues of Chlorpyrifos, Its Oxygen Analogue, and 3,5,6-Trichloro-2-pyridinol in Tissues of Cattle Fed Chlorpyrifos

Henry J. Dishburger,* Richard L. McKellar, Joan Y. Pennington, and James R. Rice

Cattle were fed chlorpyrifos daily for 30 days at levels of 3, 10, 30, and 100 ppm. Muscle, liver, kidney, omental fat, renal fat, and subcutaneous fat were collected at the end of this period. In addition, omental fat was collected by biopsy at weekly intervals for 5 weeks following withdrawal of the highest level of 100 ppm chlorpyrifos. Residues of chlorpyrifos and its oxygen analogue were determined by thermionic or flame photometric gas chromatography. The 3,5,6-trichloro-2-pyridinol moiety as the trimethylsilyl derivative was determined by electron-capture gas chromatography. The procedures were used to quantitate chlorpyrifos and its oxygen analogue down to 0.01 ppm and 3,5,6-trichloro-2-pyridinol to 0.05 ppm. Residues of chlorpyrifos were mainly in the fat tissues and averaged 0.02 ppm (<0.01–0.05 ppm) with 3 ppm in the diet and 3.28 ppm (2.28–4.70 ppm) in fat of cattle fed 100 ppm. The 3,5,6-trichloro-2-pyridinol was predominantly in the liver and kidney and averaged 0.20 ppm (0.16–0.23 ppm) in liver and 0.11 ppm (0.09–0.15 ppm) in kidney at 3 ppm; 2.41 ppm (2.16–2.61 ppm) in liver and 1.75 ppm (1.46–1.95 ppm) in kidney at the 100 ppm feeding level. No chlorpyrifos oxygen analogue was detected in any tissue at any feeding level.

DURSBAN, trademark of The Dow Chemical Company for insecticide products containing chlorpyrifos [*O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate], as the active ingredient is effective for control of numerous soil and foliar pests of crops. The many potential uses of chlorpyrifos, certain ones of which could conceivably cause residues of chlorpyrifos in cattle feeds, prompted this study. Information relating oral intake to residues in animal tissues is essential for safe utilization of the insecticide on field crops.

McKellar et al. (1972) found residues of chlorpyrifos predominantly in fat tissue and 3,5,6-trichloro-2-pyridinol in liver and kidney tissues of swine when the animals were fed chlorpyrifos in their diet for 30 days. The levels of residues were small, <0.05 ppm, even at the highest dietary concentration of 10 ppm. The residues declined rapidly to undetectable or very low levels within 7 days after withdrawal of the insecticide from the feed. Similar results were obtained when chickens were fed chloropyrifos (Dishburger et al., 1972). To define more critically the potential residue hazards that could be encountered from such uses, a feeding study was conducted with cattle. Reported herein are the results of a study to determine the level of residues of chlorpyrifos, its oxygen analogue, and the 3,5,6-trichloro-2-pyridinol moiety in tissues of cattle fed known amounts of chlorpyrifos.

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EXPERIMENTAL SECTION

Eighteen Hereford crossbred heifers were fed chlorpyrifos for a 30-day period at levels of 0, 3, 10, 30, or 100 ppm on a daily, dry matter intake basis. The cattle were subdivided into six groups of three heifers each, one for each level through 30 ppm and two groups at 100 ppm. The subdivision was based on body weight in an attempt to minimize differences between groups. The experimental animals varied in body weight from 347 to 524 lb. The cattle in each group were penned together and allowed to share a conditioning ration composed of 50% concentrate and 50% roughage for 36 days prior to the study. At the end of the acclimation period, the feed was changed to a 75% concentrate and 25% roughage ration which was fed for the duration of the study. Chlorpyrifos (commercial production lot) was administered in gelatin capsules each morning with a balling gun and the amount given was derived from the average, daily, dry matter intake of the concentrate–roughage ration. Capsules were formulated within 7 days of use by pipetting the insecticide in acetone onto a few grams of ground concentrate in the capsule, closing the capsule, and holding them in a sealed glass container at −10 °C until fed.

