JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Metabolism of [^{14}C]Chlorantraniliprole in the Lactating Goat

Venkat Gaddamidi,*[,†] Martin T. Scott,[†] R. Scott Swain,[†] David Macpherson,[§] and Chris Lowrie[§]

[†]DuPont Crop Protection, Stine-Haskell Research Center, Newark, Delaware 19714-0030, United States

[§]Charles River Laboratories, Tranent, Edinburgh EH33 2NE, United Kingdom

ABSTRACT: Metabolism of [^{14}C]chlorantraniliprole {3-bromo-*N*-[4-chloro-2-methyl-6-[(methylamino)carbonyl]phenyl]-1- (3-chloro-2-pyridinyl)-1*H*-pyrazole-5-carboxamide} was investigated in a lactating goat following seven consecutive daily single oral doses. Each dose was equivalent to 10.4 mg/kg of feed. There was no significant transfer of residues of either chlorantraniliprole or its metabolites into fat, meat, or milk. Chlorantraniliprole and its metabolites accounted for 93.57% of the administered dose and were eliminated primarily in the excreta. Residues in meat, milk, liver, and kidney together accounted for ca. 1.5% of the administered radioactivity. A total of 19 metabolites including 3 glucuronide conjugates and intact chlorantraniliprole were identified in the feces, urine, or tissues by comparison of their HPLC retention times, mass spectral fragments (LC-MS/MS), or multiple reaction monitoring (MRM) transitions to authentic synthesized standards. The major metabolic pathways of [^{14}C]chlorantraniliprole in the goat were *N*-demethylation, methylphenyl hydroxylation, and further oxidation to the carboxylic acid; loss of water from the *N*-hydroxymethyl group to yield various cyclic metabolites; and hydrolysis of *N*-methyl amides to form benzoic acid derivatives. Minor metabolic reactions involved cleavage of the amide bridge between the phenyl and heterocyclic rings of chlorantraniliprole.

KEYWORDS: Chlorantraniliprole, insecticide, identification, goat, ruminant, metabolism

INTRODUCTION

Chlorantraniliprole (DuPont Rynaxpyr) is an anthranilic diamide insecticide and marketed primarily as DuPont Coragen, DuPont Altacor, and DuPont Prevathon insecticides. Chlorantraniliprole controls almost all economically important lepidopteran insects and a number of key coleopteran, dipteran, hemipteran, and isopteran pests in fruits, vegetables, cotton, soybean, sugar cane, rice, and turf grass. Due to its novel mode of action, chlorantraniliprole shows excellent control of pest populations resistant to other insecticidal products.

Chlorantraniliprole activates insect ryanodine receptors (RyRs). Ryanodine receptors play a critical role in muscle function as selective ion channels, modulating the release of calcium. Contraction of muscle cells requires a regulated release of calcium from internal stores into the cell cytoplasm. Chlorantraniliprole binds to RyRs, causing uncontrolled release and depletion of internal calcium, preventing further muscle contraction. Insects treated with chlorantraniliprole exhibit rapid cessation of feeding, lethargy, regurgitation, and muscle paralysis, ultimately leading to death (see refs 1−6).

The main objective of this study was to determine the nature of the residues in meat and milk that may enter the human food chain. The available data on mammalian toxicology of chlorantraniliprole show that it has no significant acute toxicity via the oral, dermal, and inhalation routes of exposure. Chlorantraniliprole is not genotoxic, neurotoxic, immunotoxic, or carcinogenic, nor is it a reproductive toxicant.[7] This paper details the administration, distribution, metabolism, and elimination of chlorantraniliprole in the lactating goat. The study was conducted according to Good Laboratory Practice to meet U.S. EPA and OECD Regulatory Guidelines.

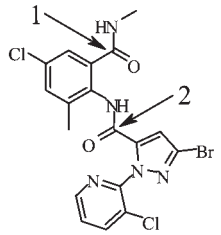
MATERIALS AND METHODS

Test Materials and Preparation of Dose Capsules. [*Benzamide carbonyl*-^{14}C]chlorantraniliprole (37.5 mg) and [*pyrazole carbonyl*-^{14}C]chlorantraniliprole labels (37.5 mg) were mixed at a ratio of 1:1 (μCi/μCi) in tetrahydrofuran (THF; Rathburn, U.K.) to prepare a stock dose solution. The positions of the radiolabels are depicted in Figure 1. The specific activity of the mixed [^{14}C]chlorantraniliprole was ca. 15.52 μCi/mg. The resulting solution was assayed by a liquid scintillation counter (LSC), and the concentration was determined (6.571 mg/mL). The dose solution (2.283 mL) was dispensed into gelatin capsules containing ca. 0.5 g of ground goat feed. THF was evaporated under a stream of nitrogen, and the capsules were left open for ca. 30 min in a fume hood to ensure complete removal of solvent. Closed capsules were stored in the dark at ca. 4 °C. A total of 10 capsules (ca. 15 mg of [^{14}C]chlorantraniliprole/capsule) for seven doses including backup and storage stability analysis were prepared.

High-Performance Liquid Chromatography (HPLC). The radiochemical purity and chemical authenticity of [^{14}C]chlorantaniliprole and authenticated analytical standards for metabolite identification were analyzed by HPLC using an Agilent 1100 series modular liquid chromatograph (Agilent Technology) consisting of a variable-wavelength detector, vacuum degasser, quaternary pump, thermostated autosampler, and thermostated column compartment connected in series to a flow scintillation analyzer (Packard 150TR or 505TR; Packard Instruments) with a 500 μL flow cell or a Gilson model 202 fraction collector (Anachem). The UV detector was set at 254 nm. HPLC data were processed using Atlas 2002 (Thermo LabSystems) product version 6.18 software.

The samples were analyzed using a Phenomenex Luna C18(2) (250 × 4.6 mm; 5 μm) column equipped with a Phenomenex Security Guard C18 (3.0 × 4.0 mm) column heated to 40 °C and eluted at a 1.0 mL/min flow rate. The mobile phase was a combination of (A) Milli-Q HPLC grade water (MilliPore Ltd., U.K.) and (B) acetonitrile (Rathburn, U.K.),

Received: September 14, 2010
Accepted: December 15, 2010
Revised: December 14, 2010
Published: January 11, 2011



1 – Position of radiolabel for [benzamide carbonyl-^{14}C]-chlorantraniliprole
2 – Position of radiolabel for [pyrazole carbonyl-^{14}C]-chlorantraniliprole

Figure 1. Chemical structure of [^{14}C]chlorantraniliprole radiolabels: 1, position of radiolabel for [*benzamide carbonyl*-^{14}C]chlorantraniliprole; 2, position of radiolabel for [*pyrazole carbonyl*-^{14}C]chlorantraniliprole.

each solvent containing 0.1% v/v formic acid (Sigma-Aldrich, U.K.). A gradient elution starting with 5% acetonitrile at 0 min, which increased to 20, 40, 65, and 100% acetonitrile at 10, 35, 45, and 50 min, respectively, was used for sample analyses.

