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OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

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HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

MEMORANDUM

Date: 11-MAY-2005

Subject: Topramezone (BAS 670 H) in/on Corn. PP# 3F6568. Summary of Analytical Chemistry and Residue Data.

DP#: 310772 Decision #: 305982
PC Code: 123009 MRID Nos.: 45902401-07
40 CFR 180. 45902409-14

From: George F. Kramer, Ph.D., Chemist
Sarah J. Levy, M.S., Chemist
Registration Action Branch 1 (RAB 1)
Health Effects Division (HED) (7509C)

Through: P.V. Shah, Ph.D. Branch Senior Scientist
RAB1/HED (7509C)

To: Joanne Miller, PM Team 23
Registration Division (RD) (7505C)

Executive Summary

BASF Corporation has submitted a petition proposing application of topramezone [3-(4,5-dihydro-isoxazol-3-yl)-4-methylsulfonyl-2-methylphenyl](5-hydroxy-1-methyl-1*H*-pyrazol-4-yl)methanone; BAS 670 336 SC (soluble concentrate) Herbicide to all corn types (field, pop, seed, sweet). BASF is proposing the establishment of permanent tolerances for residues of topramezone *per se* in/on the following raw agricultural commodities (RACs):

Corn, field, forage	0.05 ppm	Corn, sweet, forage	0.05 ppm
Corn, field, grain	0.01 ppm	Corn, sweet, kernel plus cob with husks removed	0.01 ppm
Corn, field, stover	0.05 ppm	Corn, sweet, stover	0.05 ppm
Corn, pop, grain	0.01 ppm	Cattle, kidney*	0.20 ppm
Corn, pop, stover	0.05 ppm	Cattle, liver*	0.70 ppm

* includes: hog, goat, horse, and sheep

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The subject petition is a joint review between EPA and PMRA of Canada. Primary review of all residue chemistry data was performed by PMRA.

Topramezone is a broad-spectrum, post-emergence herbicide used to control grassy and broadleaf weeds in corn. The herbicide is absorbed by the leaves, roots and shoots, then translocated to the growing points of the sensitive weeds. Topramezone belongs to the phenylpyrazolyl ketone class of chemicals. The maximum seasonal use is 0.022 lb/ai/acre. There is a maximum of two applications (at least one week apart) per season. The single application rate is 0.011 to 0.022 lb ai/acre. The product controls both broadleaf and grass weeds in all corn types (field, pop, seed, sweet) with a 45-day preharvest application interval.

The results of the topramezone metabolism studies in corn, ruminants and poultry were similar in that the parent compound was not extensively metabolized. However, significant differences were observed in the metabolic pathways. **In corn**, topramezone underwent hydrolytic cleavage to form the acid metabolite M670H05 and M670H08, while desmethylation formed M670H03. After cleavage, the pyrazole ring appeared to undergo complete catabolism and reincorporation within the carbon backbone of natural products such as starch, soluble polysaccharides and fatty acids. The phenyl ring portion of the molecule also underwent degradation, resulting in the incorporation of radioactivity into natural products. **In ruminants**, the proposed metabolic pathway involved hydroxylation of the parent compound topramezone at the 4-position of the isoxazole ring to form M670H02. The isoxazole ring was then cleaved to form the cyano metabolite (M670H01). The linkage between the phenyl and pyrazole rings remained intact in all of the metabolites identified. **In poultry**, the proposed metabolic pathway also involved hydroxylation of the parent compound topramezone at the 4-position of the isoxazole ring to form M670H02. Further N-demethylation of M670H02 occurred and formed the desmethyl hydroxy metabolite M670H04. M670H04 was only detected in the phenyl-labeled liver. The pyrazole ring of topramezone could also be cleaved to form the acid metabolite M670H05. Similar metabolic pathways were also observed in **rotational crops**, with M670H05 and M670H02 being the only metabolites identified. Topramezone *per se* is the residue of concern in crops and ruminants for purposes of tolerance enforcement and risk assessment (DP#290075, M. Rust, *et al.*).

BASF has proposed liquid chromatography (LC)/mass spectrometry (MS)/MS method No. D0007 for data-gathering and enforcement purposes for residues of topramezone and the free acid metabolite M670H05 in/on plant commodities and LC-MS/MS method D0104 for data-gathering and enforcement purposes for residues of topramezone and the metabolite M670H02 in/on livestock commodities. These methods will undergo a petition method validation (PMV) at Analytical Chemistry Laboratory/Biological and Economics Analysis Division (ACL/BEAD) (Memo S. Levy, 12/2/04; DP# 310773). Successful completion of the PMV is necessary before the proposed methods can be employed for enforcement purposes. As topramezone was not adequately recovered using any of the U.S. Food and Drug Administration (FDA) Multiresidue Methodologies (MRM) Test Protocols, MRMs would not be suitable for use as an enforcement method.

The petitioner submitted magnitude of the residue data for field corn, sweet corn, limited field rotational crops and lactating dairy cows. The field trial studies were conducted using the proposed application scenarios (with the exception of a 4.5X application rate) and in the regions

suggested in OPPTS 860.1500 for establishment of a tolerance in/on the crops requested. No processing study was required as two field corn plots treated with topramezone at exaggerated rates (12-20X) produced no quantifiable residues. The field trial data were generated using adequately-validated analytical methods (storage intervals have also been validated) and resulted in no residues greater than the limit of quantitation (LOQ) in/on any RACs. In the lactating dairy cow feeding study, conducted at 4.9X, 13X and 47X, quantifiable residues were found only in liver and kidney (all levels). Based on the magnitude of the residue and processing studies, HED concludes that the tolerances listed below in the "Recommendations" section of this document are appropriate (a revised Section F is requested; no additional field trial or processing data are necessary). The rotational-crop plantback intervals (PBIs) listed on the proposed label are supported by the data submitted for rotational crops (confined and limited field).

RESIDUE CHEMISTRY DEFICIENCIES

OPPTS 860.1340 Residue Analytical Methods

Plant commodity methods

The proposed plant enforcement methods need to pass a PMV by Agency chemists at ACL/BEAD before the methods can be deemed adequate for tolerance enforcement. Method D0007 has been forwarded to ACL/BEAD for the PMV (Memo S. Levy, 12/2/04; DP# 310773).

Livestock commodity methods

The proposed livestock enforcement methods need to pass a PMV by Agency chemists at ACL/BEAD before the methods can be deemed adequate for tolerance enforcement. Method D0104 has been forwarded to ACL/BEAD for the PMV (Memo S. Levy, 12/2/04; DP# 310773).

860.1550 Proposed Tolerances

The petitioner is requested to submit a revised Section F as specified below in the "Recommendations" section of this document. HED determined that the tolerances proposed by the registrant for livestock RACs were too high.

RECOMMENDATIONS

Provided a revised Section F is submitted and the Agency validations of the proposed plant and livestock analytical enforcement methods are successful, HED concludes there are no residue chemistry data requirements that would preclude the establishment of an unconditional registration and permanent tolerances for residues of topramezone *per se* in/on the following RACs:

Corn, field, forage	0.05 ppm	Corn, sweet, forage	0.05 ppm
Corn, field, grain	0.01 ppm	Corn, sweet, kernel plus cob with husks removed	0.01 ppm
Corn, field, stover	0.05 ppm	Corn, sweet, stover	0.05 ppm
Corn, pop, grain	0.01 ppm	Cattle, kidney*	0.05 ppm
Corn, pop, stover	0.05 ppm	Cattle, liver*	0.15 ppm

* includes: goat, horse, and sheep

A human-health risk assessment will be prepared as a separate document.

Background

BAS 670 336SC Herbicide (also known as BAS 670 00H Herbicide) is the end-use product which contains the active ingredient topramezone. It is a broad-spectrum, post-emergence herbicide used to control grassy and broadleaf weeds in corn. The herbicide is absorbed by the leaves, roots and shoots, then translocated to the growing points of the sensitive weeds. Topramezone belongs to the phenylpyrazolyl ketone class of chemicals, which inhibits carotenoid biosynthesis (4-hydroxyphenylpyruvate dioxygenase (4-HPPD) inhibitor). This causes a strong bleaching activity on the growing zones of the shoots within 2-5 days of application. Exposure to light causes necrosis of chlorotic tissues and eventual plant death within 14 days after application.

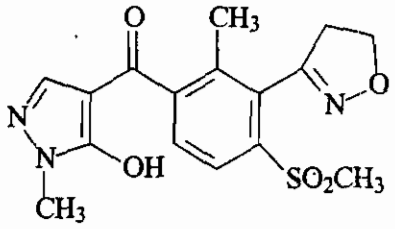
TABLE 1. Test Compound Nomenclature	
Compound	
Common name	Topramezone
Company experimental name	BAS 670 H
IUPAC name	[3-(4,5-dihydro-1,2-oxazol-3-yl)-4-mesyl-*o*-tolyl](5-hydroxy-1-methyl-1*H*-pyrazol-4-yl) methanone
CAS name	[3-(4,5-dihydro-3-isoxazolyl)-2-methyl-4-(methylsulfonyl)phenyl](5-hydroxy-1-methyl-1*H*-pyrazol-4-yl)methanone
CAS #	210631-68-8
End-use product/EP	Soluble Concentrate

TABLE 2. Physicochemical Properties of Topramezone

Parameter	Value
Melting point/range	220.9°C - 222.2°C
pH	2.9 (1% deionized water)
Density (20°C)	1.425 g/cm^3
Water solubility (20°C)	pH: 3, 5, 7, 9 — g/L: 0.06, 0.98, 15, 23.4
Solvent solubility (g/100 mL at 20°C)	Acetone <1.0; Acetonitrile <1.0; Dichloromethane 2.5 - 2.9; Ethyl acetate <1.0; Methanol <1.0; N-heptane <1.0; N,N-dimethylformamide 11.4-13.3; 1-octanol <1.0; Olive oil <1.0; 2-propanol <1.0; Toluene <1.0
Vapor pressure at 20°C and 25°C	< 1.0 x 10^{-12} hPa
Dissociation constant (pK_a)	4.06
Octanol/water partition coefficient Log(K_{ow}) at 20°C	Buffer pH: 4, 7, 9 — Log K_{ow}: – 0.81, – 1.52, – 2.34
UV/visible absorption spectrum	λ, nm: 207, 272, 300, 410 — ξ, mol^{-1}cm^{-1}: 27 077, 8601, 5800, 410

All data came from PMRA Lab Services.

860.1200 Directions for Use

TABLE 3. Summary of Directions for Use of Topramezone.

Applic. Timing, Type, and Equip.	Formulation [EPA Reg. No.]	Applic. Rate (lb ai/A)	Max. No. Applic. per Season	Max. Seasonal Applic. Rate (lb ai/A)	PHI (days)	Use Directions and Limitations
Field Corn, Popcorn, Seed Corn, Sweet Corn						
Post-emergent, Ground (ground equipment; flat nozzles) Aerial	BAS 670 336SC Post-emergent Corn Herbicide [7969-pending]	0.011 - 0.022 (12.35 - 24.7 g/ha)	2	0.022 (24.7 g/ha)	45 (forage, silage, fodder, grain)	Split applications (2) are allowed, but not to exceed 0.022 lb ai/A per growing season and allowing 7 days between sequential applications. An adjuvant AND a nitrogen fertilizer source are required to achieve optimum weed control. Plantback Restrictions: **Anytime**: All field corn types, field corn grown for seed, sweet corn, popcorn. **3 Months**: Cereal crops (wheat, barley, oats and rye, winter canola). **9 Months**: Alfalfa, cotton, canola, peanuts, sorghum, soybeans, sunflower, edible beans and peas, potato. **18 Months**: All crops not listed above.

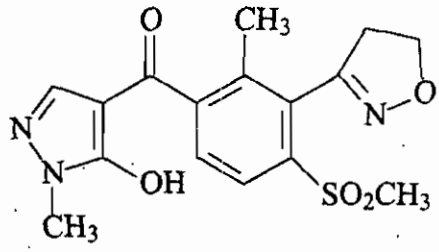
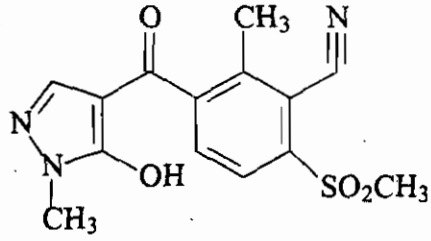
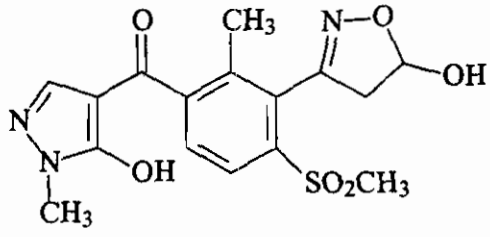
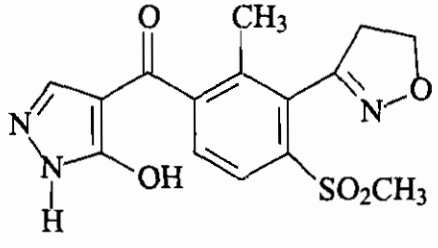
PHI = preharvest interval

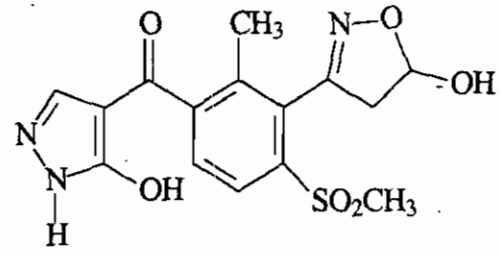
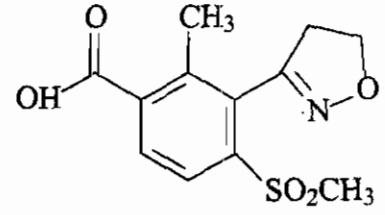
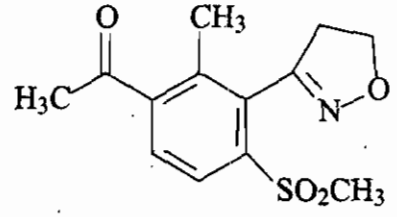
Conclusions. The label is adequate to allow evaluation of the residue data relative to the proposed use. There are no label additions/revisions/clarifications recommended by HED.

860.1300 Nature of the Residue - Plants and Livestock

45902401.der.wpd
45902402.der.wpd
45902403.der.wpd

TABLE 4. Identification of Compounds from Metabolism Studies

Common name/code Figure C.3.1 ID No.	Chemical name	Chemical structure
Topramezone	[3-(4,5-dihydro-isoxazol-3-yl)-4-methanesulfonyl-2-methylphenyl]-(5-hydroxy-1-methyl-1*H*-pyrazol-4-yl)methanone	
M670H01	cyano metabolite	
M670H02	hydroxy metabolite	
M670H03	Des-N-methyl metabolite	

M670H04	N-desmethyl hydroxy metabolite	
M670H05	3-(4,5-dihydro-isoxazol-3-yl)-4-methanesulfonyl-2-methylbenzoic acid	
M670H08	Methyl Ketone	

Corn

BASF conducted a corn metabolism study with [phenyl-U-^{14}C] topramezone and [pyrazole-4-^{14}C] topramezone. Topramezone was applied to corn plants at the 3-5 leaf growth stage (GS 13-15) at rates of 0.148 kg a.i./ha (0.132 lb a.i./A, 6.0X) for the pyrazole label or 0.146 kg a.i./ha (0.130 lb a.i./A, 5.9X) for the phenyl label as a single foliar application. The plants were grown in pots within a climate-controlled growth chamber, which was set to simulate the North American growing season. Immature plant samples were collected at 1, 9, 15 or 16, and 29 or 30 days after treatment (DAT). The corn forage samples were collected at the late dough stage (59-60 DAT), while stover and grain were harvested at maturity (77 DAT). Samples were analyzed within 5.1 months of harvest; thus, no storage stability data were collected or are necessary for this study.

The overall total radioactive residues (TRRs) in treated corn samples were determined by both combustion/liquid-scintillation counting (LSC) and by calculation (the sum of the radioactive residues in the extracts and the remaining residues in the post-extraction solids (PES)). Chromatographic determinations of radioactivity were performed by high-performance liquid chromatography (HPLC), with MS providing confirmation. Major metabolites (>10% of the TRR) were identified by co-chromatography and MS, while minor metabolites (<10% of the TRR) were identified by co-chromatography alone.

Calculated TRRs in immature corn samples treated with [phenyl-U-^{14}C] label topramezone declined from 7.64 ppm at 1 DAT to 0.468 ppm at 30 DAT. The overall TRRs in forage (59-60

DAT), stover and grain (77 DAT) were 0.534 ppm, 0.730 ppm, and 0.107 ppm, respectively. The TRRs in immature whole corn samples treated with [pyrazole-4-^{14}C] label topramezone also declined, from 7.68 ppm at 1 DAT to 0.544 ppm at 29 DAT. The overall TRRs in forage, stover and grain were lower in pyrazole-label samples at 0.294 ppm, 0.213 ppm and 0.032 ppm, respectively.

A total of 74.9-81.7% of the TRRs (0.351-6.117 ppm) of phenyl-label immature corn plants were aqueous extractable. Of the 63.9-75.8% of the TRR identified (0.299-5.117 ppm), the parent topramezone accounted for the majority of residues (55.7-69.9% of the TRR; 0.264-4.255 ppm). The desmethyl metabolite M670H03 and the acid metabolite M670H05 were considered minor metabolites (<10% of the TRR each; ≤0.717 ppm). Residues characterized as a series of minor peaks/regions comprised 5.9-13.0% of the TRR (0.052-1.00 ppm). The unextractable residues (PES) accounted for 9.9-16.5% of the TRR (0.077-0.755 ppm), for an overall accountability ranging from 89.9-93.5%.

A total of 40.1-84.1% of the TRRs (0.218-6.46 ppm) of pyrazole-label immature corn plants were aqueous extractable. Of the 35.2-84.1% of the TRR identified (0.192-6.458 ppm), the parent topramezone was the predominant metabolite at 33.2-78.7% of the TRRs (0.181-6.05 ppm). The only other metabolite identified was M670H03 (see Table 4 for structures) at <6% of the TRR (≤0.412 ppm). Residues characterized as a series of minor peaks/regions comprised 4.9-11.4% of the TRR (0.026-0.137 ppm). The unextractable residues (PES) accounted for 5.8-33.1% of the TRRs (0.18-0.475 ppm), for an overall accountability ranging from 71.3-89.9%.

A total of 78.8-82.8% of the TRRs (0.420-0.605 ppm) of phenyl-label forage and stover samples were aqueous extractable, while 83.1% of the TRR of grain (0.089 ppm) was extractable by acetonitrile (ACN):water. Of the 51.2-56.3% of the TRRs identified in forage and stover (0.273-0.412 ppm), the parent topramezone accounted for the majority of residues (40.4-40.9% of the TRR; 0.216-0.299 ppm). The metabolites M670H03, M670H05 and the methyl ketone metabolite M670H08 each comprised <11% of the TRR (≤0.075 ppm). Only 5.5% of the TRR was identified in grain (0.006 ppm), and there were more residues of M670H05 (3.4% of the TRR; 0.004 ppm) than parent (2.1% of the TRR; 0.002 ppm) identified. After exhaustive extraction/fractionation procedures, a total of 26.5-27.6% of the TRRs were characterized (0.147-0.193 ppm) in forage and stover. In grain, the majority of the residues were characterized (77.6% of the TRR, 0.083 ppm). The final unextractable residues in all matrices after fractionation accounted for 2.4-5.4% of the TRRs (0.005-0.029 ppm), for an overall accountability ranging from 84.1-87.9%.

