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Pyrolysis of [^{14}C]-Chlorantraniliprole in Tobacco

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ABSTRACT: The pyrolysis of [^{14}C]-chlorantraniliprole {3-bromo-1-(3-chloro-2-pyridinal)-*N*-[4-chloro-2-methyl-6-[(methylamino)-carbonyl]phenyl]-1*H*-pyrazole-5-carboxamide} in tobacco was examined. Typically five commercially available cigarettes were treated separately with either [*pyrazole carbonyl*-^{14}C] or [*benzamide carbonyl*-^{14}C]-chlorantraniliprole at a concentration of 20 ppm (μg chlorantraniliprole equivalent/g cigarette weight; main study) to 40 ppm (for degradate identification only). All treated cigarettes were smoked using an apparatus designed to collect mainstream (MS) and sidestream (SS) smoke through a glass fiber filter and a series of liquid traps. The material balance for recovery of applied radiolabel ranged from 92.4 to 94.9%. Unchanged chlorantraniliprole was the major component found in butt and filter extracts, averaging a total of 17.4−17.9% of the applied radioactivity. A nonpolar degradation product, 2-[3-bromo-1-(3-chloro-2-pyridinyl)-1*H*-pyrazol-5-yl]-6-chloro-3,8-dimethyl-4(3*H*)-quinazolinone, designated **1**, represented an average of 10.1−15.9% of the applied radioactivity in the [*pyrazole carbonyl*-^{14}C] or [*benzamide carbonyl*-^{14}C]-chlorantraniliprole cigarettes, respectively. $^{14}CO_2$ was the major degradate, representing an average of 32.9 and 25.1% of the applied radioactivity in pyrazole and benzamide experiments, respectively. In the pyrazole carbonyl label a polar degradate, 5-bromo-*N*-methyl-1*H*-pyrazole-3-carboxamide (**2**) was present in the filter extracts at an average of 9.5% of the applied radioactivity. The most nonpolar degradate, 2,6-dichloro-4-methyl-11*H*-pyrido[2,1*b*]quinazolin-11-one (**3**), was present in [*benzamide carbonyl*-^{14}C]-treated cigarettes only and represented an average of 14.7% of the applied radioactivity.

KEYWORDS: chlorantraniliprole, insecticide, degradates, tobacco, cigarette, smoke, pyrolysis

INTRODUCTION

Chlorantraniliprole (DuPont Rynaxpyr) is the active ingredient in the DuPont Coragen, DuPont Altacor, and DuPont Prevathon insecticides.[1] DuPont Coragen is a suspension concentrate that can be applied as a transplant water treatment or foliar spray to control tobacco budworm and tobacco hornworm.

Agrochemicals present in tobacco have been of concern to regulatory agencies worldwide as well as to the tobacco industry because of the potential toxicity of residues and degradation products formed on pyrolysis. In fact, the U.S. Environmental Protection Agency requires that thermal degradation products from agrochemicals present in tobacco at residue levels of ≥0.1 ppm be identified and quantitated.[2]

There are few examples in the literature illustrating the formation of pyrolytic degradation products from agrochemicals. Smoke condensates from dimethoate-treated tobacco have been examined for the formation of carcinogenic products,[3] and the inhalation toxicity of maleic hydrazide in cigarette smoke has been studied in hamsters.[4] In both cases treated samples gave results similar to those of controls.

Various approaches have been used to generate tobacco smoke under conditions that will allow for the identification of degradates, as reported for imidachloprid[5] and endosulfan[6] as well as for measuring toxic effects directly by introduction of smoke from tobacco treated with insecticides into the trachea of living rats.[7]

The main objective of the present study was to examine the fate and distribution of the novel insecticide [^{14}C]-chlorantraniliprole (Scheme 1) pyrolysis products in mainstream and sidestream cigarette smoke of commercially made cigarettes separately fortified with test substance radiolabeled at two different carbon positions and provide the mass balance of dosed radioactivity. The smoking apparatus utilized for the pyrolysis of [^{14}C]-chlorantraniliprole in cigarettes is shown in Figure 1. Our laboratories have previously used this apparatus successfully to conduct the pyrolysis of pesticides and addressed the EPA guideline requirement for material balance (quantitative recovery), trapping of volatile products, and identification of degradates as described in this paper.

MATERIALS AND METHODS

The cigarettes used in the study were commercially available Camel unfiltered Class A cigarettes (R. J. Reynolds Tobacco Co., Winston-Salem, NC). Prior to dosing, the cigarettes were weighed, and a mark was placed at 20 mm from the end using a black pen. The cigarettes were smoked to the mark at each experiment. The remaining section is designated as the cigarette butt and constitutes one more compartment for determination of radiocarbon.

Chemicals. Radiolabeled test substances, [*pyrazole carbonyl*-^{14}C]-chlorantraniliprole and [*benzamide carbonyl*-^{14}C]-chlorantraniliprole with specific activities of 49.57 μCi/mg (110,045 dpm/μg) and 44.5 μCi/mg (98,790 dpm/μg), respectively, were used for the tobacco pyrolysis experiments. Prior to the start of the experiment, the radiochemical purities of the test substances were determined by HPLC to be >98% for both radiocarbon labels. The positions of the ^{14}C labels in the test compound are shown in Scheme 1.

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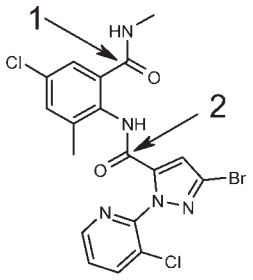
Scheme 1. Chemical Structure of [^{14}C]-Chlorantraniliprole with Radiolabel Positions Indicated



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

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

All solvents and reagents were obtained from Fisher Scientific (Pittsburgh, PA), J. T. Baker (Phillipsburg, NJ), Curtin Matheson (Pittsburgh, PA), or EMD (Gibbstown, NJ). Scintillation cocktail was Safety Solve (RPI, Mount Prospect, IL).

*Synthesis of 2-[3-Bromo-1-(3-chloro-2-pyridinyl)-1H-pyrazol-5-yl]-6-chloro-3,8-dimethyl-4(3H)-quinazolinone (Degradate **1**).* Chlorantraniliprole was stirred as a suspension in 40 mL of water containing 10% *p*-dioxane and approximately 1% of methylamine and heated to reflux at 90−95 °C for 2 h. The resulting suspension was cooled and filtered to afford 2-[3-bromo-1-(3-chloro-2-pyridinyl)-1*H*-pyrazol-5-yl]-6-chloro-3, 8-dimethyl-4(3*H*)-quinazolinone (**1**) [CAS Registry No. 438450-43-2] in high yield and purity, mp 215−216 °C. The mass spectral molecular ion at *m*/*z* 466 (M + H) and the NMR data [^{1}H NMR ($CDCl_3$) δ 2.00 (s, 3H), 3.76 (s, 3H), 6.83 (s, 1H), 7.31 (dd, 1H, J = 8.0, 5.0 Hz), 7.41 (m, 1H), 7.84 (dd, 1H, J = 8.0, 1.6 Hz] were consistent with the structure of the synthetic standard for 2-[3-bromo-1-(3-chloro-2-pyridinyl)-1*H*-pyrazol-5-yl]-6-chloro-3,8-dimethyl-4(3*H*)-quinazolinone (Scheme 2).

