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**STUDY REPORT:**

MRID No. 46801802 Rupprecht, J. K. (2006) Metabolism of [phenyl-U-$^{14}$C]-AE 0317309 in the Laying Hen: Bayer CropScience Study Identification: MEAIM012. Unpublished Bayer CropScience Report Number: MEAIM012. January 16, 2006. 93 pages.

MRID No. 46801803 Rupprecht, J. K. (2006) Metabolism of [pyrazole-3-$^{14}$C]-AE 0317309 in the Laying Hen: Bayer CropScience Lab Project Number: 04MEAIM011. Unpublished Bayer CropScience Report Number: MEAIM011. January 25, 2006. 91 pages.

## EXECUTIVE SUMMARY:

Bayer CropScience has submitted two studies investigating the metabolism of [phenyl-U-$^{14}$C] and [pyrazole-3-$^{14}$C]-pyrasulfotole (AE 0317309) in laying hens. Six laying hens were dosed orally once daily for 14 consecutive days at a dose level equal to 8.6 ppm [phenyl-U-$^{14}$C]-pyrasulfotole equivalents (specific activity of 62.4 µCi/mg) in the diet, based on the weight of feed, corresponding to 0.82 mg/kg body weight per day. Also six laying hens were dosed with 10.5 ppm [pyrazole-3-$^{14}$C]-pyrasulfotole equivalents (specific activity of 65.4 µCi/mg) in the diet, based on the weight of feed, corresponding to 0.81 mg/kg body weight per day.

Eggs were collected twice a day during the treatment period and excreta were collected daily during the treatment period. Approximately 30 minutes after the last dose, the hens were sacrificed and the edible tissues (liver, muscle, and composite fat) were collected for analysis. Identification and quantitation of the metabolites in the extractable residue was accomplished by using reverse phase high performance liquid chromatography (HPLC) and high performance liquid chromatography with electrospray ionization and tandem mass spectrometry (LC-MS/MS).

In the phenyl-label study, the total radioactive residues (TRR, expressed as pyrasulfotole equivalents) were 1.560 ppm in liver, 0.066 ppm in fat, 0.038 ppm in muscle and <0.001-0.002 ppm in eggs. The majority of the residue in the tissues and eggs was extractable (83.8-99.8% of the TRR). The majority of the residue was identified in the liver, fat and muscle matrices (97.5-99.8 % of the TRR). The predominant residue was pyrasulfotole, with lesser amounts of the pyrasulfotole-desmethyl metabolite (AE 1073910). More than 97% of the administered dose was recovered in the excreta, with less than 0.4% in tissues and eggs.

In the pyrazole-label study, TRR (expressed as pyrasulfotole equivalents) were 1.285 ppm in liver, 0.015 ppm in fat, 0.020 ppm in muscle and 0.001-0.004 ppm in eggs. The majority of the residue in the tissues was extractable (96.8-99.4% of the TRR) and approximately half of the egg residue was extractable (47.4% of the TRR). The majority of the residue was identified in the liver and muscle matrices (95.1 –99.4 % of the TRR). The predominant residue was pyrasulfotole, with lesser amounts of the pyrasulfotole-desmethyl metabolite (AE 1073910). Most of the radioactivity (>85%) was recovered in excreta, with less than 0.2% remaining in the tissues and eggs.

The metabolic fate of [phenyl-U-$^{14}$C] and [pyrazole-3-$^{14}$C]-pyrasulfotole in laying hens involved the *N*-demethylation of pyrasulfotole to yield the pyrasulfotole-desmethyl metabolite (AE 0317310).

**STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS**:

Under the conditions and parameters used in the study, the laying hen metabolism data are classified as scientifically acceptable.

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document (DP# 333412), in Canada's Regulatory Decision Document, and in Australia's Residues Evaluation Report.

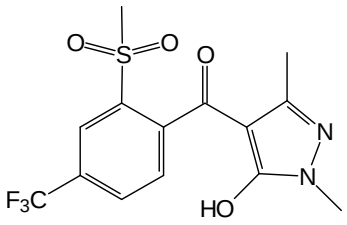
**COMPLIANCE:**

Signed and dated Good Laboratory Practice (GLP), Quality Assurance, and Data Confidentiality statements were provided. No GLP deviations were reported which would impact the study results or their interpretation.

## A. BACKGROUND INFORMATION

Pyrasulfotole, ((5-hydroxy-1,3-dimethyl-1*H*-pyrazol-4-yl)[2-(methylsulfonyl)-4-(trifluoromethyl) phenyl]methanone), is a postemergence dicot herbicide for use in cereal crops. Pyrasulfotole is an effective inhibitor of the enzyme 4-hydroxyphenylpyruvate dioxygenase (HPPDase) and consequently blocks the pathway of prenylquinone biosynthesis in plants. The end-use products are applied to the target weeds and act primarily through leaf uptake and translocation to the target site. The first symptoms appear three to five days after application. Bleaching and discoloration appear initially and symptoms progress to tissue necrosis and plant death within two weeks.

**TABLE A.1. Test Compound Nomenclature.**

| Compound | Chemical Structure |
|---|---|
| |  |
| Common name | Pyrasulfotole |
| Company experimental name | AE 0317309 |
| IUPAC name | (5-hydroxy-1,3-dimethylpyrazol-4-yl)(*α*,*α*,*α*-trifluoro-2-mesyl-*p*-tolyl)methanone |
| CAS name | (5-hydroxy-1,3-dimethyl-1*H*-pyrazol-4-yl)[2-(methylsulfonyl)-4-(trifluoromethyl)phenyl] methanone |
| CAS # | 365400-11-9 |
| End-use product/EP | *Herbicide; AE 0317309 02 SE06; AE 0317309 03 EC 23 A8* |

| **TABLE A.2. Physicochemical Properties of the Technical Grade Test Compound.** | | | |
|---|---|---|---|
| Parameter | Value | | Reference |
| Melting point | Pure: 201°C<br>No boiling point, decomposition starts at 245°C | | 1 |
| pH at 22.9°C | 3.03 | | 2 |
| Density ($g/cm^3$) | 1.53 | | 3 |
| Water solubility (g/L at 20°C) | 2.3<br>4.2<br>69.1<br>49.0 | pH 3.0 (distilled water)<br>pH 3.9 (buffer pH 4.0)<br>pH 5.4 (buffer pH 7.0)*<br>pH 5.2 (buffer pH 9.0)*<br>* exceeded buffer capacity | 4 |
| Solvent solubility (g/L at 20°C) | Ethanol<br>n-Hexane<br>Toluene<br>Dichloromethane<br>Acetone<br>Ethyl acetate<br>Dimethyl sulfoxide | 21.6<br>0.038<br>6.86<br>120-150<br>89.2<br>37.2<br>≥ 600 | 5 |
| Vapour pressure at 20°C | $2.7 \times 10^{-7}$ Pa | | 6 |
| Dissociation constant ($pK_a$) | 4.2 | | 7 |
| *n*-octanol-water partition coefficient Log($K_{OW}$) at 23°C | 0.276<br>-1.362<br>-1.580 | pH 4.0<br>pH 7.0<br>pH 9.0 | 8 |
| UV/visible absorption spectrum | $\lambda_{max}$ = 264, 241, 216 nm in water, 0.1M HCl, 0.1M NaOH respectively. | | 9 |

## B. EXPERIMENTAL DESIGN

### B.1. Livestock

B.1.1 Test System, Animal Handling and Dosing

The biological phase of this metabolism study was conducted at Southwest Bio-Labs, Inc. Six laying hens (*Gallus domesticus*, var. Leghorn) were dosed with [pyrazole-3-$^{14}$C]-pyrasulfotole (0.81 mg/kg body weight) and [phenyl-U-$^{14}$C]-pyrasulfotole (0.82 mg/kg body weight) on each of 14 consecutive days at Southwest Bio-Labs, Inc.. The treatments were administered orally via gelatin capsule using a balling gun each morning. The animals were allowed *ad libitum* access to feed and water throughout the study. General test animal information, test animal dietary regime and test animal dosing regime are given in TABLES B.1.1, B.1.2 and B.1.3.