All cattle fed 0, 3, 10, and 30 ppm and one group fed 100 ppm chlorpyrifos were sacrificed at the end of the 30-day period. Samples of muscle, liver, kidney, omental fat, renal fat, and subcutaneous fat were taken for residue analysis. At this time, chlorpyrifos was withdrawn from the second group on the 100 ppm level and the animals were allowed to continue on the concentrate–roughage ration. Omental

fat samples were collected from the three animals in this group by surgical biopsy at weekly intervals for 5 weeks after withdrawal of the insecticide.

Each sample was placed in a separate plastic bag, quick frozen, and shipped to the Residue Research Laboratory. Upon receipt the samples were partially thawed, ground, and refrozen until analyzed.

Analytical. Chlorpyrifos in beef tissues was determined by the method of Claborn et al. (1968a), and the chlorpyrifos oxygen analogue was determined by the method of Ivey and Claborn (1968). Both methods were modified slightly in the extraction portion and are described below. Samples of these same tissues were analyzed for 3,5,6-trichloro-2-pyridinol by the method of (McKellar, 1970) and for the same compound using alkaline hydrolysis (McKellar, 1971), both described in this paper.

The gas chromatograph used for determining chlorpyrifos and chlorpyrifos oxygen analogue in fat was a Hewlett-Packard Model 810, equipped with a hydrogen flame detector modified for thermionic detection by use of a cesium bromide pellet (Varian Aerograph), and for determining chlorpyrifos and chlorpyrifos oxygen analogue in muscle, liver, and kidney a Micro Tek (Tracor) Model 160, equipped with a flame photometric detector (FPD) utilizing a 526-nm phosphorus interference filter, was used. A Barber-Colman Model 5000, equipped with a ^{90}Sr electron-capture detector was used for determining 3,5,6-trichloro-2-pyridinol.

Gas Chromatography Equipment and Operating Conditions. The operating conditions of the gas chromatographs were: *Hewlett-Packard Model 810*, stainless steel column, 6 ft × 0.125 in. o.d. packed with 10% U.C. W-98 on 80/100 mesh Diataport S; carrier gas, helium, 200 mL/min; hydrogen, 20 mL/min; air, 200 mL/min; temperatures, column, 240 °C; injection port, 240 °C; detector, 265 °C; electrometer range 10, attenuation 4 × 32.

Micro Tek (Tracor) Model 160. Borosilicate glass column, 4 ft × 0.25 in. o.d. packed with 5% DC 200 on 80/100 mesh Gas-Chrom Q; carrier gas, nitrogen, 120 mL/min; hydrogen, 200 mL/min; oxygen, 40 mL/min; temperature, column, 205 °C; injection port, 220 °C; detector, 200 °C; electrometer range 10^4, attenuation 8.

Barber-Colman Model 5000. U-shaped borosilicate glass, column, 74 in. × 3 mm i.d. packed with 5% DC 200 on 80/100 mesh Gas-Chrom Z; carrier gas, nitrogen, 100 mL/min; temperatures, column, 135–140 °C; injection port, 205 °C; detector, 215 °C; detector operating voltage, 6 V; electrometer sensitivity, 3.3×10^{-10} amp full scale, chart speed 20 in./h.

Reagents. Analytical grade chlorpyrifos [*O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate], chlorpyrifos oxygen analogue [*O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphate], and 3,5,6-trichloro-2-pyridinol was obtained from the Sampling Coordinator, Agricultural Products Department, Dow Chemical U.S.A., Midland, Mich. *N,O*-Bis(trimethylsilyl)acetamide (BSA) was obtained from the Pierce Chemical Co., Rockford, Ill.

