Nature of the Residue of [^{14}C]Cloransulam-methyl in Lactating Goats

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Two lactating goats were given a daily oral dose of either [UL-*aniline*-^{14}C; AN] or [*triazolopyrimidine*-7,9-^{14}C; TP]cloransulam-methyl for 5 consecutive days. Each animal received a dietary equivalent of ∼10 mg/kg of test material, ∼2225 times the realistic maximum dietary exposure for a dairy animal. Milk, urine, and feces samples were collected in the morning and afternoon for each animal. Each goat was sacrificed within 23 h of receiving the last dose, and the liver, kidneys, samples of blood, fat, muscle, and gastrointestinal tract contents, and urine from the bladder were collected. All of these samples were analyzed for ^{14}C content. Cloransulam-methyl (CM) was rapidly excreted by the animals, with 99.9% of the recovered radioactivity appearing in the urine and feces. Radiochemical analysis showed very low residues, with the highest being in the kidneys at 0.122 and 0.128 mg equiv of CM/kg (AN and TP labeled compounds, respectively). Radioactive residues were extracted and fractionated from kidney, liver, and milk. Analysis showed ∼0.066 mg/kg CM in the kidney but <0.003 mg/kg in the liver. Only one metabolite, cloransulam, was identified (in liver, 9.5% of total radioactive residue; 0.005 mg/kg). All other metabolites were present at lower levels. Sulfonanilide bridge cleavage was not a significant degradation route for cloransulam-methyl in ruminants. These data indicated a very low bioaccumulation potential for cloransulam-methyl and its metabolites in ruminants. For a ruminant exposed to anticipated levels of cloransulam-methyl in its diet, parent and metabolites, in total, would not be expected to exceed 50 ng/kg in the kidney and liver.

Keywords: *Cloransulam-methyl; cloransulam; triazolopyrimidine sulfonanilide; ruminant metabolism*

INTRODUCTION

Biological and Physical Properties of Cloransulam-methyl. Cloransulam-methyl [*N*-(2-carbomethoxy-6-chlorophenyl)-5-ethoxy-7-fluoro[1,2,4]triazolo[1,5-*c*]-pyrimidine-2-sulfonamide] (Table 1) is a triazolopyrimidine sulfonanilide herbicide effective in the control of broadleaf weeds in soybeans through acetolactate synthase (ALS) inhibition (Dow AgroSciences, 1994; Jachetta et al., 1994; Hunter et al., 1994). The molecule is the active ingredient in FirstRate herbicide, which received Environmental Protection Agency registration for use in U.S. soybeans in October 1997 (U.S. EPA, 1997). Application methods for the product include preplant incorporation and postemergence treatment at maximum label rates of 44 and 17.5 g of active ingredient (ai)/ha, respectively. Physical properties of cloransulam-methyl have been previously summarized (Van Wesenbeeck et al., 1997), but specifics relevant to the present work are as follows. The molecular formula of cloransulam-methyl is $C_{15}H_{13}N_5O_5SFCl$, with a corresponding molecular mass of 429 g/mol. The molecule is characterized by its extremely low volatility (4×10^{-14} Pa at 25 °C) and low water solubility (3 mg/L at pH 5; 184 mg/L at pH 7) but moderate acetone (4360 mg/L) and acetonitrile (5500 mg/L) solubilities. The molecule is a weak acid, with pK_a of 4.81 at 20 °C.

Metabolism Studies on Cloransulam-methyl. The metabolism of FirstRate herbicide in a ruminant animal was studied as part of the registration package for this molecule. Two previous metabolism studies on the molecule had shown differing degradation routes. Soil metabolism studies (Wolt et al., 1996; van Wesenbeeck et al., 1997) under both laboratory and field conditions showed that the primary metabolite, formed by microbially mediated ester hydrolysis of cloransulam-methyl, is the corresponding acid, cloransulam [methyl [*N*-(2-carboxy-6-chlorophenyl)-5-ethoxy-7-fluoro[1,2,4]triazolo-[1,5-*c*]pyrimidine-2-sulfonamide] (Table 1). In contrast, the initial metabolic step in soybeans was shown to be displacement of fluorine from the 7-position by homoglutathione (G. J. DeBoer, Dow AgroSciences, personal communication, 1995). The goal of the current study was to determine the level and nature of residues in milk and tissues of lactating goats given daily oral doses of radiolabeled cloransulam-methyl for 5 consecutive days. The dose level used was ∼2200 times the realistic maximum dietary exposure level for dairy animals consuming diets with expected residue levels of cloransulam-methyl.

To determine whether sulfonanilide bridge cleavage products were formed during the metabolism of cloransulam-methyl, two radiolabeled forms of the test material were used in this study. These were [UL-*aniline*-^{14}C]cloransulam-methyl, referred to throughout this paper as the AN label, and [*triazolopyrimidine*-7,9-^{14}C]-cloransulam-methyl or TP label. The structures of these materials are shown in Figure 1.

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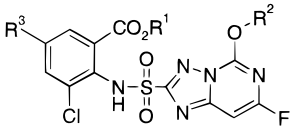
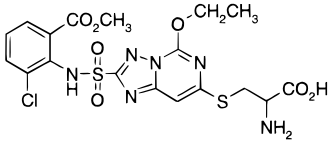
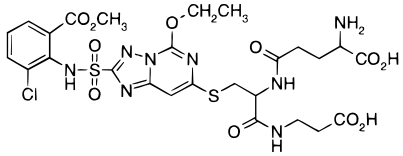
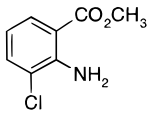
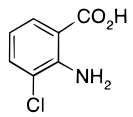
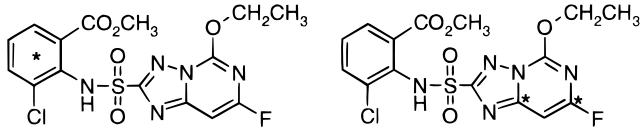
10.1021/jf9909611 CCC: $19.00
Published on Web 05/18/2000

Table 1. Summary of Reference Substances Used during Characterization of Cloransulam-methyl Goat Metabolites

Structure	R^1	R^2	R^3	Name (Percentage Purity)	Abbreviation	HPLC retention time[a]	TLC R_f[b]
	CH_3	CH_2CH_3	H	cloransulam-methyl (99.2%)	CM	39.3	0.73
	H	CH_2CH_3	H	cloransulam (96.8%)	C	34.0	0.51
	CH_3	H	H	5-hydroxy-cloransulam-methyl (95.5%)	5-HCM	23.6	0.24
	H	H	H	5-hydroxy-cloransulam (97.3%)	5-HC	21.0	Not determined
	CH_3	CH_2CH_3	OH	4-OH-phenyl-cloransulam-methyl (97%)	4-HPCM	35.4	Not determined
				cloransulam-methyl-7-cysteine (HCl salt) (95%)	CM-CYS	28.7	Not determined
				cloransulam-methyl-7-homoglutathione (HCl salt) (94%)	CM-7-hGSH	29.8	Not determined
				methyl 3-chloroanthranilate (100%)	MCA	43.2	Not determined
				3-chloroanthranilic acid (100%)	CA	33.3	Not determined

[a] In minutes, with gradient elution system. [b] Solvent system: toluene/ACN/AcOH 50:45:5.



"AN" Label "TP" Label

Figure 1. Structures of AN- and TP-labeled [^{14}C]cloransulam-methyl used in goat metabolism study (* = ^{14}C).

EXPERIMENTAL PROCEDURES

Testing Facilities. The in-life portion of this study was conducted at Hazleton Wisconsin, Inc., Madison, WI. The remaining analytical phase was performed at Dow AgroSciences' laboratories.

Test and Reference Substances. Non-radiolabeled and radiolabeled samples of cloransulam-methyl were synthesized by the Dow AgroSciences Agricultural Chemical Process Research group and Specialty Synthesis Laboratory, respectively. The non-radiolabeled cloransulam-methyl was 99% pure. The [AN-^{14}C]- and [TP-^{14}C]cloransulam-methyl samples were synthesized at specific activities of 29.9 mCi/mmol (69.7 μCi/mg; 2.58 MBq/mg) and 27.8 mCi/mmol (64.8 μCi/mg; 2.40 MBq/mg), respectively. Prior to dose capsule preparation, these radiolabeled samples were diluted to specific activities of 8.47 mCi/mmol (19.7 μCi/mg; 0.731 MBq/mg) and 8.44 mCi/mmol (19.7 μCi/mg; 0.729 MBq/mg), respectively, with non-radiola-

beled cloransulam-methyl. The radiochemical purity of each, determined as described below, was 100%. These test materials were stored below 0 °C at all times when not in use. Non-radiolabeled reference substances, used for chromatographic comparisons with extracted metabolites, are summarized in Table 1. These materials were prepared by the Specialty Synthesis Laboratory at Dow AgroSciences.

Test System. The test system consisted of three lactating goats (*Capra*) with an average weight of 64 kg. The goats were each at least 2 years of age, and each animal was assigned an identification number attached by a neck chain. Each animal was selected for the study on the basis of the level of milk production and apparent adjustment to the study stalls. The animals were acclimated to the metabolism stalls for 12 days prior to study initiation. Goat 1 was specified as the control goat and received a daily dose of a placebo capsule. Goats 2 and 3 were dosed daily with a capsule containing [AN-^{14}C]- or [TP-^{14}C]cloransulam-methyl, respectively, for 5 consecutive days. During administration of the dose, each animal was fed ~1 kg/day of hay and ~0.8 kg/day of a grain-based diet.

Calculation of Dose and Dietary Burden. Each animal was administered a dose of 10 mg/kg (= parts per million) of test material in the diet. The dose rate (milligrams per day) was targeted by monitoring the mean daily feed consumption of each of the goats during the acclimation period. The two treated goats consumed an average of 1.74 kg of feed daily and were dosed daily with an average of 18 mg of test substance, equating to a dietary level of ~10 mg/kg. The control goat consumed, on average, 1.37 kg of feed daily and was given a daily dose of a placebo capsule containing 0 mg of test substance. Soybean metabolism and magnitude of residue studies for FirstRate applied according to the product label showed no detectable levels of cloransulam-methyl in beans when assayed using a GC-MS-based method (Shackelford et al., 1996) with a limit of detection of 0.005 mg/kg. Therefore, to a first approximation, the doses used in this goat metabolism study represented ~2225 times the maximum likely dose experienced by a dairy animal on the basis of its consuming a diet containing 20% soybeans (Edwards and Zager, 1994) treated at the label rate with FirstRate.

Dose Preparation and Administration. Radiolabeled cloransulam-methyl samples (AN and TP labels) were separately dissolved in acetone to give solutions of known concentration. Aliquots of each acetone solution (7.3 mL for AN and 7.5 mL for TP) were added to each of a series of gelatin capsules, the acetone was evaporated under a stream of nitrogen gas, and the capsules were sealed. Thus, each AN-labeled capsule contained 17.96 mg (7.862×10^8 dpm), and each TP-labeled capsule contained 18.00 mg (7.850×10^8 dpm) of cloransulam-methyl. Each capsule was placed in a secondary capsule and stored below 0 °C. Each of the treated animals received a single capsule daily in the morning for 5 days. Thus, the animals received total doses of cloransulam-methyl of 3.93×10^9 dpm (AN label) and 3.92×10^9 dpm (TP label). Control capsules were prepared by spiking empty capsules with blank acetone, allowing the solvent to evaporate, then sealing and storing as for the ^{14}C-labeled capsules. The control animal received a control capsule daily.

