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Absorption, Distribution, and Metabolism of [^{14}C]Chlorpyrifos Applied Dermally to Goats

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Radiolabeled chlorpyrifos [*O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate] was applied dermally to two male weanling goats, at a dose of 22 mg/kg of body weight. A blood sample was drawn before dosing and every 4 h thereafter. The animals were sacrificed approximately 18 h after dosing, when blood radioactivity levels at 16 h had declined from maximum values (12 h) in both animals. Radioanalysis of blood and selected tissues (liver, kidney, heart, fat, muscle) indicated that radioactivity levels were, in general, very low, ranging from 0.04 ppm (chlorpyrifos equivalents) in muscle to 0.90 ppm in omental fat. Tissue extracts contained 80–96% of the ^{14}C residue, most of which was organosoluble. High-performance liquid chromatography analysis of tissue extracts showed that the predominant ^{14}C residue in liver and kidney was [^{14}C]-3,5,6-trichloro-2-pyridinol (chlorpyridinol) whereas [^{14}C]chlorpyrifos was predominant in fat and heart extracts. In muscle, in addition to approximately equal amounts of [^{14}C]chlorpyridinol and [^{14}C]chlorpyrifos, 18.6% of the radioactivity was unidentified; alkaline hydrolysis quantitatively converted the latter radioactivity to pyridinol.

Chlorpyrifos, *O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate, is the active insecticidal ingredient in Lorsban and Dursban insecticides, products of Dow Chemical U.S.A. (Midland, MI). These products have a variety of applications including dermal administration to ruminants for the control of various insect pests (Kenaga, 1974). Since such dermal exposure may result in residues of chlorpyrifos and its metabolites in edible tissues of ruminants, it is of interest to determine the magnitude and nature of those residues.

Ivey et al. (1978) reported the magnitude of residues of chlorpyrifos and its metabolite 3,5,6-trichloro-2-pyridinol (chlorpyridinol) in tissues of cattle that had a 10% chlorpyrifos-impregnated plastic band attached to each ear. Additional studies of the dermal absorption of chlorpyrifos include that of Ivey and Palmer (1979), in which residues of chlorpyrifos and pyridinol were measured in tissues of swine treated with Dursban 44 insecticide formulation and that of Nolan et al. (1983) in which the kinetics of the absorption of chlorpyrifos and pyridinol was studied following dermal administration to human volunteers. In addition, Shah et al. (1981) included [^{14}C]chlorpyrifos in their study of the comparative rates of dermal penetration of insecticides in mice.

The present report contains the results of a study conducted to determine the magnitude and nature of ^{14}C residues in tissues of goats following dermal application of [^{14}C]chlorpyrifos.

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MATERIALS AND METHODS

Chemicals. [^{14}C]Chlorpyrifos (A) (specific activity 15.7 mCi/mmol), blank Dursban 44 formulation, and nonlabeled reference standards chlorpyridinol (B), *O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphate (C), 3,5,6-trichloro-2-methoxypyridine (D), and sodium *O*-ethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate (E) were provided by the Agricultural Products Department, Dow Chemical U.S.A. The chemical structures are in Figure 1.

All solvents used were high-performance liquid chromatography (HPLC) grade; other chemicals were reagent grade from standard sources. Insta-Gel and Perma-Fluor V liquid scintillation cocktails, Carbo-Sorb carbon dioxide absorber, and Spec-Chec-^{14}C radiocarbon standard were products of Packard Instrument Co. (Downers Grove, IL).

Experimental Animal Procedures. Two male goats, weighing 15–18 kg, were purchased from Sunshine Farms, Portage, WI. The animals were acclimated to the test environment (temperature 22–25 °C, humidity 48–68%, light 12 h/day), diet, and management program for 19 days; the goats were gradually acclimated to metabolism cages during this period. From 24 h before application of chlorpyrifos, the goats were housed continuously in metabolism cages.