A total of 65.3-83.3% of the TRRs (0.178-0.193 ppm) were extractable from the aqueous extract of pyrazole-label forage and stover samples, while 77.3% of the TRRs were extractable from the ACN:water extract of grain (0.025 ppm). Of the 23.6-28.4% of the TRR identified in forage and stover (0.061-0.07 ppm), the parent topramezone accounted for the majority of residues (16.9-19.6% of the TRR; 0.042-0.05 ppm). Each of the metabolites M670H03 and M670H08 comprised <5% of the TRR (≤0.011 ppm). Only 2.5% of the TRR was identified as topramezone in grain (0.001 ppm), with no metabolites identified. After exhaustive extraction/fractionation procedures, a total of 41.7-54.9% of the TRRs were characterized (0.117-0.123 ppm) in forage and stover. In grain, the majority of the residues were characterized (74.8% of the TRR, 0.024 ppm). The final unextractable residues in all matrices after fractionation accounted for 2.7-

22.6% of the TRRs (0.006-0.066 ppm), for an overall accountability ranging from 86.4-97.2%.

The hydrolysis of grain PES indicated that radioactivity was incorporated into glucose. Saponification of oil indicated that radioactivity was also incorporated into the fatty acid constituents.

Topramezone underwent hydrolytic cleavage to form the acid metabolite M670H05 and M670H08, while desmethylation formed M670H03. Further hydrolysis and cleavage resulted in the formation of M670H08, while desmethylation resulted in M670H03. After cleavage, the pyrazole ring appeared to undergo complete catabolism and reincorporation within the carbon backbone of natural products such as starch, soluble polysaccharides and fatty acids. The phenyl ring portion of the molecule also underwent degradation, resulting in the incorporation of radioactivity into natural products.

Goat

BASF has conducted a goat metabolism study with [phenyl-U-^{14}C] topramezone and [pyrazole-4-^{14}C] topramezone. Topramezone was administered to two lactating dairy goats per label at feeding levels of 9.9 mg/kg (phenyl label, 130X) and 11.2 mg/kg (pyrazole label, 150X) by a single daily oral administration for five days. Milk was collected twice daily over the treatment period, and excreta were pooled and collected once daily. Tissue samples (muscle, bile, kidney, liver, fat and GI tract) were harvested upon termination, 21-23 hours after the final dose administration. The samples were assayed for TRRs by combustion and LSC. Extracts were characterized by thin-layer chromatography (TLC) and identified by HPLC. Confirmation of residue identification was performed by LC-MS/MS. Nuclear magnetic resonance (NMR) analysis elucidated the structures of the metabolites found in the goat urine samples. All samples other than milk were stored frozen and analyzed within four months of collection; thus, no storage stability study was conducted. Milk was stored frozen and analyzed within 11 months of collection, but no storage stability study was conducted due to low radioactivity levels.

The total applied dose recoveries of topramezone were 92.5% in pyrazole-label samples, of which 44.5% was found in the urine, 38.3% in the feces, 0.29% in the cage rinse (1.18 ppm), 2.67-5.27% in the GI tissue and contents (0.780-1.16 ppm), and negligible levels in bile (0.137 ppm). The dose recoveries in the edible tissues were predominantly in the liver at 1.28% of the administered dose (1.891 ppm), followed by kidney at 0.04% (0.282 ppm). The dose recoveries in fat, muscle and milk were <0.01% each (≤0.007 ppm). The total applied dose recoveries of topramezone were 81.9% in phenyl-label samples, of which 33.3% was found in the urine, 34.7% in the feces, 0.49% in the cage rinse (0.986 ppm), 1.20-10.2% in the GI tissue and contents (0.322-1.57 ppm), and negligible levels in bile (0.059 ppm). The dose recoveries in the edible tissues were again predominantly in the liver (1.96% of the administered dose; 2.18 ppm), followed by kidney (0.05% of the administered dose; 0.352 ppm). The dose recoveries in fat, muscle and milk were negligible at ≤0.002 ppm each.

A total of 87.7-90.8% of the TRRs (1.66-1.98 ppm) were extracted with methanol/ethyl acetate (EtOAc) from the pyrazole-label and phenyl-label liver samples. Of the 82.1-85.5% TRR identified (1.55-1.86 ppm), the parent topramezone accounted for 51.8-83.3% of the TRR (0.9799-1.817 ppm). The hydroxy metabolite M670H02 accounted for 29.6% of the TRR (0.560

ppm) in the pyrazole-label sample, but was a minor metabolite in the phenyl-label sample (2.2% of the TRR; 0.048 ppm). The cyano metabolite M670H01 was only identified in the pyrazole-label sample, at 0.67% of the TRR (0.013 ppm). Approximately 5.34-5.6% of the TRR (0.1057-0.1168 ppm) was characterized as fractions from the extracts. Approximately 3.16-3.73% of the TRR was unextractable (0.069-0.071 ppm), for an overall accountability ranging from 91.4-94.0%.

A total of 87.8-92.1% of the TRRs (0.259-0.309 ppm) were extracted with methanol/EtOAc from the pyrazole-label and phenyl-label kidney samples. Of the 84.0-90.1% TRR identified (0.2538-0.2955 ppm), the parent topramezone accounted for the vast majority of residues, at 79.5-83.2% of the TRR (0.224-0.293 ppm). M670H02 was identified as 9.95% of the TRR (0.028 ppm) in the pyrazole-label sample, but accounted for only 0.41% of the TRR (0.0014 ppm) in the phenyl-label sample. M670H01 was identified in both labels, at 0.35-0.68% of the TRR (0.0012-0.0019 ppm). Approximately 1.96-3.88% of the TRRs (0.0056-0.0137 ppm) were characterized as either a single minor peak, or as fractions from the extracts. A total of 2.45-6.71% of the TRRs were unextractable (0.0086-0.0189 ppm), for an overall accountability ranging from 90.3-98.7%.

Only samples of pyrazole-label milk were analyzed. Approximately 34.5% of the TRR (0.002 ppm) was extractable from both the methanol/water extract and the acetone extract. Of the 27.6% of the TRR identified (0.00189 ppm), the parent topramezone accounted for 25.3% of the TRR (0.0018 ppm). M670H02 and M670H01 were minor metabolites at 0.88-1.18% of the TRR (0.00006-0.00008 ppm). Approximately 0.81% of the TRR (0.00006 ppm) was characterized as a series of minor peaks, while 6.03% of the TRR (0.0004 ppm) was found in the acetone extract.

One hundred percent of the TRRs were extractable in both the pyrazole-label and phenyl-label urine samples. Of the 99.5% of the TRR identified in the pyrazole-label sample, the parent topramezone was the predominant metabolite (90.0% of the TRR). M670H02 (6.88% of the TRR) and M670H01 (2.6% of the TRR) were considered minor metabolites. The parent topramezone was the only metabolite identified in the phenyl-label sample as 96.75% of the TRR. Approximately 0.48-3.25% of the TRR was characterized as a single minor peak in both labels.

A total of 64.8-83.0% of the TRRs were extracted from the methanol/water extract of both the pyrazole-label and phenyl-label feces samples. The only metabolite identified in both labels was the parent topramezone, at 59.9-72.9% of the TRR. Approximately 4.9-10.1% of the TRR was characterized as a series of minor peaks. Approximately 15.5-22.2% of the TRR was unextractable in the PES, for an overall accountability ranging from 87.0-98.5%.

The metabolic profiles of topramezone in goats were similar between the pyrazole and phenyl treatment groups. The proposed metabolic pathway proceeded from the hydroxylation of the parent compound topramezone at the 4-position of the isoxazole ring to form M670H02. The isoxazole ring was then cleaved to form the cyano metabolite (M670H01). The linkage between the phenyl and pyrazole rings remained intact in all of the metabolites identified.

Hen

BASF has conducted a hen metabolism study with [pyrazole-4-^{14}C] topramezone and [phenyl-U-^{14}C] topramezone. Topramezone was orally administered to 10 Leghorn laying hens for each label at feeding levels of 12.3 mg/kg (pyrazole label, 1500X) or 13.4 mg/kg (phenyl label, 1700X) for 10 consecutive days. Eggs were collected twice daily, excreta were collected once daily, and cage rinse samples were collected on study day 0 and after termination. Tissue samples (liver, muscle, fat and GI tract/contents) were harvested upon termination, 21 - 23 hours after the final dose. The collected samples were assayed for TRRs by combustion and LSC. Samples were subjected to solvent extraction to recover the maximum TRR for further chromatographic characterization and elucidation of the nature of residues. Metabolite identification/characterization of the matrices was achieved by HPLC and co-chromatography and/or comparison of retention times of reference standards or isolated metabolites. All livestock matrices were analyzed within 4 months of sampling. Therefore, no storage stability data were required for this study.

The total applied dose recovery was 94.09% for the pyrazole-label, of which 92.9% was found in excreta, 0.70% in the GI tract/contents (0.796 ppm) and 0.29% in cage rinse. The dose recovery in egg, muscle and fat was <0.005 ppm each (0.0% of the TRR), while virtually all the recoveries in edible tissues were in the liver (0.22% of the TRR; 0.739 ppm). For the phenyl-label, the total applied dose recovery was 93.1%, of which 91.4% was in excreta, 0.29% in cage rinse, and 1.07% in the GI tract/contents (1.318 ppm). The dose recovery in fat, muscle and egg were each ≤0.004 ppm each (0.0% of the TRR), while the vast majority of residues in edible tissues were found in the liver (1.680 ppm).

A total of 88.6-93.4% of the TRRs (0.656-1.568 ppm) were extractable from the methanol extract (phenyl-label) or methanol/water extract (pyrazole-label) of radiolabeled liver samples. Of the 80.8-90.8% of the TRR identified in both labels (0.597-1.525 ppm), the parent topramezone accounted for the majority of residues (58.5-64.4% of the TRR; 0.475-0.982 ppm). The hydroxy metabolite M670H02 accounted for 16.4-29.9% of the TRR in both labels (0.122-0.503 ppm), while the desmethyl hydroxy metabolite M670H04 was only detected in the phenyl-label sample (2.4% of the TRR; 0.04 ppm). Residues characterized as a series of minor peaks/regions comprised 2.6-7.8% of the TRR (0.043-0.059 ppm). The unextractable residues (PES) accounted for 3.7-7.9% of the TRR (0.059-0.062 ppm).

A total of 86.0-88.5% of the TRRs were extractable from the acetone:phosphate buffer extract of both the pyrazole-label and phenyl-label excreta samples. The parent topramezone was the only metabolite identified in the pyrazole label excreta (64.0% of the TRR). Of the 70.1% of the TRR identified in the phenyl-label excreta, 59.5% was identified as the parent topramezone, while M670H02 accounted for 10.6% of the TRR. Residues characterized as a series of minor peaks/regions comprised 15.9-24.5% of the TRR. The unextractable residues (PES) accounted for 3.4-6.3% of the TRR.

A total of 32.9-42.0% of the TRRs (0.0006-0.0007 ppm) were extractable from the ACN and water extracts of both the day 7 and day 9 phenyl-label egg samples. Of the 23.2-31.7% of the TRR identified (0.0004-0.0006 ppm), the parent topramezone accounted for 6.75-13.8% of the TRR (0.0001-0.0003 ppm). M670H02 accounted for 11.6-11.9% of the TRR (0.0002 ppm each)

for both samples, while the acid metabolite M670H05 accounted for 4.6-6.3% of the TRR (0.0001 ppm each). Residues characterized as a series of minor peaks/regions comprised 9.65-10.2% of the TRR (0.0001-0.0002 ppm). The unextractable residues (PES) accounted for 21.1-23.0% of the TRR (0.0004 ppm each).

The metabolic pathway of topramezone in hens proceeded from the hydroxylation at the 4-position of the isoxazole ring to form the hydroxy metabolite M670H02. Further N-demethylation of M670H02 occurred and formed the desmethyl hydroxy metabolite M670H04. M670H04 was only detected in the phenyl labeled liver. The pyrazole ring of topramezone could also be cleaved to form the acid metabolite M670H05.

TABLE 5. Summary of Phenyl-Label (PH) and Pyrazole-Label (PY) Topramezone Metabolism Studies.

Commodity		%TRR Extractable		%Total Identified		Total TRR (ppm)		%TRR													
								Topramezone		M670H01		M670H02		M670H03		M670H04		M670H05		M670H08	
		PH	PY	PH	PY	PH	PY	PH	PY	PH	PY	PH	PY	PH	PY	PH	PY	PH	PY	PH	PY
Corn	Forage	78.8	65.3	51.2	23.6	0.534	0.294	40.4	16.9	-	-	-	-	3.0	3.1	-	-	6.6	-	1.2	3.6
	Stover	82.8	83.3	56.3	28.4	0.730	0.213	40.9	19.6	-	-	-	-	2.3	4.2	-	-	10.2	-	2.9	4.6
	Grain	83.1	77.3	5.5	2.5	0.107	0.032	2.1	2.5	-	-	-	-	-	-	-	-	3.4	-	-	-
Goat	Urine	100	100	96.8	99.5	NR[1]	NR	96.8	90.0	-	2.6	-	6.88	-	-	-	-	-	-	-	-
	Feces	64.8	83.0	59.9	72.9	NR	NR	59.9	72.9	-	-	-	-	-	-	-	-	-	-	-	-
	Kidney	87.8	92.1	84.0	90.1	0.352	0.282	83.2	79.5	0.35	0.68	0.41	9.95	-	-	-	-	-	-	-	-
	Liver	90.8	87.7	85.5	82.1	2.18	1.89	83.3	51.8	-	0.67	2.2	29.6	-	-	-	-	-	-	-	-
	Milk	-	34.5	-	27.6	0.002	0.007	-	25.3	-	0.88	-	1.18	-	-	-	-	-	-	-	-
Poultry*	Excreta (Day 1)	-	88.5	-	64.0	NA[2]	NR	-	64.0	-	-	-	-	-	-	-	-	-	-	-	-
	Excreta (Day 5)	86	-	70.1	-	NR	NA	59.5	-	-	-	10.6	-	-	-	-	-	-	-	-	-
	Liver	93.4	88.6	90.8	80.8	1.68	0.739	58.5	64.4	-	-	29.9	16.4	-	-	2.4	-	-	-	-	-
	Egg (Day 7)	58.9	-	31.7	-	0.002	NA	13.8	-	-	-	11.6	-	-	-	-	-	6.3	-	-	-
	Egg (Day 9)	52.8	-	23.2	-	0.002	NA	6.8	-	-	-	11.9	-	-	-	-	-	4.6	-	-	-

Commodity		%TRR Extractable		%Total Identified		Total TRR (ppm)		%TRR													
								Topramezone		M670H01		M670H02		M670H03		M670H04		M670H05		M670H08	
		PH	PY	PH	PY	PH	PY	PH	PY	PH	PY	PH	PY	PH	PY	PH	PY	PH	PY	PH	PY
Confined Rotational Crops (Soil aged for 34 days)	Wheat Forage	94.8	84.7	22.5	21.1	0.052	0.025	12.4	17.0	-	-	3.57	4.13	-	-	-	-	6.51	-	-	-
	Wheat Hay	82.5	81.6	20.0	21.3	0.132	0.075	11.2	21.3	-	-	2.91	-	-	-	-	-	5.96	-	-	-
	Wheat Straw	78.5	76.1	6.70	13.3	0.092	0.051	1.57	13.3	-	-	4.28	-	-	-	-	-	0.85	-	-	-
	Wheat Grain	107	117	61.2	64.4	0.021	0.007	16.3	64.4	-	-	-	-	-	-	-	-	45.0	-	-	-
	Mustard Greens	90.6	88.0	41.2	47.4	0.026	0.004	31.0	47.4	-	-	-	-	-	-	-	-	10.3	-	-	-
	Radish Top	-	-	-	-	0.010	0.002	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Radish Root	-	-	-	-	0.003	0.001	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Sorghum Forage[3]	-	-	-	-	0.004	0.001	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Sorghum Stover[3]	-	-	-	-	0.003	0.002	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Sorghum Grain[3]	-	-	-	-	0.002	0.002	-	-	-	-	-	-	-	-	-	-	-	-	-	-

1. NR = ppm values were not reported.
2. NA = commodity was not analyzed.
3. Soil aged for 99 days.

* Due to low TRRs, residues in muscle and fat were not characterized/identified.

Note: Metabolites which represent 10% or more of the TRR for a given commodity or part of a commodity are shaded.

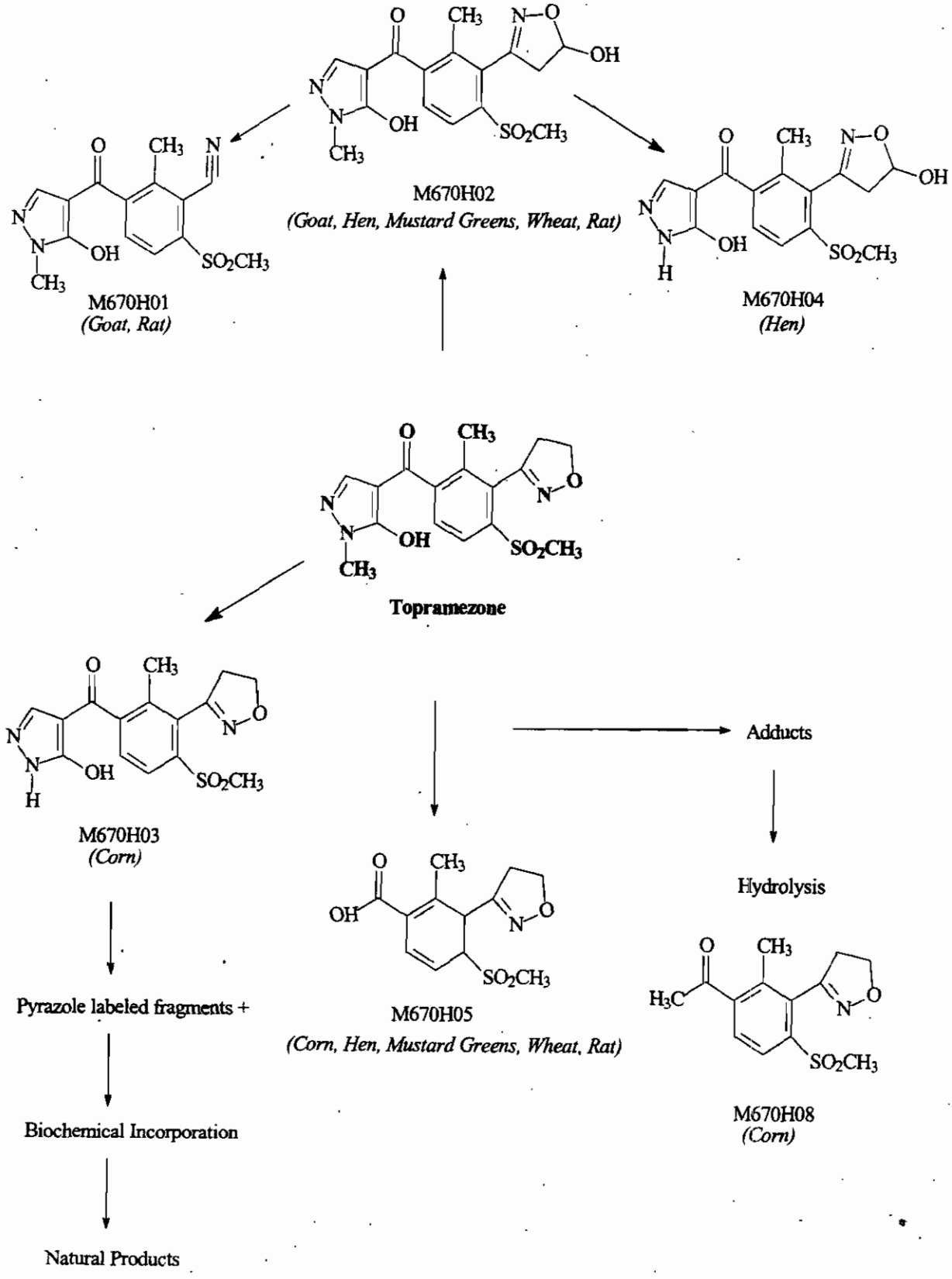
Conclusions. The results of the topramezone metabolism studies in corn, ruminants and poultry were similar in that the parent compound was not extensively metabolized. However, significant differences were observed in the metabolic pathways. **In corn**, topramezone underwent hydrolytic cleavage to form the acid metabolite M670H05 and M670H08, while desmethylation formed M670H03. After cleavage, the pyrazole ring appeared to undergo complete catabolism and reincorporation within the carbon backbone of natural products such as starch, soluble polysaccharides and fatty acids. The phenyl ring portion of the molecule also underwent degradation, resulting in the incorporation of radioactivity into natural products. **In ruminants**, the proposed metabolic pathway involved hydroxylation of the parent compound topramezone at the 4-position of the isoxazole ring to form M670H02. The isoxazole ring was then cleaved to form the cyano metabolite (M670H01). The linkage between the phenyl and pyrazole rings remained intact in all of the metabolites identified. **In poultry**, the proposed metabolic pathway also involved hydroxylation of the parent compound topramezone at the 4-position of the isoxazole ring to form M670H02. Further N-demethylation of M670H02 occurred and formed the desmethyl hydroxy metabolite M670H04. M670H04 was only detected in the phenyl-labeled liver. The pyrazole ring of topramezone could also be cleaved to form the acid metabolite M670H05. Similar metabolic pathways were also observed in **rotational crops** (see 860.1850 Confined Accumulation in Rotational Crops), with M670H05 and M670H02 being the only metabolites identified. The isoxazole ring of topramezone was not radiolabeled in any study; however, these data are not required as the isoxazole ring was not cleaved from the phenyl ring in any of the proposed metabolic pathways.