Radiochemical Purity of the Test Substances and Stability of Cloransulam-methyl in Capsules during the Dose Period. The radiochemical purity of both test substances was determined by HPLC analysis with on-line radiochemical detection prior to study initiation. In addition, the stability of cloransulam-methyl in the dose capsules during storage was demonstrated by analysis prior to dosing and after the completion of dosing. No measurable degradation took place during 7 days of frozen capsule storage.

Dose Administration and Sampling Procedures prior to Sacrifice. The encapsulated [^{14}C]cloransulam-methyl was administered to the goats using a balling gun once daily for 5 consecutive days. During the dosing period, samples of milk, feces, and urine were taken twice daily, as follows.

Milk. Animals were hand-milked twice a day, with a daytime interval of ~8 h. The teats and udder base were washed with warm water containing an iodine-based disinfectant and then dried. The milk sample was then collected into a container and the disinfecting procedure repeated on the teats. The weight of each milk sample was recorded, aliquots were taken for radioanalysis, and the remainder was stored frozen.

Urine and Feces. The total excretion of urine and feces was collected and weighed twice each day (morning and evening) during the dosing period. Samples were collected in polyethylene containers that were kept on ice in insulated containers. The urine from each collection was swirled, aliquots were taken for radioanalysis, and the remainder was stored frozen. Prior to radioanalysis, the feces were processed by homogenization as described later.

Following sacrifice, the metabolism cages were washed with a solution of 1% aqueous trisodium phosphate. The washes were collected, a subsample was taken for radioanalysis, and the remainder was stored frozen. Following washing, the cages were wiped down and the wipes were radioassayed.

Sacrifice and Tissue Collection. The goats were sacrificed using a captive-bolt pistol ~22.5 h after the last dose, and samples of the blood, muscle (round), liver (entire), kidneys (both), fat (1:1 omental/renal), urine from the bladder, and gastrointestinal contents and gastrointestinal tract were collected. Care was taken to prevent contamination of the tissues by feces or urine. After the samples were homogenized for radioanalysis and aliquots had been removed for ^{14}C determination, additional aliquots of the milk, urine, feces, fat, muscle, and liver were separated from the bulk samples. This allowed analysis of these smaller subsamples without thawing the bulk samples. All samples were stored at <0 °C before and after radioanalysis with the exception of blood, which was refrigerated until analysis was completed.

Preparation of Samples for Radioanalysis. *Blood.* Samples were homogenized by shaking, and triplicate aliquots of ~0.5 g were combusted in a sample oxidizer. The trapped $^{14}CO_2$ was then analyzed by LSC.

Cage Wash. Samples were homogenized by shaking, and triplicate aliquots of ~0.5 g were mixed with scintillation cocktail and assayed directly by LSC.

Cage Wipe. Sufficient ACN (weighed) was added to cover the wipes, and the samples were allowed to extract overnight. The extracts were then homogenized by shaking, and triplicate aliquots of ~0.5 g were mixed with scintillation cocktail and assayed directly by LSC.

Feces. Samples were homogenized using dry ice, which was then allowed to sublime. Each sample was stirred thoroughly, and a subsample of ~100 g was taken. Twice the sample weight of ACN was added, and the mixture was homogenized with a probe-type homogenizer. Triplicate aliquots of ~0.25 g of this mixture were then combusted as for the blood, described above, and analyzed by LSC. Some of the feces from goat 3 were significantly contaminated with urine due to the posture this animal adopted during excretion. In these cases, a portion of the original sample was extracted three times with ACN and centrifuged and the supernatants were combined. Aliquots of the supernatant were analyzed directly by LSC as for urine (below). Aliquots of ~0.25 g of the pellet were combusted and analyzed by LSC.

Kidney, Liver, Muscle. These tissues were homogenized with dry ice while frozen. The dry ice was allowed to sublime, and aliquots were analyzed by combustion/LSC.

Fat. Samples were homogenized as for the other tissues. Aliquots of ~0.5 g were digested in 15 mL of scintillation cocktail for ~12 h and then analyzed directly by LSC.

Urine and Milk. Samples were homogenized by shaking, and triplicate aliquots of ~0.25 g (urine) or ~0.5 g (milk) were mixed with scintillation cocktail and analyzed directly by LSC.

Gastrointestinal Tract Contents. The contents of the tract were removed, and the tract was washed with water. The entire sample was homogenized with a Polytron homogenizer, and ~1000 g was removed. This aliquot was centrifuged, and the separated pellets were extracted twice with ACN and again centrifuged. The ACN extract was pooled with the original tract homogenate supernatant, and triplicate aliquots of ~0.1 g of the resulting solution were assayed as for the urine. The

pellets were homogenized in a food grinder, and triplicate 0.25 g aliquots were assayed as for the feces pellets.

Initial Biological Samples—Validation of Radioassay Procedures. Initial tissue radioanalyses by combustion were validated by analyzing triplicate aliquots of each of control milk, urine, feces, and tissues that had been fortified with a known amount of [^{14}C]cloransulam-methyl. The overall mean recovery during combustion of these matrices was 99.2%. Therefore, the measured dpm values were not corrected for recovery.

Sources of Enzymes and Test Substrates Used in Residue Characterization. Enzyme incubation procedures were performed on the liver and kidney tissues after initial solvent extractions. Enzymes used were type VII collagenase from *Clostridium histolyticum* and pancreatin from porcine pancreas. The viability of these enzymes was verified using azo dyes bound to protein substrates. The substrates were Azocoll and Azocasein, used for collagenase and pancreatin viability, respectively. All of these materials were purchased from Sigma Chemical Co.

Chemicals Used for Metabolite Derivatization. Triethylamine and triethyloxonium tetrafluoroborate were purchased from Aldrich Chemical Co.

Sources of HPLC and TLC Stationary Phases. All HPLC analyses were performed using C_{18} Nova-Pak radial compression columns (10 cm long × 0.8 cm i.d.; 4 μm particle size) obtained from Waters, Division of Millipore. TLC was performed under normal phase conditions using 20 cm × 20 cm glass plates coated with 0.25 mm thick layers of Merck Kieselgel 60-F254.

Liquid Scintillation Counting. Radioactivity was measured using Beckman LS 6000LL or Packard Tri-Carb 2000CA liquid scintillation counters. The liquid scintillation counter automatically converted the counts per minute (cpm) to disintegrations per minute (dpm), using an empirically determined quench curve.

Sample Oxidizers. A Packard Tricarb 307, Harvey OX300, or OX500 biological sample oxidizer was used to combust aliquots of samples to carbon dioxide and water. The resulting $^{14}CO_2$ was trapped in an appropriate LSC cocktail and then quantified by liquid scintillation counting. The performance of the oxidizer was verified daily by oxidizing a known amount of a radioactive standard, measuring the recovered radioactivity and thereby calculating the percent recovery of ^{14}C. For all samples other than the initial biological matrices (i.e., extracts) the true radioactivity content of each sample aliquot combusted was determined by dividing the dpm in the sample by the percent recovery determined using the radioactive standards as described above during the performance check.

TLC Plate Analysis. Radioactive components on thin-layer plates were located using a Bioscan Imaging Scanner System 200 with autochanger. The R_f values of the radioactive components were compared with the R_f values of cochromatographed non-radiolabeled reference standards. The standards were visualized by viewing the plate under a UV light at 254 nm and observing zones of fluorescence quenching.

Chromatographic Analysis. Various chromatographic techniques were employed to analyze milk, urine, and extracts of liver and kidney tissues. Most extract fractions, both organic and aqueous, were analyzed by reverse phase HPLC. The ^{14}C component identities in the majority of organic fractions were confirmed by TLC analysis. Additionally, TLC analysis was used to screen urine fractions to determine which sample contained the highest level of cloransulam for its isolation and identification. Low-pressure silica gel column chromatography was used to provide initial purification of [^{14}C]cloransulam prior to its final purification by HPLC.

High-Pressure Liquid Chromatography. Two HPLC systems were used during the course of this study. One system consisted of Waters M-6000A and M-45 pumps, a Waters 484 UV detector and SIM box, and a radial compression module (RCM) with a C_{18} Nova-Pak reversed phase column. An Isco Retriever IV fraction collector was used to collect separated ^{14}C metabolites. The system was controlled by Maxima 820 software running on an NEC APC-IV Power Mate 1 computer. A manual Rheodyne injector (model 7125) was used to introduce samples to the flowing mobile phase. A second system, consisting of a Waters 600E controller and pumping system, a Waters 991 photodiode array detector, and a Rheodyne manual injector (model 7125), was also used. In addition, this HPLC system was equipped with a Waters C_{18} Nova-Pak reversed phase column and associated RCM column holder. Both instruments used Waters GuardPak precolumn modules ahead of the analytical column. Two main HPLC elution methods were used with solvents A [water/AcOH (99.5:0.5)] and B [ACN/AcOH (99.5:0.5)] flowing at 1.5 mL/min as follows.

Gradient Method. At $t = 0$, % B = 0, hold under these conditions for 5 min, then linear gradient to 60% B at 45 min, then linear gradient to 100% B at 50 min, hold at 100% B for 5 min, then linear gradient to 0% B at 60 min, and hold under these conditions for a further 10 min.

Isocratic Method. Hold at 75% B.

When radioactive metabolite mixtures were analyzed, the eluent from the HPLC column was collected at regular intervals (e.g., 120 × 0.5 min). The radioactivity in each fraction was determined by adding scintillation cocktail to each vial and counting by LSC, and a graphical representation of the radioactivity profile in individual analyses was plotted. The ^{14}C recovery for each analysis was checked by direct LSC of a separate aliquot of the sample solution and comparing the measured dpm with the total dpm recovered during HPLC fractionation. The average recovery from HPLC analysis of the fractions described here was 101.9%. Consequently, HPLC recovery was not factored into the calculation of individual metabolite concentrations.

Thin-Layer Chromatography. TLC was performed using Merck Kieselgel 60 F-254 silica gel plates developed in unlined chambers with toluene/acetonitrile/acetic acid (50:45:5). TLC plates bearing ^{14}C components were scanned with the BioScan Imaging plate scanner to determine the location and distribution of the ^{14}C components.

Low-Pressure Column Chromatography. A glass column (0.7 cm i.d. × 120 cm long) was packed with ~50 mL of silica gel 60 F-254 (EM Science). The ^{14}C sample was dried onto additional silica gel, applied to the top of the column, and eluted using a stepped gradient solvent system, which was delivered to the column with an FMI pump at a flow rate of ~20 mL/min. A fraction collector was used to collect 190 × 20 mL fractions, and a known aliquot was removed from each fraction for LSC analysis. The step gradient solvent system used to elute the column was as follows: hexane/AcOH (99.5:0.5; 200 mL), hexane/toluene/AcOH (90:10:0.5; 400 mL), hexane/toluene/AcOH (50:50:0.5; 400 mL), toluene/AcOH (99.5:0.5; 400 mL), toluene/EtOAc/AcOH (90:10:0.5; 400 mL), toluene/EtOAc/AcOH (50:50:0.5; 400 mL), EtOAc/AcOH (99.5:0.5; 400 mL), EtOAc/acetone/water/AcOH (80:20:2:0.5; 400 mL), acetone/water/AcOH (95:5:0.5; 400 mL), acetone/AcOH (95:5; 400 mL). Radio-TLC was used to analyze all fractions containing significant levels of radioactivity.