Approximately 10 h before dose application, an 8-in. × 10-in. patch of hair was shaved behind the right shoulder of each goat. A 4-in.-diameter rubber ring (approximately 0.5 in. in thickness) was glued to the skin with 3M CA-8 cyanoacrylate adhesive (St. Paul, MN). A sealant of Silastic medical adhesive silicone Type A, Dow Corning (Midland, MI), was applied around the outside of the rubber ring.

An appropriate amount of [^{14}C]chlorpyrifos (specific activity 15.7 mCi/mmol) was combined with nonlabeled chlorpyrifos and a blank formulation to yield a dose containing 42.2% active

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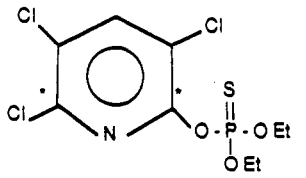
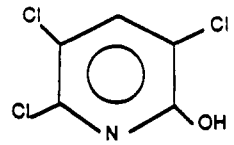
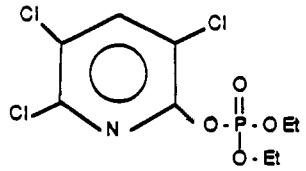


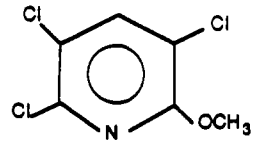
(A) chlorpyrifos * Denotes ^{14}C labels.



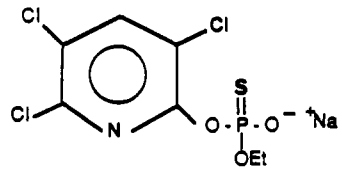
(B) chlorpyridinol



(C) 0,0-diethyl-0-(3,5,6-trichloro-2-pyridyl)phosphate



(D) 3,5,6-trichloro-2-methoxypyridine



(E) Sodium 0-ethyl-0-(3,5,6-trichloro-2-pyridyl)phosphorothioate

Figure 1. Chemical structures for standards.

ingredient and with a specific activity of approximately 2.9 mCi/mmol. Using a 2-mL glass syringe, approximately 1 mL of the solution was applied to the shaved skin exposed within the rubber ring to achieve a dose of 22 mg/kg. The dose solution was spread with a glass rod. A filter paper attached to a screen dome was glued to the rubber ring to cover the application site to prevent direct contact with any external objects.

A blood sample was drawn from the jugular vein before dosing and every 4 h thereafter. The samples were analyzed immediately for total radioactivity. The animals were sacrificed after the blood ^{14}C concentration declined in value from the previous analysis. This decline occurred in the 12- to 16-h postdose sample. This sacrifice time was based on the assumption that, at C_{max}/t_{max}, the absorption and elimination rates were equal and that the decline in blood concentrations was indicative that the rate of absorption had approached the maximum. The amount of compound in the body would then be related to the elimination rate with the best estimate of maximum amounts in tissues obtained at the sampling time (18 h) closest to C_{max} (12 h).

Radioactivity Analysis. Blood, tissue, and nonextractable tissue solids were thoroughly homogenized, and triplicate aliquots were combusted in a Packard Model 306 sample oxidizer for ^{14}C determination. Tissue extracts were analyzed directly by liquid scintillation counting using Packard Model 460CD or 4640 liquid scintillation spectrometers. Packard scintillation cocktails Perma-Fluor V and Insta-Gel were used for indirect (combusted) and direct counting, respectively.