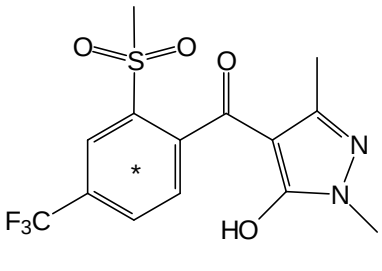
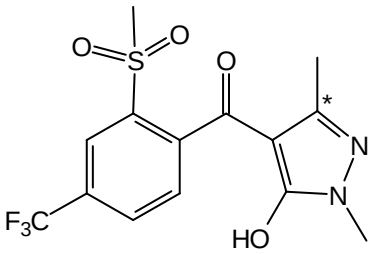
| **TABLE B.1.1. General Test Animal Information.** | | | | | |
|---|---|---|---|---|---|
| Species | Breed | Age | Weight at study initiation (kg) | Health Status | Description of housing/holding area |
| *Gallus domesticus* | Leghorn | Ca. 45 weeks | Phenyl / Pyrazole<br>1.311 / 1.635<br>1.586 / 1.485<br>1.667 / 1.720<br>1.727 / 1.690<br>1.682 / 1.350<br>1.662 / 1.645 | Overall, the animals appeared healthy throughout the course of the study as evidenced by observance of feed consumption, egg production, daily observations, body weights, and observations of tissues at necropsy. | Animals were housed in metabolism cages. The minimum/maximum temperatures and relative humidity were recorded daily throughout the study. The lighting control was set to a photoperiod of 16 hours light and 8 hours dark and was utilized throughout of the study. |

| **TABLE B.1.2. Test Animal Dietary Regime.** | | | | |
|---|---|---|---|---|
| Composition of Diet | Feed consumption (kg/day) | Water | Acclimation period | Predosing |
| *Ad libitum* access to Nutrena® Naturewise™ Layer 16 Complete Crumbs | Phenyl label: 0.148[a]<br>Pyrazole label: 0.122[a] | *Ad libitum* | Animals were brought onto the study from SBL's flock and therefore only two days were needed for acclimation in order to obtain baseline data prior to dosing. | None |

[a] This represents the average daily feed consumption (kg/day) for all the hens in this study.

| **TABLE B.1.3. Test Animal Dosing Regime.** | | | |
|---|---|---|---|
| Treatment Type | Feeding Level (ppm test material in food) | Vehicle | Timing/Duration |
| Oral | Phenyl label: 8.6<br>Pyrazole label: 10.5 | Gelatin capsule filled with alpha-lactose | 14 days |

## B.2. Test Materials

| **TABLE B.2.1. Test Material Characteristics.** | | |
|---|---|---|
| Chemical structure |  <br>* position of radiolabel |  <br>* position of radiolabel |
| Radiolabel position | [phenyl-U-$^{14}$C]-AE 0317309 | [pyrazole-3-$^{14}$C]-AE 0317309 |
| Lot No. | SEL 1319 | SEL 1320 |
| Purity | 100.0% | 100.0% |
| Dosing solutionSpecific activity | 62.4µCi/mg<br>138,528 dpm/µg | 64.5µCi/mg<br>143,190 dpm/µg |

## B.3. Sampling Information

Eggs were collected twice daily beginning at receipt and continued until the scheduled termination for each animal. Starting on Study Day 1, eggs from the afternoon and the following morning were collected, shells removed and the contents composited to form one day's sample. Excreta was collected for analysis beginning on Study Day 1 and continued until the scheduled termination for each animal. Excreta specimens collected were composited and weighed.

The animals were humanely terminated approximately 28-29 minutes following the fourteenth dose. At necropsy, tissues were dissected and examined for gross pathology or other abnormalities. Following the examination, liver, majority of muscle (a composite of leg and breast), and majority of fat (subcutaneous) were collected. Internal hard-shelled eggs were collected for the day 14-egg production. Sampling collection information and dates for treatment, sampling, extraction, and analysis of hen tissues, eggs and excreta are given in TABLE B.3.1.

**TABLE B.3.1. Sample Collection Information.**

| Eggs collected[a] | Excreta | Interval from last dose to sacrifice | Tissues harvested and analysed |
|---|---|---|---|
| Phenyl-label study | | | |
| 5.7 eggs daily post-treatment<br>5.5 eggs daily pre-treatment | 1295 g daily | 29-31 minutes | Liver, Fat and Muscle |
| Pyrazole-label study | | | |
| 4.8 eggs daily post-treatment<br>4 eggs daily pre-treatment | 1021 g daily | 28-29 minutes | Liver, Fat and Muscle |

[a] This represents the average daily egg production for all the hens in this study.

## B.4. Identification/ Characterization of Residues

### B.4.1. Sample Handling and Preparation

B.4.1.1. Sample Processing and Shipping

Each tissue from each animal was cubed, composited into one tissue sample per tissue type, weighed, double bagged and stored frozen. Each composited tissue sample was homogenized and shipped frozen to the Bayer Research Park. All samples for analysis arrived still thoroughly frozen on dry ice and were immediately placed in a freezer and stored frozen prior to analysis. Upon arrival, aliquots (0.05 to 0.6 g) of the eggs, tissues and excreta were oxidized to determine the TRR levels (TABLE C.2.1). All remaining eggs and tissues were stored frozen for later analysis.

B.4.1.2 Extraction and Analysis of Samples

Specific details related to the extraction of radioactive residues in liver, muscle, fat and eggs are given below and in FIGURE B.4.1.2.1. All solvents (HPLC grade) and reagent chemicals were obtained from commercial suppliers and were used without additional purification. Water was deionized and purified using a MILLI-Q Water System.

B.4.1.2.1 Liver

Extraction of the liver is outlined in FIGURE B.4.1.2.1. An aliquot of liver was weighed into a 200 mL centrifuge bottle. The sample was blended with 100 mL of acetonitrile/water (4:1) for 3 minutes using an Ultra Turrex. The sample was centrifuged at 2500 rpm for 10 minutes. The supernatant was decanted from the solids into a graduated cylinder. The extraction and centrifugation were repeated two times with fresh 75 mL portions of acetonitrile/water (4:1). The combined acetonitrile/water (4:1) extract was radioassayed.

The remaining solids were blended with 100 mL of acetonitrile for 3 minutes using an Ultra Turrex. The sample was centrifuged at 2500 rpm for 10 minutes. The supernatant was decanted from the solids into a graduated cylinder. The extraction and centrifugation were repeated two times with fresh 75 mL portions of acetonitrile. The combined acetonitrile extract was radioassayed.