GC-MS Conditions. GC-MS analysis was performed using a Hewlett-Packard Series II model 5890 gas chromatograph linked to a 5971 series Hewlett-Packard mass selective detector. Separations were achieved using an HP-1 column (12 m long × 0.2 mm i.d; 0.33 μm film thickness) with manual sample (2 μL) injection using a model 80378 Hamilton syringe. The column was held at 120 °C for 2 min after injection, then ramped at a rate of 20 °C/min over 10.25 min, and finally held at 325 °C for 8 min. The MS electron multiplier was set to ~1700 V.

Analytical Procedures for Goat Samples. *Preparation and Characterization of Composite Urine Samples.* Two composite urine samples (one AN-labeled and one TP-labeled) were prepared from each individual sample taken over the course of the feeding period. This was done by combining representative aliquots (by weight) from each collected sample. For example, the urine sample collected for the AN-treated goat on day 1, a.m., represented ~13% of the total urine sample by weight, collected over the 5 day test period. Therefore, the

aliquot used from this sample to form the composite sample represented ~13% of the total composite sample, by weight.

Aliquots were removed from each composite sample (AN, TP) for LSC analysis after each sample had been centrifuged at ~1600 rpm for 15 min. Each composite was profiled by HPLC (gradient method) by injecting 200 μL of sample and collecting 120 × 0.5 min fractions. Non-radiolabeled reference standards were also chromatographed under the same conditions.

Due to its high radioactivity concentration, the urine was used for isolation of a metabolite also present in the liver organic-1 fraction at much lower concentration (~0.005 mg/kg). TLC was performed on selected urine samples on the basis of their high concentration of radioactivity (dpm/g) compared with the other urine samples. The following urine samples were analyzed by TLC: AN, day 2, a.m.; AN, day 3, a.m.; AN, day 4, a.m.; AN, day 4, p.m.; AN, day 5, a.m.; AN day 5, sacrifice; TP, day 2, a.m.; TP, day 3, a.m.; TP, day 4, a.m.; and TP, day 5, a.m. Each aliquot was cospotted with ~10 μg of cloransulam-methyl and 10 μg of cloransulam non-radiolabeled reference standards. Following elution, the ^{14}C zones were located using the BioScan scanner.

Isolation and Purification of the Major Metabolite from Urine. The AN, day 5, a.m., urine sample was acidified and extracted with 3 equal volumes of EtOAc. After LSC of the resulting organic and aqueous phases, the EtOAc phase was analyzed by TLC. The sample was then evaporated to dryness and redissolved in acetone and chromatographed by low-pressure silica gel chromatography as described above. An aliquot was removed from each fraction for LSC analysis, and fractions containing radioactive components of similar polarity were pooled. The following five bands resulted: band 1 (fractions 82−93; 89.1% of eluted ^{14}C); band 2 (105−112; 7.5%); band 3 (124−131; 2.4%); band 4 (150−158; 1.7%); and band 5 (164−176; 0.7%). Each band was evaporated to dryness, redissolved in acetone, and analyzed by TLC to locate the desired metabolite for further purification.

Band 2 was evaporated to dryness and redissolved in an HPLC mobile phase consisting of ACN/water/AcOH (75:25:0.5). Aliquots of this sample were injected onto the HPLC (isocratic system), and the 18−20 min region from the HPLC eluent stream was collected. The HPLC-purified metabolite was concentrated and analyzed using the isocratic HPLC solvent elution system. In addition, an aliquot of reference standard cloransulam was separately analyzed using the same conditions.

Derivatization and GC-MS Analysis of Metabolite Isolated from Urine. The HPLC-purified urine metabolite was derivatized with TEOTFB, as follows. An aliquot of the metabolite solution was evaporated to dryness (N_2) and redissolved in acetone (500 μL). A 10 μL aliquot was removed for LSC analysis to verify that the sample had dissolved. TEA (10 μL) and TEOTFB (100 μL of a 1 M solution) were added to the acetone solution of the ^{14}C metabolite, and the sample was placed in an ultrasonic bath for 5 min and then allowed to react at room temperature for an additional 25 min. A 25 μL aliquot was removed from the crude reaction mixture for TLC analysis to verify reaction completion. The solvent was removed from the sample (N_2), and aqueous NaCl solution (1 mL of 5% w/v) and MTBE (5 mL) were then added; the sample was then placed in an ultrasonic bath for ~10 min. After centrifugation of the mixture at ~1200 rpm for 10 min, the ether phase, containing the derivatized ^{14}C metabolite, was removed, evaporated to dryness, dissolved in 50 μL of toluene, and then analyzed by GC-MS. A sample of reference standard cloransulam was derivatized concurrently under the same conditions. TLC analysis was performed on the crude reaction mixture for the non-radiolabeled reference standard, as described for the ^{14}C reaction mixture.

Characterization of Liver and Kidney Metabolites. Essentially the same fractionation methods were used for each tissue type. Initial solvent extraction and chromatographic characterization of the organic-1 and aqueous-1 phases was completed within 19 and 26 days of sacrifice, respectively. Analysis of the extracts was accomplished using gradient HPLC and/or TLC. Non-radiolabeled standards were eluted under the same conditions for any given HPLC or TLC analysis. More extensive fractionation methods for the insoluble residues were subsequently developed and used to characterize the residues as discussed below. The qualitative and quantitative data from these extractions showed that all residues were stable when the tissues were stored frozen even over periods of up to 8 months. A summary of the methods used to characterize the liver and kidney residues is given in Figures 2 and 3. Data for fractions in solid boxes are reported in Tables 4−9. Although ^{14}C data were measured for the other fractions (acetone−water soluble, pancreatin-soluble, HCl-soluble), these fractions were pooled and further fractionated prior to final analysis. Therefore, the data for these "intermediate" fractions were not reported here, for clarity.

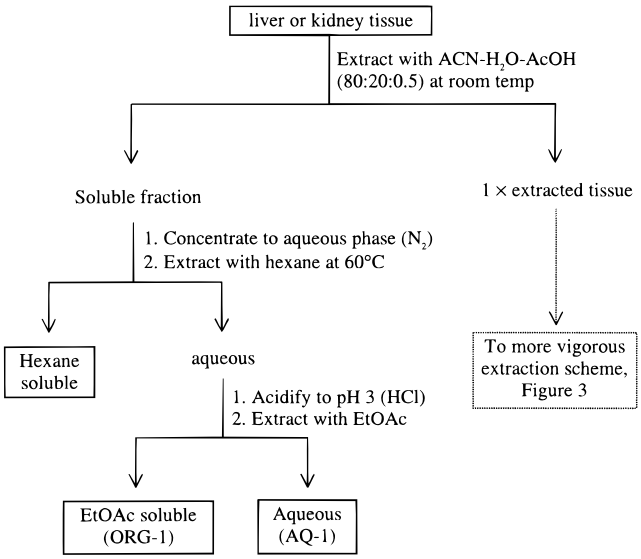


Figure 2. Scheme used for fractionation of [^{14}C]cloransulam-methyl residues in goat liver and kidney ("readily soluble" residues).

Initial Aqueous−Organic Solvent Extraction and Fractionation. Tissue samples were removed from frozen storage and allowed to partially thaw (control, AN, and TP). Control tissue was fortified with [^{14}C]cloransulam-methyl at ~0.05 mg/kg (liver) or ~0.13 mg/kg (kidney), levels similar to residue levels in the respective treated tissues. These are referred to in subsequent discussion as control spiked tissue (CS). Approximately 100 g of each liver sample and 30 g of each kidney sample were extracted with 3 successive volumes of ACN/water/AcOH (80:20:0.5; 100 mL for liver, 50 mL for kidney). Each sample was homogenized at ~20000 rpm for 2 min using a Brinkmann PT 3000 Polytron and centrifuged, and the supernatant solutions were transferred to graduated cylinders. After quantification of the ^{14}C by LSC of aliquots, the filtered extracts were concentrated (N_2) to an aqueous phase and partitioned against 3 equal volumes of hexane at ~60 °C to remove fats. The hexane phases were back-extracted with water, and the resulting aqueous extracts were combined with their corresponding main aqueous fractions. The ^{14}C in each hexane fraction was quantified by LSC: the CS liver sample was the only sample containing significant radioactivity. An aliquot of the CS liver hexane fraction was extracted with 3 equal volumes of acetonitrile. The acetonitrile phase was evaporated to dryness, redissolved in acetone, and analyzed by TLC. The defatted aqueous fractions were acidified to pH ~3 with 6 M HCl. The acidified aqueous fractions were extracted with 3 equal volumes of EtOAc, the aqueous phases were lyophilized and reconstituted with water/AcOH (99.5:0.5), and the ^{14}C was quantified by LSC. The ^{14}C in the EtOAc fractions was quantified by LSC, and then the EtOAc fractions were concentrated (N_2), redissolved in ACN/AcOH (99.5:0.5), and reassayed by LSC. The radioactive components of both the organosoluble and the aqueous soluble residues were

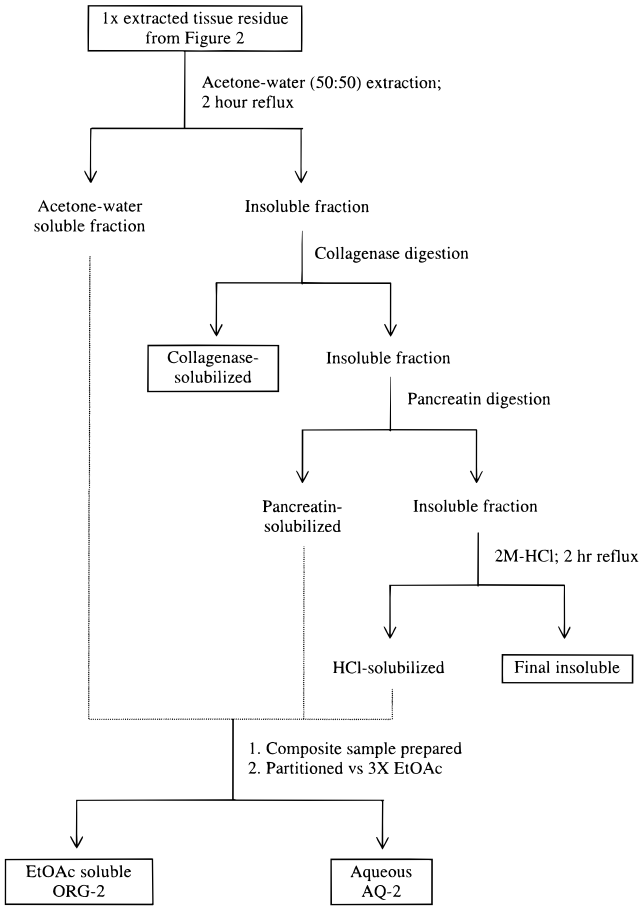


Figure 3. Scheme used for fractionation of [14C]cloransulam-methyl residues in goat liver and kidney ("not readily soluble" residues).

chromatographed by gradient HPLC. The organic fractions were, additionally, analyzed by TLC to confirm the identities of metabolites established by HPLC.

Secondary Aqueous−Organic Solvent Extraction. Acetone/water (50:50; 300 mL for liver, 60 mL for kidney) was added to each tissue residue from the initial ACN/water/AcOH extraction in an Erlenmeyer flask equipped with a water-cooled condenser. The samples were refluxed for ∼2 h, then cooled, centrifuged, and filtered through medium-porosity fritted glass Büchner funnels. The 14C in the supernatant solutions was quantified by LSC, and the samples were concentrated to an aqueous phase under nitrogen, prior to combination with pancreatin and HCl extracts (described below). The tissue pellets were treated sequentially with two enzymes in a further attempt to liberate bound residues, as described below.