Extraction and Cleanup. Tissue (5–10 g) was homogenized with 50 mL of acetonitrile–acetone (8:2) on a Polytron homogenizer (Brinkmann, Westbury, NY). The mixture was centrifuged, the phases were separated, and the solid was reextracted

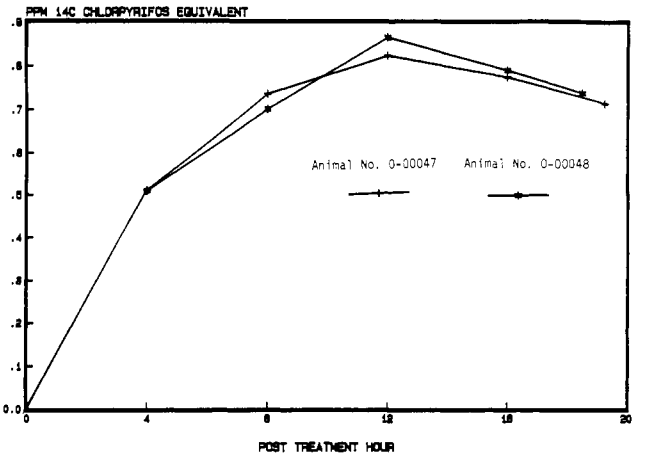


Figure 2. ^{14}C levels in blood following dermal application of chlorpyrifos to goats.

twice by the same procedures. The combined organic extract was then concentrated to dryness by rotary evaporation at reduced pressure. The residues were resuspended in 10 mL of buffer (0.02 M potassium phosphate, pH 6.5) and extracted three times with 15 mL each of dichloromethane. The combined dichloromethane extract was concentrated to dryness, and the residue was partitioned with 10 mL of hexane and 10 mL of acetonitrile. The ^{14}C content was determined for acetonitrile, hexane, and buffer fractions by direct counting and for nonextractable tissue solids by combustion.

Alkaline Hydrolysis. Matrices having high percentages of nonextractable residues (liver) or unidentified metabolites (muscle) were subjected to alkaline hydrolysis. Each sample was homogenized by a Polytron homogenizer with 20 mL of 1.0 N sodium hydroxide. The alkaline mixture was heated for 1 h at 130 °C on an oil bath, cooled to room temperature, and acidified to pH 2 with 6 N hydrochloric acid. Sodium chloride (7 g) was added, and the solution was extracted three times with 15 mL of benzene for each extraction. The concentrated benzene fraction was analyzed by HPLC.

HPLC Analysis. A metabolite profile was determined by HPLC using an ISS-100 Perkin-Elmer liquid chromatography (LC) sample system (Orwalk, CT), a Series 4 LC solvent delivery system (Norwalk, CT), and an ISCO Foxy fraction collector (Lincoln, NB). The column used was Waters Associates Radial-PAK C-18 in radial compression module (0.8 cm (i.d.) × 10 cm) (Milford, MA). The mobile phases consisted of (a) 0.02 M ammonium acetate in acetonitrile–water (20:80) and (b) 0.02 M ammonium acetate in methanol. A linear gradient of 100% (a) to 100% (b) in 20 min was used with a flow rate of 1 mL/min. The HPLC eluate was collected at 1 mL/fraction for direct ^{14}C analysis. Nonlabeled standards (Figure 1) were detected with an ultraviolet (UV) detector at 293 nm.

Thin-Layer Chromatography (TLC) Analysis. The TLC analyses were performed on precoated 5 cm × 20 cm × 0.25 mm silica gel 60 F-254 plates. The solvent system used was hexane–acetone–acetic acid (15:3:0.05). Radiolabeled standards and metabolites were located by a linear analyzer (Model LB 2842; Berthold, Nashua, NH), and nonlabeled components were located by a UV lamp.

Gas Chromatography–Mass Spectrometry (GC–MS). A Finnigan 4500 GC–MS system (San Jose, CA) equipped with a column of 0.32 mm (i.d.) × 30 m FSCC coated with 0.25-μm SPB-5 was used for chlorpyrifos structure confirmation. The GC conditions consisted of an oven temperature program of 40–300 °C at 20 °C/min and a helium flow rate of 1 mL/min. The mass unit was operated in EI mode, the mass range was 44–450 amu, and the source temperature was 105 °C.