TABLE 6. Summary of Metabolites and Degradates to be included in the Risk Assessment and Tolerance Expression

Matrix		Residues included in Risk Assessment	Residues included in Tolerance Expression
Plants	Corn	Topramezone	Topramezone
	Rotational Crops	Topramezone	not required
Livestock	Ruminant	Topramezone	Topramezone
	Poultry	tbd*	tbd
Drinking Water		Topramezone	Not Applicable

*To be determined. Note: if future proposed uses significantly increase the dietary exposure to poultry, then the poultry feeding study should include analysis for metabolite M670H02.

Figure 1- Combined metabolic pathways in plant, livestock and rat.



860.1340 Residue Analytical Methods

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Plant Matrices

BASF Corporation proposed LC-MS/MS method No. D0007 for data-gathering and enforcement purposes for residues of topramezone and the free acid metabolite M670H05 in/on plant commodities.

Residues of topramezone and M670H05 were extracted from the matrix (excluding wheat grain, corn starch, and oil) using accelerated solvent extraction (ASE) with water for non-grain matrices, or ACN:water (1:1, v:v) for grain matrices. Extraction by mechanical shaking, using the same solvents as the ASE, was also performed. Aliquots of the extract were subjected to acid and base partitioning prior to analysis with LC-MS/MS. The samples of wheat grain, corn starch, and oil were extracted, then partitioned with 1N hydrochloric acid (HCl) saturated with sodium chloride (NaCl) and dichloromethane (DCM). Basic partitioning by ammonium hydroxide (NH_4OH) occurred prior to analysis with LC-MS/MS.

Data gathering method recoveries of topramezone extracted by ASE were within the acceptable range in samples spiked at the LOQ (0.01 ppm for kernel + cob with husk removed (K+CWHR) and corn grain; 0.05 ppm for corn forage, corn stover and radish root), and at 10-fold the LOQ (0.10 and 0.50 ppm). Recoveries of M670H05 extracted by ASE were also generally within the acceptable range, except for slightly lower recoveries in two of five corn grain samples spiked at the LOQ. Several recoveries of topramezone and M670H05 extracted by mechanical shaking were lower than 70%, particularly in samples of corn matrices spiked at the LOQ. An independent laboratory validation (ILV) was conducted using maceration by Polytron homogenizer, followed by micro-partitioning. The ILV was successful for corn grain, lettuce, oilseed rape seed, and apple. Acceptable radiovalidation data were submitted for corn forage and stover.

Livestock Matrices

BASF has developed a method for determining residues of topramezone and the metabolite M670H02 in livestock tissues, milk, and eggs (method number D0104). Residues were extracted in water, acidified with HCl and partitioned with DCM. The organic phase was back-partitioned with ammonium formate, pH 10, and the aqueous phase was analyzed by LC-MS/MS. The limit of detection (LOD) was estimated at 8 pg/100 µL (0.08 ppb) injected on HPLC; the LOQs were 0.01 ppm for milk, muscle, and egg, and 0.05 ppm for liver, kidney, and fat. In the data-gathering method study, samples were spiked with both analytes at the LOQ and at 0.50 ppm. In general, recoveries of topramezone and M670H02 were within the acceptable range. The overall recoveries for topramezone and M670H02 ranged from 72-102% (SD <20%), and 62-118% (SD <20%), respectively, for all spiking levels and all matrices. The detector response was linear between 0.1 and 2.0 pg/µL (10 and 200 pg injected). No residues were detected in any control samples (<8 pg injected). The method was successfully validated in milk and liver by an independent laboratory, indicating good reproducibility and reliability. Satisfactory

radiovalidation data on goat liver were submitted. No interference testing or confirmatory method was proposed as the detector used was highly specific.

Conclusions. Under the conditions and parameters used in these studies, the residue analytical method validation data are classified as scientifically acceptable. LC-MS/MS methods D0007 and D0104 will also undergo a PMV at ACL/BEAD (Memo S. Levy, 12/2/04; DP# 310773). Successful completion of the PMV is necessary before the proposed methods can be employed for enforcement purposes.

860.1360 Multiresidue Methods

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Topramezone was analyzed by Maxim Technologies Inc. (Middleport, NY) according to the U.S. FDA MRMs Test guidelines in the Pesticide Analytical Method (PAM), Vol. 1, Third edition (January 1994) as amended by study protocol. Protocols A and B were not required as topramezone did not possess either a N-methylcarbamate structure, carboxylic acid, or phenolic moieties. Using Protocol C, injections of topramezone did not yield adequate responses to any of the DG modules. Since Protocol C failed to produce an adequate response, topramezone was not analyzed by Protocols D, E, and F. Protocol G was not required as topramezone did not possess a substituted urea structure. Topramezone was not adequately recovered using any of the MRMs.

Conclusions. The MRM residue data are classified as acceptable. These data were forwarded to the U.S. FDA for further evaluation (Memo S. Levy, 2/9/05; DP# 313151). As topramezone was not adequately recovered using any of the Protocols, MRMs would not be suitable for use as an enforcement method.

860.1380 Storage Stability

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Samples of corn forage, grain, straw and radish roots (spiked with parent topramezone, and the metabolite M670H05, at a level of 1.0 ppm) were stored at -20°C for a duration of 541 days. The LC-MS/MS BASF Method No. D0007, as outlined in the analytical methodology study, was used in the storage stability study. This method has been found to be an acceptable data-gathering and enforcement method for the analysis of parent topramezone and M670H05 in corn forage, grain, straw and radish root. Under these conditions, the decline in residues of parent and M670H05 were considered statistically insignificant ($p > 0.05$). The data indicate that residues of topramezone and the metabolite M670H05 were stable at -20°C for up to 541 days (18 months) in corn forage, grain, straw and radish roots.

Conclusions. The storage stability data are classified as scientifically acceptable. The data indicate that the overall decline of residues (topramezone and M670H05) was considered

statistically insignificant ($P > 0.05$). Thus, the residues of topramezone and the metabolite M670H05 were considered to be stable in corn forage, grain, straw and radish root up to a demonstrated interval of 541 days or 18 months. These durations adequately support the crop field trials submitted to support the petitioned uses. Corrections to residue values due to in-storage dissipation were not needed.

860.1480 Meat, Milk, Poultry, and Eggs

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The petitioner submitted a cattle feeding study with the subject petition. The maximum theoretical dietary burden (MTDB) of topramezone to livestock is presented in Table 7.

TABLE 7. Calculation of MTDB of Topramezone to Livestock.

Feed Commodity	% Dry Matter[1]	% Diet[1]	Recommended Tolerance, ppm	Dietary Contribution, ppm[2]
Beef Cattle				
Corn, field, forage	40	40	0.05	0.05
Corn, field, stover	83	25	0.05	0.015
Corn, field, grain	88	35	0.01	0.004
TOTAL BURDEN		100		**0.069**
Dairy Cattle				
Corn, field, forage	40	50	0.05	0.062
Corn, field, stover	83	15	0.05	0.009
Corn, field, grain	88	35	0.01	0.004
TOTAL BURDEN		100		**0.075**
Poultry				
Corn, field, grain	NA	80	0.01	0.008
TOTAL BURDEN		80		**0.008**
Hog				
Corn, field, grain	NA	80	0.01	0.008
TOTAL BURDEN		80		**0.008**

[1] Table 1 (OPPTS Guideline 860.1000).

[2] Contribution = [tolerance / % DM (cattle)] x % diet). Poultry and hog diets are not corrected for % dry matter.

Cattle

Topramezone was administered orally via a balling gun to 14 lactating dairy cows (*Holstein Friesian*) once daily for 29 days. Dosing was made at 0.37 mg/kg feed (4.9X), 1.04 mg/kg (14X) feed and 3.57 mg/kg (48X) feed. Residues in milk and tissue samples were extracted in water, acidified with HCl and partitioned with DCM. The organic phase was back-partitioned with ammonium formate, pH 10, and the aqueous phase was analyzed for topramezone and its metabolite M670H02 by LC-MS/MS. The fat in muscle samples was analyzed by the Soxhlet-extraction method. Samples were stored frozen and analyzed within 30 days of collection; thus, no storage stability data for livestock matrices were required. In muscle, individual recoveries of <70% were reported for both analytes, but the mean recoveries were 71.0 and 74.5% for topramezone and M670H02, respectively. Residue levels of topramezone and M670H02 in milk were below the LOQ (0.01 ppm) for every sampling period and every treatment dose level. Residue levels of both analytes were also below the LOQ for fat (0.05 ppm) and muscle (0.01 ppm). Quantifiable residue levels were seen for parent topramezone in kidney and liver, but were <LOQ (0.05 ppm) for M670H02. At the 0.37 mg/kg treatment level, the residues in liver and kidney were 0.484-0.608 ppm and 0.144-0.188 ppm, respectively. At the 1.04 mg/kg treatment level, the residues in liver and kidney were 0.808-1.30 ppm and 0.197-0.208 ppm, respectively. At the 3.57 mg/kg treatment level, the residues in liver and kidney were 1.59-1.88 ppm and 0.320-0.350 ppm, respectively. Residue levels declined 2 and 7 days after the last administered dose in both liver (1.39 ppm to 0.836 ppm) and kidney (0.178 ppm to 0.113 ppm), but were still quantifiable.

TABLE 8. Summary of Residue Data from Ruminant Feeding Study with Topramezone

Matrix	Feeding Level (ppm)	Residue Levels (ppm)*					
		n	Min.	Max.	Mean	Std. Dev.	R/F Ratio**
Milk	0.0	3	<0.01	<0.01			
	0.37	3	<0.01	<0.01	—	—	—
	1.04	3	<0.01	<0.01			
	3.57	5	<0.01	<0.01			
Liver	0.0	3	<0.05	<0.05	—	—	—
	0.37	3	0.484	0.608	0.555	0.064	1.50
	1.04	3	0.808	1.296	1.129	0.278	1.09
	3.57	3	1.588	1.882	1.782	0.168	0.499
Kidney	0.0	1	<0.05	<0.05	—	—	—
	0.37	3	0.1436	0.1876	0.159	0.025	0.430
	1.04	3	0.1972	0.2080	0.204	0.006	0.196
	3.57	3	0.3200	0.3500	0.332	0.016	0.0930
Fat	0.0	2	<0.05	<0.05			
	0.37	NA	NA	NA	—	—	—
	1.04	NA	NA	NA			
	3.57	4	<0.05	<0.05			
Muscle	0.0	1	<0.01	<0.01			
	0.37	NA	NA	NA	—	—	—
	1.04	NA	NA	NA			
	3.57	3	<0.01	<0.01			

NA - Not analyzed

* Residues of Topramezone *per se*, all values were <LOQ (0.05 ppm) for M670H02.

** Residue-to-feed ratio (mean residues divided by the feeding level)

Conclusions. The cattle feeding study residue data are adequate to satisfy data requirements for this petition. The feeding study data indicate that tolerances are needed for residues of topramezone in livestock liver and kidney (excluding hog commodities). Based on the MTDB of 0.076 ppm and the maximum residue-to-feed ratios of 1.50 and 0.430, the appropriate tolerances for topramezone in liver and kidney are 0.15 and 0.05 ppm, respectively. Also, based on the low MTDB, tolerances are not required for any hog commodity. **A revised Section F is required.**

Poultry

The petitioner did not submit a poultry feeding study with this petition, but submitted a waiver request for magnitude of the residues in poultry and eggs. The maximum combined residues of topramezone plus M670H02 observed in any poultry commodity in the hen metabolism study were 1.485 ppm in liver. Based on a 1700X dosing level in the metabolism study, the expected residues of topramezone at a 10X dosing level are 0.0089 ppm, which is below the LOD of the method. HED thus concludes that there is no reasonable expectation of finite residues (180.6(a)(3)) and that the requested waiver is appropriate for the currently-proposed uses. Should additional uses which significantly increase the poultry MTDB be proposed in the future, this conclusion will be reevaluated.

860.1500 Crop Field Trials

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TABLE 9. Summary of Residues from the Corn Field Trials (Field and Sweet) with Topramezone

Crop Matrix	Applic. Rate lb a.i./A (g a.i./ha)	PHI (days)	Residues (ppm)				
			Mean	Std. Dev.	HAFT*	Min.	Max.
Topramezone							
Field Corn (proposed use = 0.022 lb ai/A total application rate, 45-day PHI)							
K+CWHR	0.088-0.092 (99-103)	29-64	<0.01	-	-	<0.01	<0.01
Forage	0.088-0.095 (99-106)	31-91	<0.05	-	-	<0.05	<0.05
Grain	0.088-0.095 (99-106)	59-128	<0.01	-	-	<0.01	<0.01
Stover	0.088-0.095 (99-106)	59-128	<0.05	-	-	<0.05	<0.05

Crop Matrix	Applic. Rate lb a.i./A (g a.i./ha)	PHI (days)	Residues (ppm)				
			Mean	Std. Dev.	HAFT*	Min.	Max.
Sweet Corn (proposed use = 0.022 lb ai/A total application rate, 45-day PHI)							
K+CWHR	0.088-0.091 (99-102)	35-49	<0.01	-	-	<0.01	<0.01
Forage	0.088-0.091 (99-102)	35-49	<0.05	-	-	<0.05	<0.05
Stover	0.088-0.091 (99-102)	57-92	<0.05	-	-	<0.05	<0.05
BAS 670 H 05							
Field Corn (proposed use = 0.022 lb ai/A total application rate, 45-day PHI)							
K+CWHR	0.088-0.092 (99-103)	29-64	<0.01	-	-	<0.01	<0.01
Forage	0.088-0.095 (99-106)	31-91	<0.05	-	-	<0.05	<0.05
Grain	0.088-0.095 (99-106)	59-128	<0.01	-	-	<0.01	<0.01
Stover	0.088-0.095 (99-106)	59-128	<0.05	-	-	<0.05	<0.05
Sweet Corn (proposed use = 0.022 lb ai/A total application rate, 45-day PHI)							
K+CWHR	0.088-0.091 (99-102)	35-49	<0.01	-	-	<0.01	<0.01
Forage	0.088-0.091 (99-102)	35-49	<0.05	-	-	<0.05	<0.05
Stover	0.088-0.091 (99-102)	57-92	<0.05	-	-	<0.05	<0.05

* HAFT = Highest Average Field Trial.

Corn. BASF submitted crop field trial data to quantify residues of topramezone and the free acid metabolite BAS 670 H 05 (also known as M670H05) for use on field and sweet corn. During the 2000 and 2001 growing seasons, a total of 24 trials were conducted on field corn encompassing Regions 1 (PA; 1 trial), 2 (NC; 1 trial), 5 (IL; 4 trials, ID; 4 trials, MN; 5 trials, MT; 2 trials, NE; 2 trials), 5B (QC; 4 trials) and 6 (OK; 1 trial). A total of 6 trials were conducted on sweet corn encompassing Regions 1 (PA; 1 trial), 3 (FL; 1 trial), 7A (AB; 1 trial), 10 (CA; 1 trial), 11 (OR; 1 trial) and 12 (OR; 1 trial). The sweet corn RAC samples collected were forage, milk stage kernels plus cob with husks removed (K+CWHR), and stover. The field corn RAC samples collected were forage, milk stage K+CWHR, grain and stover. Field corn commodities harvested at the milk stage were extended to sweet corn. BAS 336 SC herbicide was applied 2 times sequentially (1st target application at 0.022 lb a.i./A (25 g a.i./ha) and 2nd target application at 0.067 lb a.i./A (75 g a.i./ha)) as a foliar broadcast spray over the top of the corn, with a retreatment interval (RTI) of 13 to 17 days for field corn and 12 to 15 days for sweet corn. The total seasonal rate was 0.089 lb a.i./A/season (100 g a.i./ha/season, 4.5X). An adjuvant was added to the spray mixture for all applications, which contained a crop oil concentrate (COC) and an aqueous solution of urea ammonium nitrate. Field corn was harvested at PHIs of 29-64 days for milk stage K+CWHR, 31-91 days for forage and 59-128 days for grain and stover. Sweet

corn was harvested at PHIs of 35-49 days for K+CWHR and forage, and 57-92 days for stover.

The analytical data gathering and enforcement method (D0007) was the same for topramezone and BAS 670 H 05. Residues of topramezone were extracted from non-grain crop matrices with water, and residues in seed or grain matrices were extracted using ACN:water (1:1, v:v). A 2% aliquot of the extract was removed and cleaned by partitioning with a mixture of 1 N HCl:DCM (1:5, v:v) and NaCl. The extract was centrifuged (if necessary), the aqueous layer was discarded, and residues in the DCM layer were partitioned with 0.05% NH_4OH. The final analysis of topramezone and BAS 670 H 05 was determined by LC-MS/MS. The LOQ for topramezone residues was 0.01 ppm for fresh market corn (K+CWHR) and corn grain, and 0.05 ppm for corn forage and corn stover. The LOD was 0.005 ppm for fresh corn and corn grain, and 0.025 ppm for all the other sample matrices.

Storage stability data were deemed adequate as levels of topramezone and BAS 670 H 05 in field corn were evaluated concurrently with the field trials. The actual storage duration of all the RACs fell within the 18-month demonstrated storage interval.