Collagenase Digestion. In preparation for treatment with collagenase enzyme, each previously extracted tissue pellet (AN, TP, CS) was rinsed with 0.05 M TRIS buffer plus 1 mM $CaCl_2$ (at pH 7.8) to remove any residual organic solvent. TRIS buffer solution (160 mL for liver; 50 mL for kidney) and collagenase enzyme dissolved in TRIS buffer (1 mL = 3.6 mg of enzyme/liver sample or 21 mg of enzyme/kidney sample) were added to each of the three tissue samples, and the tissues were incubated at ∼37 °C overnight using a rotary water bath shaker rotating at 130−200 rpm. After incubation, the samples were centrifuged at ∼1500 rpm for 15 min, and the aqueous supernatants were decanted. The tissue pellets were rinsed with water at pH 2, and the rinses were added to the supernatant solutions. Concentrated HCl was added dropwise to each extract to precipitate insoluble material, which was removed by centrifugation. The supernatant solutions were transferred to graduated cylinders. The 14C in each aqueous sample was determined by LSC, and the small acid-precipitated pellets were combusted to determine their 14C content. The tissue pellet rinses contained no detectable radioactivity and are not discussed further.

Pancreatin Digestion. Each tissue pellet from the collagenase treatment was rinsed with an aliquot of 0.05 M TRIS/1 mM $CaCl_2$ buffer solution (100 mL) to remove any residual enzyme from the previous treatment. Pancreatin dissolved in TRIS buffer (100 mL for liver = 1 g of enzyme; 50 mL for kidney = 0.5 g enzyme) was added to each sample, and the samples (AN, TP, CS) were incubated and worked up as described for the collagenase treatment. This generated an acidified aqueous fraction and small acid-precipitated pellets in addition to the residual tissue pellets. Radioactivity in the aqueous phases was determined by LSC, and combustion was performed on the small precipitates.

Acid Digestion. Following the enzyme treatments, the remaining liver and kidney tissue pellets were subjected to a refluxing acid treatment. The AN and TP tissues were added to Erlenmeyer flasks that were equipped with a stir bar, water-cooled condenser, and a heating/stirring mantle. Hydrochloric acid (2 M; 300 mL for liver; 100 mL for kidney) was added to each sample, and the mixture was refluxed for ∼2 h. After cooling, each sample was centrifuged. The supernatant solutions were filtered through medium-porosity fritted glass Büchner funnels and transferred to graduated cylinders. The remaining tissue pellets were dried in an oven at ∼35 °C for 3 days. The 14C in the supernatant solutions was determined by LSC of aliquots, and the final tissue pellets were assayed by combustion.

TLC Analysis of (Acetone/Water plus Pancreatin plus 2 M HCl)-Soluble Radioactive Residues. A composite sample was separately prepared from the AN and TP secondary extracts (i.e., acetone/water plus pancreatin plus 2 M HCl) of the tissues by combining 80% of the 14C in each phase. The composite samples were then extracted with 3 equal volumes of EtOAc. In the CS samples, apart from the primary aqueous/organic extract, the only extract containing significant activity was the acetone/water one, and, after removal of the acetone, this fraction was directly partitioned with EtOAc (i.e., no combination with pancreatin-soluble etc). Each EtOAc sample was evaporated to dryness under reduced pressure, redissolved in acetone, and centrifuged at ∼1200 rpm for 15 min. Aliquots were removed from the organic and aqueous fractions for direct LSC analysis and for combustion analysis, respectively. TLC analysis was performed on the acetone solutions.

Validation of Activity of Collagenase and Pancreatin Enzymes Used To Release Bound Residues. Azocoll (pink dye) and azocasein (yellow dye) were the protein-bound dye substrates used for these validations, respectively. In the assay, enzyme activity was shown by the release of dye from the protein−dye complex, thereby causing the incubation solution to become colored. Absence of the appropriate color in the filtered solution after incubation would, conversely, indicate an inactive enzyme or an interference from another component in the incubation. This latter possibility was excluded by choice of appropriate control incubations, as described below. The activity of each enzyme was verified at the time of its use. The appropriate substrate (protein−dye complex) was incubated in TRIS buffer with a known quantity of enzyme (sample referred to as "complete mix") overnight at ∼37 °C. The reaction was quenched with 10% trichloroacetic acid solution and then filtered through Whatman Puradisc 25 TF (0.45 μm) filters to remove precipitated proteins. Six validation samples were run concurrently under these conditions: a blank (TRIS buffer only), sample blank (control tissue plus buffer), enzyme blank (enzyme plus buffer), substrate blank (dye−protein substrate plus buffer), complete mix (buffer plus enzyme plus substrate), and a sample interference (control tissue plus buffer plus enzyme plus substrate). Each sample was observed for a color change after filtering. A change to the appropriate color (pink or yellow depending on the substrate used) in the complete mix and the sample interference samples demonstrated that the enzyme was active.

Determination of Maximum Cloransulam-methyl Levels in Milk Samples. Milk fractionation was completed within

2 days of sample receipt, and gradient HPLC characterization of the soluble fractions was completed within 10 days of sacrifice. Approximately 100 g of each milk sample [AN, day 4, p.m. (0.003 mg/kg) and TP, day 4, p.m. (0.002 mg/kg)] was thawed. Acetone was added to each sample to make the final acetone/aqueous milk ratio approximately 70:30, and then each sample was placed in frozen storage overnight. The samples were thawed, and the precipitated protein was removed by centrifugation at ~1000 rpm for 15 min. The aqueous acetone fraction was separated from the pellet, and the ^{14}C in the pellet was quantified by combustion of aliquots. The aqueous acetone samples were concentrated to an aqueous phase (N_2), and the ^{14}C in the resulting aqueous samples was quantified by LSC analysis. The pH of each sample was adjusted to ~3 using 6 M HCl, and the acidified aqueous samples were extracted with three successive aliquots of hexane. The ^{14}C in the hexane and aqueous phases was quantified by LSC, then the defatted aqueous phases were lyophilized and reconstituted in water/AcOH (99.5:0.5), and aliquots were assayed by LSC analysis. The radioactive components in the aqueous samples were chromatographed by HPLC.

Statistical Methods. The minimum detectable amount (MDA) of ^{14}C during LSC was calculated on the basis of typical background values and count times, according to the method of Currie (1968). This value was used to determine whether low-level LSC counts were detectable.

The MDA is the lowest level of radioactivity, in dpm, detectable above background:

$$\text{MDA (dpm)} = \frac{2.71 + (4.65 \times \sqrt{\text{BKG} \times t})}{t}$$

BKG is the dpm for a typical background sample, and t is the counting time interval (in minutes). Samples showing counts below the MDA were considered to contain no radioactivity.

RESULTS AND DISCUSSION

Residue Concentrations. A summary of the radioactivity content of the excreta and milk samples during the dosing period and the various samples collected at sacrifice is presented in Table 2. The data show that the overall recoveries of radioactivity were ~93% for the AN-labeled goat ~81% for the TP-labeled goat. Over 92% of the recovered radioactivity for each labeled form was excreted from the animals during the 5 day dosing period. Of the radiolabel remaining in the animals at sacrifice, the majority was present in the GI tract and would be expected to have passed from the animals in subsequent days under normal circumstances. Less than 0.1% of the test material consumed by the animals was present in the edible tissues or milk, demonstrating the very low potential for this compound to bioaccumulate or be retained in ruminant animals. Excretion of radiolabel followed a similar temporal pattern in each treated goat, with initiation of dose excretion in both urine and feces commencing in the afternoon following the first morning's dose and continuing regularly thereafter. Due to the posture the TP goat adopted while urinating, some problems were encountered with its urine collection. Although the total radioactivity in the feces of both animals was very similar, that in the TP urine was ~15% less than that of the AN urine, paralleling the difference in overall recovery of radioactivity. This might account in part for the lower total radioactivity recovered from the TP animal. However, (i) excretion clearly accounted for the vast majority of the test material the animals consumed, and (ii) there was no evidence that such a difference could be explained, for example, on the basis of different metabolite profiles in the urine samples (see below) resulting from a sulfonanilide bridge cleavage reaction with subsequent loss of a TP-only labeled metabolite through volatility.

Table 2. Partitioning of Radioactivity between Excreta and Tissues of Goats Receiving Five Consecutive Daily Doses of [^{14}C]Cloransulam-methyl

		% of total dose	
sample type	collection time	AN goat[a]	TP goat[b]
urine	day 1, a.m.	NA	NA
	day 1, p.m.	0.58	1.38
	day 2, a.m.	6.32	2.54
	day 2, p.m.	3.23	1.48
	day 3, a.m.	6.12	7.83
	day 3, p.m.	1.77	0.80
	day 4, a.m.	6.27	3.00
	day 4, p.m.	2.52	1.56
	day 5, a.m.	8.04	4.06
	day 5, p.m.	2.01	1.41
	day 5, sacrifice	6.26	4.47
	total	43.12	28.53
feces	day 1, a.m.	NA	NA
	day 1, p.m.	0.02	1.90
	day 2, a.m.	3.72	4.81
	day 2, p.m.	4.03	2.79
	day 3, a.m.	6.56	2.23
	day 3, p.m.	4.58	3.56
	day 4, a.m.	4.47	9.47
	day 4, p.m.	4.45	3.77
	day 5, a.m.	6.70	9.31
	day 5, p.m.	1.98	2.18
	day 5, sacrifice	6.22	5.64
	total	42.72	45.66
cage wash	day 5, sacrifice	0.81	0.64
cage wipe	day 5, sacrifice	0.04	0.03
	total excreted	86.69	74.86
milk	day 1, a.m.	NA	NA
	day 1, p.m.	0.002	0.000
	day 2, a.m.	0.000	0.000
	day 2, p.m.	0.002	0.000
	day 3, a.m.	0.000	0.000
	day 3, p.m.	0.002	0.002
	day 4, a.m.	0.000	0.000
	day 4, p.m.	0.003	0.002
	day 5, a.m.	0.002	0.000
	day 5, p.m.	0.002	0.002
	day 5, sacrifice	0.003	0.000
	total	0.015	0.007
kidneys	day 5, sacrifice	0.03	0.02
liver	day 5, sacrifice	0.06	0.05
muscle	day 5, sacrifice	0.00	0.00
fat	day 5, sacrifice	0.00	0.00
blood	day 5, sacrifice	0.00	0.00
GI tract	day 5, sacrifice	5.97	6.34
	total retained	6.07	6.42
^{14}C recovered		92.77	81.28

[a] AN total dose = 3.93×10^9 dpm. [b] TP total dose = 3.92×10^9 dpm.

The residue concentrations, expressed as milligrams of CM equivalents per kilogram, that occurred in the milk and tissues of the animals at sacrifice are summarized in Table 3. These residues resulted from a dose that was ~2225 times the maximum estimated dietary exposure for dairy animals consuming diets with soybeans containing expected levels of cloransulam-methyl and its metabolites. Even when such a greatly exaggerated dose of cloransulam-methyl was fed, tissue residue levels were very low. Kidney (0.122 and 0.128 mg/kg for AN and TP radiolabels, respectively) and liver

Table 3. Residue Concentrations[a] in Milk and Edible Tissues of Goats Receiving Five Consecutive Daily Doses of [14C]Cloransulam-methyl

matrix	AN goat	TP goat	matrix	AN goat	TP goat
milk[b]	0.002	<0.001	muscle	0.002	0.002
kidneys	0.122	0.128	fat	<0.001	0.002
liver	0.051	0.040	blood	0.029	0.043

[a] mg of CM equiv/kg. [b] Day 5 (sacrifice).