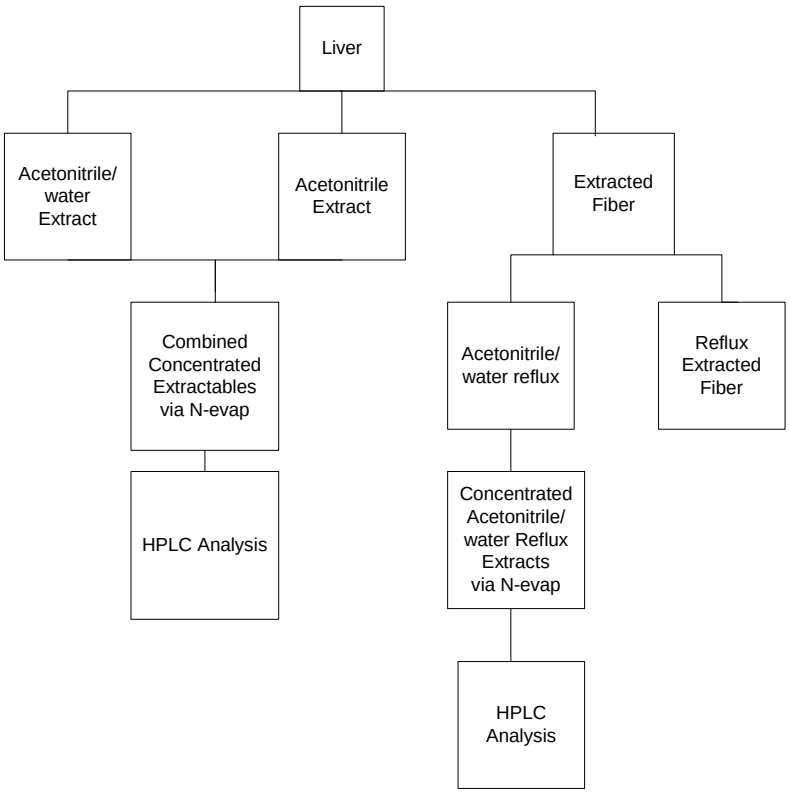
Aliquots of the acetonitrile/water extract and the acetonitrile extract were combined and concentrated to dryness using a gentle stream of nitrogen gas. The residual solids were reconstituted in acetonitrile/aqueous 0.1% trifluoroacetic acid (TFA) (1:9). The sample was radioassayed, and an aliquot was analyzed by HPLC.

The remaining acetonitrile extracted solids were suspended in acetonitrile/water (4:1, 150 mL), stirred and refluxed at 70 to 80°C for 24 hours. The suspension was cooled to room temperature

and filtered through a sinter funnel into a graduated cylinder. The resulting filter cake was rinsed three times with acetonitrile/water (4:1). The filtrate and rinses were combined and radioassayed.

An aliquot of the acetonitrile/water (4:1) reflux extract was concentrated to dryness using a gentle stream of nitrogen gas. The residual solids were reconstituted in acetonitrile/aqueous 0.1% TFA (1:9). The sample was radioassayed, and an aliquot was analyzed by HPLC. Aliquots of the pre-weighed acetonitrile/water (4:1) reflux extracted liver were taken for combustion analysis.

**FIGURE B.4.1.2.1. Summary of Extraction of Liver**



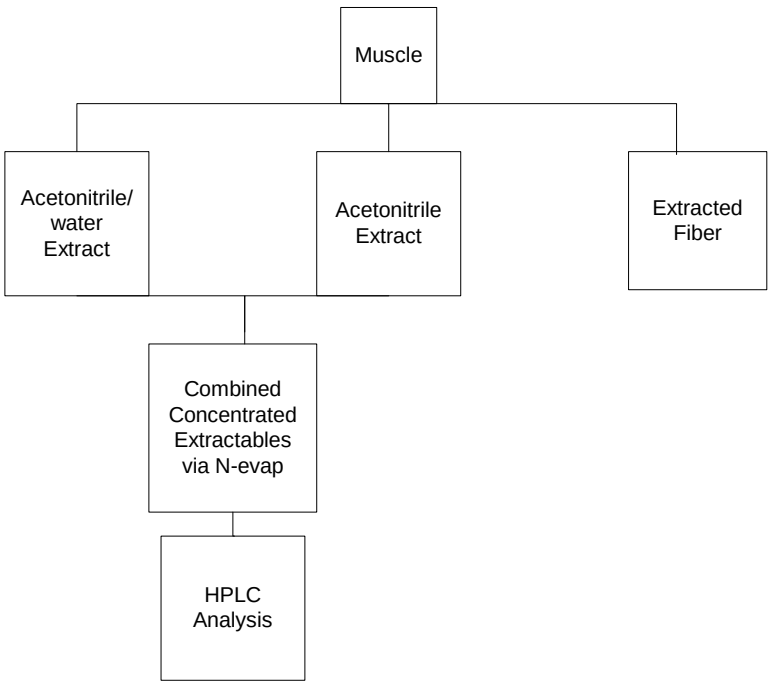
B.4.1.2.2 Muscle

Extraction of the muscle is outlined in FIGURE B.4.1.2.2. Four aliquots of muscle were weighed into separate 200 mL centrifuge bottles. Each sample was blended with 100 mL of acetonitrile/water (4:1) for 3 minutes using the Ultra Turrex. The samples were centrifuged at 2500 rpm for 10 minutes. The supernatants were decanted from the solids into a graduated cylinder. For each centrifuge bottle, the extraction and centrifugation were repeated twice with a fresh 75 mL portion of acetonitrile/water (4:1). The combined acetonitrile/water (4:1) extract was radioassayed.

An aliquot of the acetonitrile/water extract was concentrated to dryness using a gentle stream of nitrogen gas. The residual solids were reconstituted in acetonitrile/aqueous 0.1% TFA (1:9). The sample was radioassayed, and an aliquot was analyzed by HPLC.

The remaining solids were blended with 100 mL of acetonitrile for 3 minutes using an Ultra Turrex. The sample was centrifuged at 2500 rpm for 10 minutes. The supernatant was decanted from the solids into a graduated cylinder. The extraction and centrifugation were repeated two times with fresh portions of acetonitrile. The combined acetonitrile extract was radioassayed. Aliquots of the pre-weighed extracted muscle were taken for combustion analysis.