Test Animals. Two goats (British Saanen variety) were obtained from Pig Supplies and Services (PSS), Aylesbury, Bucks, U.K. Both goats were subject to veterinarian inspection upon arrival and both deemed healthy. Goats were uniquely identified by means of numbered neck collars. One goat (ca. 15 months old at dosing), with good milk-yielding capacity and in good health condition, was selected for dosing. A second goat served as a companion for the test goat throughout the in-life phase of the study and was not sacrificed. The body weight of the test goat ranged between 40 and 42.5 kg during dose administration. The test goat was offered a measured quantity (ca. 400 g) of commercially available protein concentrate twice daily at each milking. Good-quality hay was offered to the test goat *ad libitum*. Hay and concentrate consumption was monitored and recorded for the last 10 days of acclimation and throughout the duration of the dosing period. Tap water was offered to the test goat *ad libitum*.

Dosing. A single oral dose of [^{14}C]chlorantraniliprole was administered daily to the test goat for 7 consecutive days. The dosing capsules were placed in a balling gun, which was subsequently placed in the back of the animal's mouth and then released. The dose was administered immediately after morning milking prior to feeding. The actual dose received was determined with reference to the dose concentration equivalents in feed based on daily feed consumption.

Sample Collection. During the dosing period, milk and excreta samples from evening collections were added to the corresponding samples collected the following morning. The test goat was sacrificed within 24 h of the last dose administration. Kidneys, entire liver, fat (omental, renal, and subcutaneous), and muscle (composite of loin, hind, and fore quarter muscle in approximately equal proportions) were taken at sacrifice. In addition, bile was drawn from the gall bladder using a needle and syringe.

Sample Storage. Samples not analyzed immediately for levels of total radioactive residues (TRR) were stored frozen at ca. −20 °C until taken for analysis with the exception of cage wash samples (which were stored at ambient temperature for at least 24 h prior to analysis) and milk collected at afternoon milking occasions (which was stored at 4 °C overnight). All samples removed from frozen storage were allowed to thaw to room temperature for processing/analysis, and returned to storage.

Sample Processing for Total Radioactivity Analysis. The feces samples were initially soaked in acetonitrile/water (1:1 v/v) at a ratio of ca. 2:1 (w/w, feces/solvent) to soften the pellets prior to thorough homogenization using a Waring blender.

Tissue samples were processed separately with the aid of dry ice using a Hobart Vegetable Preparation Unit (VPU 206) equipped with a 6 mm grater to produce large frozen gratings that were further pulverized using a Hobart Vertical Cutter/Blender (VCB/FP-62) and stored at ca. −20 °C until analyses.

Combustion Analysis. Pulverized feces and tissue samples were weighed (in triplicate, ca. 0.5 g each) into Combustocones (Canberra Packard) and oxidized using a model 307 Tri-Carb Automatic Sample Oxidizer (PerkinElmer). The resultant $^{14}CO_2$ was absorbed in Carbosorb (PerkinElmer) and mixed automatically with PermafluorE+ scintillation fluid (PerkinElmer). Combustion efficiency and carry-over were checked at regular intervals throughout each production run by combusting blanks and quality control standards containing (i.e., Spec-Chec). Results from combustion analyses were not corrected for the actual combustion efficiency as the recoveries were in the range of 98.6−99.5%.

Radiochemical Analysis Using Liquid Scintillation Counting (LSC). Radioactivity in dose solutions, urine, diluted feces, and tissue extracts was determined by LSC analysis. Triplicate aliquots (each ca. 4 g of milk and ca. 1 g of urine and cage wash) were weighed into 20 mL scintillation vials, mixed with 10 mL of Quickszint scintillation fluid (Zinsser Analytic, Maidenhead, U.K.), and assayed by LSC for 5 min. Triplicate aliquots (ca. 0.5 g each) of omental, renal, and subcutaneous fat samples were dissolved separately in 10 mL Quickszint scintillation fluid agitated using a sonicator and assayed by LSC for 5 min. All samples were assayed using a Packard 2100 TR Liquid Scintillation Analyzer (PerkinElmer) with automatic quench correction by external standard mode together with representative blanks and standards. Representative blank sample values were subtracted from counts to give net disintegration per minute (dpm) per sample. Limits of detection (LOD) and quantification (LOQ) were calculated for measurements of total radioactivity in each sample. All values reported are in excess of the limit of quantification unless otherwise stated.

Extraction of Composite Feces. Composite processed feces sample (days 1−7) was extracted three times with ca. 3 mL/g acetonitrile/water (3:1, v/v) using a Silverson overhead homogenizer and centrifuged (4000 rpm, 10 min), and the supernatant was decanted. The extracts were combined, the total volume was measured, and duplicate aliquots were removed for LSC to determine TRR. The extracts were concentrated under a stream of nitrogen, and TRR was determined.

Duplicate subsamples of the post-extracted solids (PES) were assayed by oxidative combustion followed by LSC analysis. The PES were further extracted with ca. 3 vol/g of acetonitrile/water (3:1, v/v) in a similar manner as described above except that the sample was agitated using an ultrasonic bath (35 °C; 1 h) following initial homogenization. The extract was concentrated, assayed by LSC, and pooled with the initial extract prior to HPLC analysis.

Composite Urine Sample Processing and Analysis. The day 1−7 urine samples were combined proportionately by volume and concentrated by rotary evaporation to ca. 10% of the original volume. Radioactivity was determined (LSC assay) prior to and after rotary evaporation.

An Amersham PD-10 (Sephadex G-25M) column was conditioned using Milli-Q water (20 mL) before the composite urine concentrate (10 mL) was applied. The column was eluted with three aliquots of Milli-Q water (10 mL), the eluate collected, and the radioactive content determined by LSC analysis of known aliquots. A Varian BondElut C_8 solid phase extraction (SPE) column was conditioned with acetonitrile (10 mL) followed by 0.1% formic acid in Milli-Q water (v/v), at which time the urine sample, previously eluted from the Sephadex column, was loaded. The sample was eluted using a solvent gradient from 10 to 100% of acetonitrile diluted with Milli-Q water. Fractions were collected from each gradient concentration, and the radioactive content was determined by LSC analysis. The fractions containing 10−30% acetonitrile were adjusted to ca. pH 2 using 10% formic acid before being partitioned against 3 mL of ethyl acetate/mL fraction. The ethyl acetate layer was reduced to dryness under nitrogen, reconstituted in HPLC mobile phase, and assayed for radioactivity (LSC) prior to HPLC analysis.