*Synthesis of 5-Bromo-N-methyl-1H-pyrazole-3-carboxamide (Degradate **2**).* 3-Bromopyrazole [CAS Registry No. 14521-80-3] was protected on nitrogen to the *N*-dimethylsulfamoyl derivative using dimethylsulfamoyl chloride and triethylamine in dichloromethane. The product was treated with lithium diisopropylamide (LDA) in tetrahydrofuran (THF) at −70 °C and then quenched with carbon dioxide (g) to afford the corresponding acid. This was deprotected by dissolution in trifluoroacetic acid for 1 h at ambient temperature to give 5-bromo-1*H*-pyrazole-3-carboxylic acid (II), mp 192−194 °C, in 50% yield over three steps. The acid was esterified using methanolic HCl (formed by careful addition of thionyl chloride) to afford methyl 5-bromo-1*H*-pyrazole-3-carboxylate, mp 94−96 °C, which was then treated with excess methylamine in THF/water for 18 h to afford 5-bromo-*N*-methyl-1*H*-pyrazole-3-carboxamide (**2**), mp 198−200 °C. The mass spectral molecular ion at *m*/*z* 204 (M + H) and the NMR data [^{1}H NMR (DMSO-d_6) δ 2.76 (d, 3H, J = 4.7 Hz), 6.86 (bs, 1H), 8.46 (bs, 1H), 13.86 (bs, 1H)] confirmed the structure of the synthetic standard for 5-bromo-*N*-methyl-1*H*-pyrazole-3-carboxamide (Scheme 2).

*Synthesis of 2,6-Dichloro-4-methyl-11H-pyrido[2,1-b]quinazolin-11-one (Degradate **3**).* Approximately 100 g of an analogue of chlorantraniliprole, namely, 3-chloro-*N*-[4-chloro-2-methyl-6-[(1-methylethylamino)carbonyl]phenyl]-1-(3-chloro-2-pyridinyl)-1*H*-pyrazole-5-carboxamide [CAS Registry No. 500008-60-6],[1] was suspended in acetonitrile (42 mL) and treated with 1.2 equiv of 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU) and heated to 80 °C for 1 h. The mixture was then cooled, filtered, and rinsed with acetonitrile and then hexane to afford 2,6-dichloro-4-methyl-11*H*-pyrido[2,1-*b*]quinazolin-11-one (**3**), 93% yield, mp 233−234 °C. The mass spectral molecular ion at *m*/*z* 278 (M + H) and the NMR data [^{1}H NMR ($CDCl_3$) δ 2.74 (s, 3H), 6.80 (t, 1H, J = 7.3 Hz), 7.67 (m, 1H), 7.69 (dd, 1H, J = 7.3, 1.6 Hz), 8.24 (d, 1H, J = 2.3 Hz), 8.79 (dd, J = 7.3, 1.6 Hz)] confirmed the structure for 2,6-dichloro-4-methyl-11*H*-pyrido[2,1-*b*]quinazolin-11-one (Scheme 2).

Chlorantraniliprole and its above-described synthetic reference standards will be made available upon request.

Application. [*pyrazole carbonyl*-^{14}C]- and [*benzamide carbonyl*-^{14}C]-chlorantraniliprole fortifying solutions were prepared in acetonitrile containing 14,137 and 12,020 dpm/μL, respectively. The fortifying solutions were stored in a freezer when not in use.

For each cigarette, aliquots of 135 μL (17.3 μg) for the [*pyrazole carbonyl*-^{14}C] set and 140 μL (17.0 μg) for the [*benzamide carbonyl*-^{14}C] set from the corresponding fortifying solutions were delivered using a 250 μL glass syringe to yield ca. 20 μg/g cigarette. Additional sets of cigarettes were fortified at twice the above volumes to yield 40 mg/g cigarette for isolation and identification of degradation products. Typically five cigarettes were used per test. The average weight of the [*pyrazole carbonyl*-^{14}C] set of cigarettes was ca. 0.84 g and that of the [*benzamide carbonyl*-^{14}C] set was ca. 0.86 g. The solution was added to the cigarette by introducing the syringe needle into the cigarette and delivering the solution along the entire length of the cigarette to obtain a homogeneous distribution. Aliquots (3 × 25 μL) of the fortifying solution were taken prior to and after the application process and radioassayed (LSC) to determine the nominal applied radiocarbon and to demonstrate homogeneity of the fortifying solution.

Pyrolysis Apparatus and the Smoking Experiment. The smoking apparatus (Figure 1) consisted of a 250 mL three-neck round-bottom flask fitted with 24/40 glass joints (smoking chamber). The middle neck was used as the mainstream (MS) port, one of the side necks was used as the sidestream (SS) port, and the remaining neck was used as the air intake.

The MS port was fitted with an adapter containing a Teflon holder for the cigarette. The cigarette holder was connected to a 25 mm stainless steel filter holder for the Cambridge glass fiber filter and onto a series of traps for volatiles housed in glass washing bottles with gas scrubbing tubes. The outlet of the traps was connected to a water aspirator vacuum pump, fitted with a three-way valve to apply vacuum to the cigarette through the MS port at given time intervals.

The SS port was connected in a similar fashion as the MS to a Cambridge glass fiber filter and a set of traps containing the same trapping medium as the MS port. The water aspirator was connected at the outlet of the SS traps to apply vacuum continuously during the