The results from the field and sweet corn residue trials showed that at every PHI, the maximum residues for K+CWHR and grain for field corn were <0.01 ppm (LOQ), while the maximum residues for forage and stover for field and sweet corn were <0.05 ppm (LOQ). The formulation-bridging study between BAS 670 00 H (SC) and BAS 670 UAH (dry flowable - DF) produced residues < LOQ for topramezone or BAS 670 H 05. Residue decline studies indicated that residues of topramezone or BAS 670 H 05 remained below their respective LOQs with increasing PHIs. Exaggerated seasonal rates (503 g a.i./ha or 0.448 lb a.i./A, 20X) produced residue levels below the LOQ for field corn (forage, grain and stover).

Conclusions. The crop field trial data on corn (field and sweet) were deemed scientifically acceptable for the determination of residues of the active ingredient topramezone (the residue of concern) and the free acid metabolite BAS 670 H 05. Corn samples were stored frozen for 9 months from harvest to analysis, which is covered by the demonstrated storage interval of 18 months from the storage stability study. The field trial studies were conducted in the regions suggested in OPPTS 860.1500 for establishment of a tolerance in/on the crops requested. The residue data on corn were also acceptable when using either the SC or DF foliar treatment formulations containing topramezone. Compared to the proposed use pattern (25 g a.i./ha or 0.022 lb a.i./A), the study use pattern was conducted at an exaggerated rate of 0.089 lb a.i./A/season (100 g a.i./ha/season, 4.5X). However, since residues of topramezone and BAS 670 H 05 from the study use pattern (either the SC or DF formulations) did not exceed the LOQ (<0.01 ppm for K+CWHR and grain; <0.05 ppm for forage and stover), there is no expectation of quantifiable residues at the proposed use rate. The residue data thus support the proposed tolerances.

860.1520 Processed Food and Feed

Conclusions. No processing study was required as 2 field corn plots treated with topramezone (SC formulation) at exaggerated rates (0.306 kg a.i./ha or 0.273 lb a.i./A (12X); and 0.503 kg a.i./ha or 0.449 lb a.i./A (20X)) produced no residues above the LOQ (0.01 ppm for grain and fresh corn; 0.05 ppm for forage and stover). Separate tolerances are thus not required for processed corn commodities.

860.1850 Confined Accumulation in Rotational Crops

45902413.der.wpd

BASF conducted a confined accumulation study with [pyrazole-4-^{14}C] topramezone and [phenyl-U-^{14}C] topramezone. Topramezone was applied to bare soil at rates of 0.072-0.081 lb a.i./A (0.081-0.091 kg a.i./ha, 3.3-3.7X) for the phenyl-label, and 0.081-0.084 lb a.i./A (0.091-0.094 kg a.i./ha, 4.1-4.3X) for the pyrazole-label. Rotational crops were planted in open-bottom casks in four field plots with PBIs of 34 days (radish, mustard greens, and wheat), 99 days (mustard greens and sorghum) and 393 days (mustard greens only). Swiss chard was planted at the 34-day PBI, but the crop failed to grow. Due to climatic conditions, sorghum was planted at the 99-day PBI instead of wheat.

If the TRRs in a RAC sample were below 0.01 ppm, then no further planting of the crop was conducted. RAC samples with TRRs greater than 0.01 ppm were subjected to further analysis to characterize the radioactive residues. The range of overall TRRs in pyrazole- and phenyl-label crop RAC samples at the 34-day PBI were 0.0039-0.0256 ppm for mustard greens; 0.0021-0.0095 ppm in radish tops; 0.0010-0.0030 ppm in radish roots; 0.0753-0.1321 ppm for wheat grain; 0.0510-0.0920 ppm for wheat straw; 0.0249-0.0518 ppm for wheat forage; and 0.0073-0.0206 ppm for wheat hay. The range of overall TRRs in pyrazole- and phenyl-label crop RAC samples at the 99-day PBI were 0.001-0.004 ppm for sorghum forage; 0.0016-0.0032 ppm for sorghum stover; 0.0019-0.0021 ppm for sorghum grain; and 0.0025-0.0113 ppm for mustard green RACs. The overall TRRs in the phenyl-label mustard green RAC at the 393-day PBI was 0.003 ppm. All plant samples were analyzed within 3 months of harvest; therefore, no storage stability data were generated.

A total of 88.0-90.6% of the TRRs (0.0034-0.0231 ppm) were extractable from the combined MeOH/water extracts of pyrazole-label and phenyl-label mustard greens from the 34-day PBI. The parent topramezone accounted for the majority of residues (31.0% of the TRRs; 0.0079 ppm) in the phenyl-label mustard greens, while the acid metabolite M670H05 accounted for 10.3% of the TRR (0.0026 ppm). The parent topramezone was the only metabolite identified in the pyrazole-label sample (47.4% of the TRR; 0.0018 ppm). Residues characterized as a series of minor peaks/regions comprised 40.6-49.3% of the TRRs (0.0016-0.0126 ppm). The unextractable residues (PES) accounted for 12.7-17.3% of the TRRs (0.0007-0.0033 ppm), for an overall accountability ranging from 103-105%.

A total of 76.1-117% of the TRRs (0.0085-0.1092 ppm) were extractable from the combined

MeOH/water extracts in phenyl- and pyrazole-label wheat matrices (34 day PBI). The parent compound, topramezone, was either the predominant residue or the only residue identified in the pyrazole-label wheat matrices (13.3-64.4% of the TRRs; 0.0043-0.0160 ppm), and in phenyl-label forage and hay (11.2-12.4% of the TRRs; 0.0064-00.0147 ppm). M670H05 was the predominant metabolite identified in the phenyl-label grain (45.0% of the TRR; 0.0093 ppm). In the other radiolabeled wheat matrices, the metabolites M670H02 and M670H05 comprised less than 7% of the TRR (<0.01 ppm). After exhaustive extraction/fractionation procedures, a total of 45.5-72.3% of the TRRs were characterized in the phenyl-and pyrazole-label wheat RAC extracts (0.0038-0,0828 ppm). The final unextractable residues in all the wheat matrices after fractionation were ≤20.2% of the TRRs (≤0.0182 ppm), for accountabilities ranging from 92.4-126%.

The proposed metabolism of topramezone involved the hydrolysis of the parent to form the free acid M670H05. The pyrazole moiety was catabolized after cleavage, then reincorporated into natural products (as seen in the corn metabolism study). Parent-related adducts were formed from the des-methylation of the pyrazole ring. Hydroxylation of the isoxazole ring formed M670H02 (as seen in the goat metabolism study), then reincorporation into natural products occurred after further ring degradation.

TABLE 10. Summary of Characterization and Identification of Radioactive Residues in Rotational Crop Matrices Following Application of Radiolabeled Topramezone.

Compound	34-d PBI (Phenyl Label)		34-d PBI (Pyrazole Label)	
	% TRR	ppm	% TRR	ppm
Mustard Greens				
Topramezone	31.0	0.0079	47.4	0.0018
M670H02	—	—	—	—
M670H05	10.3	0.0026	—	—
Wheat Forage				
Topramezone	12.4	0.0064	17.0	0.0043
M670H02	3.57	0.0018	4.13	0.0010
M670H05	6.51	0.0034	—	—
Wheat Hay				
Topramezone	11.2	0.0147	21.3	0.0160
M670H02	2.91	0.0038	—	—
M670H05	5.96	0.0079	—	—
Wheat Straw				
Topramezone	1.57	0.0014	13.3	0.0068
M670H02	4.28	0.0039	—	—
M670H05	0.85	0.0008	—	—
Wheat Grain				
Topramezone	16.3	0.0034	64.4	0.0047
M670H02	—	—	—	—
M670H05	45.0	0.0093	—	—

Conclusions. The confined rotational crop data are classified as scientifically acceptable. The residue of concern was determined to be topramezone *per se* (Table 6). The total residues of concern were <0.01 ppm in radish and mustard greens planted at a 34-day PBI and sorghum planted at a 99-day PBI.

860.1900 Field Accumulation in Rotational Crops

45902414.der.wpd

BASF has submitted rotational crop field trial data for topramezone and the free acid metabolite BAS 670 H 05 (also known as M670H05) on radish (root and top), soybean (forage, hay and seed), spinach leaves, sorghum (forage, grain and stover) and winter wheat (forage, hay, grain, straw). Six limited rotational crop field trials were conducted in two principal corn growing regions in the United States encompassing Regions 1 (PA) and 5 (IA) during the 2000 growing season. A supplementary limited rotational grain sorghum field trial was conducted at the PA site in 2001. BAS 670 00H Herbicide was applied two times sequentially (1st nominal application at 0.022 lb a.i./A (25 g a.i./ha) and 2nd nominal application at 0.067 lb a.i./A (75 g a.i./ha)) as a foliar broadcast spray over the top of the field corn (year 2000 trials) or sweet corn (year 2001 trial). A retreatment period of 12 to 17 days was used, with a total application rate of 0.089 lb a.i./A/season (100 g a.i./ha/season, 4.5X). The study was conducted with PBIs of 28 and 90 days for radish (root and top); 28-29 days for soybean (forage, hay, and seed) and sorghum (forage, grain and stover); and 90 days for spinach leaves and winter wheat (forage, hay, grain and straw). An adjuvant was added to the spray mixture for all applications, which contained a COC and an aqueous solution of urea ammonium nitrate.

The analytical data-gathering and enforcement method (D0007) was the same for topramezone and BAS 670 H 05. Residues of topramezone and BAS 670 H 05 were extracted from non-grain crop matrices with water, and residues in seed or grain matrices were extracted using ACN:water (1:1, v:v). A 2% aliquot of the extract was removed and cleaned by partitioning with a mixture of 1N HCl:DCM (1:5, v:v) and NaCl. Wheat grain samples were extracted with 1N HCl:DCM (2:5, v:v) saturated with NaCl. The extract was centrifuged (if necessary), the aqueous layer was discarded, and residues in the DCM layer were partitioned with 0.1% NH_4OH. The final analysis of topramezone and BAS 670 H 05 was determined by LC-MS/MS. The LOQ for topramezone and BAS 670 H 05 residues was 0.05 ppm for radish (root and top), soybean (forage, hay and seed), spinach leaves, sorghum (forage, grain and stover) and winter wheat (forage, hay, grain, straw). The LOD in these matrices was 0.025 ppm.

The storage stability of topramezone and BAS 670 H 05 in this study was evaluated concurrently with the field trials for radish, soybean, spinach, sorghum and winter wheat matrices. The demonstrated maximum storage stability interval of 18 months for radish (root) and corn (forage, grain, straw) from the storage stability study encompasses the actual storage intervals in this study, as the corn storage stability data can be translated to the different rotational crop matrices.

All residue levels of topramezone and BAS 670 H 05 were less than the LOQ (0.05 ppm) at both the 28- to 29-day and/or 90-day PBIs for radish (root and top), soybean (forage, hay and seed),

spinach leaves, sorghum (forage, grain and stover) and winter wheat (forage, hay, grain, straw).

TABLE 11. Summary of Topramezone Residues from the Field Accumulation in Rotational Crops*

Crop Matrix	Applic. Rate lb ai/A (g ai/A)	PHI (days)	Residues (ppm)				
			Mean	Std. Dev.	HAFT	Min.	Max.
28-29-day PBI							
Radish Root	0.089-0.091 (100 - 102)	54-57	<0.05	—	—	<0.05	<0.05
Radish Top		54-57	<0.05	—	—	<0.05	<0.05
Soybean Forage		62-64	<0.05	—	—	<0.05	<0.05
Soybean Hay		77-89	<0.05	—	—	<0.05	<0.05
Soybean Seed		127-159	<0.05	—	—	<0.05	<0.05
Sorghum Forage		113-122	<0.05	—	—	<0.05	<0.05
Sorghum Grain		127-172	<0.05	—	—	<0.05	<0.05
Sorghum Stover		127-172	<0.05	—	—	<0.05	<0.05
90-day PBI							
Radish Root	0.089-0.091 (100 - 102)	130-137	<0.05	—	—	<0.05	<0.05
Radish Top		130-137	<0.05	—	—	<0.05	<0.05
Spinach Leaves		137-154	<0.05	—	—	<0.05	<0.05
Winter Wheat Forage		137-320	<0.05	—	—	<0.05	<0.05
Winter Wheat Hay		354-363	<0.05	—	—	<0.05	<0.05
Winter Wheat Grain		385-397	<0.05	—	—	<0.05	<0.05
Winter Wheat Straw		385-397	<0.05	—	—	<0.05	<0.05

* Identical results were also obtained for the metabolite BAS 670 H 05

Conclusions. The crop rotational field trial data were acceptable for the determination of residues of topramezone and BAS 670 H 05. Compared to the proposed use pattern (25 g a.i./ha or 0.022 lb a.i./A), the study use pattern was conducted at an exaggerated rate of 100 g a.i./ha (0.089 lb a.i./A, 4.5X). With this exaggerated use pattern, residues of topramezone and BAS 670 H 05 did not exceed the LOQ (<0.05 ppm). Based on the data provided, the minimum PBIs are 28 days for all non-labeled crops.

860.1550 Proposed Tolerances

There are currently no established Codex, Canadian, or Mexican maximum residue limits (MRLs) for topramezone. An International Residue Limit Status sheet is attached to this review.

TABLE 12. Tolerance Summary for Topramezone

Commodity	Proposed Tolerance (ppm)	Recommended Tolerance (ppm)	Comments (correct commodity definition)
Corn, field, forage	0.05	0.05	
Corn, field, stover	0.05	0.05	
Corn, field, grain	0.01	0.01	
Corn, pop, grain	0.01	0.01	
Corn, pop, stover	0.05	0.05	
Corn, sweet, kernel plus cob with husks removed	0.01	0.01	
Corn, sweet, forage	0.05	0.05	
Corn, sweet, stover	0.05	0.05	
Cattle, kidney	0.20	0.05	
Cattle, liver	0.70	0.15	
Goat, kidney	0.20	0.05	
Goat, liver	0.70	0.15	
Horse, kidney	0.20	0.05	
Horse, liver	0.70	0.15	
Sheep, kidney	0.20	0.05	
Sheep, liver	0.70	0.15	
Hog, kidney	0.20	Not Required	
Hog, liver	0.70	Not Required	

cc: G. Kramer (RAB1), S. Levy (RAB1)
RDI: P.V. Shah (2/16/05), RAB1 Chemists (2/16/05), ChemSAC (3/2/05)
G.F. Kramer:806T:CM#2:(703)305-5079:7509C:RAB1

Template Version March 2003

INTERNATIONAL RESIDUE LIMIT STATUS

Chemical Name:	Common Name:	☒ Proposed tolerance ☐ Reevaluated tolerance ☐ Other	Date:
[3-(4,5-dihydro-3-isoxazolyl)-2-methyl-4-(methylsulfonyl)phenyl](5-hydroxy-1-methyl-1H-pyrazol-4-yl)methanone	Topramezone (BAS 670 H)		2/8/05

Codex Status (Maximum Residue Limits)	U. S. Tolerances
X No Codex proposal step 6 or above ☐ No Codex proposal step 6 or above for the crops requested	Petition Number: 3F6568 DP #: 310772 Other Identifier:
Residue definition: N/A	Reviewer/Branch: G.F. Kramer
	Residue definition: parent only

Crop (s)	MRL (mg/kg)	Crop(s)	Tolerance (ppm)
		Corn, field, forage	0.05
		Corn, field, stover	0.05
		Corn, field, grain	0.01
		Corn, pop, grain	0.01
		Corn, pop, stover	0.05
		Corn, sweet, kernal plus cob with husks removed	0.01
		Corn, sweet, stover	0.05
		Cattle*, kidney	0.20
		Cattle*, liver	0.70
		* includes: goat, horse, and sheep	

Limits for Canada	Limits for Mexico
X No Limits ☐ No Limits for the crops requested	X No Limits ☐ No Limits for the crops requested
Residue definition: N/A	Residue definition: N/A

Crop(s)	MRL (mg/kg)	Crop(s)	MRL (mg/kg)

Notes/Special Instructions:.
S.Funk, 02/09/05.

Rev 1998

Evaluator

Robert Vogrig — Date: March 21, 2005
Robert Vogrig
Evaluation Officer
FREAS, PMRA

Suzan Mathew — Date: Mar 21/05
Suzan Mathew
Evaluation Officer
FREAS, PMRA

Peer Reviewer

George F. Kramer — Date: 4-12-05
George F. Kramer, Ph.D.
Chemist
RAB1, HED, EPA

Approved by

Louise G. Croteau — Date: March 22/05
Louise G. Croteau
Senior Evaluation Officer
FREAS, PMRA

Ariff Ally — Date: March 29/05
Ariff Ally, Ph.D.
Section Head
FREAS, HED, PMRA

P.V. Shah — Date: 4/18/05
P.V. Shah, Ph.D.
Branch Senior Scientist
RAB1, HED, EPA

STUDY REPORTS:

MRID No. 45902414. Versoi, P.L., Ellenson, J.L. (2002). Limited Field Rotational Crop Study For BAS 670 H. BASF Study Number: 63884. Unpublished study prepared by BASF Agro Research. 126 pages.

EXECUTIVE SUMMARY:

BASF Agro Research has submitted rotational crop field trial data for topramezone and the free acid metabolite BAS 670 H 05 (also known as M670H05) on radish (root and top), soybean (forage, hay and seed), spinach leaves, sorghum (forage, grain and stover) and winter wheat (forage, hay, grain, straw). Six limited rotational crop field trials were conducted in two principal corn growing regions in the United States encompassing Regions 1 (Pennsylvania) and 5 (Iowa) during the 2000 growing season. A supplementary limited rotational grain sorghum field trial was conducted at the Pennsylvania site in 2001. BAS 670 00H Herbicide (a soluble-concentrate (SC) formulation containing the active ingredient topramezone) was applied two times sequentially (1st nominal application at 25 g a.i./ha (0.022 lb a.i./A) and 2nd nominal application at 75 g a.i./ha (0.067 lb a.i./A)) as a foliar broadcast spray over the top of the field corn (year 2000 trials) or sweet corn (year 2001 trial). A retreatment period of 12 to 17 days was used, with an application rate of 100 g a.i./ha/season (0.089 lb a.i./A/season). The study was conducted with plantback intervals (PBIs) of 28 and 90 days for radish (root and top); 28-29 days for soybean (forage, hay, and seed) and sorghum (forage, grain and stover); and 90 days for spinach leaves and winter wheat (forage, hay, grain and straw). An adjuvant was added to the spray mixture for all applications, which contained a crop oil concentrate and an aqueous solution of urea ammonium nitrate.

The analytical data-gathering and enforcement method (D0007) was the same for topramezone and BAS 670 H 05. Residues of topramezone and BAS 670 H 05 were extracted from non-grain crop matrices with water, and residues in seed or grain matrices were extracted using acetonitrile:water (1:1, v:v). A 2% aliquot of the extract was removed and cleaned by partitioning with a mixture of 1N HCl:dichloromethane (DCM) (1:5, v:v) and NaCl. Wheat grain samples were extracted with 1N HCl:DCM (2:5, v:v) saturated with NaCl. The extract was centrifuged (if necessary), the aqueous layer was discarded, and residues in the DCM layer were partitioned with 0.1% ammonium hydroxide (NH_4OH). The final analysis of topramezone and BAS 670 H 05 was determined by high-performance liquid chromatography with dual mass-selective detectors (LC-MS/MS). The limit of quantitation (LOQ) for topramezone and BAS 670 H 05 residues was 0.05 ppm for radish (root and top), soybean (forage, hay and seed), spinach leaves, sorghum (forage, grain and stover) and winter wheat (forage, hay, grain, straw). The limit of detection (LOD) in these matrices was 0.025 ppm.