RESULTS AND DISCUSSION

The radioactivity concentration in the blood of two goats dosed dermally with [^{14}C]chlorpyrifos (22 mg/kg) reached maximum values (C_{max}) of 0.824 and 0.866 ppm (chlorpyrifos equivalents) 12 h postdose (t_{max}) (Figure 2). Based on these C_{max} values and an assumption of 70.5 mL of

Table I. ^{14}C Residue Level in Goat Tissues following Dermal Application of [^{14}C]Chlorpyrifos at 22 mg/kg

matrix	chlorpyrifos equivalents, ppm	
	animal 0-00046	animal 0-00047
liver	0.60	0.36
kidney	0.73	0.44
heart	0.48	0.25
omental fat	0.83	0.40
skeletal muscle	0.09	0.05

Table II. Extraction Distribution of ^{14}C Residues in Goat (0-00046) Tissues following Dermal Application of [^{14}C]Chlorpyrifos at 22 mg/kg

tissue	total residue, %					
	hexane	aqueous (pH 6.5)	aceto-nitrile	total extractable	solid	total recovery
liver	3.9	3.9	72.0	79.8	13.4	93.2
kidney	2.2	5.1	77.9	85.2	7.7	92.9
heart	2.3	2.2	85.1	89.6	4.9	94.5
fat	5.4	0.6	89.3	95.3	2.2	97.5
muscle	3.3	0.6	91.9	95.8	6.0	101.8

Table III. HPLC Retention Times and TLC R_f Values for Analytical Standards

anal. std	HPLC ret time, min	TLC R_f
A	24	0.52–0.63
B	6.8	0.19–0.26
C	21	0.22
D	23	0.76
E	10.4	0.025

blood/kg of body weight (Altman and Dittner, 1964), it is estimated that less than 0.3% of the applied dose was present in the systematic circulation at t_{max}. Shah et al. (1981), studying the dermal absorption of chlorpyrifos in mice, reported that approximately 2.7% of the radioactivity was present in the blood 8 h after treatment at 1 mg/kg. Nolan et al. (1983) studied the dermal absorption of chlorpyrifos in human subjects and reported that a small fraction (less than 5%) of the applied dose (0.5 mg/kg) was absorbed. It is apparent that chlorpyrifos is absorbed to a low degree in goats, mice, and humans, with the lowest absorption occurring in goats.

The levels of radioactivity in the examined tissues are summarized in Table I. Although all residue levels were less than 1 ppm, the levels of ^{14}C in the tissues of one goat (animal no. 0-00046) were almost twice those of the other goat. Of the tissues analyzed, omental fat had the highest average level (0.62 ppm) and skeletal muscle had the lowest (0.07 ppm).

The tissue samples that had the highest radioactivity levels (animal no. 0-00046) were extracted for characterization of residues. The extraction procedure yielded 80–96% extractable radioactivity, with liver having the lowest amount and muscle the highest. The distribution of the radioactivity in the various fractions is shown in Table II. Most of the radioactivity was found in the acetonitrile fraction; hexane and aqueous fractions each contained approximately 5% or less of the total radioactivity.

Several radioactive components were detected in the tissue acetonitrile extracts using HPLC and TLC methods. The retention measurements for HPLC and TLC analysis of the standard mixtures are in Table III. Graphs of the HPLC profiles for chlorpyrifos standard and the tissue acetonitrile fractions are in Figure 3a–f. The two major components in the tissue extracts were identified (see below) as chlorpyrifos (test material) and chlorpyridinol (primary metabolite). The minor radioactive components exhibited chromatographic properties different from those