Extraction of Bile Sample. Prior to LC-MS analysis, the bile sample (0.5 mL) was mixed with concentrated hydrochloric acid (20 μL) and the pH measured as pH 1−2 using indicator paper. The acidified bile

sample was partitioned against diethyl ether (10 mL) and then centrifuged (1500 rpm, 5 min). The organic liquid phase was removed, and duplicate aliquots were submitted for LSC analysis prior to evaporation under nitrogen. The residues were then reconstituted in acetonitrile/water (1:1, v/v), and the procedural recovery was determined by LSC analysis. The residual bile sample was dried under nitrogen and then mixed with ethyl acetate (10 mL). The sample was sonicated for 15 min and then centrifuged (1500 rpm, 5 min). The organic liquid phase was removed and reduced to dryness under nitrogen. The residues were then reconstituted in acetonitrile/water (9:1, v/v), and the procedural recovery was determined by submitting aliquots for LSC analysis.

Extraction of Composite Milk. Two SPE cartridges (20 mL, Isolute HM-N) (Biotage) were placed in series and conditioned by eluting with ethyl acetate (3 × 20 mL) followed by water (3 × 20 mL). The composite (days 1−7) milk sample (ca. 20 g) was added to the first of two cartridges and left to equilibrate for ca. 20 min. The cartridges were eluted with ethyl acetate (160 mL) and duplicate aliquots of the pooled eluates submitted for LSC analysis. The extract was concentrated to dryness by rotary evaporation, and the residues were reconstituted in acetonitrile/water (3:1, v/v; 5 mL). The procedural recovery was determined by LSC analysis.

Extraction of Fat and Muscle Sample. Homogenized fat and muscle samples (ca. 50 g each) were each extracted separately three times with ca. 1.5−2 mL of acetonitrile/water (9:1, v/v)/g sample. On each occasion, the samples were macerated using a Silverson overhead homogenizer and centrifuged (4000 rpm, 10 min), and the supernatant was decanted. The extracts were combined for each matrix, the total volume was measured, and duplicate aliquots were submitted for LSC analysis. The extracts were concentrated using a stream of nitrogen and the residues transferred to a vial with the aid of acetonitrile. The reconstituted extracts were centrifuged (2000 rpm, 5 min), and the radioactivity in the supernatants was determined by LSC analysis. Duplicate subsamples of each tissue PES (ca. 0.3 g) were removed and submitted for combustion analysis to determine TRR.

Extraction of Kidney and Liver. Kidney and liver samples (ca. 20 g each) were extracted with ca. 3 mL of acetonitrile/water (9:1, v/v) per gram of tissue by pulverizing with a Silverson overhead homogenizer, centrifuging (4000 rpm, 15 min), and decanting the supernatant. The extraction process was repeated two more times, and the extracts were pooled and assayed by LSC. The extract was concentrated by rotary evaporation and transferred to a centrifuge tube with the aid of acetonitrile. The reconstituted extracts were centrifuged (3000 rpm, 10 min), and the procedural recovery was determined by LSC analysis. Unextracted radioactivity in the PES (ca. 0.3 g) was determined by combustion analysis.

Pepsin Digestion of PES of Liver. Pepsin (porcine gastric mucosa; Sigma-Aldrich; ca. 3 g) was added to the liver PES, mixed with hydrochloric acid (0.1 N; 80 mL), and then homogenized using a Silverson overhead homogenizer. The sample was then incubated (37 °C, 24 h) in a shaking water bath. At the end of incubation, methanol (90 mL) was added and the sample stored at ca. −20 °C for ca. 3.5 h to aid precipitation. The sample was then centrifuged (10000 rpm, 30 min), the supernatant decanted, and the radioactive content determined by LSC analysis. The extract was concentrated by rotary evaporation, and the residues were transferred to a centrifuge tube with the aid of acetonitrile/water (1:1, v/v). The reconstituted extract was centrifuged (3000 rpm, 10 min) and the procedural recovery determined by LSC analysis.

Protease Digestion of PES of Liver. To release unextracted bound residues, a fresh sample (not digested with pepsin) of liver PES was treated with protease, a nonspecific protein digesting enzyme. Protease (type 1 bovine pancreas; Sigma-Aldrich; ca. 1 g) was added to the liver PES and mixed with Milli-Q water (40 mL) before being homogenized using a Silverson overhead homogenizer. The sample was then incubated (37 °C, 24 h) in a shaking water bath. At the end of incubation, methanol (40 mL) was added and the sample stored at ca. −20 °C overnight to aid precipitation.

Table 1. Distribution of Total Radioactive Residues in Milk, Excreta, and Tissues Following Oral Administration of [^{14}C]Chlorantraniliprole to the Lactating Goat[a]

	goat total radioactive residues	
sample	TRR, mg/kg	% total dose[b]
feces	NA[c]	78.93
urine	NA	10.73
cage wash	NA	3.91
bile	2.406	0.07
milk[d]	0.067	0.79
liver	0.640	0.45
kidney	0.090	0.01
muscle	0.017	NA
fat	0.070	NA
total recovery	NA	94.89

[a] Specific activity = 15.52 μCi/mg. [b] Feces, urine, and cage wash data are the sum of recovered radioactivity over day 1 through day 7 and expressed as percentage of total administered dose. [c] NA, not applicable. [d] TRR value corresponds to concentration determined for the day 1−7 composite sample. The % administered dose value corresponds to total recovery of radioactivity from milk collected over the entire study period.

The sample was then centrifuged (10000 rpm, 30 min), the supernatant decanted, and the radioactive content determined by LSC analysis. The extract was concentrated by rotary evaporation, and the residues were transferred to a centrifuge tube with the aid of acetonitrile/water (1:1, v/v). The reconstituted extract was centrifuged (3000 rpm, 10 min) and the procedural recovery determined by LSC analysis.