The storage stability of topramezone and BAS 670 H 05 in this study was evaluated concurrently with the field trials for radish, soybean, spinach, sorghum and winter wheat matrices. The demonstrated maximum storage stability interval of 18 months for radish (root) and corn (forage, grain, straw) from the storage stability study encompasses the actual storage intervals in this study, as the corn storage stability data can be translated to the different rotational crop matrices.

All residue levels of topramezone and BAS 670 H 05 were less than the LOQ (0.05 ppm) at both the 28- to 29-day and/or 90-day PBIs for radish (root and top), soybean (forage, hay and seed), spinach leaves, sorghum (forage, grain and stover) and winter wheat (forage, hay, grain, straw).

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the rotational crop field trial residue 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# 310772] and in Canada's Regulatory Decision Document.

COMPLIANCE:

Signed and dated Good Laboratory Practice (GLP), Quality Assurance and Data Confidentiality statements were provided. The following protocol deviations were noted:

- The timing between the 1st and 2nd applications was two days longer at one site (Germansville/PA/2000).
- Crop maintenance pesticides were applied pre-emergent to soybeans at one plot in the 2000 Pennsylvania site which was not approved until after use.
- Soybean sample weights at one plot in the 2000 Iowa site were 1.0-2.0 lbs instead of 2.2 lbs.
- The weight of control radish roots at one plot in the 2000 Iowa site was 3.5 lbs instead of 4.5 lbs.
- Cold weather prevented the normal production of sorghum at one plot in the 2000 Pennsylvania site. Immature seed samples were substituted for that site, and data from a supplementary rotational grain sorghum trial conducted concurrently with the supervised residue trial study was used for mature sorghum grain matrix.
- Wheat grain matrices were to be analysed slightly differently from non-grain matrices, yet the sample preparation of wheat grain was conducted in the same manner as non-grain matrices.

The deviations listed did not appear to have an impact on the regulatory decision.

A. BACKGROUND INFORMATION

BAS 670 336SC (soluble concentrate) Herbicide (also known as BAS 670 00H Herbicide) is the end-use product which contains the active ingredient topramezone. It is a broad-spectrum, post-emergence herbicide used to control grassy and broadleaf weeds in corn. The herbicide is absorbed by the leaves, roots and shoots, then translocated to the growing points of the sensitive weeds. Topramezone belongs to the triketone class of chemicals, which inhibits carotenoid biosynthesis (4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitor). This causes a strong bleaching activity on the growing zones of the shoots within 2-5 days of application. Exposure to light causes necrosis of chlorotic tissues and eventual plant death within 14 days after application.

TABLE A.1. Test Compound Nomenclature

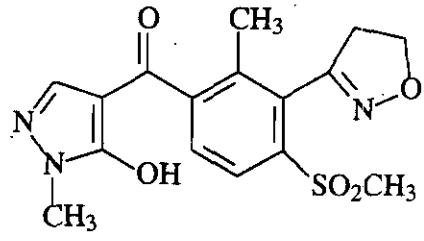
Compound	Chemical Structure 
Common name	Topramezone
Company experimental name	BAS 670 H
IUPAC name	[3-(4,5-dihydro-1,2-oxazol-3-yl)-4-mesyl-*o*-tolyl](5-hydroxy-1-methyl-1*H*-pyrazol-4-yl) methanone
CAS name	[3-(4,5-dihydro-3-isoxazolyl)-2-methyl-4-(methylsulfonyl)phenyl](5-hydroxy-1-methyl-1*H*-pyrazol-4-yl)methanone
Molecular Formula	$C_{16}H_{17}N_3O_5S$
Molecular Mass	363.39 g/mol
CAS #	210631-68-8
End-use product/EP	Soluble Concentrate

TABLE A.2. Physicochemical Properties of Topramezone

Parameter	Value
Melting point/range	220.9°C - 222.2°C
pH	2.9 (1% de-ionized water)
Density (20°C)	1.425 g/cm^3
Water solubility (20°C)	pH: 3, 5, 7, 9 g/L: 0.06, 0.98, 15, 23.4
Solvent solubility (g/100 mL at 20°C)	Acetone <1.0 Acetonitrile <1.0 Dichloromethane 2.5 - 2.9 Ethyl acetate <1.0 Methanol <1.0 N-heptane <1.0 N,N-dimethylformamide 11.4-13.3 1-octanol <1.0 Olive oil <1.0 2-propanol <1.0 Toluene <1.0
Vapour pressure at 20°C and 25°C	< 1.0 x 10^{-12} hPa
Dissociation constant (pK_a)	4.06
Octanol/water partition coefficient Log(K_{OW}) at 20°C	Buffer pH: 4, 7, 9 Log K_{ow}: – 0.81, – 1.52, – 2.34
UV/visible absorption spectrum	λ, nm: 207, 272, 300, 410 ε, mol^{-1}cm^{-1}: 27 077, 8601, 5800, 410

All data came from PMRA Lab Services.

B. EXPERIMENTAL DESIGN

B.1. Study Site Information

TABLE B.1.1 Trial Site Conditions

Trial Identification (City, State/Year)	Soil characteristics				Meteorological data	
	Type	%OM	pH (1:1, soil:water)	CEC meq/100 g	Overall rainfall range/ Irrigation method	Overall T°C range (°F)
Germansville/PA/2000 (3 plots)	Clay Loam	2.2	6.7	8.3	NP/ Overhead Sprinklers	16.6-22.0 (61.8-71.6)
Richland/IA/2000 (3 plots)	Silty Clay Loam	3.3	6.2	19.5	NP/ Overhead sprinklers	26.1-33.3 (79-92)
Germansville/PA/2001 (1 plot)	Silt Loam	NP	NP	NP	Below Normal/ Drip	Normal

OM- Organic matter
CEC- Cation exchange capacity
NP- Not provided

Wind speed during application was between zero and 9 mph. No actual or historical data were provided by the petitioner, except for actual temperatures at the year 2000 sites during the study period. Irrigation was not used unless otherwise indicated.

TABLE B.1.2. Study Use Pattern

Location (City, State/Year)	EP [1]	Application					Tank Mix Adjuvants [4]
		Method/Timing	Vol GPA [2]	Rate g a.i./ha (lb a.i./A)	RTI [3] days	Total Rate g a.i./ha (lb a.i./A)	
Germansville/PA/ 2000 (3 plots)	BAS 670 00H (SC)	Application #1 - Fourth to 6th leaf vegetative stage. Plants 4 to 8 inches tall.	35.4	25 (0.022)	17	101 (0.090)	Booster Plus E Crop oil concentrate + Urea Ammonium Nitrate
		Application #2 - Eighth to 9th leaf vegetative stage. Plant ~24 inches tall.	35.4	76 (0.068)			
Richland/IA/2000 (3 plots)		Application #1 - Sixth to 7th leaf vegetative stage. Plants 18 inches tall.	13.6	24 (0.021)	14	100 (0.089)	Premium Crop oil concentrate + 28% Urea Ammonium Nitrate
		Application #2 - Ninth leaf vegetative stage. Plant 37 inches tall.	10.2	76 (0.068)			
Germansville/PA/ 2001 (1 plot - supplementary)		Application #1 - Second to 4th leaf vegetative stage. Plants 4 to 6 inches tall.	43.8	26 (0.023)	12	102 (0.091)	Booster Plus E & Urea Ammonium Nitrate
		Application #2 - Fourth to 6th leaf vegetative stage. Plants 12 to 18 inches tall.	43.3	76 (0.068)			

[1] EP = End-use Product
[2] Gallons per acre
[3] Retreatment Interval - Days after 1st application until the day of the 2nd application.
[4] The adjuvant rate was 1.25% v/v each.

B.2. Sample Handling and Preparation

Approximately 12-24 radish roots were collected from each plot. Radish tops from 12 separate plants were also collected from each plot. Soybeans were collected at forage (plants 6-8 inches tall), hay (mid-to-full bloom stage), and seed stages. Sorghum was collected at forage (soft-to-hard dough stage), mature grain and stover stages. Winter wheat was collected at forage (plants 6-8 inches tall), hay (between milk and soft dough stage), mature straw and grain stages. Samples were collected and placed into separate labelled sample bags. The untreated control plot was sampled prior to the treated plot(s) on the same day to avoid contamination. All samples were promptly transferred to freezers on the date of collection. Samples remained frozen during storage and shipment to BASF Agro Research (Research Triangle Park, NC). The sample preparation for wheat grain matrices was the same as for non-wheat grain matrices. All samples were placed in a freezer (<-10°C) upon arrival at BASF Agro Research, and remained there until homogenization and analysis. Rotational crop matrices were homogenized with dry ice to a consistency appropriate for analysis. Samples were stored frozen in plastic bags until analysis. The moisture content of selected control and treated rotational crop samples was determined with a Kett Infrared Moisture Determination Balance FD620.

B.3. Analytical Methodology

Residues of topramezone and BAS 670 H 05 (M670H05) in radish (roots and tops), soybean (forage, hay and seed), spinach leaves, sorghum (forage, grain and stover), and winter wheat (forage, hay, grain and straw) matrices were extracted using the data gathering analytical method D0007. Residues were extracted using an accelerated solvent extraction (ASE) process. Residues of topramezone were extracted from non-grain rotational crop matrices with water, while residues in seed or grain matrices (excluding wheat grain) were extracted using acetonitrile:water (1:1, v:v). A 2% aliquot of the extract was removed and cleaned by partitioning with a mixture of 1N HCl:DCM (1:5, v/v) and NaCl. The extract was centrifuged (if necessary), the aqueous layer was discarded, and residues in DCM layer were partitioned into 0.1% NH_4OH. Residues in wheat grain samples were extracted with 1N HCl:DCM (2:5, v:v) saturated with NaCl. Following phase separation, the aqueous layer was discarded and residues in the organic layer were partitioned into 0.1% NH_4OH. The final chromatographic analysis of topramezone and BAS 670 H 05 residues in crop matrices was performed by LC-MS/MS.

Stock solutions were made fresh every 3 months and refrigerated during their use in the study. The LOQ for method D0007 for topramezone and BAS 670 H 05 residues was reported as 0.05 ppm. The LOD of 0.025 ppm was defined as the lowest standard level injected with the analysis set with a signal-to-noise (SIN) ratio between 3-5. Detector response was linear in the range of 0.2 pg/μL (4 pg) to 10 pg/μL (200 pg) for topramezone (coefficient of determination r^2 of 0.9994) and BAS 670 H 05 (coefficient of determination r^2 of 0.9998). Samples (including LOQ) were diluted to fall within the area of the standard curves. The analytical standards of topramezone and BAS 670 H 05 were 99.9% pure. Sample chromatograms of control, spiked, and unknown samples were provided and were free of interference.

C. RESULTS AND DISCUSSION

A total of 7 rotational crop trials were conducted using field or sweet corn with radish, soybean, spinach, grain sorghum, and winter wheat conducted during the 2000 and 2001 growing season. BAS 670 00H (SC formulation) was applied as a foliar broadcast spray over the top of field and sweet corn, using commercial ground application equipment. No actual or historical data were provided by the petitioner about rainfall, and only limited temperature data were provided for the 2000 trial sites. The temperature and rainfall (TABLE B.1.1.) were in some cases below normal in the 2001 trial site, but had no impact on this study. Irrigation was used as needed. Production of sorghum was terminated at one site due to cold weather. Immature sorghum samples were collected and analysed, while a supplementary sorghum rotational crop field trial (Germansville/PA/2001) was used to determine residues in mature sorghum grain.

Method validation for the active ingredient topramezone and BAS 670 H 05 was performed at various spiking levels on radish (root and top), soybean (forage, hay and seed), spinach leaves, sorghum (forage, grain and stover) and winter wheat (forage, hay, grain and straw) and reported in TABLE C.1. Procedural recoveries were corrected for residues detected in the control samples analysed simultaneously. Individual recoveries for topramezone in the different rotational crop

matrices ranged from 68% to 121% (n = 38). Individual recoveries for BAS 670 H 05 in the different rotational crop matrices ranged from 65 to 119% (n = 38). Although some individual recoveries were less than 70% or greater than 120%, the mean and standard deviations were within the acceptable range.

Storage stability data (TABLE C.2) for topramezone were evaluated concurrently with the field trials in radish (root and top - 9 months), soybean (forage - 8 months; hay - 7 months; seed - 5 months), spinach (leaves - 9 months), sorghum (forage - 6 months; grain and stover - 5 months) and winter wheat (forage - 5 months; hay, grain and straw - 2 months) matrices. The maximum actual storage interval in this study was 9 months. The storage stability study was conducted on radish (root) and corn (forage, grain, straw). The data from the storage stability study on corn grain can be translated to soybean seed, sorghum grain and winter wheat grain; and corn forage can be translated to soybean forage, sorghum forage, winter wheat forage, spinach leaves and radish tops. The data from the storage stability study on corn straw can be translated to soybean hay, sorghum straw, winter wheat hay and winter wheat straw. The validated storage interval from the storage stability study for corn matrices and radish root was 18 months. Thus, the storage stability of the different crop matrices in this study were covered by the storage stability study.

The residue levels of topramezone and BAS 670 H 05 in the rotational crop matrices are reported in TABLE C.3. Residue values were not corrected for apparent residues in the untreated controls, or for procedural recoveries. The petitioner provided data for the three required representative crops (roots and tuber vegetables - radish; leafy vegetables - spinach and soybean; small grains - winter wheat and sorghum). A summary of the residue data following 2 applications of BAS 670 00H (SC formulation) is presented in TABLE C.4.1. The maximum residues of topramezone and BAS 670 H 05 were <0.05 ppm (LOQ) for all the rotational crop matrices with PBIs of 28-29 days (radish -root and top; soybean - forage, hay and seed; sorghum - forage, grain and stover) and 90 days (spinach - leaves; winter wheat - forage, hay, grain and straw; radish - root and top).

TABLE C.1. Summary of Concurrent Recoveries of Topramezone from Radish, Soybean, Spinach, Sorghum and Winter Wheat

RAC	Matrix	Spike level (mg/kg)	Sample size (n)	Recoveries (%)		Mean ± Std Dev (%)	
				Topramezone	BAS 670 H 05	Topramezone	BAS 670 H 05
Radish	Root	0.05	3	68, 86, 101	65, 65, 94	85 ± 17	75 ± 17
		0.5	3	77, 88, 95	75, 77, 94	87 ± 9	82 ± 10
	Tops	0.05	2	82, 101	83, 103	92	93
		0.5	2	81, 97	81, 94	89	88
Soybean	Forage	0.05	1	121	102	-	-
		0.5	1	92	87	-	-
	Hay	0.05	1	94	78	-	-
		0.5	1	81	86	-	-
	Seed	0.05	1	98	119	-	-
		0.5	1	95	98	-	-
Spinach	Leaves	0.05	1	110	97	-	-
		0.5	1	101	100	-	-
Sorghum	Forage	0.05	1	82	66	-	-
		0.5	1	79	83	-	-
	Grain	0.01	1	104	99	-	-
		0.05	1	90	89	-	-
		0.10	1	103	91	-	-
		0.5	1	85	83	-	-
	Stover	0.05	1	88	92	-	-
		0.5	1	87	90	-	-
Winter Wheat	Forage	0.05	2	82, 97	78, 82	90	80
		0.5	2	84, 97	89, 93	91	91
	Hay	0.05	2	87, 105	84, 85	96	85
		0.5	2	89, 90	79, 87	90	83
	Grain	0.05	1	104	79	-	-
		0.5	1	93	91	-	-
	Straw	0.05	1	76	70	-	-
		0.5	1	90	92	-	-

Note: The recoveries are less than 70% for radish (root, spike level 0.05 ppm for BAS 670 H 05 and topramezone) and sorghum (forage, spike level of 0.05 ppm for BAS 670 H 05).
- % recovery values for wheat (hay and straw) samples at the 0.05 ppm spiking levels have been corrected.

TABLE C.2. Summary of Storage Conditions

RAC	Extract	Storage Temp. (°C)	Actual Storage Duration (months)	Interval of Demonstrated Storage Stability (months)
Radish root	Root	<-10	9	18 (using translations from corn matrices within the storage stability study)
	Top	<-10	9	
Soybean	Forage	<-10	8	
	Hay	<-10	7	
	Seed	<-10	5	
Spinach	Leaves	<-10	9	
Sorghum	Forage	<-10	6	
	Grain	<-10	5	
	Stover	<-10	5	
Winter wheat	Forage	<-10	5	
	Hay	<-10	2	
	Grain	<-10	2	
	Straw	<-10	2	

TABLE C.3. Topramezone Residues in Rotational Crops

Location (City, State/Year)	Region	Crop/Variety	Commodity	Total Rate g a.i./ha (lb a.i./A)	Harvest DAP[1]	PBI[2] (days)	Residues (ppm)	
							Topramezone	BAS 670 H 05
Germansville/PA/ 2000	1	Radish/ Johnny's - Altglobe	Root	101 (0.090)	26	28	<0.05	<0.05
			Top					
			Root		40	90	<0.05	<0.05
			Top					
		Soybean/ Mycogen 5420N 95778	Forage		34	28	<0.05	<0.05
			Hay		61-64			
			Seed		131			
		Spinach/ Stokes - Melody	Leaves		64	90	<0.05	<0.05
		Sorghum/ Cargill - 647 CAR P800623	Forage		94	28	<0.05	<0.05
			Grain		144			
			Stover		144			
		Winter Wheat/ Maryland Certified - Madison	Forage		230	90	<0.05	<0.05
			Hay		273-276			
			Grain		295			
			Straw					
Richland/IA/ 2000	5	Radish/ French Breakfast	Root	100 (0.089)	28	29	<0.05	<0.05
			Top					
		Radish/ Crimson Giant	Root		47	90	<0.05	<0.05
			Top					
		Soybean/ Pioneer 93B45	Forage		35	29	<0.05	<0.05
			Hay		48-52			
			Seed		98			
		Spinach/ Bloomsdale	Leaves		47	90	<0.05	<0.05
		Sorghum/ Pioneer 87G57	Forage		84	29	<0.05	<0.05
			Grain		98			
			Stover					
		Winter Wheat/ Willcross 738	Forage		47	90	<0.05	<0.05
			Hay		264-291			
			Grain		307			
			Straw					

TABLE C.3. Topramezone Residues in Rotational Crops

Location (City, State/Year)	Region	Crop/Variety	Commodity	Total Rate g a.i./ha (lb a.i./A)	Harvest DAP[1]	PBI[2] (days)	Residues (ppm)	
							Topramezone	BAS 670 H 05
Germansville/PA /2001	1	Sorghum	Forage	102 (0.091)	104	28	NA	NA
			Grain		156		<0.05	<0.05
			Stover		156		NA	NA

[1] DAP = Days After Planting is the number of days between planting and harvesting of the rotational crop.
[2] PBI = Plant-Back Interval is the number of days between the last application of topramezone to the primary crop (field corn) and planting of the rotational crop.
NA - Not analysed
Immature sorghum seed heads were collected and analysed, but were supplemented by another sorghum rotational crop field study (Germansville/PA/2001).