**FIGURE B.4.1.2.2 Summary of Extraction of Muscle**



B.4.1.2.3 Fat

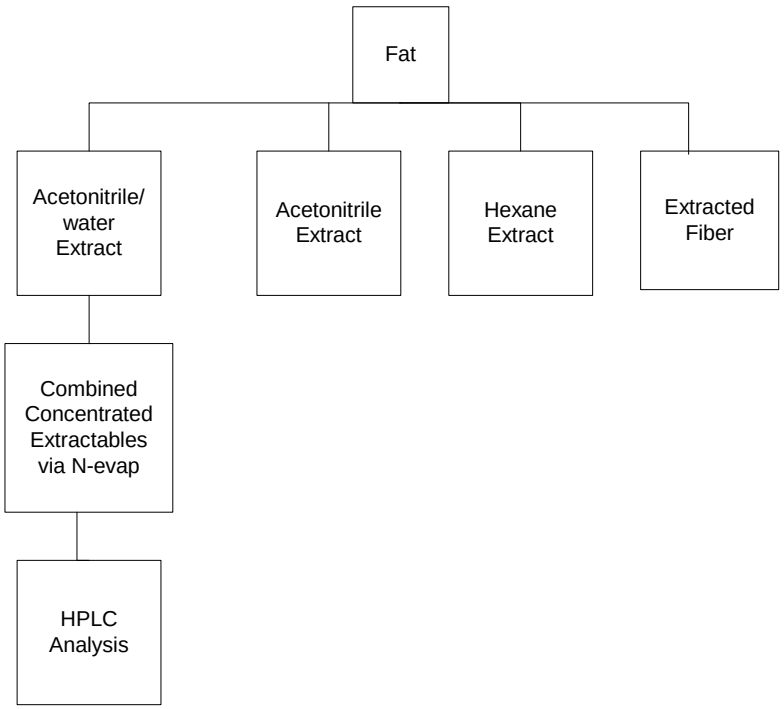
The extraction of fat is outlined in FIGURE B.4.1.2.3. An aliquot of fat was weighed into a 200 mL centrifuge bottle. The sample was blended with 100 mL of acetonitrile/water (4:1) for 3 minutes using the Ultra Turrex. The sample was centrifuged at 2500 rpm for 10 minutes. The supernatant was decanted from the solids into a graduated cylinder. The extraction and centrifugation were repeated twice with a fresh portion of acetonitrile/water (4:1). The combined acetonitrile/water (4:1) extract was radioassayed.

The remaining solids were blended with 100 mL of acetonitrile for 3 minutes using an Ultra Turrex. The sample was centrifuged at 2500 rpm for 10 minutes. The supernatant was decanted from the solids into a graduated cylinder. The extraction and centrifugation were repeated two times with fresh 100 mL portions of acetonitrile. The combined acetonitrile extract was radioassayed.

An aliquot of the acetonitrile/water extract was concentrated to dryness using a gentle stream of nitrogen gas. The residual solids were reconstituted in acetonitrile/aqueous 0.1% TFA (1:9). The sample was radioassayed, and an aliquot was analyzed by HPLC.

Aliquots of the pre-weighed extracted fat were taken for combustion analysis.

**FIGURE B.4.1.2.3 Summary of Extraction of Fat**

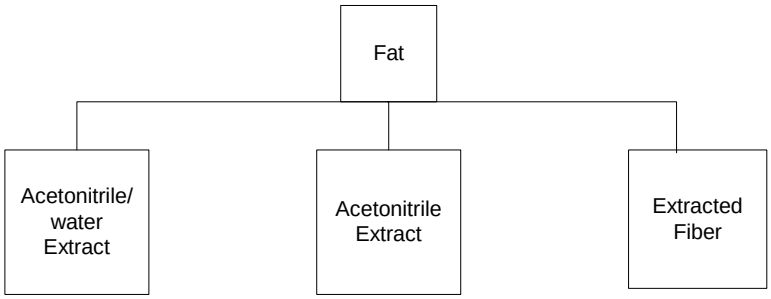



B.4.1.2.4 Eggs

The extraction of the Day 14 egg sample is summarized in FIGURE B.4.1.2.4. An aliquot of the Day 14 egg sample was weighed into a 200 mL centrifuge bottle. The sample was blended with 100 mL of acetonitrile/water (4:1) for 3 minutes using the Ultra Turrex. The sample was centrifuged at 2500 rpm for 10 minutes. The supernatant was decanted from the solids into a graduated cylinder. The extraction and centrifugation were repeated twice with a fresh 75 mL portion of acetonitrile/water (4:1). The combined acetonitrile/water (4:1) extract was radioassayed.

The remaining solids were blended with 100 mL of acetonitrile for 3 minutes using an Ultra Turrex. The sample was centrifuged at 2500 rpm for 10 minutes. The supernatant was decanted from the solids into a graduated cylinder. The extraction and centrifugation were repeated two times with fresh portions of acetonitrile. The combined acetonitrile extract was radioassayed. Aliquots of the pre-weighed extracted egg were taken for combustion analysis.

**FIGURE B.4.1.2.4 Summary of Extraction of Eggs**




B.4.1.2.5 Excreta

Aliquots of the pre-weighed excreta were taken for combustion analysis.

## B.4.2. Analytical Methodology

B.4.2.1 Measurement of Radioactivity

Liquid samples (0.010 mL to 2.00 mL) were mixed with 6 or 20 mL of Ultima Gold™ scintillation fluid and radioassayed in a Beckman Model LS6000LL, or LS6500 liquid scintillation counter (LSC). Data were processed with Beckman data reduction software. Aliquots of solid samples were oxidized and radioassayed.

B.4.2.2 Chromatography

B.4.2.2.1 Radio-High Performance Liquid Chromatography (HPLC)

HPLC analysis was performed with a Beckman System Gold Chromatographic system consisting of a Beckman Model 128 solvent module and a Beckman Model 166 variable wavelength detector. The chromatographic system was connected to a radioactivity detector. Data were collected and analyzed by a Beckman Gold Nouveau Chromatography Workstation. Samples were dissolved in acetonitrile/0.1% aqueous acetic acid (1:9), except as noted, prior to HPLC analysis.

**TABLE B.4.2. Limits of Detection and Quantitation.**

| Study | Background | Counting Efficiency | | Specific Activity | Sample Volume | Aliquot Size | LSC LOQ | Combustion LOQ | HPLC LOD |
|---|---|---|---|---|---|---|---|---|---|
| | | Combustion | LSC | dpm/µg | mL | g | ng | ppm | ng |
| Phenyl-label | 25 dpm | 81% | 84% | 138,528 | 1 | 0.1 | 0.43 ng | 0.00049 | 4.1 |
| Pyrazole-label | 25 dpm | 81% | 84% | 143,190 | 1 | 0.1 | 0.42ng | 0.00043 | 4.0 |

B.4.2.2.2 Mass Spectrometry

Mass spectral analyses were performed with a TSQ 7000 mass spectrometer. The spectrometer was connected to an HPLC system consisting of a P4000 quaternary gradient solvent pump, an autosampler, and a Zorbax Rx C8 (5µ, 250 mm x 4.6mm) reverse phase column. For negative ion electrospray LC-MS analyses, solvent A (0.1% formic acid) and solvent B (methanol) were used in combination as the mobile phase at a flow rate of 0.8 mL/min. The flow from the column was split to deliver 0.2 mL/min to the electrospray interface and 0.6 mL/min to a radiodetector. The solvent gradient program was a linear ramp from 5% solvent B to 100% solvent B over 11 minutes.

Daughter ion spectra were produced by liquid chromatography/mass spectrometry/mass spectrometry (LC-MS/MS). The first quadrapole of the TSQ 7000 was used to isolate a precursor ion, which, in the negative ion mode, was a deprotonated $(M-1)^+$ ion. The second stage of the instrument was used to induce fragmentation of the precursor ion by collision with argon gas at approximately 2.3-mTorr and collision energy of about 20 eV. The second quadrapole of the instrument was used to measure the mass spectra of the resultant molecular fragments.

## C. RESULTS AND DISCUSSION

TRR in hen egg and tissues is reported in TABLE C.2.1. The pharmacokinetics of the radiolabels in excreta and eggs are depicted in FIGURES C.2.1 and C.2.1.1. The distribution of radioactivity in hen commodities is reported in TABLE C.2.2. Characterization and identification of radioactive residues is summarized in TABLE C.2.3.