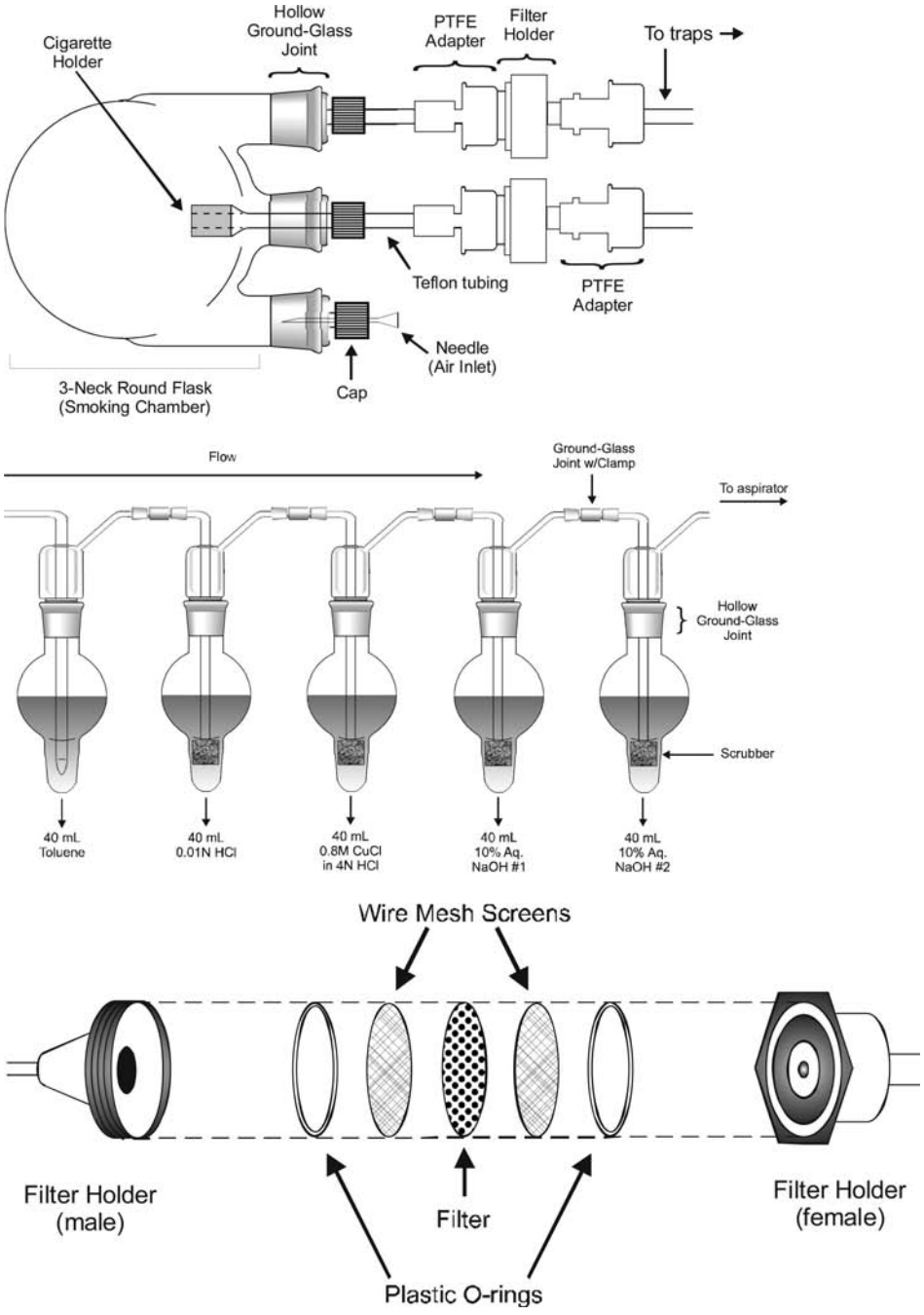


Figure 1. Total recovery smoking apparatus.

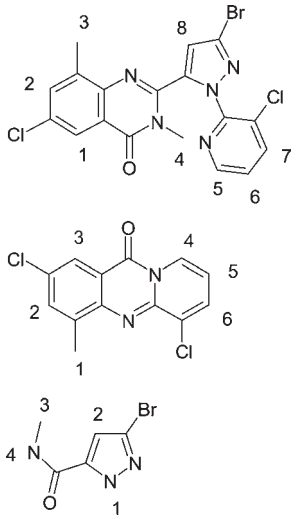
experiment, except at the puffing intervals, when the vacuum was diverted to the MS port.

The air intake was fitted with a screw-cap adapter with a Teflon-lined silicon septum. A needle was threaded through the septum to provide an air intake for the sample during the experiment. To light the cigarettes, a lit match was introduced through this open port while the vacuum pump was open to both MS and SS ports. After the cigarette had been lit, the match was immediately removed and the screw-cap adapter was put in place for the remainder of the experiment. To extinguish the cigarette, a nitrogen line was connected to the needle and nitrogen flowed through the system until the cigarette was completely extinguished.

The trapping solutions used for the experiments (both MS and SS) consisted of a toluene (benzamide carbonyl label) or methanol (pyrazole carbonyl label) trap, placed in a methanol/dry ice bath and maintained at approximately −30 °C, a 0.01 M (aq) hydrochloric acid (HCl) trap, a 0.8 M cuprous chloride (CuCl) in 4 M (aq) HCl carbon monoxide trap, and two 10% aqueous sodium hydroxide (NaOH) carbon dioxide traps.

After fortification, the cigarette was placed under a gentle stream of nitrogen to evaporate the solvent to dryness, and then the fortified dry cigarette was placed in the cigarette holder. The flows through the MS and SS traps were measured and adjusted to between 1000 and 1500 mL/min. The smoking chamber was connected to the MS and SS ports, and the cigarette was ignited with a match. The match was removed and the air vent put in place immediately after the cigarette was lit. The flow through the MS port was stopped via the three-way valve,

Scheme 2. Chemical Structures of Chlorantraniliprole Synthetic Standards with Proton Numbers



leaving the SS flow on. The flow through the MS port was opened every 30 s for a 2 s interval (which closed the SS flow) and then closed again to simulate a cigarette puff. The total smoking time to the 20 mm mark was recorded for each cigarette. The cigarette was extinguished by allowing a nitrogen gas stream into the smoking chamber through the air vent for about 1 min. After the cigarette had been extinguished, the MS and SS flows were left open for an additional 5 min to flush all headspace through the traps.

The remainder (cigarette butt) was extracted by placing it in 15 mL of acetonitrile and shaking in a Wrist Action shaker for 20 min, allowed to stand at room temperature for at least 4 h in the extraction solvent, and filtered to remove the butt. Aliquots (3 × 0.5 mL) of the filtrate were radioassayed by liquid scintillation counter (LSC). The extracted cigarette butt was dried, and remaining radioactivity was determined by combustion analysis. MS and SS glass fiber filters were also extracted into acetonitrile (15 mL) in the same fashion as the cigarette butts followed by LSC radioassay of the supernatant and combustion of the extracted filters. MS and SS port tubing and filter holders were rinsed with acetonitrile. The smoking chamber was rinsed with acetonitrile and combined with the cigarette ashes. Volumes of the rinses were measured, and aliquots (3 × 0.5 mL) of all rinses were radioassayed by LSC. Trap solutions were measured, and aliquots (3 × 50 μL for the CuCl traps and 3 × 1.0 mL for all other traps) were radioassayed by LSC. Volumes of all solutions were determined using graduated cylinders. Extracts containing significant radiocarbon were analyzed by HPLC analysis. Combustion analysis of cigarette butts was carried out using a Harvey OX-600 Biological Oxidizer, and the $^{14}CO_2$ generated was trapped with Carbon 14 Cocktail (R. J. Harvey Instrument Corp., Hillsdale, NJ). The ^{14}C content was determined by LSC.