TABLE C.4. Summary of Residue Data in Rotational Crops Following Primary Treatment with Topramezone

Crop	Commodity	Applic. Rate g a.i./ha (lb a.i./A)	PBI (days)	Residue Levels (ppm)						
				n	Min.	Max.	HAFT*	Median	Mean	Std. Dev.
Topramezone										
Radish	Root	100 - 102 (0.089-0.091)	28 and 90	4	<0.05	<0.05	—	—	—	—
	Top									
Soybean	Forage		28-29	2	<0.05	<0.05	—	—	—	—
	Hay			2	<0.05	<0.05	—	—	—	—
	Seed			2	<0.05	<0.05	—	—	—	—
Spinach	Leaves		90	2	<0.05	<0.05	—	—	—	—
Sorghum	Forage		28-29	2	<0.05	<0.05	—	—	—	—
	Grain			3	<0.05	<0.05	—	—	—	—
	Stover			2	<0.05	<0.05	—	—	—	—
Winter Wheat	Forage		90	2	<0.05	<0.05	—	—	—	—
	Hay			2	<0.05	<0.05	—	—	—	—
	Grain			2	<0.05	<0.05	—	—	—	—
	Straw									
BAS 670 H 05										
Radish	Root	100 - 102 (0.089-0.091)	28 and 90	4	<0.05	<0.05	—	—	—	—
	Top									
Soybean	Forage		28-29	2	<0.05	<0.05	—	—	—	—
	Hay			2	<0.05	<0.05	—	—	—	—
	Seed			2	<0.05	<0.05	—	—	—	—
Spinach	Leaves		90	2	<0.05	<0.05	—	—	—	—
Sorghum	Forage		28-29	2	<0.05	<0.05	—	—	—	—
	Grain			3	<0.05	<0.05	—	—	—	—
	Stover			2	<0.05	<0.05	—	—	—	—
Winter Wheat	Forage		90	2	<0.05	<0.05	—	—	—	—
	Hay			2	<0.05	<0.05	—	—	—	—
	Grain			2	<0.05	<0.05	—	—	—	—
	Straw									

* HAFT = Highest Average Field Trial

D. CONCLUSION

The crop rotational field trial data on radish (root and top), soybean (forage, hay and seed), spinach leaves, sorghum (forage, grain and stover) and winter wheat (forage, hay, grain, straw) were deemed acceptable for the determination of residues of topramezone and BAS 670 H 05 when using BAS 670 00H (SC formulation) for foliar treatment on field and sweet corn. The maximum study seasonal application rate was 100 g a.i./ha (0.089 lb a.i./A), with PBIs of 28-29 days (radish -root and top; soybean - forage, hay and seed; sorghum - forage, grain and stover) and 90 days (spinach - leaves; winter wheat - forage, hay, grain and straw; radish - root and top). With this use pattern, residues of the active ingredient topramezone and BAS 670 H 05 are not expected to exceed the LOQ (<0.05 ppm).

E. REFERENCES

Abdel-Baky, S (2002). Validation and Accountability of BASF Method Number D0007 titled "Method for the Determination of BAS 670 H and Its Metabolite (M670H05) Residues in Plant Matrices Using LC/MS/MS." BASF Study Number: 56542. Unpublished study prepared by BASF Corporation Agro Research. 93 pages.

Ellenson, James L. (2002). Final Report: Confined Rotational Crop Study With [^{14}C]-BAS 670 H. BASF Study Number 56739. Unpublished study prepared by BASF Agro Research. 146 p.

Jordan, J., (2002). Storage Stability of BAS 670 H and its Cleaved Acid Metabolite M670H05 in Plant Matrices. BASF Study Number: 56750. Unpublished study prepared by BASF Corporation Agro Research. 67 pages.

F. DOCUMENT TRACKING

RDI: P.V. Shah (3/2/05), RAB1 Chemists (12/8/04), ChemSAC (3/2/05)
G.F. Kramer:806T:CM#2:(703)305-5079:7509C:RAB1
Petition Number: 3F6568
DP #: 310772
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Template Version September 2003

Evaluators

Suzan Mathew — Date: Mar 21/05
Evaluation Officer
FREAS, HED, PMRA

for Stephanie Wille — Date: Mar 21/05
Evaluation Officer
FREAS, HED, PMRA

Peer Reviewer

Sarah Levy, M.S. — Date: May 11, 2005
Chemist
RAB1, HED, EPA

Approved by

Louise G. Croteau — Date: March 22/05
Senior Evaluation Officer
FREAS, HED, PMRA

Ariff Ally, Ph.D. — Date: March 29/05
Section Head
FREAS, HED, PMRA

George F. Kramer, Ph.D. — Date: 4-12-05
Chemist
RAB1, HED, EPA

STUDY REPORTS:

MRID No. 45902413. Ellenson, James L. (2002). Final Report: Confined Rotational Crop Study With [^{14}C]-BAS 670 H. BASF Study Number 56739. Unpublished study prepared by BASF Agro Research. 146 p.

EXECUTIVE SUMMARY:

BASF Agro Research conducted a confined accumulation study with [pyrazole-4-^{14}C] topramezone (5.76 MBq/mg specific radioactivity; >98% radiochemical purity) and [phenyl-U-^{14}C] topramezone (4.65 MBq/mg specific radioactivity; >98% radiochemical purity). Topramezone (also known as BAS 670 H) was applied to bare soil at rates of 0.081-0.091 kg a.i./ha (0.072-0.081 lb a.i./A) for the phenyl-label, and 0.091-0.094 kg a.i./ha (0.081-0.084 lb

a.i./A) for the pyrazole-label. Rotational crops were planted in open-bottom casks in four field plots with plantback intervals (PBIs) of 34 days (radish, mustard greens, and wheat), 99 days (mustard greens and sorghum) and 393 days (mustard greens only). Swiss chard was planted at the 34 day PBI, but the crop failed to grow. Due to climatic conditions, sorghum was planted at the 99 day PBI instead of wheat.

Total radioactive residues (TRRs) in the harvested rotational crop commodities were determined by combustion/LSC (Beckman Liquid Scintillation Counter Model LS9800). The samples were extracted sequentially with water and methanol (MeOH), and the combined MeOH/water extracts were analyzed by high-performance liquid chromatography (HPLC) for the purpose of identification and characterization. All plant samples were analyzed within 3 months of harvest; therefore, no storage stability data were generated. Fractionation procedures of the post-extraction solids (PES) involved extraction with aqueous ammonia solution, acidification with phosphoric acid (H_3PO_4), extraction with dimethylsulfoxide (DMSO), and water and ethanol (EtOH), refluxing with sodium hydroxide (NaOH), acidification with hydrochloric acid (HCl), and drying of the precipitate. The reported limits of quantitation (LOQs) were 0.00258 ppm for phenyl-label samples and 0.00208 ppm for pyrazole-label samples.

At a soil depth of 0-15 cm, the TRRs in both phenyl- and pyrazole-label soil samples declined from 0.452-0.481 ppm (0 days after treatment - DAT) to 0.024 ppm (phenyl-label) by 393 DAT. At the 15-30 cm soil depth, the overall TRRs in both labels reached their highest levels by 85 DAT (pyrazole-label; 0.015 ppm) and 118 DAT (phenyl-label; 0.081 ppm) before declining to near negligible levels by 230 DAT. If the TRRs in a raw agricultural commodity (RAC) sample were below 0.01 ppm, then no further planting of the crop was conducted. RAC samples with TRRs greater than 0.01 ppm were subjected to further analysis to characterize the radioactive residues. The range of overall TRRs in pyrazole- and phenyl-label crop RAC samples at the 34 day PBI were 0.0039-0.0256 ppm for mustard greens; 0.0021-0.0095 ppm in radish tops; 0.0010-0.0030 ppm in radish roots; 0.0753-0.132 ppm for wheat grain; 0.0510-0.092 ppm for wheat straw; 0.0249-0.0518 ppm for wheat forage; and 0.0073-0.0206 ppm for wheat hay. The range of overall TRRs in pyrazole- and phenyl-label crop RAC samples at the 99 day PBI were 0.001-0.004 ppm for sorghum forage; 0.0016-0.0032 ppm for sorghum stover; 0.0019-0.0021 ppm for sorghum grain; and 0.0025-0.0113 ppm for mustard green RACs. The overall TRR in the phenyl-label mustard green RAC at the 393 day PBI was 0.003 ppm.

A total of 88.0-90.6% of the TRRs (0.0034-0.0231 ppm) were extracted with MeOH/water from pyrazole-label and phenyl-label mustard greens from the 34 day PBI. The parent topramezone accounted for the majority of residues (31.0% of the TRRs; 0.0079 ppm) in the phenyl-label mustard greens, while the acid metabolite M670H05 accounted for 10.3% of the TRRs (0.0026 ppm). The parent topramezone was the only metabolite identified in the pyrazole-label sample (47.4% of the TRRs; 0.0018 ppm). Residues characterized as a series of minor peaks/regions comprised 40.6-49.3% of the TRRs (0.0016-0.0126 ppm). The unextractable residues (PES) accounted for 12.7-17.3% of the TRRs (0.0007-0.0033 ppm), for an overall accountability ranging from 103-105%.

A total of 76.1-117% of the TRRs (0.0085-0.109 ppm) were extracted with MeOH/water from phenyl- and pyrazole-label wheat matrices (34 day PBI). The parent compound, topramezone, was either the predominant residue or the only residue identified in the pyrazole-label wheat matrices (13.3-64.4% of the TRRs; 0.0043-0.0160 ppm), and in phenyl-label forage and hay (11.2-12.4% of the TRRs; 0.0064-00.0147 ppm). M670H05 was the predominant metabolite identified in the phenyl-label grain (45.0% of the TRRs; 0.0093 ppm). In the other radiolabelled wheat matrices, the metabolites M670H02 and M670H05 comprised less than 7% of the TRRs (<0.01 ppm). After exhaustive extraction/fractionation procedures, a total of 45.5-72.3% of the TRRs were characterized in the phenyl-and pyrazole-label wheat RAC extracts (0.0038-0.0828 ppm). The final unextractable residues in all the wheat matrices after fractionation were ≤20.2% of the TRRs (≤0.0182 ppm), for accountabilities ranging from 92.4-126%.

The proposed metabolism of topramezone involved the hydrolysis of the parent to form the free acid M670H05. The pyrazole moiety was catabolized after cleavage, then reincorporated into natural products. Parent-related adducts were formed from the des-methylation of the pyrazole ring. Hydroxylation of the isoxazole ring formed M670H02, then reincorporation into natural products occurred after further ring degradation.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the confined rotational crop residue data are classified as scientifically acceptable. No second spectroscopic method was used to confirm identification data in this study.

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document [DP# 310772] and in Canada's Regulatory Decision Document.

COMPLIANCE:

Signed and dated good laboratory practice (GLP), Quality Assurance (not dated), and Data Confidentiality statements were provided. Deviations from the protocol included minor editorial changes; replacing the study director; correction of a specified treatment date; revision of the study design to both better represent normal agricultural practices, and the addition of extra plots. These deviations do not impact the validity of the study.

A. BACKGROUND INFORMATION

BAS 670 336SC (soluble concentrate) Herbicide (also known as BAS 670 00H Herbicide) is the end-use product which contains the active ingredient topramezone. It is a broad-spectrum, post-emergence herbicide used to control grassy and broadleaf weeds in corn. The herbicide is absorbed by the leaves, roots and shoots, then translocated to the growing points of the sensitive weeds. Topramezone belongs to the triketone class of chemicals, which inhibits carotenoid biosynthesis (HPPD (4-hydroxyphenylpyruvate dioxygenase) inhibitor)). This causes a strong bleaching activity on the growing zones of the shoots within 2-5 days of application. Exposure to light causes necrosis of chlorotic tissues and eventual plant death within 14 days after application.

TABLE A.1. Test Compound Nomenclature

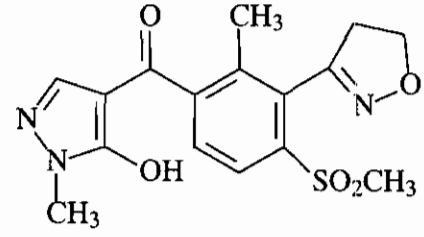
Compound	Chemical Structure 
Common name	Topramezone
Company experimental name	BAS 670 H
IUPAC name	[3-(4,5-dihydro-1,2-oxazol-3-yl)-4-mesyl-*o*-tolyl](5-hydroxy-1-methyl-1*H*-pyrazol-4-yl) methanone
CAS name	[3-(4,5-dihydro-3-isoxazolyl)-2-methyl-4-(methylsulfonyl)phenyl](5-hydroxy-1-methyl-1*H*-pyrazol-4-yl)methanone
Molecular Formula	$C_{16}H_{17}N_3O_5S$
Molecular Mass	363.39 g/mol
CAS #	210631-68-8
End-use product/EP	Soluble Concentrate

TABLE A.2. Physicochemical Properties of the Technical Grade Test Compound

Parameter	Value (all data from PMRA Lab Services)
Melting point/range	220.9°C-222.2°C
pH	2.9 (1% de-ionized water)
Density	1.425 g/cm^3
Water solubility (20°C)	pH / g/L 3 / 0.06 5 / 0.98 7 / 15 9 / 23.4
Solvent solubility (g/100 mL at 20°C)	Solvent / Solubility acetone / < 1 acetonitrile / < 1 dichloromethane / 2.5 –2.9 ethyl acetate / < 1 methanol / < 1 n-heptane / < 1 N,N-dimethylformamide / 11.4-13.3 1-octanol / < 1 olive oil / < 1 2-propanol / < 1 toluene / < 1
Vapor pressure at 20°C and 25°C	< 1 x 10^{-12} hPa
Dissociation constant (pK_a)	4.06 at 20°C
Octanol/water partition coefficient Log(K_{OW})	Buffer pH / Log K_{ow} 4 / – 0.81 7 / – 1.52 9 / – 2.34
UV/visible absorption spectrum	λ, nm / ξ, mol^{-1}cm^{-1} 207 / 27 077 272 / 8601 300 / 5800 410 / 410

B. EXPERIMENTAL DESIGN

B.1. Test Site and Crop Information

Plants were maintained using normal agricultural practices. The study was conducted in five open-bottom casks [measuring 1.22 m x 2.44 m x 1.22 m (48.0 in. x 96.1 in. x 48.0 in.)] and placed in the ground such that the cask walls were approximately 30 cm above ground. The casks were filled with soil to ground level. Following direct application to the soil, the soil was aged for nominal targets of 30, 90, and 360 days (actual aging period was 34, 99, and 393 days). During the aging period, water losses were compensated by adding additional water through irrigation. An initial application of fertilizer was pre-plant incorporated into the soil, along with subsequent applications done according to common agricultural practices. After the aging period and prior to planting, plowing was simulated by hand-tilling the plots to a depth of about 10 cm. Planting was accomplished by hand-sowing seeds into shallow furrows and then covering the seeds with soil.

Limited temperature and precipitation data during the study period were provided. Temperatures were normal, and rainfall was slightly lower than normal over the test period. Irrigation was conducted when necessary.

TABLE B.1.1. Test Site Information

Testing Environment and location	Soil characteristics						
	Type	% Sand	% Silt	% Clay	%OM	pH	CEC
Open-bottom casks placed in outdoor test plots - Plots 11and 12 (pyrazole), plots 14 and 15 (phenyl)	Loamy sand (plot 11) Sandy Loam (plots 12, 14, 15)	76 - 78	12 - 14	8 - 10	1.3 - 1.6	4.5 - 5.6	4.9 - 5.1

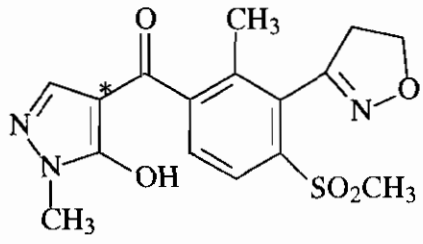
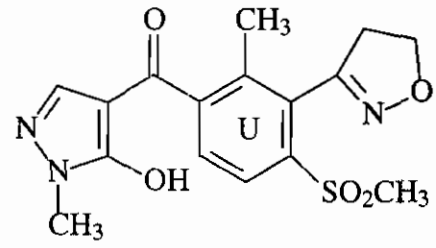
OM = organic matter
CEC = cation exchange capacity

TABLE B.1.2. Crop Information

Crop/crop group	Variety	Plant-back intervals (PBIs) (days)	Growth stage at harvest	Harvested RAC	Harvesting procedure
Radish	*Sparkler*	34	Immature, Mature	Radish tops, Radish roots	Cut by hand
Mustard	*Florida Broad Leaf*	34 99 393	Immature, Mature	Mustard greens	Cut by hand
Wheat	*Coker 9835*	34	Immature, Forage, Mature	Forage, Grain, Hay, Straw	Cut by hand
Sorghum	*G820*	99	Immature, Forage, Mature	Forage, Grain, Stover	Cut by hand

B.2. Test Materials

TABLE B.2.1. Test Material Characteristics

Chemical structure		
Radiolabel position	pyrazole-4-^{14}C	uniformly labelled phenyl ring
Lot No.	706-1013	714-1026
Purity	>98% (by HPLC)	>98% (by HPLC)
Specific activity (Bq)*	2.88 MBq/mg	2.33 MBq/mg

*Bq = disintegrations per second

B.3. Study Use Pattern

TABLE B.3.1. Use Pattern Information

Chemical name	Topramezone
Application method	Applied to bare soil by brass sprayer with Tee-Jet fan spray nozzle.
Application rate	Phenyl - 0.081-0.091 kg a.i./ha (0.072-0.081 lb a.i./A) Pyrazole - 0.091-0.094 kg a.i./ha (0.081-0.084 lb a.i./A)
Number of applications	1
Timing of applications	Pre-plant
PHI (days)	N/A

B.4. Identification/Characterization of Residues

B.4.1. Sample Handling and Preparation

All plant samples were cut by hand, sealed in plastic bags, packed in dry ice, and immediately shipped to BASF Agro Research. Soil cores were obtained from plots 11, 12, 14, and 15 via a plastic soil sampling tube submerged into the soil surface. Soil samples were sealed in plastic bags, placed in coolers, and transported directly to the research facility. All samples were stored frozen (<-10°C) at the facility until needed for preparation and analysis.

All samples were finely ground under liquid nitrogen (Brinkmann Polytron homogenizer fitted with a PTA 36/3 generator). Homogenized aliquots of solid samples were combusted (Packard System 307 Robotic Combustor or a manual Harvey Biological Oxidizer). The $^{14}CO_2$ generated was trapped either in Harvey Cocktail (manual combustor) or Carbosorb (robotic combustor), then combined with Permaflour scintillator for measuring by LSC (Beckman LSC Model LS9800). Liquid samples were mixed with a scintillator and quantified by LSC. Oxidizer efficiency (determined by a ^{14}C-Spec-Check supplied by Packard) was used to correct measurements of radioactivity.

The homogenized plant material was extracted with water, followed by a MeOH extraction. The combined aliquots from the MeOH:water extract were concentrated by a rotational evaporator, filtered, then analysed by LSC and radio-HPLC. The concentrated samples were diluted with the solvent mixture used as the HPLC mobile phase. Solutions of reference standards were similarly prepared and analysed with the same HPLC gradient systems. The retention times were compared with the retention time from the peaks of interest. Losses due to evaporation or precipitation of matrix components were monitored. Different subsamples were used in each extraction step.

Further characterization was conducted if residues remaining after liquid extraction were greater than 0.01 ppm. Radioactivity in the PES of the crop matrices were fractionated into different classes of natural products using schemes based on the procedure of Honeycutt and Adler (1975). Samples were analysed sequentially, and involved extraction with aqueous ammonia solution; acidification to pH 4.2 with 2N H_3PO_4 (protein fraction); extraction with DMSO and water (9:1, v:v); addition of EtOH to the DMSO/water extract (starch fraction); further extraction of solids by refluxing and washing with a 10% NaOH solution (cellulose fraction) for 3 hours before drying; acidification to pH 1 with concentrated HCl (polysaccharide fraction); then the drying of the remaining precipitate (lignin). All subsample supernatants and hydrolysates from each step were filtered and analysed by LSC.