Analysis of Tissues for Chloropyrifos and Chlorpyrifos Oxygen Analogue. The preparation for gas chromatography was the same as described by Claborn et al. (1968a) and Ivey and Claborn (1968). Both methods were modified in that the extraction of liver and kidney tissues was carried out using methylene chloride instead of acetone.

3,5,6-Trichloro-2-pyridinol Extraction from Tissues. Ten grams of tissue was blended 3 min with 40 mL of methanol and 5 g of filter aid on a Lourdes multimixer. The spindle was rinsed with methanol. The bottle was capped and shaken for 5 min. The sample was filtered through a 0.5-cm pad of filter aid. The pad was washed with 10 mL-aliquots of methanol to a total of 100 mL. A 20-mL aliquot was pipetted into a 100-mL centrifuge tube. Fifteen milliliters of water, 8 g of sodium chloride, and 0.1 mL of 40:60 (v/v) hydrochloric acid–water solution were added, and the sample was shaken for 5 min with 20 mL of benzene and centrifuged.

Cleanup. A chromatographic column was prepared by adding 3.5 cm of Woelm acidic alumina, activity grade I (stored at 130 °C) in a 17 cm × 10 mm i.d. chromatographic tube having a coarse-sintered glass disk. The benzene phase was transferred to the column and allowed to run completely through. The column was washed with 10 mL of methanol (gravity flow). The receiver was changed (12 dram vial) and the 3,5,6-trichloro-2-pyridinol eluted with 20 mL of 40:60 (v/v) concentrated hydrochloric acid–water solution, using air pressure to regulate flow to 5–7 mL/min. One milliliter of concentrate hydrochloric acid and 9 mL of benzene were added to the eluate and shaken for 5 min, then centrifuged. The benzene phase was decanted. The eluate was partitioned again with 9 mL of benzene. The two extracts were combined and diluted to 20 mL with benzene. Five milliliters of benzene solution was transferred to a 10-mL volumetric flask and 80 μL of BSA was added. The solution was mixed well and diluted to volume with benzene, and 4 μL of the solution was injected into the gas chromatograph.

Samples of muscle, liver, kidney, and fat were reanalyzed for total residues of 3,5,6-trichloro-2-pyridinol by a method (McKellar, 1971) which includes an alkaline hydrolysis of the tissues to free "bound or conjugated" residues. Any chlorpyrifos present is hydrolyzed to 3,5,6-trichloro-2-pyridinol so that the analysis is for total pyridinol (including that contributed by chlorpyrifos).

Ten grams of tissue was heated for 25 min in a 130 °C oven in the presence of 40 mL of methanol and 5 mL of 10% sodium hydroxide contained in a 4-oz bottle and covered with a watch glass. After cooling, 4 g of filter aid was added and the sample was blended for 3 min on a Lourdes multimixer. The spindle was rinsed with methanol. The sample was capped, shaken for 15 min, then filtered through a 0.5-cm pad of filter aid. The pad and bottle were rinsed with 10-mL aliquots of methanol to a total volume of 100 mL. A 20-mL aliquot was pipetted into a 4-oz bottle and the methanol was evaporated on a hot plate to 1–3 mL. The residue was taken up in 10 mL of water. Eight grams of sodium chloride and 4 mL of concentrated hydrochloric acid were added to the sample. The sample was partitioned two times with 20-mL portions of benzene and the benzene phases combined.

Cleanup. A chromatographic column was prepared by adding 2 cm of Woelm acidic alumina, activity grade I (stored at 130 °C) in a 17 cm × 10 mm i.d. chromatographic tube having a coarse-sintered glass disk. The benzene phase was transferred to the column and allowed to run completely through. The 3,5,6-trichloro-2-pyridinol was eluted with 100 mL of diethyl ether saturated with pH 6.5 buffer solution (Handbook of Chemistry and Physics, 1963) with gravity feed. The eluate was partitioned two times with 100 mL of 0.25 M sodium bicarbonate, shaking the sample 5 min each time. The sodium bicarbonate phases were combined and washed with 50 mL of benzene by shaking the sample for 3 min, then discarding the organic phase. Six milliliters of concentrated hydrochloric acid was carefully added to the sodium bicarbonate with swirling to fully release the carbon dioxide. The sample was then partitioned with 2–9-mL portions of benzene