Distribution of total radioactive residues:

A total of 97.5% and 85.2% of the administered dose was recovered for [phenyl-U-$^{14}$C] and [pyrazole-3-$^{14}$C]-pyrasulfotole studies, respectively. The majority of the recovered radioactivity was in the excreta at 97.2% (phenyl-label) and 85.2% (pyrazole-label). Over the course of the 14-day treatment period, the pharmacokinetics of the phenyl and pyrazole-label studies were within normal biological deviations. The TRRs in eggs plateaued after 8 days of treatment at 0.002 ppm (phenyl-label) and 0.003 ppm (pyrazole-label). The TRR reported for the [phenyl-U-$^{14}$C] study in liver, fat and muscle were 1.560 ppm, 0.066 ppm and 0.038 ppm, respectively. Residue levels in the eggs sampled daily ranged from 0.000 to 0.002 ppm, for a total of 0.019 ppm. The TRR reported for the [pyrazole-3-$^{14}$C] study in liver, fat and muscle were 1.285 ppm, 0.015 ppm, and 0.020 ppm, respectively. Residue levels in the eggs sampled daily ranged from 0.000 ppm to 0.004 ppm, for a total of 0.033 ppm.

Characterization and identification of TRRs:

The major component of the combined acetonitrile/water, acetonitrile, and acetonitrile/water reflux extracts was isolated by preparative HPLC and subjected to mass spectrometry. Negative ion LC-MS showed a parent ion at m/z 361 $(M-1)^+$. The negative ion LC-MS/MS daughter ion spectrum of the m/z 361 and HPLC retention time were both identical to an authentic pyrasulfotole standard (MW=362). The pyrasulfotole-desmethyl (AE 1073910) metabolite was identified based on its HPLC retention time relative to an authentic standard of AE 1073910. Also, the negative ion LC-MS showed an ion at m/z 347 $(M-1)^+$. The negative ion LC-MS/MS daughter ion spectrum of m/z 347 and HPLC retention time were both identical to an authentic AE 1073910 standard (MW=348).

*Phenyl-label study*. Extraction of liver with acetonitrile/water (4:1) and acetonitrile released 92.9% of the TRR (1.449 ppm). Acetonitrile/water reflux released an additional 6.9% of the TRR (0.107 ppm). The remaining solids contained 0.2 % of the TRR (0.004 ppm). The major component of the combined acetonitrile/water (4:1) and acetonitrile liver extract was positively identified as pyrasulfotole (86.5% of the TRR, 1.350 ppm). The minor component was identified as the pyrasulfotole-desmethyl metabolite (6.4 % of the TRR, 0.099 ppm). The acetonitrile/water reflux liver extract was comprised of pyrasulfotole (6.9% of the TRR, 0.107 ppm) and pyrasulfotole-desmethyl (0.1 % of the TRR, 0.002 ppm). Total identification of the radioactive residues in liver was pyrasulfotole at 93.3% (1.456 ppm) with an additional 6.5% of the TRR (0.101 ppm) identified as pyrasulfotole-desmethyl.

Extraction of muscle with acetonitrile/water released 97.5% of the TRR (0.037 ppm). Acetonitrile extraction released an additional 1.3% of the TRR (0.001 ppm) and was not analyzed further. The remaining solids contained 1.2% of the TRR (<0.001 ppm). The major component (95.3% of the TRR, 0.036 ppm) of the combined acetonitrile/water (4:1) muscle extract was identified as pyrasulfotole. The minor component (2.2 % of the TRR, 0.001 ppm) was identified as pyrasulfotole-desmethyl. Total identification of the radioactive residues in muscle was pyrasulfotole at 95.3% (0.018 ppm) with an additional 2.2% of the TRR (0.001 ppm)

as pyrasulfotole-desmethyl.

Extraction of fat with acetonitrile/water released 98.9% of the TRR (0.065 ppm). Acetonitrile and hexane extraction released only an additional 0.5% of the TRR (<0.001 ppm) and was not analyzed further. The remaining solids contained 0.6% of the TRR (<0.001 ppm). The component of the acetonitrile/water (4:1) fat extract was identified as pyrasulfotole (97.1% of the TRR, 0.064 ppm), with the minor component (1.8 % of the TRR, 0.001 ppm) identified as the pyrasulfotole-desmethyl. Total identification of the radioactive residues in fat was pyrasulfotole at 97.1% (0.064 ppm) with an additional 1.8% of the TRR (0.001 ppm) as pyrasulfotole-desmethyl.

Extraction of eggs with acetonitrile/water released 80.9% of the TRR (<0.001 ppm). Further extraction with acetonitrile released an additional 2.9% of the TRR (< 0.001 ppm). The total extractable residue was < 0.01 ppm; therefore the acetonitrile/water and acetonitrile extracts were not analyzed further for identification purposes. The remaining solids contained 16.2% of the TRR (< 0.001 ppm).

The overall accountability of the TRR in tissues and eggs was 100%.

*Pyrazole-label study*: Extraction of liver with acetonitrile/water (4:1) and acetonitrile released 90.1% of the TRR (1.158 ppm). Acetonitrile/water reflux released an additional 9.3% of the TRR (0.119 ppm). The remaining solids contained 0.6% of the TRR (0.008 ppm). The major component of the combined acetonitrile/water (4:1) and acetonitrile liver extract was identified as pyrasulfotole (85.3% of the TRR, 1.096 ppm) with a minor component (4.8% of the TRR, 0.062 ppm) identified as the pyrasulfotole-desmethyl metabolite. The single component of the acetonitrile/water reflux liver extract (9.3% of the TRR, 0.119 ppm) was identified as the parent ion. A total of 94.6% (1.215 ppm) of the TRR in liver was identified as pyrasulfotole, with an additional 4.8% of the TRR (0.062 ppm) as pyrasulfotole-desmethyl.

Extraction of muscle with acetonitrile/water released 95.1% of the TRR (0.019 ppm). Acetonitrile extraction released only an additional 1.7% of the TRR (<0.001 ppm) and was not analyzed further. The remaining solids contained 3.2% of the TRR (0.001 ppm). The major component of the combined acetonitrile/water (4:1) muscle extract was identified as pyrasulfotole (92.9% of the TRR, 0.018 ppm), with a minor component (2.2 % of the TRR, <0.001 ppm) as the pyrasulfotole-desmethyl.

Extraction of fat with acetonitrile/water (87% of the TRR, 0.013 ppm) and acetonitrile (1.8% of the TRR, < 0.001 ppm) and hexane (8.9% of the TRR, 0.001 ppm) released a total of 97.7% of the TRR (0.014 ppm), of which was predominantly identified as pyrasulfotole (AE 0317309). The remaining solids contained only 2.3% of the TRR (<0.001 ppm).

Extraction of eggs with acetonitrile/water and acetonitrile released 47.4% of the TRR (0.002 ppm). Since the total extractable residue was <0.01 ppm, the sample was not analyzed further for identification purposes. The remaining solids contained 52.6% of the TRR (0.002 ppm).

The overall accountability of the TRR was 100% in tissues and 133% in eggs.

## C.1. Storage Stability

Animal tissues were frozen immediately after dissection. Eggs and excreta samples were frozen after collection. Samples were stored frozen prior to analysis. The tissues were shipped to Bayer CropScience on dry ice. Upon arrival at Bayer CropScience, the samples remained frozen solid and were stored frozen prior to analysis. All tissues were extracted and the metabolic profile determined within 2 to 6 months (TABLE C.1). Metabolites in the extractable residue of liver and muscle were profiled within 3 to 4 months (123 days) of necropsy. The high accountability of the TRR as identified metabolites and the similarity of the profile between tissues support stability over the short storage period.