Acid Hydrolysis of Enzyme-Digested Solid. Following protease digest, the PES were subjected to acid hydrolysis by addition of hydrochloric acid (100 mL, 6 M) and homogenization using a Silverson overhead homogenizer and then incubated at 60 °C for 3 h in a shaking water bath. At the end of the incubation, the pH of the sample was adjusted to pH 3 (6 M sodium hydroxide), the sample was transferred to an Amberlite glass jar, and dimethoxypropane (500 mL) added. The sample was then stirred for ca. 3 h and centrifuged (4000 rpm, 10 min) and the supernatant decanted. The total volume of extract was measured and the radioactive content determined by LSC analysis. The extract was concentrated by rotary evaporation, and the residues were transferred to a centrifuge tube with the aid of acetonitrile/water (1:1, v/v). The reconstituted extract was centrifuged (4000 rpm, 10 min), the supernatant decanted, and the remaining pellet resuspended in acetonitrile/water (1:1, v/v, 20 mL). The supernatant was removed and combined with the reconstituted extract before being concentrated further by rotary evaporation. The sample was centrifuged (4000 rpm, 10 min) and the procedural recovery determined by submitting duplicate aliquots for LSC analysis. No HPLC analysis was performed due to significant losses of radioactivity during processing.

Metabolite Identification by LC-MS Analysis. LC-MS experiments were performed on a quadrupole time of flight (Q-ToF) mass spectrometer (Waters/Micromass U.K., Ltd.), and a triple-stage quadrupole (TSQ) TSQ7000 API 2 mass spectrometer (Thermo Finnigan). Both instruments were used in conjunction with an Agilent series 1100 liquid chromatograph (Agilent Technologies) and either a Packard 150TR or a Packard 505TR radiochemical detector (Perkin-Elmer), operating Flo-one software (version 3.65).

Q-ToF Mass Spectrometry. The mass spectrometer was used in positive ion electrospray ionization (ESI+) mode with capillary voltage of 4.0 kV, cone voltage of 20 V, extraction cone voltage of 1.0 V, source temperature of 100 °C, and desolvation temperature of 350 °C. The full-scan analysis was set at 100−900 amu continuous with a scan rate of 1 s.

Table 2. Chlorantraniliprole and Metabolites Present in the Goat with HPLC Retention Times and Multiple Reaction Monitoring (MRM) Transitions, Protonated Ions, and Fragment Ions

metabolite code	confirmation method[a]	UV t_R (min)	TIC t_R (min)	MRM 1	MRM 2	protonated molecular ion	fragment ions
chlorantraniliprole [b]	C	44.95	45.29	545 → 504	547 → 506	484	453, 286
I[c]	C	39.50	39.79	451 → 451	453 → 453	500	407
II	C	32.76	33.17	498 → 284	500 → 286	500	482, 469, 451, 415, 286, 197
III	B	26.56	26.93	467 → 413	469 → 415	516	489
IV	A	27.7	28.02	484 → 284	486 → 286	486	468, 286
V[d]	A	52.73	53.03	464 → 464	466 → 466	466	*d*
VI[d]	A	50.65	50.84	450 → 450	452 → 452	452	416, 323, 286
VII	intermediate for metabolite VIII	42.25		468 → 451	470 → 453	470	453, 286
VIII	B	47.08	47.36	469 → 284	471 → 286	471	
IX	B	46.23	46.48	480 → 426	482 → 428	482	428
X[d]	C	41.15	41.30	466 → 466	468 → 468	468	450, 414
XI	B	36.47	36.74	512 → 284	514 → 284	514	483, 286
XII	C	49.18	49.42	494 → 476	496 → 478	496	478, 443, 414, 361
XIII	XIII was not observed, but its glucuronide conjugate (metabolite XVIII) was tentatively identified by LC-MS/MS analysis						
XIV	C	30.53	30.81	343 → 284	345 → 286	304	286, 260, 205, 177, 151
XV	B	24.07	24.69	229 → 198	231 → 200	229	198
XVI	A	20.45	20.64	NA	NA	241	198
XVII[e]	C	NA[f]	35.90	NA	NA	658	482, 464, 444, 428
XVIII[e]	C	NA	24.14	NA	NA	676	469, 286
XIX[e]	C	NA	29.16	NA	NA	644	NA

[a] Metabolite is present in species type (A, cochromatography; B, MRM transition; C, full-scan MS with fragmentation. [b] The molecular ion cluster $[M + H]^+$ for chlorantraniliprole was observed at *m/z* 482/484/486; the ions observed at *m/z* 545 and 547 are sodium and acetonitrile adducts $[M + Na + ACN]^+$. The adduct ions were chosen for MRM transition experiments because they offered greater specificity. [c] The molecular ion cluster $[M + H]^+$ for metabolite I was observed at *m/z* 498/500/502; the ions observed at *m/z* 451 and 453 are source-induced fragments. These fragment ions were chosen for MRM transition experiments because they offered greater specificity. [d] At this collision energy these compounds did not fragment and only the parent ion was observed. [e] Metabolites XVII, XVIII, and XIX are glucuronide conjugates of metabolites II, XIII, and X, respectively. [f] NA, not applicable; UV retention times for multiple reaction monitoring transitions experiments were not established as the reference standards for glucuronides were not synthesized.

The MS/MS analysis was set at 100−700 amu continuous with a scan rate of 1 s. MassLynx V4.0 software (Waters) was used for instrument operation and mass spectral data processing.

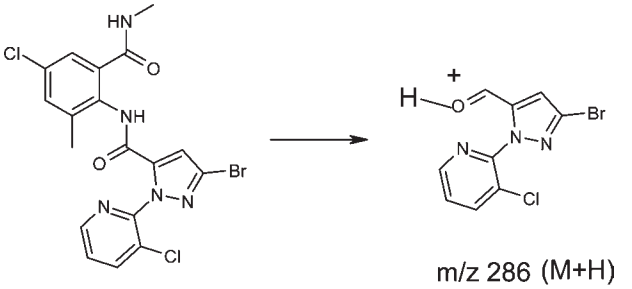
TSQ Mass Spectrometry. The mass spectrometer was used in positive ion electrospray ionization (ESI+) mode with probe position 2, spray voltage of 4.5 kV, capillary temperature of 350 °C, sheath gas of 80 psi, and auxiliary gas of 40 psi. Full-scan analysis (100−1000 amu) at a scan rate of 1 s was used. ESI-MS/MS collision gas pressure was ca. 2.2 mTorr utilizing argon. Xcalibur software (Thermo Finnigan) was used for instrument operation and mass spectral data processing.

RESULTS

Excretion of Administered Dose and Mass Balance. The administered daily single dose of ca. 10.4 mg [^{14}C]chlorantraniliprole equiv/kg of feed was at least 10 times greater than the worst case estimated dietary burden. Approximately 93.6% of the total dose was accounted for in feces, urine, and cage washes (Table 1). Milk and edible tissues contained <1.5% of the administered dose, indicating no significant potential for transfer of residues to meat and milk due to livestock exposure to chlorantraniliprole.