Table I. Recovery of Chlorpyrifos and Its Oxygen Analogue Added to Tissues of Cattle

	Chlorpyrifos				Chlorpyrifos oxygen analogue			
			% recovery				% recovery	
Tissue	ppm added	No. deter.	Range	Av	ppm added	No. deter.	Range	Av
Muscle	0.01-0.10	16	75-95	86	0.01	16	75-90	82
Liver	0.01-0.08	12	73-95	78	0.01	5	70-85	78
Kidney	0.01-0.03	10	70-80	74	0.01	4	80-95	90
Fat[a]	0.01-0.10	26	70-95	88	0.01 0.01-0.05	20	70-95	86

[a] Omental, renal, and subcutaneous.

Table II. Recovery of 3,5,6-Trichloro-2-pyridinol Added to Tissues of Cattle

	Without hydrolysis				With hydrolysis			
			% recovery				% recovery	
Tissue	ppm added	No. deter.	Range	Av	ppm added	No. deter.	Range	Av
Muscle	0.05-5	12	84-96	89	0.10	2	92-94	92
Liver	0.05-5	14	70-100	88	0.10	2	98-104	101
Kidney	0.05-5	12	66-98	82	0.10	2	84-88	86
Fat[a]	0.05-5	13	68-96	81	0.10	1		98

[a] Omental, renal, and subcutaneous.

which were combined in a 25-mL graduated cylinder and diluted to 20 mL total volume with benzene. Five milliliters of the benzene solution was transferred to a 10-mL volumetric flask and 10 μL of *N,O*-bis(trimethylsilyl)-acetamide was added. The sample was mixed well, diluted to volume with benzene, and 4 μL was injected into the gas chromatograph.

Typical chromatograms for chlorpyrifos, its oxygen analogue, and 3,5,6-trichloro-2-pyridinol as the trimethylsilyl derivative in muscle, liver, kidney, and fat are shown in Figures 1, 2, and 3. The retention times of chlorpyrifos and chlorpyrifos oxygen analogue as indicated by the chromatograms are different for the fat sample analysis because they were done at an earlier date using a different gas chromatograph than the muscle, liver, and kidney determinations. The numbers reported under the typical chromatograms in Figures 1, 2, and 3 are gross values, whereas numbers reported in the tables are corrected values.

Standard Curves. Standard solutions of chlorpyrifos and its oxygen analogue were prepared in acetone. The 3,5,6-trichloro-2-pyridinol silyl derivative used to determine response curves was prepared in benzene 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 of 3,5,6-trichloro-2-pyridinol–benzene standard into a 10-mL volumetric flask. With a 100-μL syringe transfer 80 μL of 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 (Claborn, 1968a) over the concentration range of 0.01 to 0.10 ppm, its oxygen analogue (Ivey and Claborn, 1968) over the concentration range of 0.01 to 0.05 ppm, 3,5,6-trichloro-2-pyridinol (McKellar, 1970) over the concentration range of 0.05 to 5 ppm, and 3,5,6-trichloro-2-pyridinol by hydrolysis (McKellar, 1971) at 0.10 ppm was determined by fortifying control samples with the appropriate chemical and analyzing them as described. The results are shown in Tables I and II.