B.4.2. Analytical Methodology

Chromatographic determinations were conducted using a Hewlett Packard (series 1050) HPLC, with a Model 2B IN/US ^{14}C detector. Either Columbus or PRP-1 columns were used, with the eluants being 10mM Phosphate buffer (pH 7.0) and MeOH. For the liquid cell, the scintillant

cocktail (ULTIMA-FLOW) was mixed 3:1 with the column eluant. Solid cells (containing Li-doped glass as the scintillant) of differing internal volumes were used. Reference and study samples were injected/applied under similar conditions for characterization and identification.

HPLC methods used in this study are summarized in TABLE B.4.2.1.

TABLE B.4.2.1. Summary of HPLC Methods

HPLC Method Number	Column	Eluents		Flow Rate [mL/min]	Gradients		
		A	B		Time [min]	%A	%B
1 (CLMBSS TD.M)	Columbus	10mM Phosphate Buffer; pH 7.00	MeOH	1	0-20 21-26 28-35	95-20 5 95	5-80 95 5
2 (PRP-1-10.M)	PRP-1	10mM Phosphate Buffer; pH 7.00	MeOH	1.5	0-20 21-26 28-35	95-20 5 95	5-80 95 5

The identification of the metabolite residues was via co-HPLC and/or comparison of retention times of reference standards or isolated metabolites.

All matrices were extracted and analysed within 3 months of harvest; thus, no storage stability studies were conducted on the stored matrices or extracts.

Experimental deviations from typical retention times were noted, which may have been due to the nature of the matrix (i.e., condition, constituents, ratio, and radioactivity), temperature changes, small variations in the eluant composition, or different column batches used within the study. The LOQ was calculated by the formula:

2 * Background /(aliquot weight * Specific Activity)

Reported LOQs were 0.00258 ppm for phenyl-label samples and 0.00208 ppm for pyrazole-label samples.

C. RESULTS AND DISCUSSION

When overall detected residues in the RAC were below 0.01 ppm, no further extraction work was conducted on the sample. The residue levels in mustard greens were above 0.01 ppm at both the 34 and 99 day PBI. Wheat matrices had residues above 0.01 ppm at the 34 day PBI, but due to climatic conditions sorghum was planted at the 99 day PBI in its place. No extraction work was performed on radish matrices at the 34 day PBI, on sorghum matrices at the 99 day PBI or mustard green RACs at the 393 day PBI (residues <0.01 ppm).

The residues in this study were identified by only one analytical technique (HPLC) with no

confirmatory spectroscopic data. The residues were identified by the comparison of retention times to known standards using HPLC chromatography with two different column phases - a polymer-based reverse-phase column (PRP-1, HPLC method 2) and a silica-based reverse-phase column (Columbus, HPLC method 1). Each column used MeOH and 10 mM phosphate buffer (pH 7) as eluants. Although the two methods had different flow rates (1.5 mL/min for PRP-1, and 1.0 mL/min for the Columbus), they both had the same mobile phase and gradient program. Both methods were able to identify all of the metabolites. HPLC method 1 was used to identify and characterize residues in the 34 day PBI mustard green and wheat RAC matrices. Co-chromatography (co-HPLC), using radiolabelled reference samples and the 2 different HPLC methods, confirmed the identities of parent topramezone, M670H02 and M670H05 in the 34-day PBI mustard green and wheat RAC samples.

The distribution of overall TRRs for soil and plant matrices are reported in TABLE C.2.1. At a soil depth of 0-15 cm, the TRRs in both phenyl- and pyrazole-label soil samples declined from 0.452-0.481 ppm (0 DAT) to 0.024 ppm (phenyl-label) by 393 DAT. At the 15-30 cm soil depth, the overall TRRs in both labels reached their highest levels by 85 DAT (pyrazole-label; 0.015 ppm) and 118 DAT (phenyl-label; 0.081 ppm) before declining to levels ≤0.001 ppm by 230 DAT. This result indicated that topramezone degraded quickly in soil, consistent with the petitioner's findings that the half-life of topramezone (from a soil metabolism study) is 31 days in sandy loam soil. Thus, there would be fewer residues available to be taken up by the plants at longer PBIs.

The overall TRRs in both phenyl- and pyrazole-label crop samples were highest in the early immature stages of radish (54 DAT; 0.0139-0.0202 ppm); sorghum (118 DAT; 0.008-0.0190 ppm); and mustard greens (54 DAT; 0.0276-0.0492 ppm and 118 DAT; 0.0104-0.0229 ppm). Residue levels declined in the mature RAC samples of these crops, as TRRs ranged from 0.0021-0.0095 ppm in radish tops; 0.0010-0.0030 ppm in radish roots; 0.001-0.004 ppm in sorghum forage; 0.0016-0.0032 ppm in sorghum stover; 0.0019-0.0021 ppm in sorghum grain; and 0.0039-0.0256 ppm and 0.0025-0.0113 ppm in the 85 and 161 DAT mustard green samples, respectively. The exception was wheat, where the highest residues were seen in mature grain (118 DAT; 0.0753-0.132 ppm) and mature straw (161 DAT; 0.0510-0.0920 ppm). The TRRs in immature wheat (54 and 74 DAT) and forage (118 DAT) ranged from 0.0249-0.0650 ppm, while the TRRs in hay were only 0.0073-0.0206 ppm (161 DAT). Slow uptake of the chemical by the maturing wheat stalk may have been the cause of the higher TRRs reported in grain compared to immature samples. As per FIGURES C.2.2.-C.2.6, a greater percentage of the TRRs were attributed to the phenyl-label samples compared to the pyrazole-label samples.

The majority of both the phenyl-label and pyrazole-label residues were in the water extracts of the mustard green samples (34 day PBI) at 83.0-89.9% of the TRRs (0.0035-0.0213 ppm), while less than 9% of the TRRs were extracted with MeOH/water (≤0.0023 ppm). The parent topramezone was identified from the combined MeOH/water extract as 31.0-47.4% of the TRRs (0.0018-0.0079 ppm). M670H05 was identified only in the phenyl-label samples as 10.3% of the TRRs (0.0026 ppm), while M670H02 was not identified in either label. Approximately 40.6-49.3% of the TRRs were characterized as a series of minor peaks/regions (0.0016-0.0126 ppm).

The unextractable residues comprised 12.7-17.3% of the TRRs (0.0007-0.0033 ppm), for accountabilities ranging from 103-105% (TABLE C.2.3.1). While the unextractable residues were greater than 10% of the TRRs, the absolute residue levels were well below 0.05 ppm, so no further extraction measures were necessary. For the 99 day PBI samples (both labels), the majority of extractable residues were found in the water extract (60.2-70.3% of the TRRs; 0.0015-0.0079 ppm), while less than 16% of the TRRs were found in the MeOH extract (≤0.0012 ppm). Approximately 17.3-32.6% of the TRRs (0.0008-0.0019 ppm) remained unextractable. No further analyses by HPLC were conducted on the 99 day PBI samples.

As per TABLE C.2.2.2, the majority of residues were found in the water extract of phenyl-label wheat matrices (29.3-71.4% of the TRRs; 0.0147-0.0637 ppm), while less than 7% of the TRRs (≤0.0077 ppm) were found in the MeOH extracts. The parent topramezone was the predominant metabolite identified from the composite MeOH/water extracts for forage and stover (11.2-12.4% of the TRRs; 0.0064-0.0147 ppm). M670H02 and M670H05 were considered minor metabolites in forage, hay, and straw (0.85-6.51% of the TRRs; 0.0008-0.0079 ppm). In wheat grain, M670H05 accounted for nearly one-half of identified residues (45.0% of the TRRs; 0.0093 ppm), while the parent topramezone accounted for 16.3% of the TRRs (0.0034 ppm). M670H02 was not identified in grain. Approximately 11.4-26.4% of the TRRs (0.0044-0.0240 ppm) were characterized as a series of minor peaks/regions for all wheat matrices. Unextractable residues from combustion/LSC were 26.3-80.2% of the TRRs (0.0054-0.0768 ppm). The PES were subjected to fractionation procedures to further characterize residues. The procedures were exhaustive, resulting in the further characterization of 24.9-60.4% of the TRRs (0.0052-0.0588 ppm). Generally, residue levels were highest in the ammonia soluble fraction (5.24-19.9% of the TRRs; 0.0011-0.0263 ppm), and in the soluble polysaccharides from the NaOH soluble fraction (5.67-19.3% of the TRRs; 0.0012-0.01 ppm). Residue levels were also high in the protein fraction of the hay sample (11.4% of the TRRs; 0.0151 ppm) and the DMSO soluble fraction of the straw sample (14.7% of the TRRs; 0.0135 ppm). Starch, lignin and cellulose contributed to less than 7% of the TRRs that were characterized (≤0.0057 ppm). These results indicate that topramezone underwent extensive degradation before being re-incorporated into various natural products. The remaining bound residues accounted for up to 19.8% of the TRRs (≤0.0180 ppm) after fractionation, for an overall accountability ranging from 94.8-109% (TABLE C.2.3.2).

As per TABLE C.2.2.3, the majority of residues were found in the water extract of pyrazole-label wheat matrices (24.2-51.0% of the TRRs; 0.0037-0.0273 ppm), while less than 9.0% of the TRRs (≤0.0035 ppm) were found in the MeOH extracts. The parent topramezone was the predominant metabolite identified from the composite MeOH/water extracts in wheat forage (17.0% of the TRRs; 0.0043 ppm), and was the only metabolite identified in hay, straw, and grain (13.3-64.4% of the TRRs; 0.0047-0.0160 ppm). M670H02 was identified in forage alone at 4.13% of the TRRs (0.001 ppm), and M670H05 was not identified in any matrix. Approximately 2.03-9.21% of the TRRs (0.0005-0.0058 ppm) were characterized as a series of minor peaks/regions for all wheat matrices. Unextractable residues contributed to 54.7-81.0% of the TRRs (0.0040-0.0519 ppm). The residues characterized after fractionation accounted for 46.4-60.8% of the TRRs (0.0033-0.0395 ppm). The highest residue levels from fractionation were in the soluble polysaccharides fraction for forage and hay (25.8-29.8% of the TRRs;

0.0074-0.0194 ppm). The highest residues in straw were in the DMSO soluble fraction (13.6% of the TRRs; 0.0069 ppm), and in the starch fraction for grain (20.6% of the TRRs; 0.0015 ppm). High residue levels were also characterized in the ammonia soluble fraction for all the wheat matrices (5.74-15.5% of the TRRs; 0.0004-0.0116 ppm). Lignin and cellulose contributed to less than 10% of the TRRs that were characterized (≤0.0051 ppm). These results indicate that topramezone underwent extensive degradation before being re-incorporated into various natural products. The remaining bound residues accounted for 6.96-20.2% of the TRRs (0.0007-0.0124 ppm) after fractionation, for an overall accountability ranging from 92.4-126% (TABLE C.2.3.3).

Fewer metabolites were identified in the pyrazole-label samples when compared to the phenyl-label samples in wheat, and an increased number of bound residues were reported in the pyrazole-label samples. This result could indicate that the pyrazole ring was more readily broken down and reincorporated into natural products compared to the phenyl ring. Except for phenyl-label straw and grain, the parent topramezone was the predominant metabolite identified in the mustard green and wheat RAC matrices of both labels (FIGURES C.2.7. and C.2.8). Topramezone was generally the only metabolite identified in the pyrazole-label samples, while more of the metabolites M670H02 and M670H05 were identified in the phenyl-label samples. Because the structure of M670H05 involved the pyrazole ring being cleaved, the metabolite was not radiochemically visible in the pyrazole-label samples.

The majority of residues in the wheat and mustard green RACs (both labels) were found in the water extract, rather than the MeOH extract. This result indicated high water solubility for topramezone.

The proposed metabolism of topramezone involves the hydrolysis of the parent to form the free acid M670H05. Des-methylation of the pyrazole ring causes the formation of parent-related adducts. Hydroxylation of the isoxazole ring (at the 4th position) forms the metabolite M670H02. Further degradation of the phenyl and isoxazole rings results in reincorporation into sugars and amino acids.

C.1. Storage Stability

TABLE C.1. Summary of Storage Conditions

Matrix	Plantback Interval (days)	Storage Temp. (°C)	Actual Storage Duration	Interval of Demonstrated Storage Stability (months)
Mustard greens, Wheat forage, Wheat hay, Wheat grain, Wheat straw	34	<-10	0.92-2.76 months (28-84 days)	18 (translated from corn matrices with the storage stability study)

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

TABLE C.2.1. Total Radioactive Residues (TRRs) in Soil, Radish, Wheat, Sorghum and Mustard Greens

Matrix	Days after treatment (DAT)	Pyrazole	Phenyl
		ppm	ppm
Soil	0	0.481	0.452
	34 (0-15 cm)	0.078	0.081
	(15-30 cm)	0.003	0.002
	54 (0-15 cm)	0.081	0.071
	(15-30 cm)	0.000	0.003
	74 (0-15 cm)	0.032	0.046
	(15-30 cm)	0.000	0.002
	85 (0-15 cm)	0.113	0.145
	(15-30 cm)	0.015	0.029
	99 (0-15 cm)	0.037	0.045
	(15-30 cm)	0.000	0.004
	118 (0-15 cm)	0.073	0.032
	(15-30 cm)	0.005	0.081
	161 (0-15 cm)	0.029	0.036
	(15-30 cm)	0.001	0.002
	230 (0-15 cm)	0.016	0.019
	(15-30 cm)	0.001	0.000
	393 (0-15 cm)	Not Taken	0.024
Radish:			
34 day PBI			
Immature	54	0.0139	0.0202
	74	0.0021	0.0055
Tops	85	0.0021	0.0095
Roots	85	0.0010	0.0030

Matrix	DAT	Pyrazole	Phenyl
		ppm	ppm
Wheat:			
34 day PBI			
Immature	54	0.0388	0.0650
	74	0.0266	0.0308
Forage	118	0.0249	0.0518
Hay	118	0.0753	0.1321
Grain	161	0.0073	0.0206
Straw	161	0.0506	0.0918
Sorghum:			
99 day PBI			
Immature	118	0.0080	0.0190
	140	0.0030	0.0060
Forage	161	0.001	0.004
Stover	230	0.0016	0.0032
Grain	230	0.0021	0.0019
Mustard Greens:			
34 day PBI			
Immature	54	0.0276	0.0492
	74	0.0098	0.0216
Mustard Greens RAC	85	0.0039	0.0256
99 day PBI			
Immature	118	0.0104	0.0229
	140	0.0056	0.0182
Mustard Greens RAC	161	0.0025	0.0113
393 day PBI			
Mustard Greens RAC	442	Not planted	0.003

TABLE C.2.2.1 Distribution of the Parent and the Metabolites in Rotational Crop Mustard Greens Matrices when Dosed with Pyrazole and Phenyl-label Topramezone*

Metabolite Fraction	34 day PBI -Pyrazole (0.0039 ppm)		34 day PBI -Phenyl (0.0256 ppm)		99 day PBI-Pyrazole (0.0025 ppm)		99 day PBI -Phenyl (0.0113 ppm)	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
Water Extract	89.9	0.0035	83.0	0.0213	60.2	0.0015	70.3	0.0079
MeOH extract	4.86	0.0002	8.93	0.0023	15.7	<0.001	10.5	0.0012
Combined MeOH/H_2O extract	76.6	0.0030	90.5	0.0232	50.4	0.013	64.0	0.0072
Topramezone	47.4	0.0018	31.0	0.0079	NA	NA	NA	NA
M670H02	—	—	—	—	NA	NA	NA	NA
M670H05	—	—	10.3	0.0026	NA	NA	NA	NA
Unknown Pyrazole: 20 peaks, each ≤0.0003 ppm Phenyl: 16 peaks, each ≤0.0066 ppm	40.6	0.0016	49.3	0.0126	NA	NA	NA	NA
Unextractable (PES)	17.3	0.0007	12.7	0.0033	32.6	0	17.3	0.0019

* Different subsamples were used in each extraction step.
NA - not analysed

TABLE C.2.2.2 Distribution of the Parent and the Metabolites in Rotational Crop Wheat Matrices when Dosed with Phenyl-label Topramezone at the 34 day PBI*

Metabolite Fraction	Wheat Forage (0.0518 ppm)		Wheat Hay (0.1321 ppm)		Wheat Straw (0.0920 ppm)		Wheat Grain (0.0206 ppm)	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
Water Extract	63.5	0.0329	48.3	0.0637	29.3	0.027	71.4	0.0147
MeOH extract	3.7	0.0019	5.8	0.0077	3.81	<0.010	6.83	0.001
Combined MeOH/H_2O extract	48.9	0.0253	38.1	0.0503	18.1	0 017	81.8	0.0169
Topramezone	12.4	0.0064	11.2	0.0147	1.57	<0.010	16.3	0.003
M670H02	3.57	0.0018	2.91	0.0038	4.28	<0.010	—	—
M670H05	6.51	0.0034	5.96	0.0079	0.85	<0.010	45	0.009
Unknown **Forage:** 19 peaks/regions, each at ≤0.0016 ppm **Hay:** 25 peaks/regions, each at ≤0.0028 ppm **Straw:** 5 peaks/regions, each at ≤0.0074 ppm **Grain:** 7 peaks/regions, each at ≤0.0015 ppm	26.4	0.0137	18.1	0.024	11.4	0.011	20.6	0.004
Unextractable (PES)[1]	45.9	0.0238	58.2	0.0768	80.2	0.074	26.3	0.005
Ammonia Soluble Fraction	18.7	0.0097	19.9	0.0263	18.1	0.017	5.24	0.001
Protein (ammonia pellet)	1.34	0.001	11.4	0.0151	0.44	<0.001	2.52	<0.001
DMSO Soluble Fraction	4.95	0.0026	4.13	0.0055	14.7	0.014	5.0	0.001
Starch (DMSO precipitate)	3.13	0.0016	0.72	0.001	4.66	<0.01	5.22	0.001
Soluble Polysaccharides (NaOH Soluble Fraction)	19.3	0.01	6.87	0.0091	10.3	0.01	5.67	0.001
Lignin (insoluble residue)	3.07	0.0016	0.72	0.001	6.2	0 01	0.86	<0.001
Cellulose	0.97	0.001	0.57	0.001	6.04	0.01	0.36	<0.001
Final Unextractable [2]	0	0	13.8	0.018	19.8	0.018	1.42	<0.001

* Different subsamples were used in each extraction step

1 PES subsample used for fractionation and further analysis

2 Residues remaining after exhaustive extractions

TABLE C.2.2.3. Distribution of the Parent and the Metabolites in Rotational Crop Wheat Matrices when Dosed with Pyrazole-label Topramezone at the 34 day PBI*

Metabolite Fraction	Wheat Forage (0.0249 ppm)		Wheat Hay (0.0753 ppm)		Wheat Straw (0.0510 ppm)		Wheat Grain (0.0073 ppm)	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
Water Extract	48.4	0.0121	36.2	0.0273	24.2	0.012	51	0.0037
MeOH extract	3.79	0.001	4.64	0.0035	4.37	<0.01	8.91	0.0007
Combined MeOH/H_2O extract	31.6	0.0079	29	0.0218	15.4	0.01	70.9	0.0052
Topramezone	17	0.0043	21.3	0.016	13.3	0.01	64.4	0.0047
M670H02	4.13	0.001	—	—	—	—	—	—
M670H05	—	—	—	—	—	—	—	—
Unknown **Forage:** 11 peaks/regions, each at ≤0.0004 ppm **Hay:** 5 peaks/regions, each at ≤0.0034 ppm **Straw:** 2 peaks/regions, each at ≤0.0007 ppm **Grain:** 1 peak/region at 0.0005 ppm	9.21	0.0024	7.66	0.0058	2.03	<0.01	6.53	0.0005
Unextractable (PES) [1]	61.3	0.0153	68.9	0.0519	81	0.041	54.7	0.0040
Ammonia Soluble Fraction	13.3	0.0033	15.5	0.0116	12.0	0.01	5.74	0.0004
Protein (ammonia pellet)	2.04	0.001	3.60	0.0027	5.91	<0.01	3.06	0.0002
DMSO Soluble Fraction	7.26	0.0018	5.36	0.004	13.6	0.01	5.74	0.0004
Starch (DMSO precipitate)	0.52	<0.001	0.69	0.001	3.27	<0.01	20.6	0.0015
Soluble Polysaccharides (NaOH Soluble Fraction)	29.8	0.0074	25.8	0.0194	11.1	0.01	9.97	0.0007
Lignin (insoluble residue)	1.28	<0.001	1.15	0.001	9.97	0.01	0.48	0.0000
Cellulose	0.17	<0.001	0.53	<0.001	4.96	<0.01	0.80	0.0001
Final Unextractable [2]	6.96	0.0019	16.3	0.0124	20.2	0.01	8.28	0.0007

* Different subsamples were used in each extraction step
1 PES subsample used for fractionation and further analysis
2 Residues remaining after exhaustive extractions

Table C.2.3.1 Summary of Characterization and Identification of Radioactive Residues in Rotational Crop Mustard Green Matrices Following Application of Phenyl-label Topramezone at 0.081-0.091 kg a.i./ha (0.072-0.081 lb a.i./A), and Pyrazole-label Topramezone at 0.091-0.094 kg a.i./ha (0.081-0.084 lb a.i./A)

Compound	34 day PBI - Pyrazole (0.0039 ppm)		34 day PBI - Phenyl (0.0256 ppm)	
	% TRR	ppm	% TRR	ppm
Total identified	47.4	0.0018	41.2	0.0105
Topramezone	47.4	0.0018	31	0.0079
M670H02	—	—	—	—
M670H05	—	—	10.3	0.0026
Total characterized	40.6	0.0016	49.3	0.0126
Total extractable	88.0	0.0034	90.6	0.0231
Unextractable (PES)	17.3	0.0007	12.7	0.0033
Accountability [1]	105		103	

[1] Accountability = (Total extractable + Total unextractable)/(TRRs from combustion analysis) * 100.