Analytical Reference Standards. Predicted metabolite reference standards were synthesized by DuPont Crop Protection. The synthetic reference standards will be made available upon request. Retention times (UV and mass spectral total ion current), full-scan and product ion spectra, and specific multiple reaction monitoring (MRM) transitions were established for each reference standard (Table 2).

Mass Spectral Fragmentation of Chlorantraniliprole. Chlorantraniliprole analytical standard was analyzed by LC-MS in positive ion mode. The mass spectrum of chlorantraniliprole in positive ion mode (Figure 2A) shows a characteristic isotope pattern of a compound containing one bromine and two chlorines. The exact monoisotopic mass of chlorantraniliprole is 481. The isotopic abundance of chlorine (37) and bromine (81) contributes to the M + 2 peak enhancement as shown in Figure 2. Therefore, the most abundant protonated ion in a positive ion mode for chlorantraniliprole is *m/z* 484 ($M + H^+$). The major fragment ion seen at *m/z* 286 (Figure 2B) is proposed to be the pyrazole−pyridine carbonyl ion as depicted below.



The molecular mass for the pyrazole−pyridine fragment is 283. The fragment ion at *m/z* 286 ($M + H^+$) is due to the

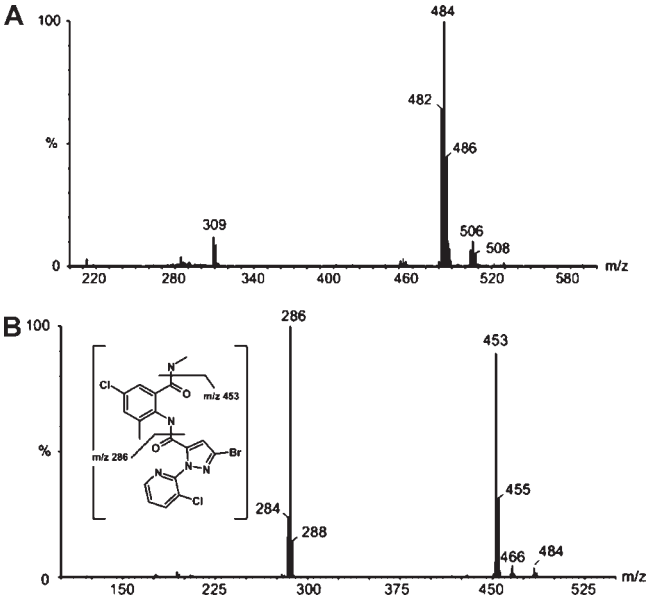


Figure 2. Mass spectra of chlorantraniliprole: (A) full-scan spectrum of chlorantraniliprole; (B) product ion spectrum of chlorantraniliprole.

isotopic abundance of chlorine and bromine atoms in the fragment ion (Figure 2B). The majority of goat metabolites contained the intact chlorantraniliprole skeleton. The protonated and unprotonated ions for these metabolites were observed in the positive and negative ion mode, respectively. The isotope pattern observed correlates to the proposed number of bromine and chlorine atoms.

Identification of Metabolites. Full-scan spectra and, when possible, product ion spectra were produced for each of the reference standards (Table 2). In addition, specific MRM transitions were established for each reference standard. MRM transitions were used to confirm assignments made by cochromatography when a low concentration of metabolites present in samples necessitated the improved sensitivity offered by this technique. The feces and urine extracts contained the highest concentration of chlorantraniliprole metabolites and were used for metabolite identification. Metabolites I−XVI were identified in excreta using HPLC and LC-MS/MS (Q-ToF and MRM transitions) by comparing the retention times, molecular weights, and fragmentation patterns of the authenticated analytical reference standards. The retention times of metabolites, mass spectral fragment ions, and MRM transitions are provided in Table 2. Representative HPLC radiochromatograms and mass spectral data are provided in Figures 3 and 4. A total of 19 metabolites including 3 glucuronide conjugates and intact chlorantraniliprole were identified from feces, urine, bile, or tissues (Figures 5 and 6).

Glucuronide conjugates of metabolites XIII, II, and X were identified as metabolites XVII, XVIII, and XIX, respectively, in the bile. Metabolite XVII, the glucuronide conjugate of metabolite II, had a retention time of 39.9 min (Table 2) in the total ion current (TIC) chromatogram of the mass spectral analysis. Its full-scan spectrum (Figure 4A) showed a molecular ion at m/z 676. Its product ion spectrum showed fragment ions at m/z (M + H), 484, 469, and 286 (Figure 4B). The fragmentation pattern in the product ion spectrum of the glucuronide conjugate (metabolite XVII, Figure 4B) closely matched that of metabolite II (Figure 4C). Therefore, metabolite XVII was tentatively identified as the glucuronide conjugate of metabolite II. The structures for metabolites I−XVI and conjugates XVII−XIX are provided in Figures 5 and 6,

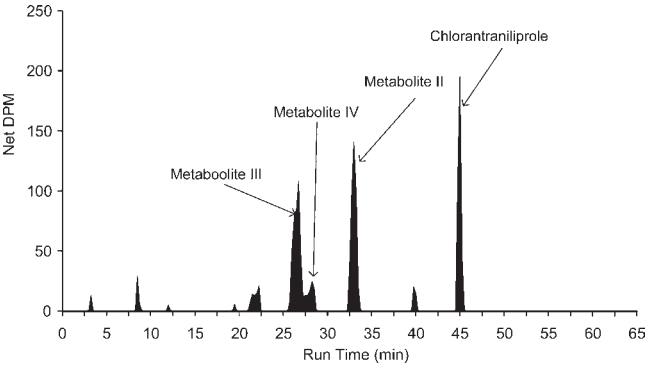


Figure 3. HPLC radiochromatogram of extracts of composite milk samples at residue plateau.