RESULTS AND DISCUSSION

Residues of chlorpyrifos were predominantly in the fatty tissues and averaged 0.02 (<0.01–0.05 ppm) and 3.28 ppm

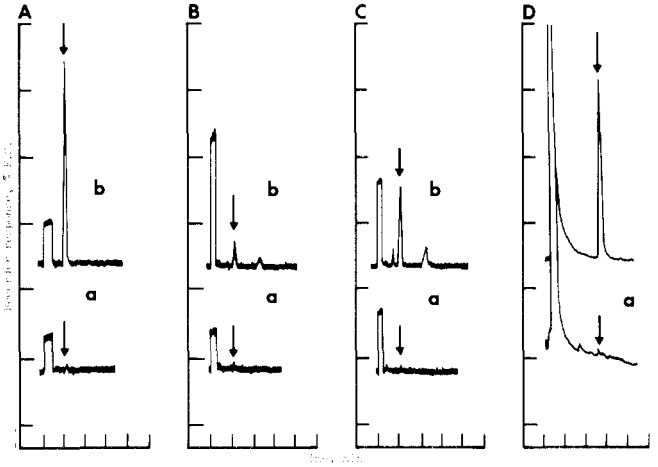

Figure 1. Typical chromatograms from the determination of chlorpyrifos in tissues of beef cattle: (A) muscle: (a) control, 0.00 ppm; (b) 100 ppm diet, 0.14 ppm, 5× dilution; (B) liver: (a) control, 0.00 ppm; (b) 100 ppm diet, <0.01 ppm; (C) kidney: (a) control, 0.00 ppm; (b) 100 ppm diet, 0.01 ppm; (D) fat: (a) control, 0.00 ppm; (b) 100 ppm diet, 2.0 ppm, 100× dilution.

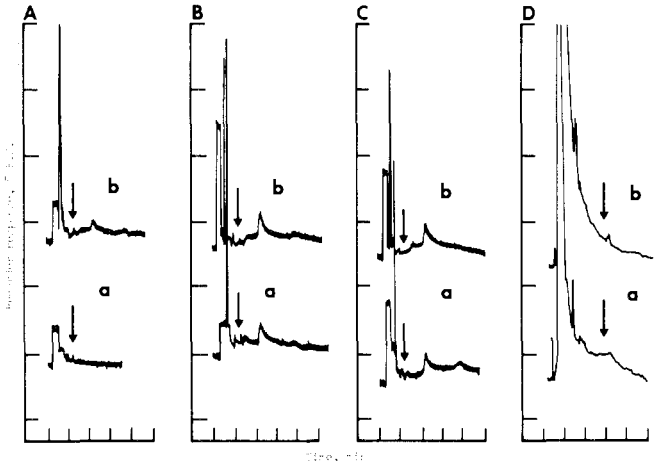

Figure 2. Typical chromatograms from the determination of chlorpyrifos oxygen analogue in tissues of beef cattle: (A) muscle: (a) control, 0.00 ppm; (b) 100 ppm diet, 0.00 ppm; (B) liver: (a) control, 0.00 ppm; (b) 100 ppm diet, 0.00 ppm; (C) kidney: (a) control, 0.00 ppm; (b) 100 ppm diet, 0.00 ppm; (D) fat: (a) control, 0.00 ppm; (b) 100 ppm diet, 0.00 ppm.

Table III. Residues of Chlorpyrifos and 3,5,6-Trichloro-2-pyridinol in Body Tissues from Cattle Fed Chlorpyrifos for 30 Days