(0.051 and 0.040 mg/kg) were the only tissues having levels >0.002 mg/kg. Levels in milk were also extremely low at ~0.003 mg/kg when averaged over the highest residue levels in AN and TP radiolabeled milk occurring during the dosing regime. Simple extrapolation of the measured (exaggerated) tissue residue levels to those resulting from consumption of a 0.02 mg/kg level of cloransulam-methyl residues in soybeans would predict that total residues in both kidney and liver would be on the order of 50 ng/kg and probably undetectable by conventional analytical techniques.

In general, the residue level in any given tissue was independent of the site of radiolabeling of [14C]cloransulam-methyl. These data are consistent with a metabolic pathway in which sulfonanilide bridge cleavage does not play a significant role in the degradation of the compound. As discussed below, this was reflected in the chromatographic profiles of metabolites from both edible tissues and urine. In the following discussion, identified metabolites are referred to by name. Table 1 gives a list of structures and abbreviations for standards used to assist in metabolite identification. Several of these metabolites (C, 5-HCM, 5-HC) had been identified in soil metabolism studies (Wolt et al., 1996). The homoglutathione pathway adducts CM-7-hGSH (DeBoer, personal communication, 1995) and CM-CYS (Lewer et al., unpublished data) were identified as major CM soybean metabolites and, although these compounds were not expected to be formed in the goat, the structurally similar CM-7-glutathione and CM-7-*N*-acetylcysteine analogues were plausible metabolites. Although metabolic sulfonanilide bridge cleavage reactions have been generally rare for this molecule, if such reaction were to occur, likely initial products would be MCA and CA. Thus, these materials were used as standards to check for this metabolic event.

Several discrete but low-level (<10% of TRR or <0.01 mg/kg) components were separated chromatographically and thereby characterized but not identified. These are referred to in the discussion and tables as LIV-1, KID-2, etc., with the lower numbered components having higher polarity as judged by earlier elution during reversed phase HPLC analysis. Because these components were unidentified, it was not possible to conclude whether specific LIV-*n* and KID-*n* metabolites were the same, and no such implication is intended to be made by this numbering system. In some cases, 14C metabolites cochromatographed with synthetic standards and were tentatively identified on this basis. In such cases, HPLC-based identification was always substantiated by TLC-based identification. However, their levels were generally extremely low, and no more definitive identification was pursued. Throughout this discussion, all individual metabolite concentrations are expressed as milligrams of CM equivalents per kilogram.

Liver Residues. Liver samples contained 0.06 and 0.05% of the administered 14C activity for the AN and TP tissues, respectively, with corresponding concentrations of 0.051 and 0.040 mg/kg (Tables 2 and 3). The tissues were extracted using the scheme summarized in Figures 2 ("readily soluble" metabolites) and 3 ("not readily soluble" metabolites). The first step, extraction with ACN/water/AcOH (80:20:0.5), released 38.7% (0.020 mg/kg) and 42.3% (0.017 mg/kg) of the TRR in the respective tissues (AN, TP). These extracts were concentrated, acidified, defatted using hexane, and then extracted with EtOAc, resulting in organosoluble and aqueous fractions referred to as ORG-1 and AQ-1, the readily soluble fractions. Only minimal radioactivity, ≤4.9% (≤0.002 mg/kg) of the TRR for the AN and TP samples, was extracted into the hexane (Tables 4 and 5), and this was not investigated further

Table 4. Summary of Fractionation of Liver Residues[a,b] from Goats Receiving Five Consecutive Daily Doses of [14C]Cloransulam-methyl (AN Label)

	components					
fraction	CM	C	5-HCM	LIV-2[c]	other	fraction total
ORG-1	2.0% (0.001)	9.7% (0.005)	1.6% (<0.001)	ND	3.8%[d] (0.002)	17.1% (0.009)
ORG-2	1.1% (<0.001)	ND	ND	ND	17.8%[d] (0.009)	18.9% (0.010)
AQ-1	ND	ND	ND	6.8% (0.003)	5.0%[d] (0.003)	11.8% (0.006)
component total	3.1% (0.002)	9.7% (0.005)	1.6% (<0.001)	6.8% (0.003)	71.3%[e] (0.036)[e]	92.5%[f] (0.047)[f]

[a] In all cases, % = percent of TRR. [b] All parenthetical values are in mg of CM equiv/kg. [c] Unidentified metabolites are designated LIV-*n*. [d] Not a single component but the sum of several chromatographically separable components. [e] Sum of ORG-1 + ORG-2 + AQ-1 + AQ-2 (17.1%, 0.009) + hexane (4.9%, 0.002) + collagenase (1.6%, <0.001) + insoluble (21.1%[g], 0.011) "other" fractions. [f] Sum of ORG-1 + ORG-2 + AQ-1 + AQ-2 (17.1%, 0.009) + hexane (4.9%, 0.002) + collagenase (1.6%, <0.001) + insoluble (21.1%[g], 0.011) "fraction totals". [g] Insoluble fraction contains final insoluble pellet plus insoluble pellets from enzyme treatments.

The ORG-1 fractions represented 17.1% (0.009 mg/kg) and 19.5% (0.008 mg/kg) of the TRR for the AN and TP tissues, respectively. Reversed phase HPLC of these fractions showed the samples to be multicomponent and to have very similar metabolite profiles (Figure 4). The largest radioactive component, representing 9.7% (0.005 mg/kg) and 9.3% (0.004 mg/kg) of the TRR for the AN and TP tissues, respectively, showed a retention time similar to that of cloransulam. This metabolite was subsequently identified as the major metabolite in the urine (see discussion below) and, in view of its very low level in the liver, was identified from this tissue only on the basis of cochromatography (HPLC, TLC) with an authentic standard. Cloransulam-methyl comprised only 2.0% (0.001 mg/kg) and 5.9% (0.002 mg/kg) of the TRR for the AN and TP ORG-1 samples. Likewise, the identity of residual 14C-labeled parent was confirmed by HPLC and TLC cochromatography with authentic cloransulam-methyl. A third, more polar, radioactive component was present at ≤1.6% (<0.001 mg/kg) of the TRR in each of the AN and TP-labeled samples. This component had an HPLC retention time and TLC R_f value similar to those of 5-hydroxycloransulam-methyl (5-HCM; Table 1) and, on the basis of the chromatographic data and its occurrence in both labeled forms, was tentatively identified as the 5-hydroxy metabolite.

The AQ-1 fractions represented 11.8% (0.006 mg/kg) and 15.7% (0.006 mg/kg) of the TRR for the AN and TP tissues, and these were chromatographed using reversed phase HPLC analysis. The data (Figure 5) showed one major component, LIV-2, present at ~0.003 mg/kg.

Table 5. Summary of Fractionation of Liver Residues[a,b] from Goats Receiving Five Consecutive Daily Doses of [^{14}C]Cloransulam-methyl (TP Label)

fraction	components						fraction total
	CM	C	5-HCM	LIV-1[c]	LIV-2	other	
ORG-1	5.9% (0.002)	9.3% (0.004)	1.1% (<0.001)	ND	ND	3.2%[d] (0.001)	19.5% (0.008)
ORG-2	1.3% (<0.001)	ND	ND	ND	ND	16.9%[d] (0.007)	18.2% (0.007)
AQ-1	ND	ND	ND	1.7% (<0.001)	8.5% (0.003)	5.5%[d] (0.002)	15.7% (0.006)
component total	7.2% (0.003)	9.3% (0.004)	1.1% (<0.001)	1.7% (<0.001)	8.5% (0.003)	60.1%[e] (0.024)[e]	87.9%[f] (0.035)[f]

[a] In all cases, % = percent of TRR. [b] All parenthetical values are in mg CM equiv/kg. [c] Unidentified metabolites are designated LIV-*n*. [d] Not a single component but the sum of several chromatographically separable components. [e] Sum of ORG-1 + ORG-2 + AQ-1 + AQ-2 (18.8%, 0.008) + hexane (2.3%, <0.001) + collagenase (1.5%, <0.001) + insoluble (11.9%[g], 0.005) "other" fractions. [f] Sum of ORG-1 + ORG-2 + AQ-1 + AQ-2 (18.8%, 0.008) + hexane (2.3%, <0.001) + collagenase (1.5%, <0.001) + insoluble (11.9%[g], 0.005) "fraction totals". [g] Insoluble fraction contains final insoluble pellet plus insoluble pellets from enzyme treatments.

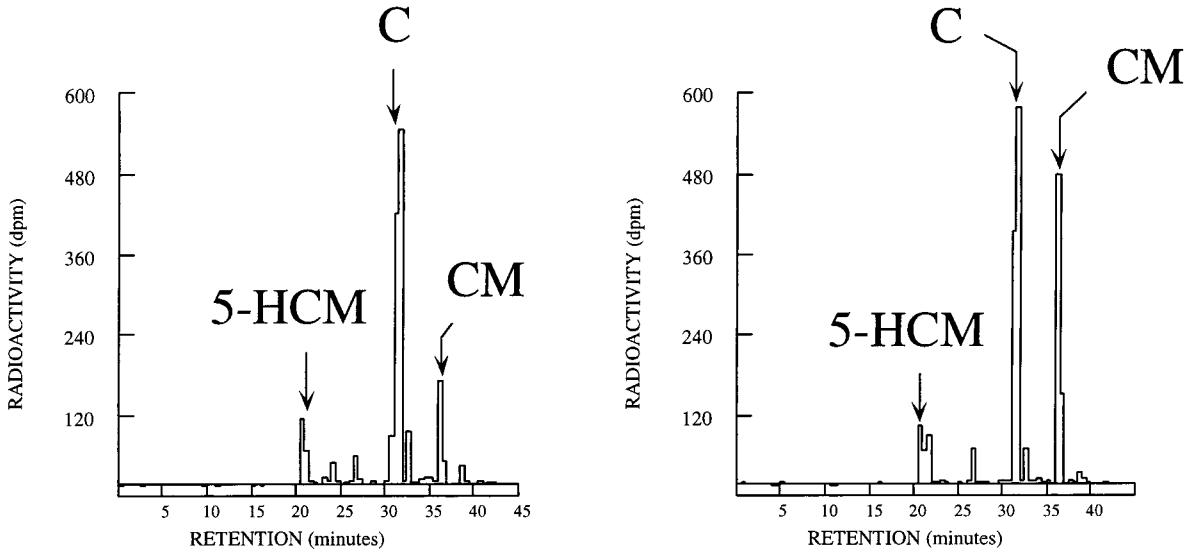


Figure 4. HPLC radiohistograms of AN- and TP-liver "organic-1" extracts (gradient elution method).