**TABLE C.1. Summary of Storage Conditions.**

| Matrix | Storage Temp.(°C) | Actual Storage Duration (days) | | Interval of Demonstrated Storage Stability (days) |
|---|---|---|---|---|
| | | Phenyl | Pyrazole | |
| Excreta | <-20 | 97 | 132 | Not required [a] |
| Liver | <-20 | 64 | 123 | Not required |
| Muscle | <-20 | 62 | 61 | Not required |
| Fat | <-20 | 62 | 90 | Not required |
| Eggs | <-20 | 28 | 61 | Not required |

[a]. Storage stability data should not normally be required for samples analyzed within 4 to 6 months of collection.[10]

## C.2. Identification, Characterization, and Distribution of Residues

**TABLE C.2.1. Total Radioactive Residues (TRR) in Eggs, Tissue and Excreta.**

| Matrix | Collection Timing | [Phenyl-U-$^{14}$C]-AE 0317309 | | [Pyrazole-3-$^{14}$C]-AE 0317309 | |
|---|---|---|---|---|---|
| | | ppm | % AD | ppm | % AD |
| Excreta | Day 1 | 5.049 | 6.903 | 5.695 | 4.984 |
| | Day 2 | 5.230 | 7.515 | 10.896 | 7.448 |
| | Day 3 | 5.245 | 7.332 | 7.518 | 6.860 |
| | Day 4 | 5.643 | 7.616 | 7.650 | 7.485 |
| | Day 5 | 5.151 | 7.282 | 6.743 | 7.626 |
| | Day 6 | 4.953 | 7.237 | 6.272 | 7.583 |
| | Day 7 | 5.529 | 6.633 | 6.228 | 6.921 |
| | Day 8 | 6.546 | 7.664 | 6.309 | 6.730 |
| | Day 9 | 6.401 | 7.292 | 7.613 | 7.612 |
| | Day 10 | 6.016 | 7.000 | 3.903 | 4.327 |
| | Day 11 | 6.589 | 7.923 | 4.884 | 4.992 |
| | Day 12 | 7.033 | 9.067 | 4.262 | 4.559 |
| | Day 13 | 6.174 | 7.459 | 6.970 | 7.312 |
| | Day 14 | 4.075 | 0.235 | 6.828 | 0.795 |
| Excreta | Total | 79.634 | 97.158 | 91.771 | 85.234 |
| Muscle | At sacrifice | 0.038 | 0.050 | 0.020 | 0.048 |
| Fat | At sacrifice | 0.066 | 0.005 | 0.015 | 0.016 |
| Liver | At sacrifice | 1.560 | 0.307 | 1.285 | 0.108 |
| Eggs | Day 1, pm + am | 0.000 | < 0.001 | | < 0.001 |
| | Day 2, pm + am | 0.000 | < 0.001 | 0.000 | < 0.001 |
| | Day 3, pm + am | 0.001 | < 0.001 | 0.001 | < 0.001 |
| | Day 4, pm + am | 0.001 | < 0.001 | 0.001 | < 0.001 |
| | Day 5, pm + am | 0.001 | < 0.001 | 0.002 | < 0.001 |
| | Day 6, pm + am | 0.001 | < 0.001 | 0.002 | < 0.001 |
| | Day 7, pm + am | 0.001 | < 0.001 | 0.003 | < 0.001 |
| | Day 8, pm + am | 0.002 | < 0.001 | 0.002 | < 0.001 |
| | Day 9, pm + am | 0.002 | < 0.001 | 0.003 | < 0.001 |
| | Day 10, pm + am | 0.002 | < 0.001 | 0.003 | < 0.001 |
| | Day 11, pm + am | 0.002 | < 0.001 | 0.003 | < 0.001 |
| | Day 12, pm + am | 0.002 | < 0.001 | 0.003 | < 0.001 |
| | Day 13, pm + am | 0.002 | < 0.001 | 0.004 | < 0.001 |
| | Day 14, pm + am | 0.002 | < 0.001 | 0.003 | < 0.001 |
| | | | | 0.003 | |
| | Total | 0.019 | 0.006 | 0.033 | 0.005 |
| Upper GI tract | Not Applicable | Not Applicable | | Not Applicable | |
| Lower GI tract | Not Applicable | Not Applicable | | Not Applicable | |
| Other | Not Applicable | Not Applicable | | Not Applicable | |
| Sum of % Administered Dose | | 97.526% | | 85.411% | |

**FIGURE C.2.1. Pharmacokinetics of [Phenyl-U-$^{14}$C]-AE 0317309 and [Pyrazole-3-$^{14}$C]- AE 0317309 in Excreta of the Laying Hen**

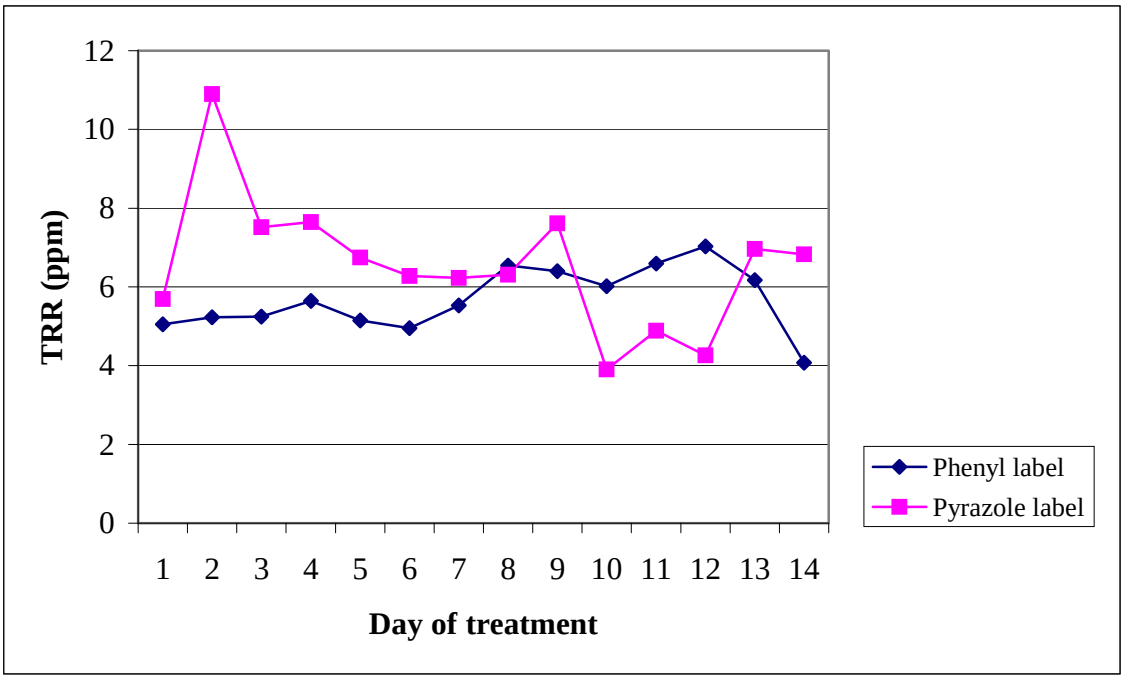



**FIGURE C.2.1.1. Pharmacokinetics of [Phenyl-U-$^{14}$C]-AE 0317309 and [Pyrazole-3-$^{14}$C]-AE 0317309 in Eggs of the Laying Hen**