Animal no.	Chlorpyrifos rate fed, ppm	Residue found, ppm					
		Muscle	Liver	Kidney	Omental fat	Renal fat	Subcutaneous fat
				Chlorpyrifos			
801	0	0.00	0.00	0.00	0.00	0.00	0.00
806	0	0.00	0.00	0.00	0.00	0.00	0.00
809	0	0.00	0.00	0.00	0.00	0.00	0.00
802	3	<0.01	<0.01	<0.01	0.02	0.01	0.01
817	3	<0.01	<0.01	<0.01	0.05	0.03	0.03
819	3	<0.01	<0.01	<0.01	<0.01	0.01	<0.01
804	10	0.02	0.02	<0.01	0.11	0.16	0.16
807	10	<0.01	<0.01	<0.01	0.08	0.10	0.07
813	10	<0.01	<0.01	<0.01	0.11	0.15	0.08
805	30	<0.01	<0.01	<0.01	0.43	0.46	0.21
812	30	0.01	0.01	<0.01	0.85	1.09	0.59
820	30	0.02	0.02	<0.01	0.35	0.46	0.26
808	100	0.14	0.01	0.03	2.89	3.47	3.52
811	100	0.23	0.03	0.01	2.72	4.70	4.37
815	100	0.34	<0.01	<0.01	2.28	2.62	2.92
				3,5,6-Trichloro-2-pyridinol			
801	0	0.00	<0.05	<0.05	0.00	0.00	0.00
806	0	0.00	<0.05	<0.05	0.00	0.00	0.00
809	0	0.00	<0.05	<0.05	0.00	0.00	0.00
802	3	<0.05	0.16	0.10	<0.05	<0.05	<0.05
817	3	<0.05	0.23	0.15	<0.05	<0.05	<0.05
819	3	<0.05	0.22	0.09		<0.05	<0.05
804	10	<0.05	0.42	0.35	0.20	0.11	0.06
807	10	<0.05	0.39	0.29	<0.05	<0.05	<0.05
813	10	<0.05	0.49	0.54	<0.05	0.05	<0.05
805	30	0.05	1.28	0.76	0.09	0.07	0.08
812	30	0.07	1.67	0.29	0.12	0.14	0.13
820	30	0.06	1.62	1.05	0.11	0.07	0.12
808	100	0.17	2.61	1.95	0.25	0.23	0.36
811	100	0.12	2.16	1.46	0.31	0.22	0.31
815	100	0.13	2.45	1.83	0.25	0.25	0.23

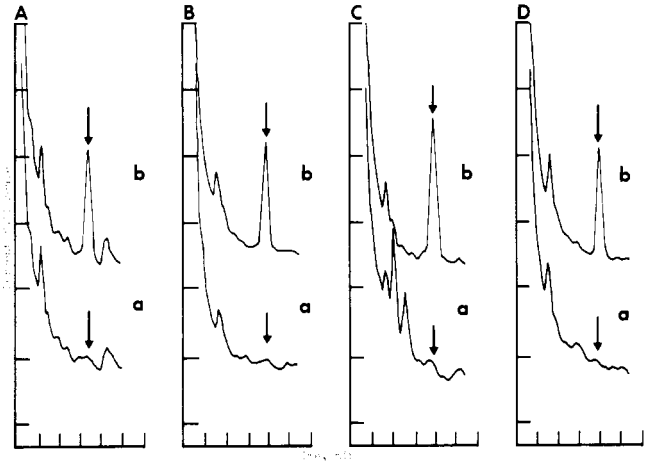

Figure 3. Typical chromatograms from the determination of 3,5,6-trichloro-2-pyridinol in tissues of beef cattle: (A) muscle: (a) control, 0.02 ppm; (b) 100 ppm diet, 0.13 ppm; (B) liver: (a) control, 0.02 ppm; (b) 100 ppm diet, 2.61 ppm, 10× dilution; (C) kidney: (a) control, 0.02 ppm; (b) 100 ppm diet, 1.95 ppm, 10× dilution; (D) fat: (a) control, 0.01 ppm; (b) 100 ppm diet, 0.25 ppm, 2× dilution.