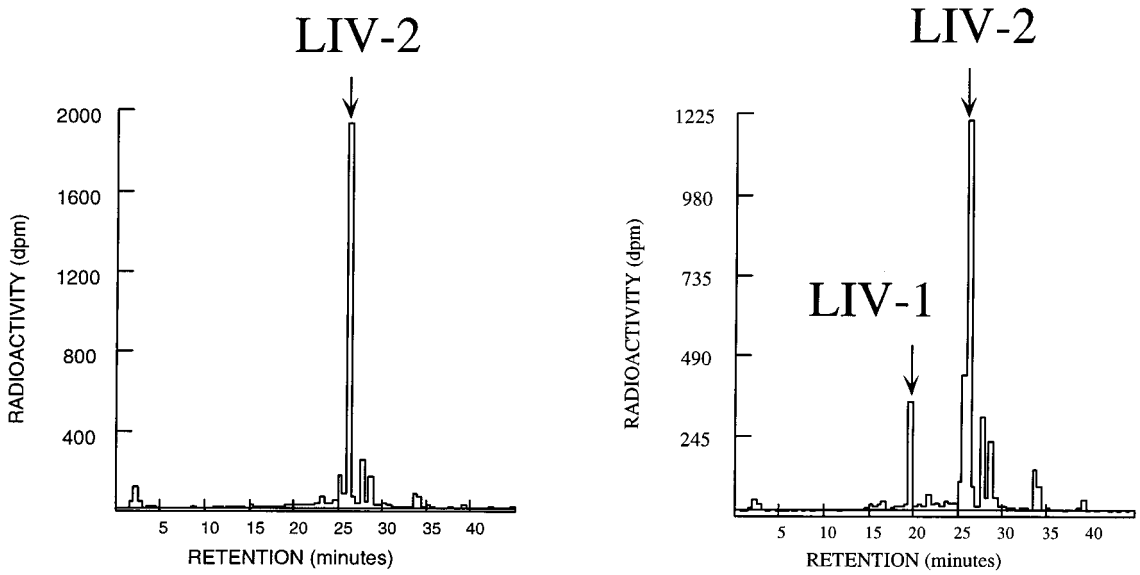
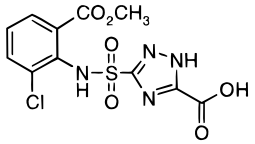


Figure 5. HPLC radiohistograms of AN- and TP-liver "aqueous-1" extracts (gradient elution method).

Because this component was present in each sample, it was "bridge-intact". However, it did not chromatograph with any of the standards and remained unidentified. The remaining several components totaled 0.003 mg/kg and were not further investigated.

In addition to the initial solvent extraction, attempts were made to release insoluble residues by using refluxing 50% aqueous acetone followed by a selection of enzymes and, finally, an acid treatment as summarized in Figure 3. Although not specifically demonstrated

during this work, CM was expected to be stable during the refluxing aqueous acetone and enzyme steps because the molecule is relatively stable under weakly acidic and neutral conditions. Products, which are formed slowly under these conditions, include 5-HCM and CM-TA (S. Erhardt-Zabik, Dow AgroSciences, personal communication, 1995).



CM-TA

Even during the relatively vigorous final acid treatment, only minor degradation would be expected. For example, a 2 h treatment of CM with refluxing 2 M HCl gave low levels of CM-TA (13%) and C (8%) as the only degradates (Lewer, unpublished data).

The refluxing 50% aqueous acetone extraction liberated 8.5% (0.004 mg/kg) and 9.8% (0.004 mg/kg) of the TRR for the AN and TP tissues, respectively. In trial incubations, protease was shown to liberate only minor (<8% of TRR; <0.003 mg/kg) amounts of ^{14}C-labeled residues from the aqueous−organic insoluble fraction. Therefore, as an alternative approach, the insoluble fractions were incubated with a two-step enzymic treatment designed to provide maximum likelihood of cleavage of any cloransulam-methyl residues covalently bound to the matrix, under mild conditions. Thus, collagenase was used first in an attempt to partially digest the collagen strands in the tissues and "open up" the tissue matrix. This should also help in the release of any physically entrapped CM residues. Subsequently, the matrix was treated with pancreatin, an enzyme mixture including protease, lipase, amylase, and DNase activities, in an attempt to release [^{14}C]cloransulam-methyl residues via nonspecific hydrolysis of macromolecular covalent bonds. The collagenase treatment resulted in the solubilization of ≤1.6% (<0.001 mg/kg) TRR for each radiolabeled form (Tables 4 and 5). The subsequent pancreatin treatment solubilized an additional 10.5% (0.005 mg/kg) and 7.3% (0.003 mg/kg) of the TRR in the AN and TP samples, respectively, with the secondary pellets containing 2.6% (0.001 mg/kg) and 0.4% (<0.001 mg/kg) of the TRR. Acid treatment (refluxing 2 M HCl) of the residual tissue matrix released the majority of the remaining residues: 23.4% (0.012 mg/kg) and 25.8% (0.010 mg/kg) of the TRR in the AN and TP tissues. The final insoluble pellets contained only 18.3% (0.009 mg/kg) and 11.3% (0.005 mg/kg) of the TRR for each of the respective tissues: therefore, the majority of the residue was solubilized by this procedure.

Due to the difficulty in separately analyzing each of the solubilized fractions, some of which contained very low ^{14}C levels, a composite sample was prepared that enabled a fractionation into EtOAc followed by TLC analysis of this organosoluble material. Organic extraction of the (acetone/H_2O plus pancreatin plus 2 M HCl) composite resulted in organosoluble and aqueous phases referred to as ORG-2 and AQ-2. Radioactivity in these samples was considered to be "not readily soluble" because more vigorous conditions were used to release the residues. The ORG-2 fractions contained 18.9% (0.010 mg/kg) and 18.2% (0.007 mg/kg) of the TRR in the AN and TP samples, whereas the AQ-2 fractions contained 17.1% (0.009 mg/kg) and 18.8% (0.008 mg/kg) of the TRR (Tables 4 and 5). TLC analysis of the ORG-2 fractions showed the samples to be multicomponent, with no single ^{14}C component representing >6.7% (0.003 mg/kg) of the TRR. ^{14}C cochromatographing with cloransulam-methyl represented <1.3% (<0.001 mg/kg) of the TRR. Overall, then, metabolism of cloransulam-methyl in the liver was extensive, with little parent remaining and with the formation of several low-level products.

Table 6. Summary of Fractionation of Radiolabel from Control Goat Liver Fortified with [^{14}C]Cloransulam-methyl

fraction[a]	components		fraction total
	CM	other	
ORG-1	62.8%[b] (0.032)[c]	0.5%[d] (<0.001)	63.3% (0.032)
ORG-2	4.8% (0.002)	0.3%[d] (<0.001)	5.1% (0.003)
AQ-1	ND	0.8% (<0.001)	0.8% (<0.001)
AQ-2	ND	0% (0.000)	0% (0.000)
hexane	15.1% (0.008)	2.3%[d] (0.001)	17.4% (0.009)
collagenase		0.9% (<0.001)	0.9% (<0.001)
pancreatin		1.7% (<0.001)	1.7% (<0.001)
insoluble		6.2% (0.003)	6.2% (0.003)
component total	82.7% (0.042)	12.7% (0.006)	95.4% (0.049)

[a] Fortification level = 0.051 mg/kg (purity of [^{14}C]CM = 95.3%). [b] In all cases, = percent of TRR. [c] All parenthetical values are in mg of CM equiv/kg. [d] Not a single component but the sum of several chromatographically separable components; in no case was [^{14}C]C detected.

To determine the extraction efficiency and stability of the parent compound during the extraction conditions employed for the AN and TP tissues, a sample of control liver (referred to as the CS sample) was fortified with [^{14}C]cloransulam-methyl. The fortification level was comparable to the total radioactive residue levels in the AN and TP samples, that is, ~0.051 mg/kg. This sample was then extracted and fractionated, and the fractions were chromatographed as for the AN and TP samples, with minor changes noted below. Of the ^{14}C added to the tissue, ~99.8% was recovered in the ACN/water/AcOH and acetone/water extracts. Because the ^{14}C levels present in the collagenase and pancreatin extracts were so low (Table 6), only the acetone−water-soluble material was partitioned into ORG-2 and AQ-2 fractions. Also, because essentially all of the ^{14}C had been extracted following pancreatin treatment, the acid digestion step was omitted. After further fractionation, 68.5% of the added ^{14}C was present in the ORG-1 and ORG-2 fractions, whereas 16.9% (0.009 mg/kg) had partitioned into the hexane fraction. Back-extraction of the hexane phase with ACN recovered all of the hexane-soluble radioactivity and enabled TLC analysis of this fraction, which proved to be largely cloransulam-methyl, as were the ORG-1 and -2 phases, as summarized in Table 6. Of the original ^{14}C added to the tissue sample, 82.7% was shown chromatographically to be cloransulam-methyl. Another 12.7% of the added ^{14}C was distributed over seven fractions (Table 6), and because the [^{14}C]cloransulam-methyl used to fortify the tissue

Table 7. Summary of Fractionation of Kidney Residues[a,b] from Goats Receiving Five Consecutive Daily Doses of [^{14}C]Cloransulam-methyl (AN Label)

	components								fraction
fraction	CM	C	KID-4[c]	KID-5	KID-6	KID-7	KID-8	other	total
ORG-1	51.3% (0.063)	2.3% (0.003)	ND	ND	1.5% (0.002)	ND	1.1% (0.001)	0.4%[d] (<0.001)	56.6% (0.069)
ORG-2	0.9% (0.001)	ND	ND	ND	ND	ND	ND	3.5%[d] (0.004)	4.4% (0.005)
AQ-1	ND	ND	0.9% (0.001)	2.8% (0.003)	ND	0.8% (<0.001)	ND	1.8%[d] (0.002)	6.3% (0.008)
component total	52.2% (0.064)	2.3% (0.003)	0.9% (0.001)	2.8% (0.003)	1.5% (0.002)	0.8% (<0.001)	1.1% (0.001)	20.3%[e] (0.025)[e]	81.9%[f] (0.100)[f]

[a] In all cases, % = percent of TRR. [b] All parenthetical values are in mg of CM equiv/kg. [c] Unidentified metabolites are designated KID-*n*. [d] Not a single component but the sum of several chromatographically separable components. [e] Sum of ORG-1 + ORG-2 + AQ-1 + AQ-2 (7.7%, 0.009) + hexane (3.4%, 0.004) + collagenase (0.9%, 0.001) + insoluble (2.6%[g], 0.003) "other" fractions. [f] Sum of ORG-1 + ORG-2 + AQ-1 + AQ-2 (7.7%, 0.009) + hexane (3.4%, 0.004) + collagenase (0.9%, 0.001) + insoluble (2.6%[g], 0.003) "fraction totals". [g] Insoluble fraction contains final insoluble pellet plus insoluble pellets from enzyme treatments.

Table 8. Summary of Fractionation of Kidney Residues[a,b] from Goats Receiving Five Consecutive Daily Doses of [^{14}C]Cloransulam-methyl (TP Label)

	components											fraction
fraction	CM	C	KID-1[c]	KID-2	KID-3	KID-4	KID-5	KID-6	KID-7	KID-8	other	total
ORG-1	51.0% (0.065)	1.3% (0.002)	ND	ND	6.0% (0.008)	ND	ND	0.7% (<0.001)	ND	0.5% (<0.001)	0.2%[d] (<0.001)	59.7% (0.076)
ORG-2	0.7% (<0.001)	ND	ND	ND	ND	ND	ND	ND	ND	ND	3.8%[d] (0.005)	4.5% (0.006)
AQ-1	ND	ND	1.9% (0.002)	0.7% (<0.001)	ND	0.6% (<0.001)	2.7% (0.003)	ND	0.6% (<0.001)	ND	1.2%[d] (0.002)	7.7% (0.010)
component total	51.7% (0.066)	1.3% (0.002)	1.9% (0.002)	0.7% (<0.001)	6.0% (0.008)	0.6% (<0.001)	2.7% (0.003)	0.7% (<0.001)	0.6% (<0.001)	0.5% (<0.001)	18.1%[e] (0.023)[e]	84.8%[f] (0.109)[f]

[a] In all cases, % = percent of TRR. [b] All parenthetical values are in mg of CM equiv/kg. [c] Unidentified metabolites are designated KID-*n*. [d] Not a single component but the sum of several chromatographically separable components. [e] Sum of ORG-1 + ORG-2 + AQ-1 + AQ-2 (7.1%, 0.009) + hexane (2.6%, 0.003) + collagenase (0.7%, < 0.001) + insoluble (2.5%[g], 0.003) "other" fractions. [f] Sum of ORG-1 + ORG-2 + AQ-1 + AQ-2 (7.1%, 0.009) + hexane (2.6%, 0.003) + collagenase (0.7%, < 0.001) + insoluble (2.5%[g], 0.003) "fraction totals". [g] Insoluble fraction contains final insoluble pellet plus insoluble pellets from enzyme treatments.

was 95.3% pure (i.e., contained ~4.7% total impurities), this meant that (12.7% − 4.7% =) 8% degradation had taken place during the extraction and fractionation process. However, no significant amounts of individual soluble breakdown products were detected and, specifically, no cloransulam—the major metabolite identified in the AN and TP tissues discussed above—was detected. Overall, the data showed that cloransulam-methyl spiked into liver was extractable in good yield and did not decompose to a significant extent under the extraction conditions employed.