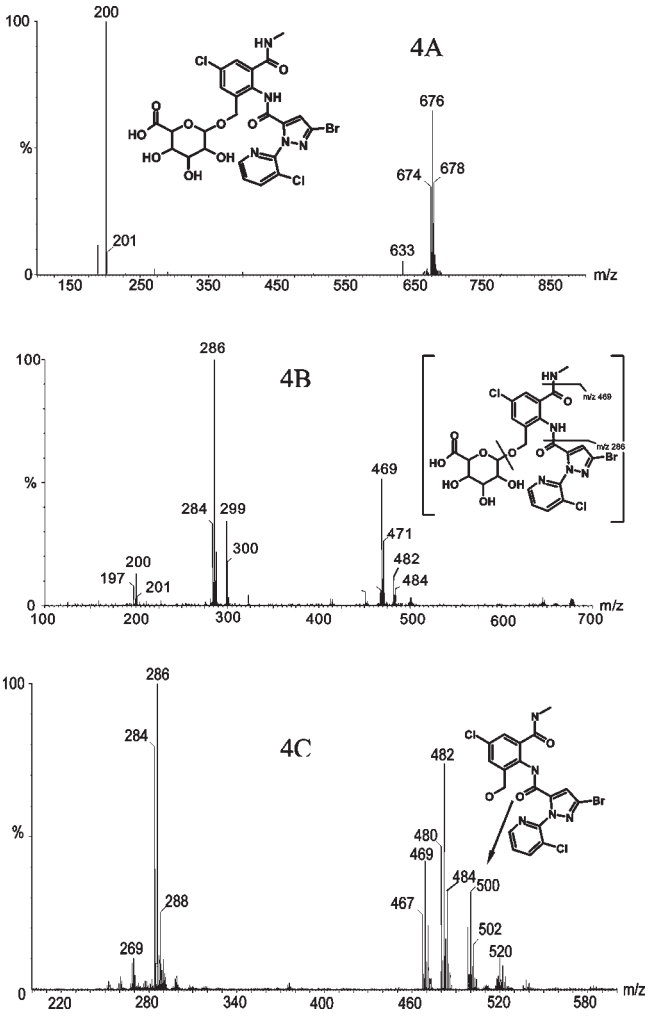


Figure 4. (A) Full-scan spectrum of metabolite II glucuronide. (B) Product ion spectrum of metabolite II glucuronide. (C) Full-scan spectrum of metabolite II.

respectively. The metabolite levels and the matrices in which these metabolites were observed are provided in Table 3.

DISCUSSION

The lactating goat was used as a typical ruminant species, which was a surrogate of the dairy cow. The test goat was in good

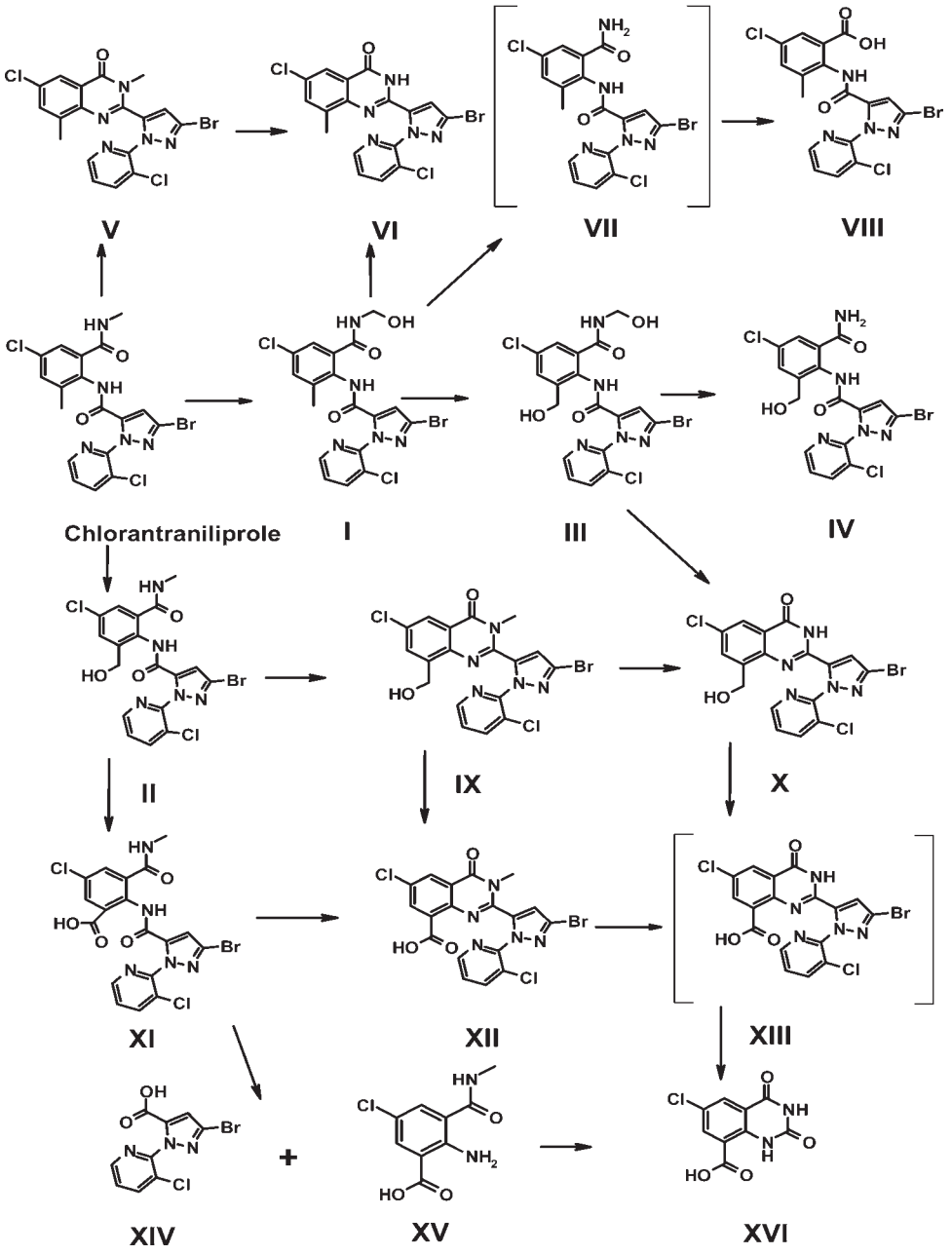


Figure 5. Proposed metabolic pathways of chlorantraniliprole in the lactating goat.

health throughout the acclimation and dosing period. The feed consumption, milk-yielding capacity, and body weight were relatively constant throughout the study. The body weight of the test goat ranged between 40 and 42.5 kg during the acclimation and dosing periods. The average daily feed consumption and milk yield were ca. 1.46 and ca. 1.83 kg, respectively, during the 7 day dosing period. Radiolabels in the benzamide and pyrazole carbonyl positions were found to be stable and were chosen for the metabolism study. To minimize use of experimental animals, the two radiolabels, [*benzamide carbonyl*-^{14}C]- and [*pyrazole carbonyl*-^{14}C]-chlorantraniliprole,were mixed at a ratio of 1:1 (μCi/μCi) to prepare the dose, in lieu of dosing two goats with different radiolabels.