The radioassay of extracts and solutions by LSC utilized 5 or 15 mL of Safety Solve scintillation cocktail (Research Products International Corp.) in 7 or 20 mL standard polyurethane counting vials and Beckman LS 6000 IC, LS 6000 SC, or LS 6500 liquid scintillation spectrometers. Typical counting time was 5 min with a counting efficiency of 96%. Computer-constructed quench curves, derived from a series of 10 sealed quenched standards, automatically converted counts per minute (cpm) to disintegrations per minute (dpm).

Extracts were analyzed by high-performance liquid chromatography (HPLC) utilizing the Agilent 1100 and 1200 models equipped with diode array detectors, degassers, and column temperature control and operated by Chemstation system. Chromatographic separation was accomplished on an Alltech Alltima C18, 250 × 4.6 mm i.d., 5 μm particle size, PTRL column (342, serial no. 01101848.1). The UV was set at 254 nm. The solvents were 0.1% formic acid in HPLC grade water (solvent A) and 0.1% formic acid in HPLC grade acetonitrile (solvent B). The column was eluted at a flow rate of 1.0 mL/min linear gradient, starting with 5% solvent B at 0 min and increasing to 100% in 40 min.

Aliquots of the dilute test substance solutions in acetonitrile were co-injected with the chlorantraniliprole reference standard for the radiochemical purity checks. Extracts and rinses were co-injected with reference standard solutions. Detection of ^{14}C was done by collection of 0.5 min fractions and radioassay by LSC. Peak assignments were made by comparison to the UV trace for each run. HPLC column recoveries were quantitative. Typical retention times (t_R) were as follows: chlorantraniliprole, 27.5 min; **1**, 34.6 min; **2**, 13.2 min; and **3**, 38.7 min.

The isolated degradates and selected reference standards were analyzed by liquid chromatography−mass spectrometry (LC-MS) using a Thermo Scientific LCQ Fleet mass spectrometer with electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) probes. Chromatographic separation was accomplished using a Michrom Magic C18, 150 × 2 mm, 5 μm, column heated to 40 °C. The UV detector was set at 254 nm. The mobile phase was a combination of Milli-Q HPLC grade water (MilliPore Ltd., U.K.) and acetonitrile, each solvent containing 0.1% v/v formic acid (Sigma-Aldrich, U.K.). A linear gradient elution with a flow rate of 0.2 mL/min, starting with 5% acetonitrile at 0 min and increasing to 100% acetonitrile in 40 min, was used for sample analyses.

Degradates **1** and **3** were isolated from the benzamide carbonyl-labeled samples and degradates **1** and **2** were isolated from the pyrazole carbonyl-labeled samples for LC-MS analysis and confirmation of identity by cochromatography (HPLC and/or TLC) with authentic standards.

Pyrolysis of [*pyrazole carbonyl*-^{14}C]-Chlorantraniliprole. The MS filter extract from [*pyrazole carbonyl*-^{14}C] cigarette 4 was filtered through a 0.45 μm filter and concentrated under nitrogen to a final volume of approximately 200 μL. The concentrated extract was injected on HPLC, and fractions of the eluate were collected. The fractions corresponding to **1** (69−70) from [*pyrazole*-^{14}C]-treated cigarette extracts were combined and analyzed by LC-MS, and its mass spectral characteristics were compared to those of the synthetic standards.

For degradate **2** isolation, two additional cigarettes were fortified with [*pyrazole carbonyl*-^{14}C]-chlorantraniliprole at approximately 40 μg/g cigarette (twice the original concentration) and only the MS and SS filters were collected. The filters from both cigarettes were combined and extracted with acetonitrile (80 mL). The extract was filtered (0.45 μm filter) and concentrated under reduced pressure and nitrogen to approximately 2 mL, and then 12 mL of deionized water was added to the concentrate. This sample was loaded on a preconditioned C18 SPE column (Varian BondElut, 1 g, 6 mL), and the eluate was collected. The eluate contained degradate **2**. It was then acidified and extracted with ethyl acetate (2 × 20 mL). The ethyl acetate extract was concentrated under reduced pressure and nitrogen to dryness and reconstituted in acetonitrile/water (4:1, v/v, 0.3 mL). The sample was injected on HPLC, and fractions of the eluate were collected. The fractions (29−30) that corresponded to degradate **3** were combined for LC-MS analysis.

Thin-layer chromatography (TLC) analysis utilized precoated TLC plates containing fluorescent indicator (silica gel, F_{254}, 20 × 20 cm, 0.25 mm thickness). Extracts were cospotted with authentic reference standards and developed in one or two dimensions using the following solvent systems: system A, hexane/ethyl acetate/acetic acid 10:20:1 (v/v/v); system B, chloroform/acetone, 2/:1 (v/v); system C, methanol/toluene saturated with formic acid, 1:1, v/v; system D, dichloromethane/ethyl acetate, 1:3 (v/v). Solvent systems A and B were used

for 2D-TLC confirmation of chlorantraniliprole in sample extracts, and solvent systems C and D were used for 2D-TLC confirmation of **2** by cochromatography with the authentic standard. Compound **2** exhibited R_f values of 0.40 (solvent system C) and 0.66 (solvent system D). All TLC plates were subjected to radioscanning with a Molecular Dynamics Storm 820 optical scanner (Piscataway, NJ).

Pyrolysis of [*benzamide carbonyl*-^{14}C]-Chlorantraniliprole. For LC-MS analysis of degradate **1**, the MS filter extract from [*benzamide carbonyl*-^{14}C]- cigarette 5 was passed through a 0.45 μm filter and concentrated under nitrogen to approximately 200 μL. The resulting concentrated extract was then injected on HPLC, and fractions of the eluate were collected in 8 mL glass amber vials. The fractions corresponding to degradate **1** (fractions 68−69) were combined and evaporated under nitrogen to <300 μL. The isolated **1** was analyzed by LC-MS.

The MS filter extracts from cigarettes 4, 6, and 7 of [*benzamide carbonyl*-^{14}C]-chlorantraniliprole treatments were combined in a pear-shaped flask, and the solvent was evaporated under reduced pressure to approximately 1.5 mL. The concentrated extract was diluted with 30 mL of HPLC grade water and eluted through a preconditioned C18 SPE column (Varian BondElut 500 mg, 6 mL), and then the column was rinsed with 22 mL of water. Both eluates were discarded, and the column was eluted with 4 mL of acetonitrile. The solvent was concentrated under nitrogen to approximately 600 μL, filtered through a 0.45 μm filter, and analyzed by HPLC, and the fractions were collected. The fractions corresponding to **3** (75−76) were combined and concentrated under nitrogen to evaporate the acetonitrile, leaving the sample dissolved mostly in water. The aqueous sample was extracted with hexane (2 × 1 mL), and the extracts were combined. The combined extract was loaded onto a preconditioned silica SPE column (Varian, BondElut-Si, 500 mg, 3 mL), and the column was rinsed with hexane and then eluted with 4 mL of hexane/diethyl ether (3:1, v/v). The eluate was then evaporated to dryness under nitrogen, and the sample was reconstituted with 200 μL of acetonitrile. The resulting isolated **3** was analyzed by LC-MS and compared to the synthetic standard.