In summary, the data from the liver fractionation (Tables 4 and 5) showed that neither cloransulam-methyl nor its metabolites accumulated to significant levels in the liver. Cloransulam, present at ~9.5% (0.005 mg/kg) of the TRR, when averaged over the two radiolabeled forms, was the most abundant metabolite identified. Cloransulam-methyl itself represented <7.2% (0.003 mg/kg) of the TRR. The remainder of the residue fractionated into multiple minor components, with no individual component >0.01 mg/kg. No significant metabolites arising from only the aniline or only the triazolopyrimidine moieties were detected; therefore, sulfonanilide bridge cleavage was not significant in the liver.

Kidney Residues. Kidney samples contained 0.03 and 0.02% of the administered ^{14}C activity for the AN and TP tissues, respectively, with corresponding concentrations of 0.122 and 0.128 mg/kg (Tables 2 and 3). The tissues were extracted using the same scheme as for the liver, summarized in Figures 2 and 3. The first step, extraction with ACN/water/AcOH acid (80:20:0.5) released 74.3% (0.091) TRR and 81.4% (0.104 mg/kg) TRR for the AN and TP tissues, respectively. These extracts were worked up as described for the liver samples, generating the ORG-1 and AQ-1 (readily soluble) fractions. The ORG-1 fractions represented the majority of the easily soluble residues with 56.6% (0.069 mg/kg) and 59.7% (0.076 mg/kg) TRR for AN and TP, respectively (Tables 7 and 8). Subsequent HPLC analysis (Figure 6) showed these fractions to be predominantly composed of a radioactive component representing 51.3% (0.063 mg/kg) and 51.0% (0.065 mg/kg) of the TRR, respectively, with an HPLC retention time similar to that of cloransulam-methyl. A radioactive component present at 2.3% (0.003 mg/kg) and 1.3% (0.002 mg/kg) of the TRR was tentatively identified as cloransulam by comparison of its HPLC retention time with that of a non-radiolabeled standard. As with the liver ORG-1 samples, TLC analysis confirmed the presence of cloransulam-methyl and cloransulam in both the AN and TP samples on the basis of their R_f values. The AQ-1 fractions represented only 6.3% (0.008 mg/kg) and 7.7% (0.010 mg/kg) TRR for the AN and TP tissues. Reverse phase HPLC (Figure 7) showed these to be multicomponent, with no single radioactive component present in either sample at >2.8% (0.003 mg/kg) TRR. None of these low-level metabolites cochromatographed with any of the standards and remained unidentified.

As described for the liver, extensive attempts were made to release additional insoluble residues (Figures 2 and 3). The refluxing 50% aqueous acetone extraction solubilized 2.8% (0.004 mg/kg) of the TRR for the AN and TP tissues. The collagenase treatment solubilized very little ^{14}C (≤0.9% TRR; ≤0.001 mg/kg). Pancreatin treatment of the resulting tissue matrices solubilized

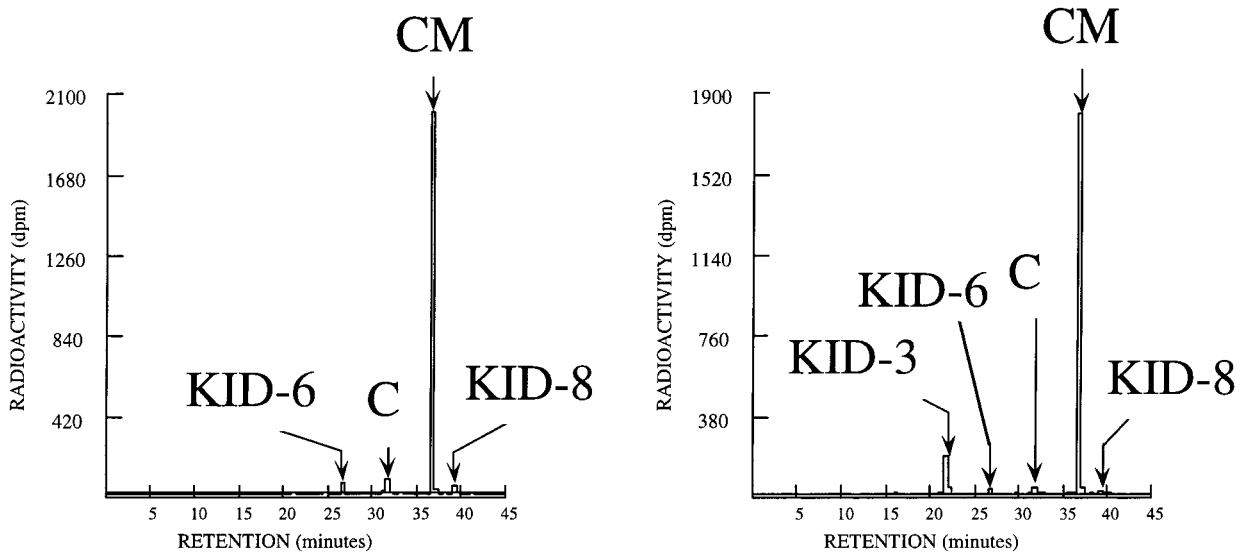


Figure 6. HPLC radiohistograms of AN- and TP-kidney "organic-1" extracts (gradient elution method).

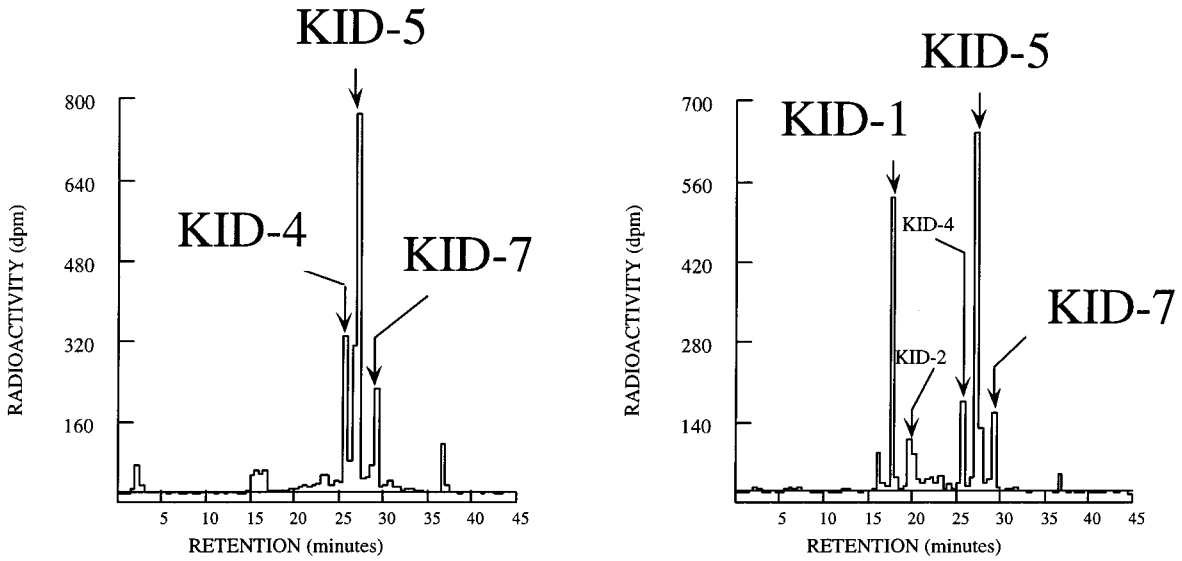


Figure 7. HPLC radiohistograms of AN- and TP-kidney "aqueous-1" extracts (gradient elution method).

an average of 6.4% (0.008 mg/kg) of the TRR. Acid treatment of the remaining insoluble pellet released an additional 3.3% (0.004 mg/kg) and 2.5% (0.003 mg/kg) of the TRR for the AN and TP samples, respectively. The final insoluble pellets contained ≤1% (0.001 mg/kg) of the TRR for each sample; therefore, this extraction procedure had succeeded in solubilizing at least 99% of the kidney residues for each radiolabeled form.

As for the liver samples, composites of the AN and TP acetone−water plus pancreatin plus 2 M HCl soluble fractions were prepared by combining 80% of each extract. The composites were then extracted with EtOAc, and the organosoluble components were chromatographed on TLC to further characterize the "not readily soluble" residues. The ORG-2 fractions contained an average of 4.5% (0.006 mg/kg) of the TRR, whereas the AQ-2 fractions contained an average of 7.4% (0.009 mg/kg) of the TRR. TLC of the ORG-2 fractions showed the samples to be multicomponent with no single ^{14}C component representing >3% (0.004 mg/kg) of the TRR. Radioactivity cochromatographing with cloransulam-methyl represented an average of 0.8% (0.001 mg/kg) of the TRR.

The extraction efficiency and stability of the parent compound during the extraction conditions employed for the AN and TP tissues was determined in a fashion similar to that used for the liver samples. Thus, a sample of control kidney (CS sample) was fortified with [^{14}C]cloransulam-methyl at a fortification level comparable to the total radioactive residue levels in the AN and TP samples, that is, ~0.13 mg/kg. This sample was then extracted and fractionated, and the fractions were chromatographed as for the AN and TP samples (Table 9), with minor changes noted below. Of the ^{14}C added to the tissue, ~104.7% was recovered in the ACN/water/AcOH extract. After further fractionation, 96.0% of the added ^{14}C was present in the ORG-1 fraction. Chromatographic analysis (HPLC and TLC) showed this radioactivity to be largely unchanged cloransulam-methyl, and a total of 93.4% of the originally added ^{14}C was shown chromatographically to be cloransulam-methyl (Table 9). Because the majority of the radioactivity recovered was in the form of the parent molecule, the acid digestion and acetone−water/pancreatin-solubilized compositing steps were omitted for the kidney CS sample. Approximately 7.8% of the added activity

Table 9. Summary of Fractionation of Radiolabel from Control Goat Kidney Fortified with [^{14}C]Cloransulam-methyl

fraction[a]	components		fraction total
	CM	other	
ORG-1	93.0%[b] (0.121)[c]	3.0%[d] (0.004)	96.0% (0.125)
AQ-1	0.4% (<0.001)	0.7%[d] (<0.001)	1.1% (0.001)
hexane		2.5% (0.003)	2.5% (0.003)
acetone−water (50:50)		0.7% (<0.001)	0.7% (<0.001)
collagenase		0.6% (<0.001)	0.6% (<0.001)
pancreatin		0% (0.000)	0% (0.000)
insoluble		0.3% (<0.001)	0.3% (<0.001)
component total	93.4% (0.121)	7.8% (0.010)	101.2% (0.131)

[a] Fortification level = 0.130 mg/kg (purity of [^{14}C]CM = 95.3%). [b] In all cases, % = percent of TRR. [c] All parenthetical values are in mg of CM equiv/kg. [d] Not a single component but the sum of several chromatographically separable components; in no case was [^{14}C]C detected.

was distributed over seven other fractions, and because the [^{14}C]cloransulam-methyl used to fortify the tissue was 95.3% pure (i.e., 4.7% total impurities), this meant that (7.8% − 4.7% =) 3.1% degradation had taken place during the extraction and fractionation process. Again, no significant amounts of individual breakdown products were detected when the extracts were chromatographed. Overall, the data showed that cloransulam-methyl spiked into kidney was extractable in good yield and did not decompose to a significant extent under the extraction conditions employed.