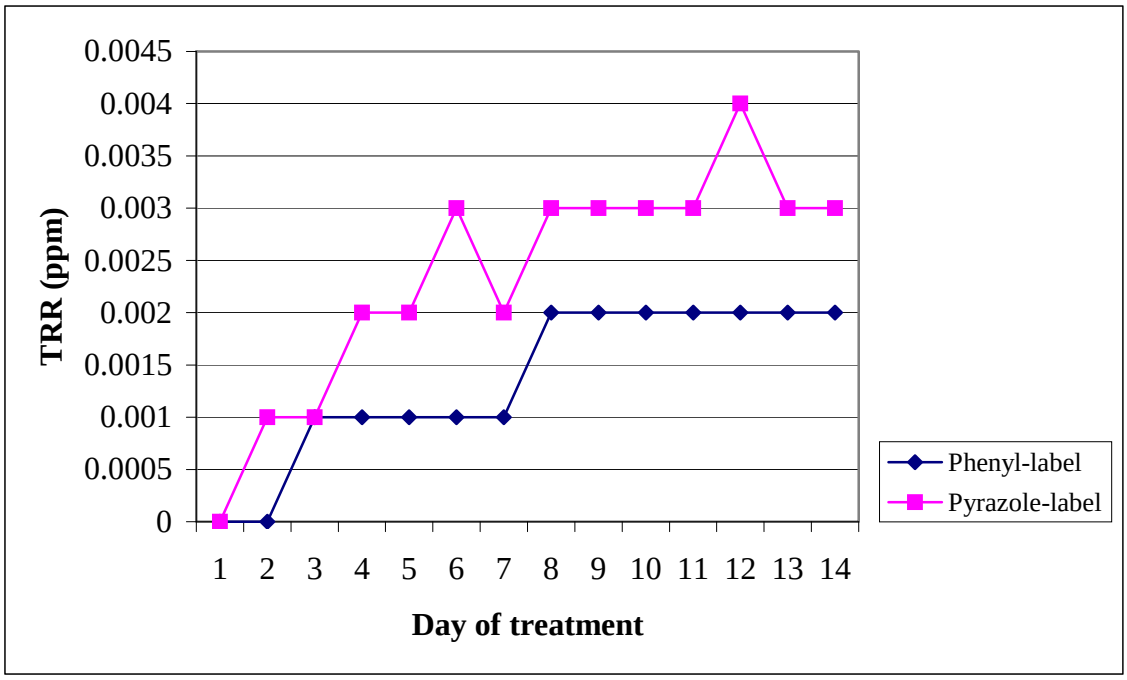


**TABLE C.2.2. Distribution of the Parent and the Metabolites in Livestock Matrices when Dosed with [phenyl-U-$^{14}$C] and [pyrazole-3-$^{14}$C]-AE 0317309.**

| Metabolite Fraction | Excreta | | Muscle | | Fat | | Liver | | Eggs | |
|---|---|---|---|---|---|---|---|---|---|---|
| | %TRR | ppm | %TRR | ppm | %TRR | ppm | %TRR | ppm | %TRR | ppm |
| Phenyl-label study | | | | | | | | | | |
| ACN/Water Ext.[a] | NA | NA | 97.5 | 0.037 | 98.9 | 0.065 | 92.9 | 1.449 | 80.9 | 0.001 |
| AE 0317309 | - | - | 95.3 | 0.036 | 97.1 | 0.064 | 86.5 | 1.350 | - | - |
| AE 1073910 | - | - | 2.2 | 0.001 | 1.8 | 0.001 | 6.4 | 0.099 | - | - |
| Hexane Extract | NA | NA | NA | NA | 0.2 | <0.001 | NA | NA | NA | NA |
| AE 0317309 | - | - | | | - | - | - | - | - | - |
| Acetonitrile Reflux | NA | NA | NA | NA | NA | NA | 6.9 | 0.107 | 2.9 | <0.001 |
| AE 0317309 | - | - | - | - | - | - | 6.8 | 0.106 | - | - |
| AE 1073910 | - | - | - | - | - | - | 0.1 | 0.002 | - | - |
| Pyrazole-label study | | | | | | | | | | |
| ACN/Water Ext.[a] | NA | NA | 95.1 | 0.019 | 88.8 | 0.013 | 90.1 | 1.158 | 47.4 | 0.002 |
| AE 0317309 | - | - | 92.9 | 0.018 | 88.8 | 0.013 | 85.3 | 1.096 | - | - |
| AE 1073910 | - | - | 2.2 | <0.001 | NA | NA | 4.8 | 0.062 | - | - |
| Hexane extract | NA | NA | | | 8.9 | 0.001 | | | | |
| AE 0317309 | - | - | | | 8.9 | 0.001 | | | | |
| Acetonitrile Reflux | NA | NA | - | - | - | - | 9.3 | 0.119 | - | - |
| AE 0317309 | - | - | - | - | - | - | 9.3 | 0.119 | - | - |

[a] ACN/Water extract or combined ACN/Water + ACN extract
NA = not analyzed

**TABLE C.2.3. Summary of Characterization and Identification of Radioactive Residues in Livestock Matrices Following Application of Radiolabeled AE 0317309 at a rate of 8.6 ppm [phenyl-U-$^{14}$C]-AE 0317309 equivalents in the diet and 10.5 ppm [pyrazole-3-$^{14}$C]-AE 0317309 equivalents in the diet.**

| Phenyl-label study | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Compounds | Muscle TRR = 0.038 ppm | | Fat TRR = 0.066 ppm | | Liver TRR = 1.560 ppm | | Eggs TRR = 0.002 ppm | |
| | %TRR | ppm | %TRR | ppm | %TRR | ppm | %TRR | ppm |
| AE 0317309 | 95.3 | 0.036 | 97.1 | 0.064 | 93.3 | 1.456 | - | - |
| AE 1073910 | 2.2 | 0.001 | 1.8 | 0.001 | 6.5 | 0.101 | - | - |
| Total identified | 97.5 | 0.037 | 98.9 | 0.065 | 99.8 | 1.557 | - | - |
| Total characterized | 1.3 | 0.001 | 0.5 | < 0.001 | | | 83.8 | <0.001 |
| Total extractable | 98.8 | 0.037 | 99.4 | 0.065 | 99.8 | 1.557 | 83.8 | <0.001 |
| Nonextractable (PES)[a] | 1.2 | < 0.001 | 0.6 | < 0.001 | 0.2 | 0.004 | 16.2 | < 0.001 |
| Accountability [b] | 100 | | 100 | | 100.1 | | 100 | |
| Pyrazole-label study | | | | | | | | |
| Compounds | Muscle TRR = 0.020 ppm | | Fat TRR = 0.015 ppm | | Liver TRR = 1.285pm | | Eggs TRR = 0.003 ppm | |
| | %TRR | ppm | %TRR | ppm | %TRR | ppm | %TRR | ppm |
| AE 0317309 | 92.9 | 0.018 | 97.7 | 0.014 | 94.6 | 1.215 | - | - |
| AE 1073910 | 2.2 | < 0.001 | - | - | 4.8 | 0.062 | - | - |
| Total identified | 95.1 | 0.018 | - | - | 99.4 | 1.277 | - | - |
| Total characterized | 1.7 | < 0.001 | - | - | - | - | 47.4 | 0.002 |
| Total extractable | 96.8 | 0.019 | 97.7 | 0.014 | 99.4 | 1.277 | 47.4 | 0.002 |
| Nonextractable (PES)[a] | 3.2 | 0.001 | 2.3 | < 0.001 | 0.6 | 0.008 | 52.6 | 0.002 |
| Accountability [b] | 100 | | 100 | | 100 | | 133.3 | |