Isolated degradate **1** from cigarettes treated with [*benzamide carbonyl*-^{14}C]-chlorantraniliprole and reference standards for chlorantraniliprole and **1** were analyzed using APCI in positive mode. Isolated degradate **3** and its suspected reference standard were analyzed using an ESI probe in positive mode. The retention time of ^{14}C peaks in the instrument was established by diverting the eluate and collecting fractions (0.5 min) followed by LSC of collected fractions in separate runs, carried out the same day of LC-MS analysis.

Method Validation, Sensitivity, and Detection Limit. Chromatographic methods were validated with authentic standards achieving the necessary resolution and sensitivity. Products with yields as low as 1% could be reliably quantified.

(i) Combustion. The limits of detection for combustion samples are determined according to the following equation:

$$\frac{2 \times \text{background (dpm)}}{\text{averagesamplesize (g)}} \times \frac{1}{\text{specificactivity (dpm}/\mu\text{g)}} = \text{ppm}$$

(ii) HPLC Radiochromatograms. The limits of detection (LOD) for individual degradates observed in the HPLC radiochromatograms are determined by the dpm injected and the LSC detection limit. Thus, peaks containing 2 times background dpm are considered to be real. Typical detection limits were as follows: for a background of 35 dpm, and a sample size of 5000 dpm (matrix containing 10% of applied radioactivity or 2.0 ppm):

$$\frac{70 \text{ dpm}}{5000 \text{ dpminjected}} \times 2.0 \text{ ppm} = 0.028 \text{ ppm}$$

(iii) Liquid Scintillation Counting. The limits of detection for individual aliquots are determined by the following equation:

$$\text{LSCLOD} = \frac{\text{lowercountinglimit } (2 \times \text{background})}{\text{dpmapplied}} \times 100$$

For a lower counting limit of 2 times background or 70 dpm and 4.0×10^6 dpm applied, the detection limit for LSC counting is calculated as

$$\frac{70 \text{ dpm}}{4.0 \times 10^6 \text{ dpmapplied}} \times 100 = 0.002\%$$

(iv). Relative standard deviations were generally <5% for all experiments.

RESULTS

Radiochemical Purity of Test Substances. The positions of carbon-14 substitutions in [*pyrazole carbonyl*-^{14}C]-chlorantraniliprole and in [*benzamide carbonyl*-^{14}C]-chlorantraniliprole are indicated in Scheme 1. The radiochemical purities for both labels were determined by HPLC prior to the start of the experiment as >98%. LSC radioassay of aliquots of the fortifying solutions taken throughout the fortifying processes resulted in relative standard deviations of 5.1% of the (n = 5) for the pyrazole carbonyl set and 2.9% (n = 6) for the benzamide carbonyl set, confirming homogeneity of the fortifying solutions during application. The postapplication radiochemical purities of the dose solutions for [*pyrazole carbonyl*-^{14}C]-chlorantraniliprole and [*benzamide carbonyl*-^{14}C]-chlorantraniliprole were unchanged after dosing, confirming the stability of the test substances under conditions of administration.

Synthesis of Analytical Reference Standards. The analytical reference standards for chlorantraniliprole,[1] **1**, **2**, and **3** were synthesized at DuPont Crop Protection. All compounds gave spectral data consistent with their structures. The mass spectral and NMR data of synthetic standards were consistent with the structures (Scheme 2). The synthetic reference standards are available upon request.

Application of [^{14}C]-Chlorantraniliprole. Cigarettes were treated separately with either [*pyrazole carbonyl*-^{14}C]-chlorantraniliprole or [*benzamide carbonyl*-^{14}C]-chlorantraniliprole. The final concentration of the test substance in the cigarettes for all applications ranged from 19.3 to 21.2 μg/g cigarette. For isolation of degradates, cigarettes were fortified with [^{14}C]-chlorantraniliprole at approximately 40 μg/g cigarette concentrations.

Accountability of Radiocarbon following Pyrolysis of [^{14}C]-Chlorantraniliprole. The material balance for the study was determined as the sum of radiocarbon in the butt extracts, filter extracts, tubing and flask rinses, volatile traps, and bound residues remaining in the cigarette and filters and is expressed as percent of applied radiocarbon based on aliquots of the fortifying solution. The mass balance for each test substance is summarized in Table 1 for pyrazole- and benzamide-labeled sets.

Generally, results presented refer to averages of experiments involving five cigarettes, but in some cases additional cigarettes were smoked either to improve the material balance or to generate enough material for identification purposes.

Pyrolysis of [*pyrazole carbonyl*-^{14}C]-Chlorantraniliprole. The material balance for the five cigarettes for the pyrazole-labeled set averaged 92.4 ± 2.0% of the applied radioactivity (Table 1). Total average radiocarbon from all compartments recovered in the mainstream and sidestream averaged 23.5 and 34.5% of the applied radioactivity, respectively (Table 1).

Table 1. Accountability of Radiocarbon in the Mainstream (MS) and Sidestream (SS) Smoke and Cigarette Butt following Pyrolysis of either [*pyrazole carbonyl*-^{14}C]-Chlorantraniliprole- or [*benzamide carbonyl*-^{14}C]-Chlorantraniliprole-Treated Cigarettes

	extracted	unextracted	tube/flask rinse[a]	organic cold trap	0.01 M HCl	0.8 M CuCl	NaOH trap	% recovery
				[*pyrazole carbonyl*-^{14}C]-Chlorantraniliprole				
MS av recovery	13.7	0.4	1.5	0.3	<0.1	<0.1	7.6	23.5
SS av recovery	7.4	0.3	0.5	1.0	<0.1	<0.1	25.4	34.5
cigarette butt	19.9	11.4	3.1	NA	NA	NA	NA	34.4
total recovery as % applied								92.4
				[*benzamide carbonyl*-^{14}C]-Chlorantraniliprole				
MS av recovery	15.7	0.5	1.5	<0.1	<0.1	<01	5.2	22.9
SS av recovery	12.6	0.4	0.7	0.2	<0.1	<01	20.3	34.0
cigarette butt	22.4	11.0	4.6	NA	NA	NA	NA	38.0
total recovery as % applied								94.9

[a] Rinses from cigarette butt rinses are from the flask including ashes and MS and SS rinses are from the tube.