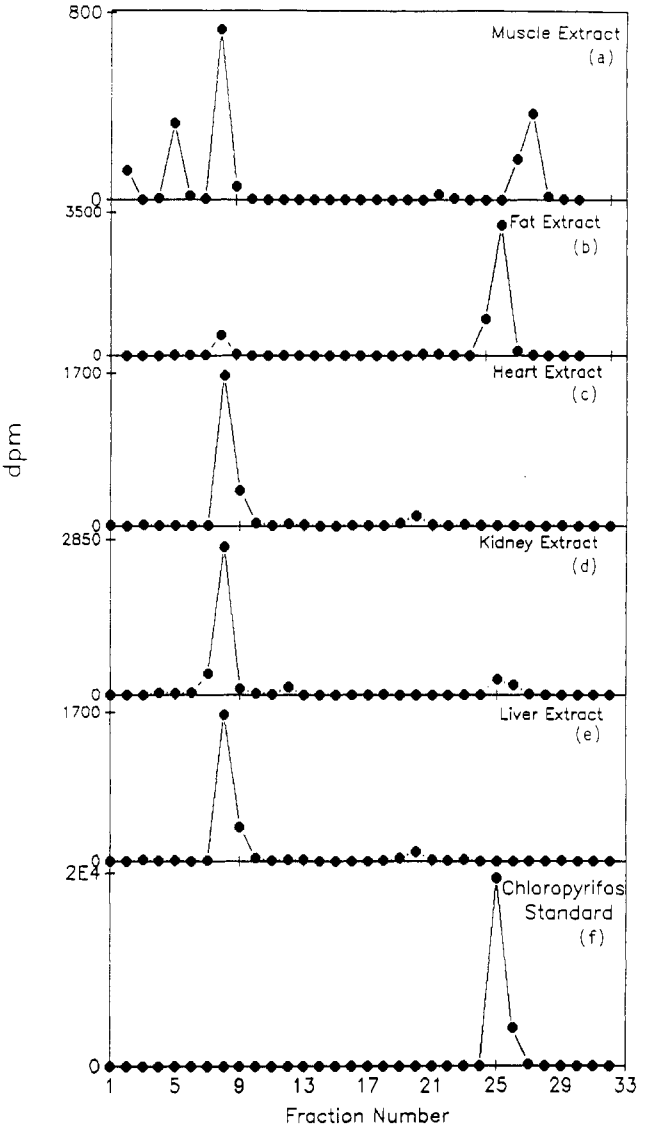


Figure 3. HPLC radioactivity profiles for standard [^{14}C]chlorpyrifos (a) and for acetonitrile fractions of tissue extracts in liver (b), kidney (c), heart (d), fat (e), and muscle (f).

Table IV. HPLC Metabolite Profile of ^{14}C Residues in Goat (0-00046) Tissues following Dermal Application of [^{14}C]Chlorpyrifos at 22 mg/kg

matrix[a]	chlorpyridinol		chlorpyrifos		unidentified	
	%[a]	ppm[b]	%[a]	ppm[b]	%[a]	ppm[b]
liver	54.1	0.33	ND[c]	ND	3.90	0.03
kidney	59.7	0.44	8.72	0.06	2.57	0.02
heart	21.5	0.10	52.6	0.25	2.94	0.01
fat	9.2	0.08	78.4	0.65	ND	ND
muscle	32.4	0.03	22.1	0.02	18.6	0.02

[a] Percent of total residue. [b] Microgram equivalents of chlorpyrifos per gram of tissue. [c] Not detected.

of the nonlabeled standards (Figure 1).

Radioactive chlorpyrifos was isolated from the fat extract by HPLC. The HPLC fraction was collected, dried, redissolved in dichloromethane, and analyzed by GC–MS. The mass spectra of the chlorpyrifos standard and the chlorpyrifos isolated from the fat extract were similar. Both mass spectra showed molecular ions of low intensity at m/e 349. Fragment ions included m/e 314 (equivalent to a loss of one chlorine atom from the molecular ion) and 197 (equivalent to the pyridinol ion). Isotope clusters characteristic of chlorine ion were present in each spectrum. Radioactive chlorpyridinol was identified by co-