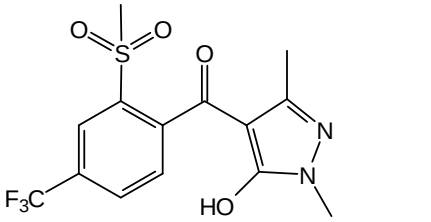
[a] Residues remaining after exhaustive extractions

[b] Accountability = (Total extractable (ppm) + Total unextractable (ppm))÷(TRR (ppm) from combustion analysis)* 100

## C.3. Proposed Metabolic Profile

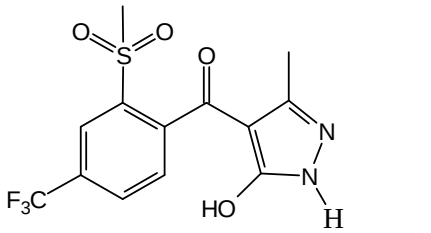
The proposed metabolic pathway for the [phenyl-U-$^{14}$C]-pyrasulfotole and [pyrazole-3-$^{14}$C]-pyrasulfotole in laying hens is shown in FIGURE C.3.1. Pyrasulfotole was not metabolized to a great extent in the laying hen. The only metabolic pathway involved the N-demethylation of pyrasulfotole (AE 0317309), resulting in the pyrasulfotole-desmethyl metabolite (AE 1073910).

**FIGURE C.3.1. Proposed Metabolic Pathway of [Phenyl-U-$^{14}$C] and [Pyrazole-3-$^{14}$C]-Pyrasulfotole in Laying Hens.**



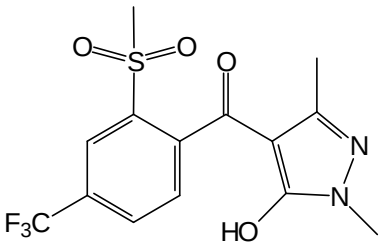
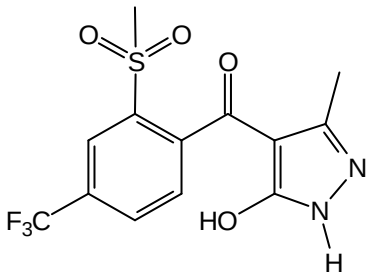

AE 0317309

N-Demethylation




AE 1073910

**TABLE C.3.1. Identification of Compounds from Metabolism Study.**

| Common name/code FIGURE C.3.1 ID No. | Chemical name | Chemical structure |
|---|---|---|
| pyrasulfotole AE 0317309 | (5-hydroxy-1,3-dimethylpyrazol-4-yl)(2-mesyl-4-trifluoromethylphenyl) methanone |  |
| pyrasulfotole-desmethyl AE 1073910 | (5-hydroxy-1*H*-pyrazol-4-yl)[2-mesyl-4-(trifluoromethyl)phenyl]methanone |  |

## D. CONCLUSION

The metabolic fate of pyrasulfotole in tissues and eggs has been studied in laying hens dosed orally for 14 consecutive days with [phenyl-U-$^{14}$C] and [pyrazole-3-$^{14}$C]-pyrasulfotole. Pyrasulfotole was the predominant residue in tissues and eggs. Pyrasulfotole is not extensively metabolized in laying hens. The metabolic profile involved N-demethylation of the parent pyrasulfotole to afford the pyrasulfotole-desmethyl metabolite (AE 1073910).

## E. REFERENCES

1. MRID 46801701 Franke, J. (2004). Melting point, boiling point, thermal stability of AE 0317309: substance, pure code: AE 0317309 00 1B99 0001. Document No. C042370. Bayer CropScience Report No. 20040374.01

2. MRID 46801701 Mühlberger, B. and Strunk, B. (2003). Determination of the pH-Value of AE 0317309. Document Number C033462. Bayer CropScience Report Number PA03/011.

3. MRID 46801701 Mühlberger, B. and Lemke, G. (2003). AE 0317309 - Relative density. Bayer CropScience Report Number PA03/040.

4. MRID 46801701 Mühlberger, B. (2003). Water solubility of AE 0317309 at pH 4, pH 7, pH 9 and bidistilled water. Document Number C028268. Bayer CropScience Report Number PA03/008.

5. MRID 46801701 Mühlberger, B. (2003). AE 0317309: Solubility in Organic Solvents. Document Number C034280. Bayer CropScience Report Number PA03/009.

6. MRID 46801701 Franke, J. (2004). AE 0317309: Vapour Pressure. Document Number C042368. Bayer CropScience Report Number 20040374.02.

7. MRID 46801701 Mühlberger, B. and Eyrich, U. (2005, amended 2006). AE 0317309: Determination of the Dissociation Constant. Bayer CropScience Report Number PA03/045.

8. MRID 46801701 Mühlberger, B. (2003). AE 0317309: Partition coefficient 1-octanol/water. Document Number C030789. Bayer CropScience Report Number PA03/010.

9. MRID 46801701 Wiche, A. and Mühlberger, B. (2003). AE 0317309: Spectral data (UV/VIS, IR, $^{1}$H-NMR, $^{13}$C-NMR, MS) and molar extinction coefficient. Document Number C036440. Bayer CropScience Report Number PA03/023.

10. Zager, E. and Edwards, D. (1992). EPA Memorandum: Additional guidance for conducting plant and livestock metabolism studies. Pesticide registration rejection rate analysis for residue chemistry.

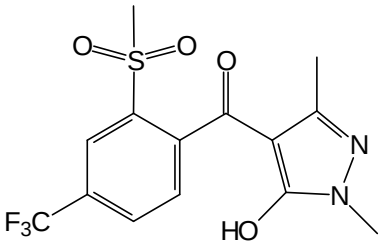
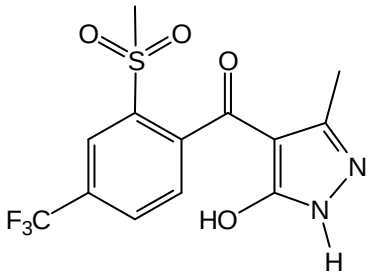
## F. DOCUMENT TRACKING

RDI: Louise G Croteau (6 September 2006); RAB1 Chemists (15 November 2006); George Kramer (15 November 2006)
Petition Number: 6F7059
DP#: 333412

Template Version June 2005.

## APPENDIX 1

Reference Standards.

| Common name/code | Chemical name | Chemical structure |
|---|---|---|
| pyrasulfotole<br>AE 0317309 | (5-hydroxy-1,3-dimethylpyrazol-4-yl)(2-mesyl-4-trifluoromethylphenyl) methanone |  |
| pyrasulfotole-desmethyl<br>AE 1073910 | (5-hydroxy-1*H*-pyrazol-4-yl)[2-mesyl-4-(trifluoromethyl)phenyl]methanone |  |