Table 2. Product Distribution of [^{14}C]-Chlorantraniliprole and Degradates in Cigarette Butt and Filter Extracts by HPLC, Expressed as Percentage of Applied Radioactivity

		degradation product as % of applied dose			
sample	av % recovery	chlorantraniliprole	degradate **1**	degradate **2**	others[a]
av pyrazole-^{14}C butt extract	19.9	16.2	1.6	1.8	0.4
av pyrazole-^{14}C MS filter	13.7	0.7	5.4	5.4	2.2
av pyrazole-^{14}C SS filter	8.8	0.6	2.9	3.1	2.3
av pyrazole-^{14}C flask rinse	5.7	0.3	1.5	2.2	1.7
av benzamide-^{14}C butt extract	22.5	16.8	2.5	2.5	0.6
av benzamide-^{14}C MS filter	16.4	0.5	6.5	7.3	2.0
av benzamide-^{14}C SS filter	12.1	0.4	4.2	4.5	3.1
av benzamide-^{14}C flask rinse	5.5	0.2	1.8	1.9	1.5

[a] Others in this column comprised multiple components, none >1.4% of dose.

Radiocarbon in the butt extracts averaged 19.9% of the applied radioactivity in the five cigarettes tested. Unextracted radiocarbon remaining in the butts averaged 11.4%. The smoking chamber, which also included the cigarette ashes, was rinsed at the end of each experiment. An average of 3.1% of the applied radioactivity was recovered in the combined rinse/ash extract. Total average radiocarbon in the cigarette butts is given in Table 1.

Pyrolysis of [*benzamide carbonyl*-^{14}C]-Chlorantraniliprole. The material balance for the benzamide-labeled set (six cigarettes used) averaged 94.9 ± 3.1% of the applied radioactivity (Table 1). Total average radiocarbon from all compartments recovered in the mainstream and sidestream averaged 22.9 and 34.0% of the applied radioactivity, respectively (Table 1).

Radiocarbon in the butt extracts averaged 22.4% of the applied radioactivity in the six cigarettes tested, and the unextracted radiocarbon was 11%. The smoking chamber was rinsed at the end of each experiment, and the cigarette ashes were combined with this rinse for radioassay. Radiocarbon in the flask rinses averaged 4.6% of the applied radioactivity. Total average radiocarbon in the cigarette butts is given in Table 1.

Identification of the Pyrolysis Products of [^{14}C]-Chlorantraniliprole. Product distribution of chlorantraniliprole and degradates in extracts and rinses are presented in Table 2. The product balance for the analytes in combined compartments is summarized in Table 3 for pyrazole- and benzamide-labeled sets.

Table 3. Product Balance following the Pyrolysis of either [*pyrazole carbonyl*-^{14}C]-Chlorantraniliprole- or [*benzamide carbonyl*-^{14}C]-Chlorantraniliprole-Treated Cigarettes

	degradation product as % of applied dose				
sample	chlorantraniliprole	degradate **1**	degradate **2** or **3**	$^{14}CO_2$	others[a]
av pyrazole-^{14}C	17.4	10.1	9.5 (2)[b]	32.9	22.1
av benzamide-^{14}C	17.9	15.9	14.7 (3)[c]	25.1	20.4

[a] Others refers to the sum of the organic solvent and HCl traps (**3**) (MS and SS), MS and SS tubing rinses, remaining in MS and SS extracted filters, extracted butt, flask rinse, and minor degradates observed in HPLC analysis that do not coelute with the reference standard or one of the degradates above. [b] Degradate **2** was present only in the [*pyrazole carbonyl*-^{14}C]-chlorantraniliprole-treated cigarettes. [c] Degradate **3** was present only in the [*benzamide carbonyl*-^{14}C]-chlorantraniliprole-treated cigarettes.

Butt extracts, MS and SS filter extracts, and flask with ash rinses containing >4% of the applied radioactivity were analyzed by HPLC.

Representative radiochromatograms for [*pyrazole carbonyl*-^{14}C]-chlorantraniliprole and [*benzamide carbonyl*-^{14}C]-chlorantraniliprole pyrolysates are presented in Figures 2 and 3, respectively.

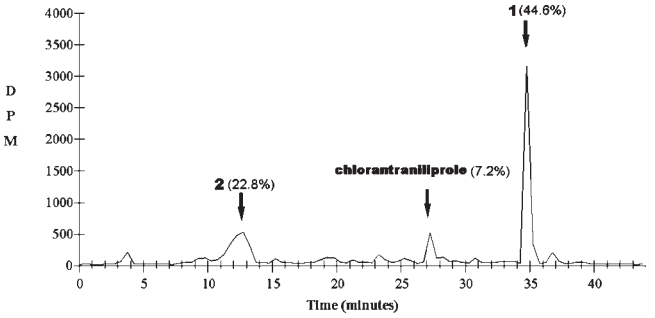


Figure 2. Representative HPLC radiochromatogram of [*pyrazole carbonyl*-^{14}C]-chlorantraniliprole sidestream filter extract.

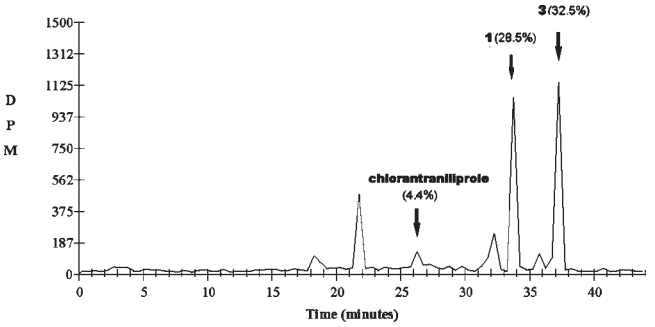


Figure 3. Representative HPLC radiochromatogram of [*benzamide carbonyl*-^{14}C]-chlorantraniliprole sidestream filter extract.

Chlorantraniliprole was identified in extracts by HPLC cochromatography with chlorantraniliprole reference standard.

The presence of $^{14}CO_2$ in the MS and SS caustic traps (yields are given in Table 3) was confirmed by treating aliquots of selected traps with a saturated $BaCl_2$ solution to precipitate the trapped carbon dioxide as barium carbonate. Essentially 100% of the trapped radiocarbon in the caustic solutions precipitated as $Ba^{14}CO_3$, thus confirming the identity of $^{14}CO_2$.

Identification of [*pyrazole carbonyl*-^{14}C]-Chlorantraniliprole Pyrolysis Products. The pyrolysis of [*pyrazole carbonyl*-^{14}C]-chlorantraniliprole in cigarettes resulted primarily in degradation to $^{14}CO_2$, representing a combined average of 32.9% of the applied radioactivity (MS and SS combined). Unchanged chlorantraniliprole, averaging a total of 17.4% of the applied radioactivity in butt and filter extracts for MS and SS combined, was present in the second highest quantity following the pyrolysis of five cigarettes (Table 2). Chlorantraniliprole was the major component extracted from the unsmoked butt extract, representing an average of 16.2% of applied radioactivity. In contrast, it was only present as <1% of the applied radioactivity in MS and SS filter extracts (Table 2).