The TRR eliminated in the excreta were expressed as percent of the total administered dose. The TRR in milk and edible tissues were expressed as milligrams of chlorantraniliprole equivalents per kilogram of matrix (Table 1).

Radioactive Residues in Milk and Tissues. Elimination of radioactivity in milk reached a steady state level within 3 days after the first dose. The highest levels of TRR in milk were observed between 2 and 3 days at 0.081 mg/kg and decreased to 0.047 mg/kg in the day 7 milk sample. The decrease of milk residues from the plateau level of 0.081 mg/kg to 0.047 mg/kg in the day 7 sample was presumably a result of enzyme induction, which generated metabolites that were more easily eliminated. However, this fact was not established experimentally. TRR in edible tissues were low (0.02−0.09 mg/kg), except for liver, which contained 0.640 mg/kg. Solvent-extractable TRR in liver was low (25.7%) due to association of chlorantraniliprole and its metabolites with tissue protein structural matrix. These residues were

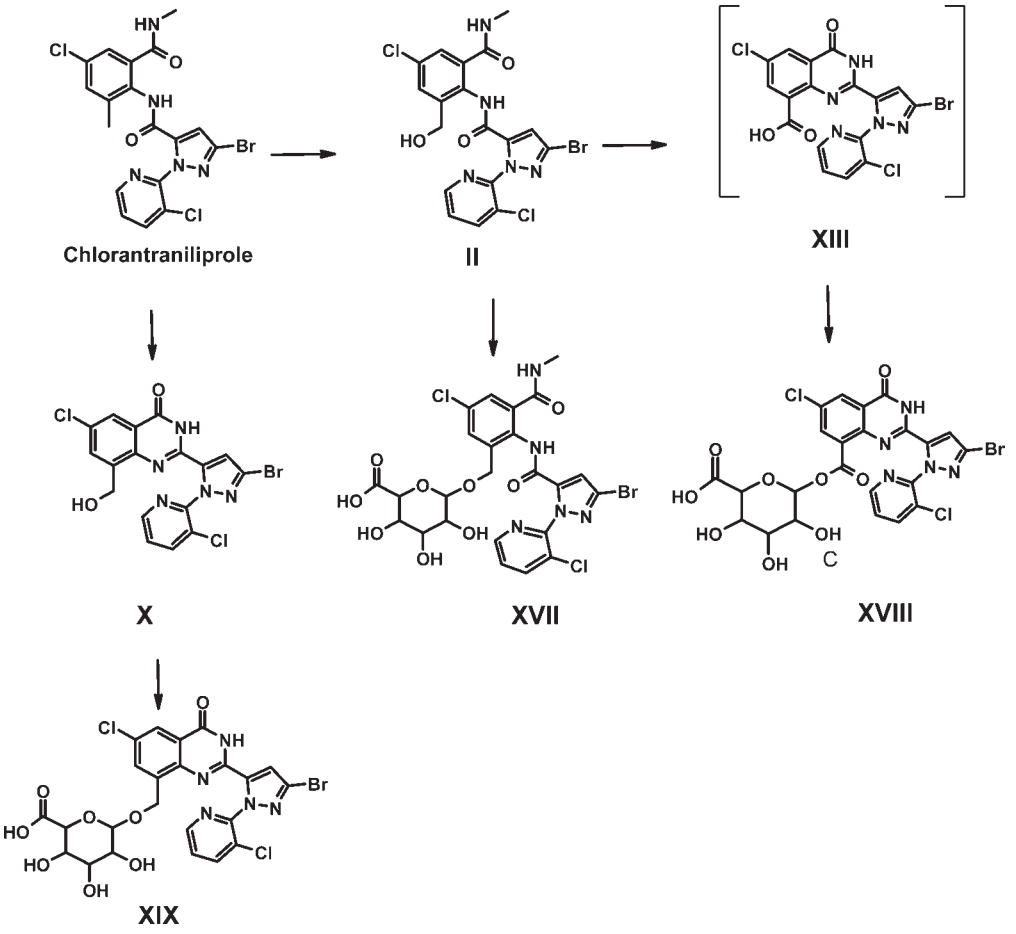


Figure 6. Proposed phase II metabolic pathways of primary oxidation products of chlorantraniliprole.

liberated following treatment with digestive enzymes and acid hydrolysis. A sequential treatment of PES with pepsin, protease, and acid treatment liberated 31.7, 5.0, and 20.6% of the liver total radioactive residues, respectively. Chlorantraniliprole was found in all tissues together with trace levels of oxidized metabolites. Unchanged chlorantraniliprole was the major terminal residue identified in fat (0.007−0.009 mg/kg) and liver (0.046 mg/kg). A proposed enzymatic hydroxylation of the *N*-methyl carbon of chlorantaniliprole followed by elimination of the $-CH_2OH$ group gave an amide, metabolite VII. However, the amide was not detected in tissues or excreta as it undergoes deamination resulting in an anthranilic acid derivative designated metabolite VIII. Metabolite VIII was the major component in liver, representing 0.056 mg/kg. Several minor metabolites (I, II, IV−VII, IX−XII, XIV, and XV) were identified in tissues including liver and kidney at concentrations ranging from <0.001−0.035 mg/kg.

Radioactive Residues in Excreta and Bile. The majority (ca. 95%) of administered radioactivity was recovered in excreta with 10.73% in urine and 82.84% in the feces and cage wash. The HPLC profile of the composite urine sample contained at least 10 radiolabeled components (Table 3). Unchanged chlorantraniliprole was not detected in urine, but accounted for 23.3% of applied dose in the feces. Two significant urine components were identified as metabolite X (1.8% dose) and the glucuronide conjugate of metabolite II (metabolite XVII, 3.1% dose). Bile was analyzed to study the phase II metabolites to understand the detoxification pathways of chlorantraniliprole. Two additional glucuronides, designated metabolites XVIII and XIX, were also tentatively identified from urine as conjugates of metabolites XIII and X, respectively (Tables 2 and 3). Glucuronide metabolites XVII and XIX were also present in bile. Two significant feces components were metabolites III (8.6% dose) and II (26.6% dose). Table 3 shows a complete list of metabolites identified from urine, feces, and bile.