Table IV. Residues of Chlorpyrifos and 3,5,6-Trichloro-2-pyridinol in Omental Fat from Cattle after Feeding 100 ppm Chlorpyrifos for 30 Days (Withdrawal Data)

Animal no.	Withdrawal, days	Residue found, ppm	
		Chlorpyrifos	3,5,6-Trichloro-2-pyridinol
814	7	1.15	<0.05
816	7	0.98	<0.05
818	7	0.66	<0.05
814	14	0.67	<0.05
816	14	0.15	<0.05
818	14	0.26	<0.05
814	21	0.58	
816	21	0.13	
818	21	0.09	
814	28	0.15	
816	28	0.07	
818	28	0.02	
814	35	0.04	
816	35		
818	35	<0.01	

(2.28–4.70 ppm) in fat of cattle fed 3 and 100 ppm chlorpyrifos, respectively, for 30 days with no withdrawal (Table III). The average residue in the fat declined to 0.93 ppm (0.66–1.15 ppm) at 7 days and 0.02 ppm (<0.01–0.04 ppm) at 35 days after withdrawal of the insecticide at the highest feeding level (Table IV). No differences were apparent in the amounts of residues found in the three types of fat. Low residues of chlorpyrifos were found in the other tissues: muscle, 0.24 ppm (0.14–0.34 ppm); liver, 0.02 ppm (<0.01–0.03 ppm); and kidney, 0.02 ppm (< 0.01–0.03 ppm) of the animals fed 100 ppm of the chemical for 30 days.

No residue of the oxygen analogue was detected in any tissue at any feeding level. Recovery studies showed the compound to be unstable in liver and kidney tissues.

Table V. Comparison of Residues of 3,5,6-Trichloro-2-pyridinol in Body Tissues from Cattle with and without Alkaline Hydrolysis

Animal no.	Chlorpyrifos in feed, ppm	Total 3,5,6-trichloro-2-pyridinol, ppm: No hydrolysis (McKellar, 1970)	Total 3,5,6-trichloro-2-pyridinol, ppm: Alkaline hydrolysis (McKellar, 1971)
Muscle			
801	0	0.01	0.00
808	100	0.24	0.24
811	100	0.24	0.14
815	100	0.32	0.17
Liver			
809	0	0.01	0.00
808	100	2.6	2.9
811	100	2.2	1.9
815	100	2.4	2.7
Kidney			
801	0	0.02	0.00
808	100	2.0	1.3
811	100	1.5	1.8
815	100	1.8	1.7
Subcutaneous fat			
801	0	0.01	0.00
808	100	2.3	1.6
811	100	2.7	2.5
815	100	1.9	2.3

When samples of the tissues were fortified with the oxygen analogue and allowed to sit for 1 h before extraction, recoveries dropped to 0–40%. These data corroborate results reported previously (Claborn et al., 1968b). A chemical or enzymatic reaction appears to take place in the fortified tissues and results in decomposition of the oxygen analogue. Thus, it is unlikely that any residues of the oxygen analogue would be present in these tissues.

Unlike chlorpyrifos, the 3,5,6-trichloro-2-pyridinol moiety was found predominantly in the liver and kidney tissues. Residues of 3,5,6-trichloro-2-pyridinol were present in the liver and kidney of animals at all feeding levels. The average residue of the pyridinol in these tissues of animals fed 3 ppm chlorpyrifos for 30 days was 0.20 ppm (0.16–0.23 ppm) in liver and 0.11 ppm (0.09–0.15) in kidney. Chlorpyrifos fed at a level of 100 ppm showed residues of 3,5,6-trichloro-2-pyridinol of 2.41 ppm (2.16–2.61 ppm) in liver and 1.75 ppm (1.46–1.95 ppm) in kidney. Low residues of 3,5,6-trichloro-2-pyridinol were found in the other tissues and ranged from an average of <0.05 at the 3 ppm feeding level to an average of 0.27 ppm (0.22–0.36 ppm) in fat and 0.14 ppm (0.12–0.17 ppm) in muscle when 100 ppm chlorpyrifos was fed. The average residue in the fat declined to <0.05 ppm in 7 days and nondetectable at 35 days withdrawal (Table IV).

The data in Table V show that no additional 3,5,6-trichloro-2-pyridinol was found over the amount determined initially when selected samples of muscle, liver, kidney, and omental fat at the 100 ppm feeding level were subjected to an alkaline hydrolysis and analyzed for 3,5,6-trichloro-2-pyridinol.

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