The second largest degradate (after carbon dioxide) observed in the [*pyrazole carbonyl*-^{14}C]-labeled set was degradate **1**, comprising an average of 10.1% of the applied radioactivity following pyrolysis (Table 2). Degradate **1** was isolated in high purity by HPLC and identified by LC-MS by comparison of the APCI (positive) spectrum (Figure 4) with that of the synthetic standard. The only difference observed was the relative abundance of the quasimolecular (M + H) ions for **1** at *m*/*z* 464/466/468/470 (arising from Cl, Br, and ^{14}C), which in the corresponding synthetic standard lacked the ^{14}C label. Degradate **1** was present in the MS and SS filter and butt extracts with 5.4, 2.9, and

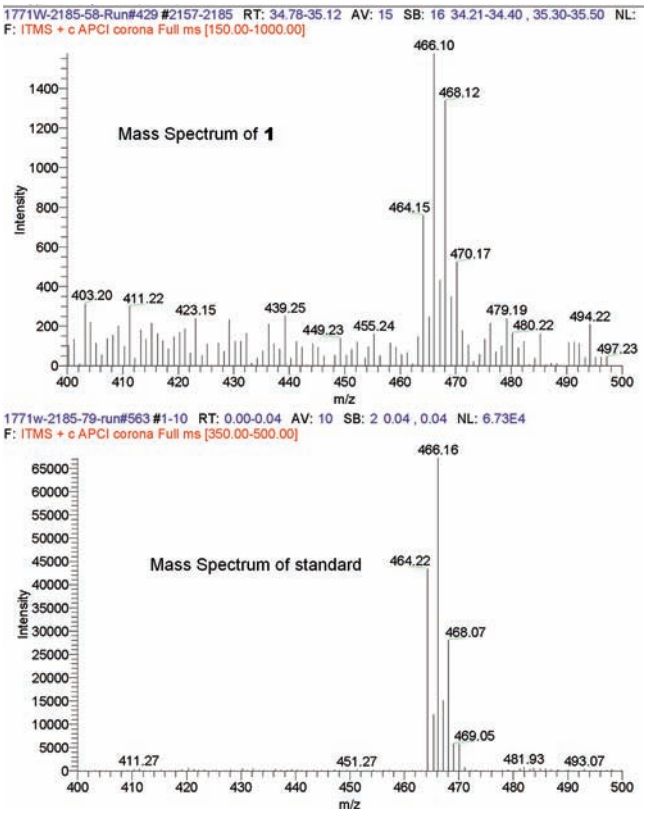


Figure 4. Mass spectra of degradate **1** and its corresponding synthetic reference standard.

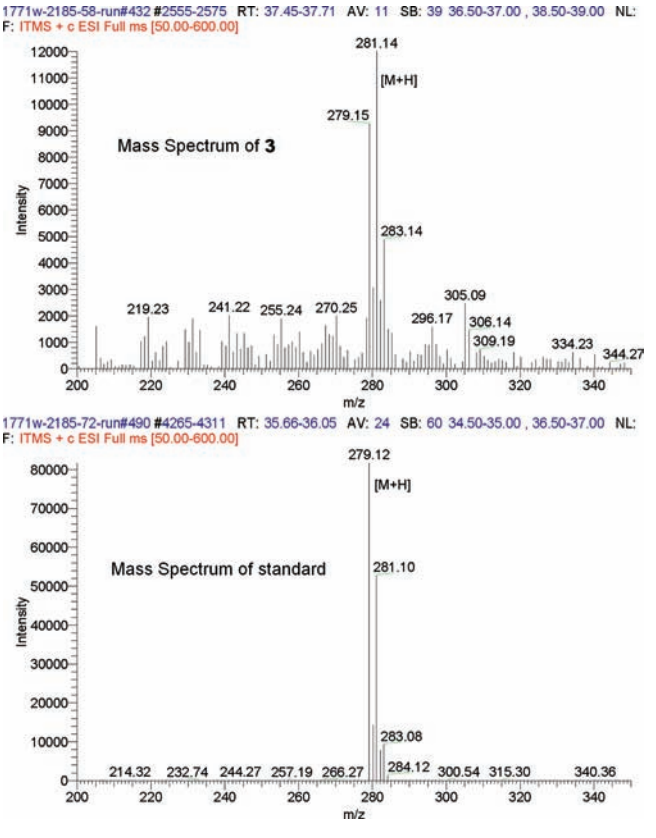


Figure 5. Mass spectra of degradate **3** and its synthetic reference standard.

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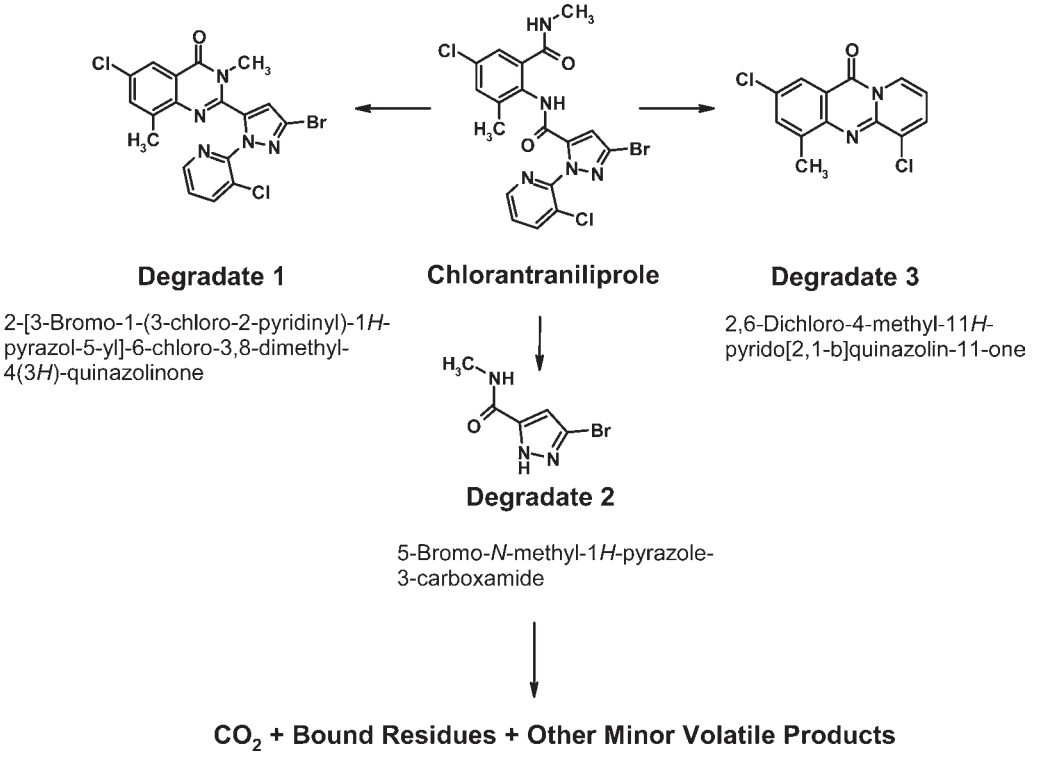
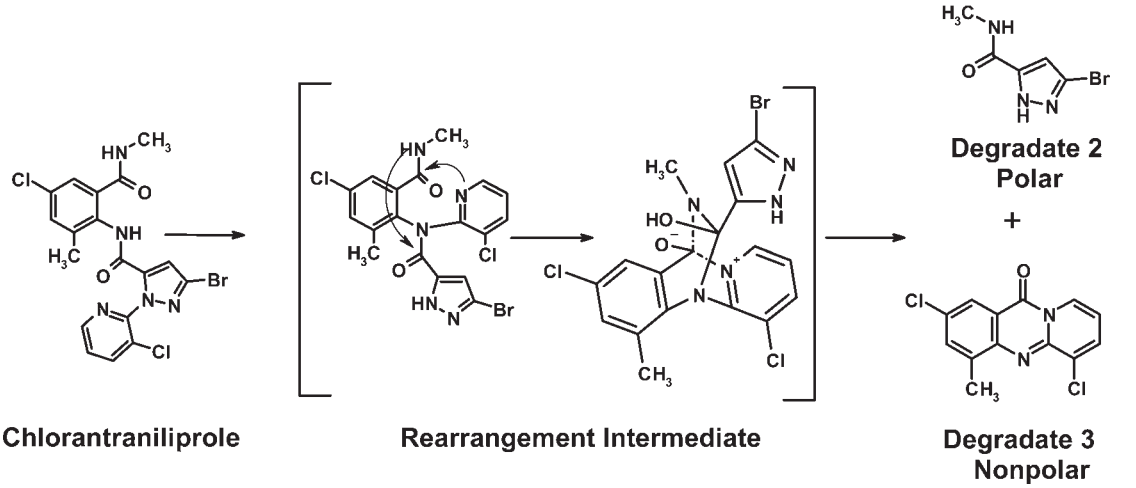
Scheme 3. Proposed Pyrolysis Pathway of Chlorantraniliprole