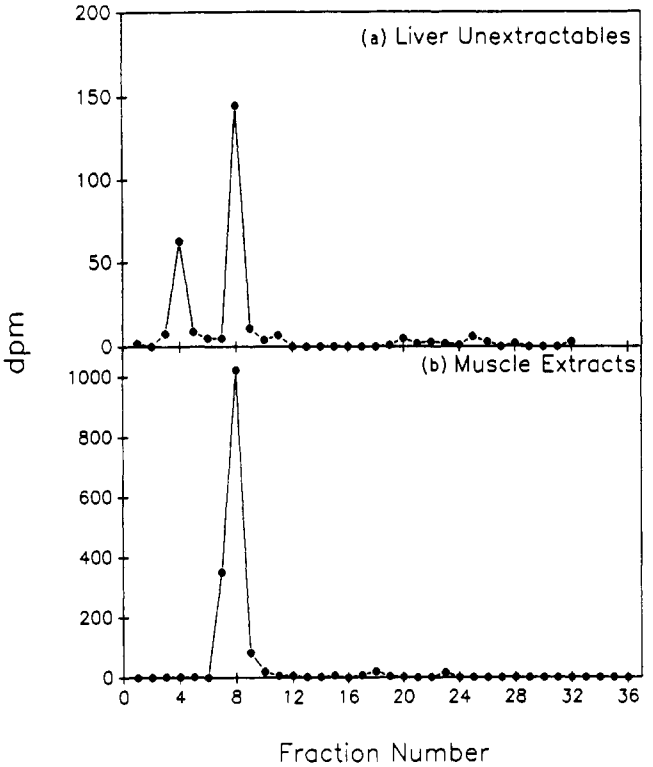


Figure 4. HPLC radioactivity profile for benzene extracts of alkaline hydrolysates: (a) liver nonextractables; (b) muscle extract.

chromatography with nonlabeled authentic standard by HPLC and TLC methods.

The quantitative results of the HPLC metabolite profiles are summarized in Table IV. Chlorpyrifos was the predominant component in fat (78.4%, 0.65 ppm) and heart (52.6%, 0.25 ppm). Chlorpyridinol was the predominant component in liver (54.1%, 0.33 ppm [^{14}C]chlorpyrifos equivalents) and kidney (59.7%, 0.44 ppm [^{14}C]chlorpyrifos equivalents). Muscle contained approximately equal amounts of chlorpyrifos (22.1%, 0.02 ppm) and chlorpyridinol (32.4%, 0.03 ppm [^{14}C]chlorpyrifos equivalents).

Residues of chlorpyrifos and chlorpyridinol in tissues of swine and cattle dermally treated with chlorpyrifos were reported by Ivey and Palmer (1979) and Ivey et al. (1978), respectively. For both species, the highest residue levels were found in fat, with both chlorpyrifos and chlorpyridinol being present; in liver or kidney, chlorpyridinol was detected but chloropyrifos was not. Similarly, studies on the bioaccumulation and metabolism of chlorpyrifos in different animal species (turkeys, laying hens, cattle) following oral treatments (Dishburger et al., 1969, 1972, 1977) showed that fat contained chlorpyrifos and liver and kidney contained chlorpyridinol.

Goat liver contained the largest amount of nonextracted radioactivity (13.4%; Table II), and muscle extract contained the largest amount of unidentified extractable radioactivity (18.6%; Table IV). The alkaline hydrolysis of these samples yielded organosoluble ^{14}C, the HPLC profiles for which are in Figure 4a,b. The overall recovery of radioactivity from the alkaline hydrolysis of liver nonextractables was low, but approximately 20% was converted to chlorpyridinol. Alkaline hydrolysis of muscle extract yielded quantitative conversion of radioactivity to chlorpyridinol. These results indicated that metabolic dechlorination of chlorpyrifos was not involved in the metabolism pathway and that the unidentified components were most likely test material related or were pyridinol conjugates that yielded chlorpyridinol after alkaline hydrolysis.

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Registry No. Chlorpyrifos, 2921-88-2; chlorpyridinol, 6515-38-4.

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