In summary, the fractionation data showed (Tables 7 and 8) that cloransulam-methyl was present in the kidney at 52.2% (0.064 mg/kg) and 51.7% (0.066 mg/kg) of the TRR, in the AN and TP tissues, respectively. Cloransulam was tentatively identified on the basis of its HPLC and TLC behavior, at a level of ~2.3% (0.003 mg/kg) of the TRR. The remainder of the residue fractionated into multiple minor components, with no individual component >7.7% (0.009 mg/kg) of the TRR. As with the liver, no significant metabolites arising from only the aniline or only the triazolopyrimidine moieties were detected: therefore, sulfonanilide bridge cleavage was not significant in the kidney.

Components in Urine and Identification of Cloransulam as the Only Significant Metabolite. The urine contained 43.1 and 28.5% of the administered AN and TP ^{14}C dose for the respective goats. Reversed phase HPLC analysis of composite urine samples (Figure 8) showed the major radioactive component present in urine to be cloransulam-methyl, as identified by retention time comparison with a non-radiolabeled reference standard. Cloransulam-methyl accounted for 80.3% of the AN and 85.7% of the TP urine composite sample ^{14}C. The next largest radioactive component (HPLC retention range of 32−32.5 min) present in the urine samples (6.8% of the AN and 4.4% of the TP) had a retention time on HPLC and an R_f on TLC similar to those of a standard of cloransulam. In addition, this metabolite was present in both AN- and TP-labeled urine samples, showing that the sulfonanilide bridge was intact in this metabolite. No other single radioactive component was present at >3.4% in either the AN or TP urine composite samples. The metabolite that eluted at 32 min on HPLC had chromatographic properties similar to those of the major metabolite in both liver and kidney, where it was present at much lower concentrations (≤0.007 mg/kg). Therefore, the urine was used as an isolation source for this metabolite so that its structure could be confirmed spectroscopically.

TLC analysis, performed on aliquots of the highest radioactivity urine samples, showed that the AN, day 5, a.m., urine sample contained the maximal metabolite level, at 9.8% of the total ^{14}C in this sample. On this basis this urine sample was selected for isolation of the putative acid metabolite, as follows. Ethyl acetate extraction of the acidified urine gave 96% of the urine ^{14}C in the organic phase. The organic extract was chromatographed on a column of silica gel, using a stepped hexane/toluene/EtOAc/acetone/water gradient elution system. This gave five major radioactive bands of increasing polarity in the ratio 89.1:7.5:2.4:1.7:0.7. TLC analysis showed that band 2 contained the putative cloransulam metabolite. This band was further purified using preparative isocratic HPLC, and the purified metabolite was derivatized using TEOTFB. The product, *N*-ethylcloransulam-ethyl ester (MW = 471; Wolt et al., 1996), did not show an M^+ ion at *m*/*z* 471 under the electron impact conditions used. However, structurally diagnostic ions were observed as a result of molecular ion fragmentation. Thus, loss of 245 amu, which corresponded to the triazolopyrimidine part of the molecule, gave the *N*-ethyl(2-carboethoxy-6-chlorophenyl) fragment ion with a characteristic *m*/*z* 226. A reference standard of cloransulam was analyzed under the same conditions and yielded a product with the same TLC R_f, the same GC retention time, and the same mass spectrum during GC-MS analysis. In summary, comparison of the chromatographic (HPLC, TLC, and GC) and mass spectral data for the ^{14}C-labeled metabolite with those for the non-radiolabeled cloransulam standard confirmed that the metabolite isolated from urine was cloransulam. These data confirmed the structure of the major goat metabolite as cloransulam, the acid derivative of cloransulam-methyl.

Milk Residues. The radioactivity levels were extremely low in the milk samples, averaging only 0.002 and <0.001 mg/kg in the AN and TP samples, respectively, over the 5 day dosing period. Therefore, the samples containing the highest concentration of radioactivity (AN, day 4, p.m.; TP, day 4, p.m.) were selected for this analysis, thereby representing a worst case. Acetone was added to the AN (0.003 mg/kg) and TP (0.002 mg/kg) milk samples to precipitate the protein. The soluble fractions contained 39% (0.001 mg/kg) and 57.4% (0.001 mg/kg) of the TRR for the AN and TP samples, respectively, whereas the protein contained 55.3% (0.002 mg/kg) and 29.6% (<0.001 mg/kg) of the TRR, by combustion. The soluble fractions were defatted with hexane and then concentrated. The hexane samples contained ≤2.7% TRR, whereas the aqueous concentrates contained 28.2% (<0.001 mg/kg) and 41.6% (<0.001 mg/kg) TRR in the AN and TP samples. Subsequent HPLC performed on the aqueous concentrates showed each to be multicomponent with cloransulam-methyl comprising only approximately 5 and 8.7% TRR for the AN and TP samples, respectively. Therefore, even at this highly exaggerated feeding level, the maximum level of unmetabolized cloransulam-

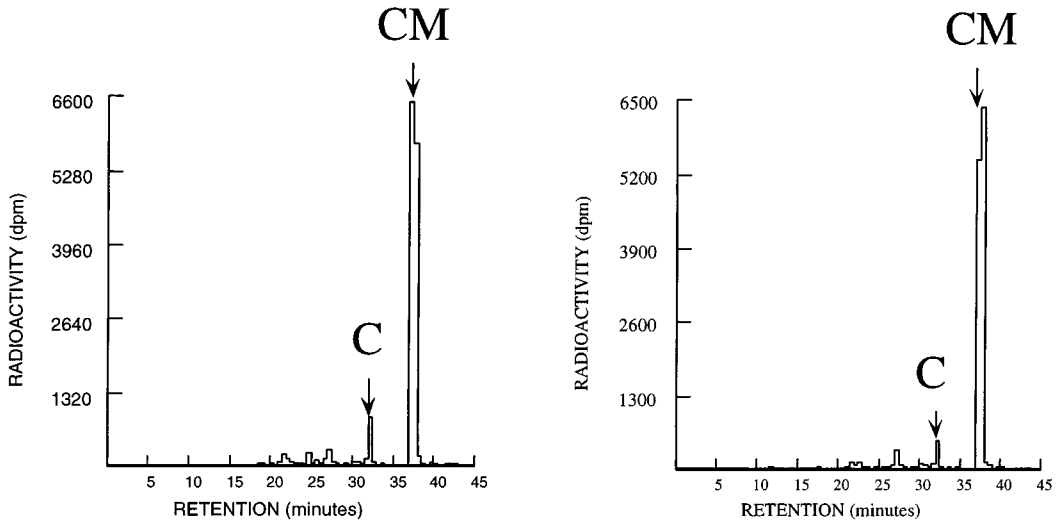


Figure 8. HPLC radiohistograms of AN- and TP-urine composites (gradient elution method).

methyl in the milk, averaged over the two AN and TP labels, was ~6.9% (0.0002 mg/kg).

Metabolite Overview. In summary, the only significant metabolites of CM in the goat were the acid C and the de-ethylated 5-HCM. This combination of metabolites most closely resembles that formed under microbial conditions in soil (Wolt et al., 1996). Significantly, neither aniline fragment metabolites such as MCA or its hydrolysis product CA (Table 1), which might result from sulfonanilide bridge cleavage, was observed, nor were conjugation products such as the glutathione/mercapturate series. In this regard, the pathway is clearly different from that observed in foliarly treated soybeans. Under those conditions, the dominant pathway is displacement of the 7-fluorine atom to give the homoglutathione adduct CM-7-hGSH (Table 1; DeBoer, personal communication, 1995), followed by degradation of the peptide moiety to the cysteine adduct CM-CYS (Table 1; Lewer et al., unpublished data).

CONCLUSIONS

Two goats were orally dosed with [AN-^{14}C]- or [TP-^{14}C]cloransulam-methyl at the equivalent of 10 mg/kg in the diet for 5 consecutive days. Oral dosing was used because oral ingestion was determined to be the most likely route of exposure for this molecule. The test substance was 100% radiochemically pure, and the administered dose of cloransulam-methyl was ~2225 times the realistic maximum dietary exposure for a dairy animal. During this time ~99.9% of the recovered compound was excreted, approximately equally split between urine and feces, whereas only 0.1% was retained in edible tissues (kidney, liver, muscle, and fat). Radiochemical analysis of the edible tissues showed very low residues, with the highest concentrations of 0.122 and 0.128 μg equiv of cloransulam-methyl/g found in the AN and TP kidneys and concentrations found in the liver of only 0.051 and 0.040 mg/kg, respectively. Muscle and fat concentrations were $\leq$0.002 mg/kg in each radiolabeled form. Milk samples contained average concentrations of 0.002 and <0.001 mg/kg, with peak concentrations of 0.003 and 0.002 mg/kg, respectively, for the AN and TP labels. The low residue levels in the milk and edible tissues, combined with the rapid rate of excretion, indicated a low propensity for cloransulam-methyl to bioconcentrate in ruminant animals.

Extraction methods were developed that were successful in releasing >99% of the kidney residues and >81% of the liver residues, leaving <0.001 mg/kg insoluble ^{14}C kidney residue and <0.009 mg/kg insoluble ^{14}C liver residue. Chromatographic analysis of the extracted residues showed that the primary residues were parent cloransulam-methyl, in the kidney, and cloransulam, in the liver. Approximately 0.066 mg/kg cloransulam-methyl was detected in the kidney, and <0.003 mg/kg was detected in the liver. The major metabolite, cloransulam, formed by de-esterification of the parent, represented ~0.005 mg/kg in the liver. Although this was present at too low a level to be isolated from the tissues, it was isolated and identified from a urine sample, and its identity was confirmed by GC-MS analysis of an ethylated derivative. No metabolites were present above 10% of the TRR or 0.01 mg/kg in either the liver or kidney.

The behavior of cloransulam-methyl residues in goat tissues, milk, and urine during extraction, fractionation, and chromatographic analysis demonstrated no significant differences between aniline- and triazolopyrimidine-labeled goats. Therefore, these ring systems did not become separated during metabolism in the animal; that is, sulfonanilide bridge cleavage was not a significant degradation route for cloransulam-methyl in ruminants.

ABBREVIATIONS USED

HPLC, high-performance liquid chromatography; TLC, thin-layer chromatography; LSC, liquid scintillation counting; dpm, disintegrations per minute; ACN, acetonitrile; EtOAc, ethyl acetate; AcOH, acetic acid; GC-MS, combined gas chromatography−mass spectrometry; TEOTFB, triethyloxonium tetrafluoroborate; TEA, triethylamine; NaCl, sodium chloride; MTBE, methyl *tert*-butyl ether; TRIS, tris[hydroxymethyl]aminomethane; TRR, total radioactive residue; ND, not detected.

ACKNOWLEDGMENT

We thank Mike Bodden for performing the in-life portion of the study before his retirement from Hazelton labs.

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Received for review August 26, 1999. Revised manuscript received March 28, 2000. Accepted April 2, 2000.

JF9909611