Scheme 4. Proposed Thermal Rearrangement of Chlorantraniliprole to Degradates 2 and 3



1.6% of applied radioactivity, respectively (Table 2), and an average of 1.5% recovered in the flask rinses.

The other major degradate present following pyrolysis of [*pyrazole carbonyl*-^{14}C]-chlorantraniliprole in cigarettes was a polar component, **2**, which eluted at 12.5 min and represented a combined average of 9.5% of the applied radioactivity (Table 2). Degradate **2** was not observed in the pyrolysis experiments on the benzamide carbonyl-labeled cigarettes, indicating that its structure did not contain the benzamide carbonyl carbon. It was purified by HPLC and identified by cochromatography with authentic synthetic standard by HPLC (Figure 3) and TLC. Degradate **2** was present as a major component in MS filter extracts, representing an average of 5.4% of the applied radioactivity. Degradate **2** represented an average of 3.1, 1.8, and 2.2% of the applied in the extracts of SS filter and butt and in the flask rinses, respectively. Results are summarized in Table 2. Several minor products were also present in the butt and filter extracts, but none accounted for greater than an average of 1.4% of the applied radioactivity.

Identification of [*benzamide carbonyl*-^{14}C]-Chlorantraniliprole Pyrolysis Products. The results found in the benzamide-labeled experiments were similar to the results observed in the experiments using pyrazole-labeled test substance. The pyrolysis of [*benzamide carbonyl*-^{14}C]-chlorantraniliprole in cigarettes

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resulted primarily in degradation to $^{14}CO_2$, representing a combined average of 25.1% of the applied radioactivity (MS and SS combined) (Table 3). Unchanged chlorantraniliprole, averaging a total of 17.9% of the applied radioactivity in butt and filter extracts for MS and SS combined, was present in the second highest quantity following the pyrolysis of five cigarettes (Table 3). Chlorantraniliprole was the major component extracted from the unsmoked butt extract, representing an average of 16.8% of the applied radioactivity. In contrast, it was only present as <1% of the applied dose in MS and SS filter extracts or flask rinses (Table 2).

A degradate was observed eluting at 34 min by HPLC, which represented an average of 15.9% of the applied dose. HPLC and LC-MS characteristics of this component were identical with degradate **1**, which was identified in the [*pyrazole carbonyl*-^{14}C]-chlorantraniliprole-treated cigarettes and synthetic reference standard (Figures 2−4). This degradate was present mainly in the MS and SS filter extracts (6.5 and 4.2% of the applied dose, respectively), with 2.5% detected in the cigarette butt extracts and an average of 1.8% in the flask rinses.

The second largest degradate observed in the benzamide set was degradate **3**, which comprised an average of 14.7% of the applied radioactivity following pyrolysis (Table 3). This degradate was not observed in the pyrazole set, indicating that the pyrazole carbonyl carbon was absent in its structure. Degradate **3** was identified by LC-MS of the HPLC-purified fraction by comparison of authentic synthetic standard spectra (Figure 5). The quasimolecular ion cluster at m/z 279/281/283 for degradate **3** was consistent with that of synthetic standard, except that the latter lacked the ^{14}C contribution. Degradate **3** was present in the MS filter, SS filter, and butt extracts at an average of 7.3, 4.5, and 2.5% of dose, respectively (Table 2). Approximately 1.9% was recovered in the flask rinses. Several minor products were also present in the butt and filter extracts, but none accounted for greater than an average of 1.3% of the applied radioactivity.

DISCUSSION

A proposed chlorantraniliprole pyrolysis degradation pathway is shown in Scheme 3. Chlorantraniliprole undergoes dehydration by cyclization to yield degradate **1**. The mechanism of thermal degradation of chlorantraniliprole, involving an initial Smiles rearrangement[8] of the pyridyl ring to the adjacent amide nitrogen atom followed by cyclization, methyl amine transfer, and fragmentation to **2** and **3**, is presented in Scheme 4. This initial rearrangement product can be isolated in certain circumstances and was found to be thermally labile toward concurrent fragmentation into the two observed products. A hypothetical bicyclic transition state is shown in Scheme 4.

The results obtained with the tobacco pyrolysis apparatus described, which effectively separates the main- and side-stream smoke, confirm that it is possible to account for the totality of the radiocarbon applied and to identify chlorantraniliprole degradates, thus satisfying the regulatory agencies' requirements. This approach may prove useful in the examination of the pyrolytic degradation processes of other agrochemicals used in tobacco.

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ABBREVIATIONS USED

dpm, disintegrations per minute; ESI, electrospray ionization; HPLC, high-performance liquid chromatography; LSC, liquid scintillation counting; MM, mainstream; SS, sidestream; TLC, thin layer chromatography.

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