Proposed Metabolic Pathway. Overall, 19 metabolites including three glucuronide conjugates and unchanged chlorantraniliprole were identified/characterized from milk, tissues, and excreta (Tables 2 and 3 and Figures 2 and 3). Hydroxylation of the *N*-methyl and methylphenyl carbons resulting in metabolites I and II, respectively, was a major metabolic pathway for chlorantraniliprole in the goat. Further hydroxylation of either metabolite I or II yielded the dihydroxy metabolite III. *N*-Demethylation of metabolite I gave metabolite VII, whereas loss of the hydroxymethyl group from metabolite III gave metabolite IV.

Cyclization of chlorantraniliprole (through the benzamide nitrogen and the pyrazole carbonyl) with concomitant loss of water was proposed for the formation of metabolite V. Metabolite VI was formed through cyclization of metabolite I with loss of water or *N*-demethylation of metabolite V. Similar cyclization reactions are proposed for the transformation of metabolite II to IX, II to X, and XI to XII. Oxidative metabolism was also an alternative pathway for conversion of metabolites IX and X to the carboxylic acids metabolites XII and XIII, respectively. *N*-Demethylation of metabolite XII would also yield metabolite XIII.

Table 3. Summary of [^{14}C]-Labeled Residues in Various Goat Matrices after Dosing with [^{14}C]Chlorantraniliprole Once per Day for 7 Consecutive Days[a]

	radioactive residues present in matrix								
component	feces, % dose	urine, % dose	bile, mg/kg	milk, mg/kg	liver, mg/kg	digested liver, mg/kg	kidney, mg/kg	muscle, mg/kg	fat,[b] mg/kg
chlorantraniliprole	23.32	ND[c]	0.028	0.016	0.025	0.002	0.016	0.007	0.040
metabolite I	3.15	<0.01	<0.001	<0.001	0.005	<0.001	0.002	0.001	<0.001
metabolite II	26.63	0.40	0.004	0.018	0.005	<0.001	0.003	0.002	0.001
metabolite III	8.60	0.68	0.092	0.017	<0.001	<0.001	0.002	<0.001	<0.001
metabolite IV	2.54	0.40	0.018	0.004	<0.001	0.005	<0.001	<0.001	<0.001
metabolite IV	<0.01	<0.01	0.058	<0.001	<0.001	<0.001	<0.001	<0.001	0.005
metabolite VI	<0.01	<0.01	0.018	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
metabolite VII	metabolite VII was an intermediate that undergoes deamination to generate metabolite VIII								
metabolite VIII	<0.01	0.30	0.065	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
metabolite IX	0.95	<0.01	0.124	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
metabolite X	<0.01	1.77	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
metabolite XI	<0.01	<0.01	<0.001	<0.001	0.004	<0.001	<0.001	<0.001	<0.001
metabolite XII	<0.01	0.21	0.073	<0.001	<0.001	<0.001	0.005	<0.001	0.003
metabolite XIII	XIII was not detected; however, its glucuronide was tentatively identified as XVIII								
metabolite XIV	<0.01	0.38	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
metabolite XV	<0.01	<0.01	<0.001	<0.001	0.048	0.004	0.001	<0.001	<0.001
metabolite XVI	1.57	0.65	0.176	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
metabolite XVII	<0.01	3.08	1.403	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
metabolite XVIII	<0.01	0.74	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
metabolite XIX	<0.01	0.62	0.060	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[a] The LOD for quantitiative analysis of these residues was calculated as follows: 1, for LSC analysis a background of 30−40 dpm was subtracted from each count rate; 2, for HPLC analysis a background from the void volume was collected and subtracted from the rest of the chromatogram. [b] Concentrations represent a mean of the results obtained from all three fat types analyzed (subcutaneous, renal, and omental). [c] ND, not detected.

Hydrolysis of the amide bridge linking the benzamide and pyrazole rings of chlorantraniliprole gave metabolite XIV and XV, which was a minor metabolic pathway. Glucuronide conjugation of metabolites II, X, and XIII to form metabolites XVII, XVIII, and XIX, respectively, was also one of the detoxification metabolic pathways of chlorantraniliprole (Figure 6) in the lactating goat.

Conclusions. Chlorantraniliprole is extensively metabolized presumably through cytochrome P450 catalyzed mono-oxygenase oxidation reactions, such as hydroxylation followed by *N*-demethylation or conjugation. Other metabolic reactions involved dehydration followed by cyclization and oxidative deamination. Phase II metabolites, glucuronide conjugates, were minor metabolic products. The administered chlorantraniliprole and its metabolites were readily excreted with a mass balance of 93% of the administered dose. There is no reasonable expectation of significant transfer of chlorantraniliprole or its metabolites to meat, milk, and fat when goat and other ruminant animals such as beef and dairy cattle are exposed to it under normal agricultural practices.

ABBREVIATIONS USED

ESI, electrospray ionization; LSC, liquid scintillation counting; MRM, multiple reaction monitoring; PES, post-extracted solids; Q-ToF, quadrupole time of flight; TIC, total ion current; TSQ, triple-stage quadrupole; TRR, total radioactive residues.

AUTHOR INFORMATION

Corresponding Author

*Phone: (302) 451-3582; e-mail venkat.gaddamidi@usa.dupont.com.

ACKNOWLEDGMENT

Dr. William T. Zimmerman is gratefully acknowledged for syntheses of reference standards.

REFERENCES

(1) Coronado, R.; Morrissette, J.; Sukhareva, M.; Vaughan, D. M. *Am. J. Physiol.* **1994**, *266*, C1485.

(2) Rogers, E. F.; Koniuszy, F. R.; Shavel, J., Jr.; Folkers, K. *J. Am. Chem. Soc.* **1948**, *70*, 3086.

(3) Folkers, K.; Rogers, E.; Heal, R. E. U.S. Patent 2,400,295, 1946.

(4) Sattelle, D.; Cordova D.; Cheek, T. R. *Invertebr. Neurosci.* **2008**, *8*, 107−119.

(5) Cordova, D.; Benner, E. A.; Sacher, M. D.; Rauh, J. J.; Sopa, J. S.; Lahm, G. P.; Selby, T. P.; Stevenson, T. M.; Flexner, L.; Gutteridge, S.; Rhoades, D. F.; Wu, L.; Smith, R. M.; Tao, Y. *Pestic. Biochem. Physiol.* **2006**, *84*, 196−214.

(6) Lahm, G. P.; Cordova, D.; Barry, J. D. New insecticides functioning at the ryanodine receptor. *Bioorg. Med. Chem.* **2009**, *17*, 4127–4133.

(7) Bentley, K. S.; Fletcher, J. L.; Woodward, M. D. Chlorantraniliprole: an insecticide of the anthranilic diamide class. *Hayes Handbook of Pesticide Toxicology*; 2010, Chapter 102, pp 2231−2242