Table C.2.3.2 Summary of Characterization and Identification of Radioactive Residues in Rotational Crop Wheat Matrices Following Application of Phenyl-label Topramezone at 0.081-0.091 kg a.i./ha (0.072-0.081 lb a.i./A) for the 34 day PBI

Compound	Wheat Forage (0.0518 ppm)		Wheat Hay (0.1321 ppm)		Wheat Straw (0.0920 ppm)		Wheat Grain (0.0206 ppm)	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
Total identified	22.5	0.0116	20.0	0.0264	6.70	0.0061	61.2	0.0127
Topramezone	12.4	0.0064	11.2	0.0147	1.57	0.0014	16.3	0.0034
M670H02	3.57	0.0018	2.91	0.0038	4.28	0.0039	—	—
M670H05	6.51	0.0034	5.96	0.0079	0.85	0.001	45.0	0.0093
Total characterized	72.3	0.0375	62.5	0.0828	71.8	0.0662	45.5	0.0096
Final extractable	94.8	0.0491	82.5	0.1092	78.5	0.0723	107	0.0223
Final unextractable (PES) [1]	0	0	13.8	0.018	19.8	0.0182	1.42	0.0002
Accountability [2]	94.8%		96.3%		98.4%		109%	

[1] Residues remaining after exhaustive extractions

[2] Accountability = (Total extractable + Total unextractable)/(TRRs from combustion analysis) * 100.

Table C.2.3.3 Summary of Characterization and Identification of Radioactive Residues in Rotational Crop Wheat Matrices Following Application of Pyrazole-label Topramezone at 0.091-0.094 kg a.i./ha (0.081-0.084 lb a.i./A) for the 34 day PBI

Compound	Wheat Forage (0.0249 ppm)		Wheat Hay (0.0753 ppm)		Wheat Straw (0.0510 ppm)		Wheat Grain (0.0073 ppm)	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
Total identified	21.1	0.0053	21.3	0.016	13.3	0.0068	64.4	0.0047
Topramezone	17	0.0043	21.3	0.016	13.3	0.0068	64.4	0.0047
M670H02	4.13	0.001	—	—	—	—	—	—
M670H05	—	—	—	—	—	—	—	—
Total characterized	63.6	0.0158	60.3	0.0453	62.8	0.032	52.9	0.0038
Final extractable	84.7	0.0211	81.6	0.0613	76.1	0.0388	117	0.0085
Final unextractable (PES) [1]	6.96	0.0019	16.3	0.0124	20.2	0.0104	8.28	0.0007
Accountability [2]	92.4%		97.9%		96.5%		126%	

[1] Residues remaining after exhaustive extractions

[2] Accountability = (Total extractable + Total unextractable)/(TRRs from combustion analysis) * 100.

FIGURE C.2.1. TRRs in Soil (0-15 cm depth) Treated with Radiolabelled Topramezone

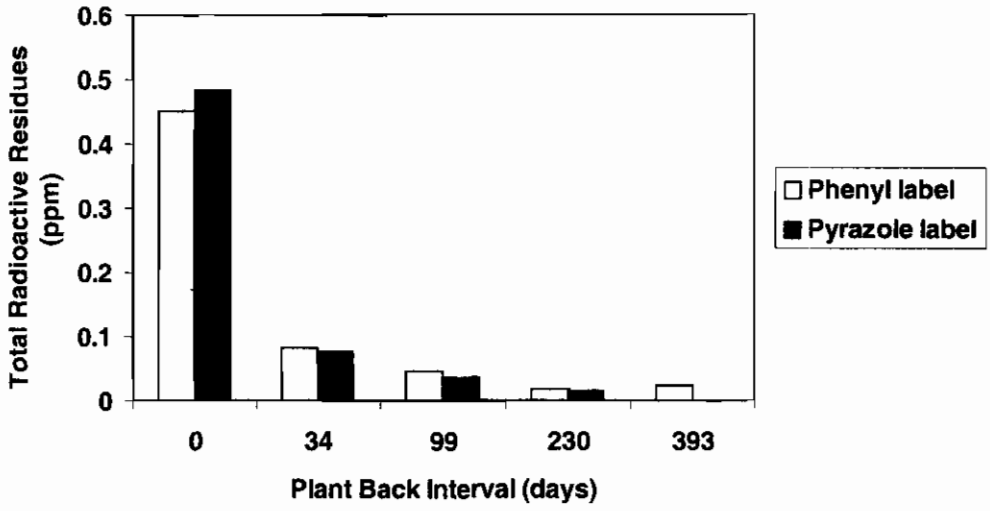


FIGURE C.2.2. TRRs in Radish Matrices (at the 34 Day PBI) after Soil Treatment with Radiolabelled Topramezone

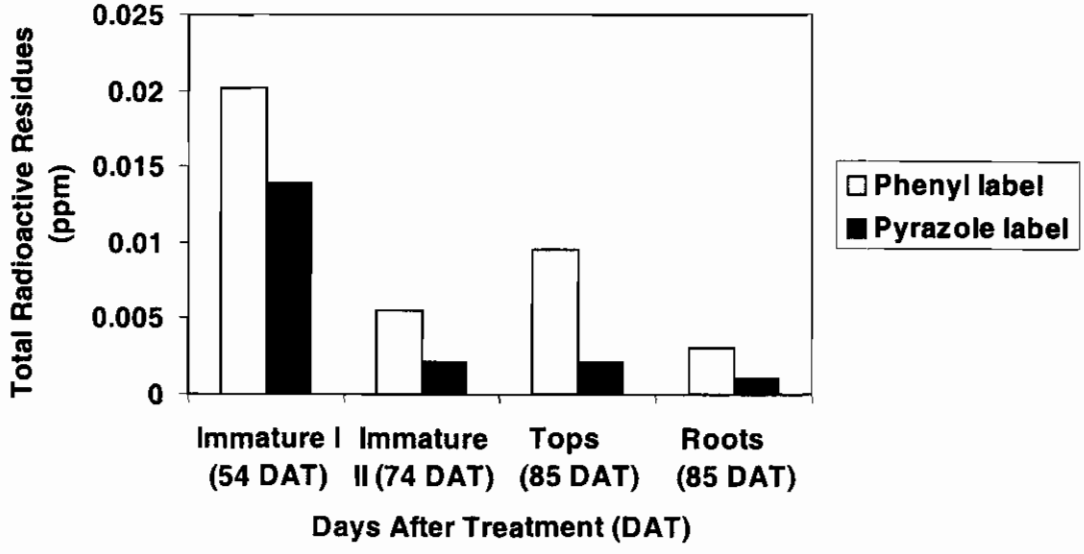


FIGURE C.2.3. TRRs in Wheat Matrices (at the 34 Day PBI) after Soil Treatment with Radiolabelled Topramezone

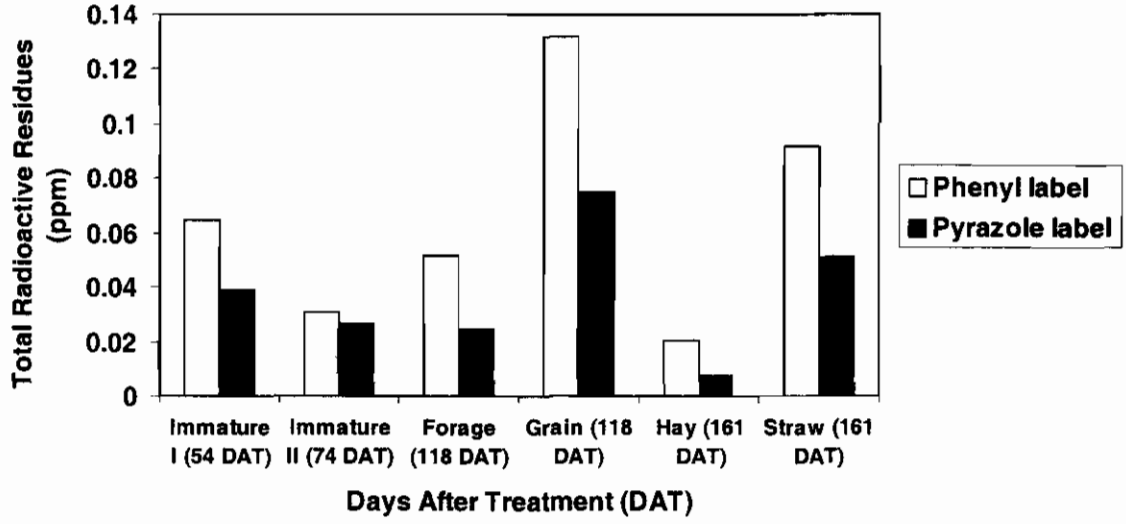


FIGURE C.2.4. TRRs in Sorghum Matrices (at the 99 Day PBI) after Soil Treatment with Radiolabelled Topramezone

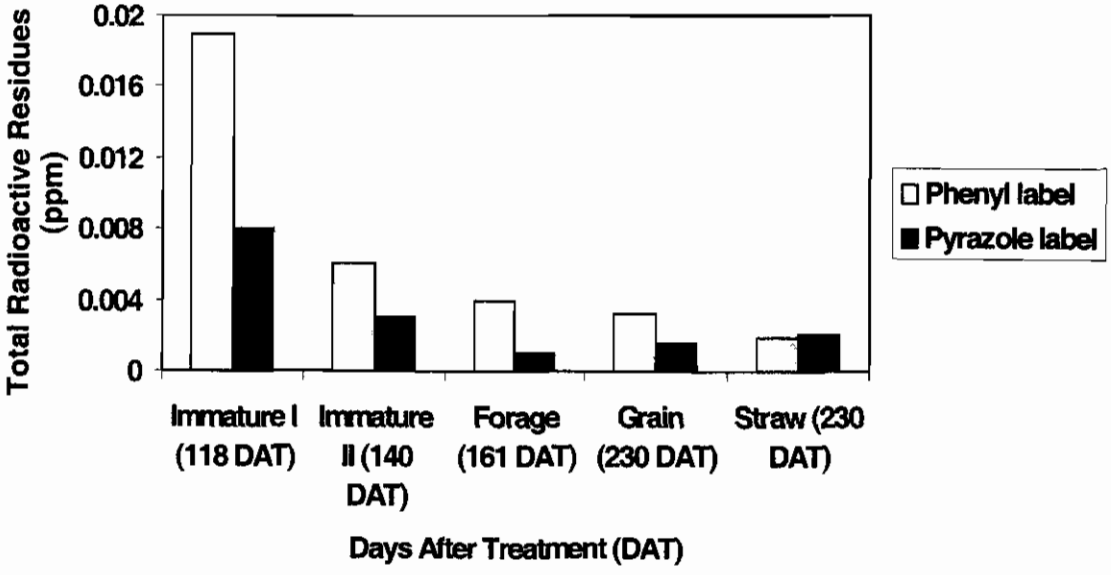


FIGURE C.2.5. TRRs in Mustard Green Matrices (at the 34 Day PBI) after Soil Treatment with Radiolabelled Topramezone

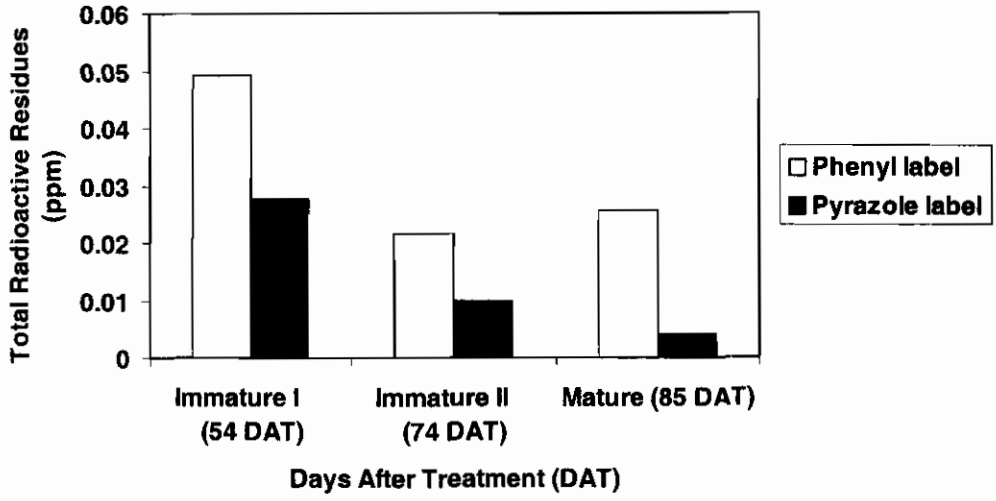


FIGURE C.2.6. TRRs in Mustard Green Matrices (at the 99 Day PBI) after Soil Treatment with Radiolabelled Topramezone

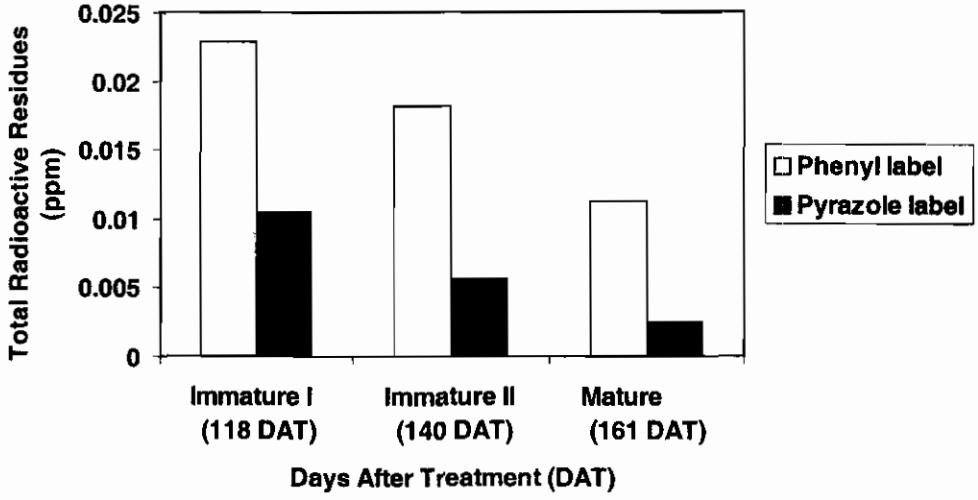


FIGURE C.2.7. Distribution of Topramezone and Minor Metabolites in Mustard Green Matrices (34 Day PBI) following One Soil Application of Radiolabelled Topramezone

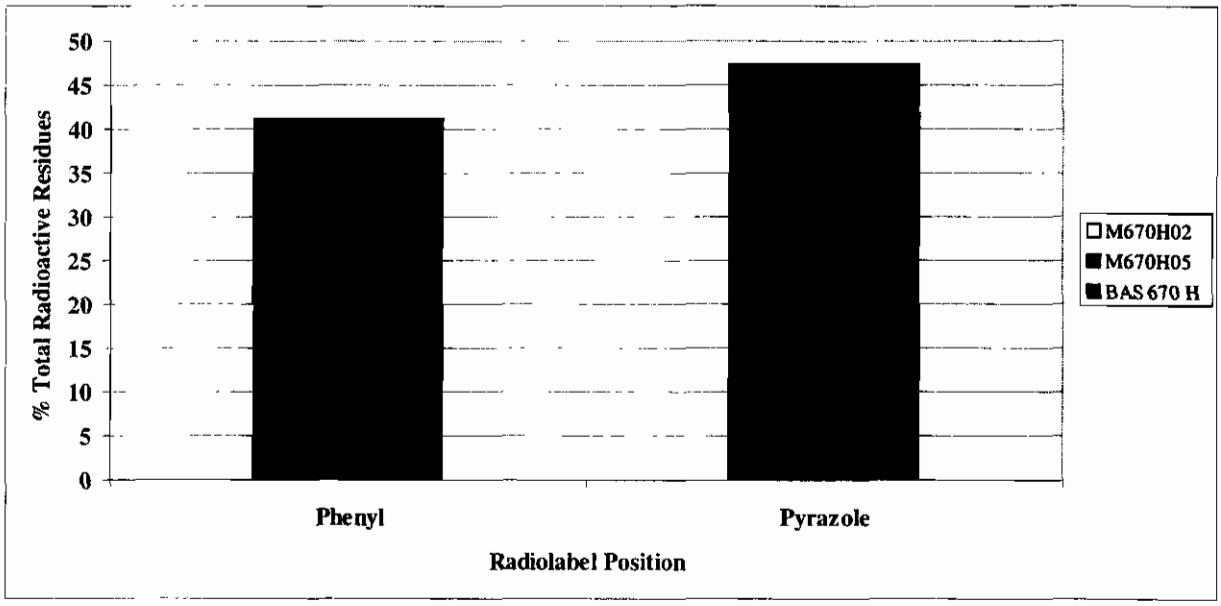
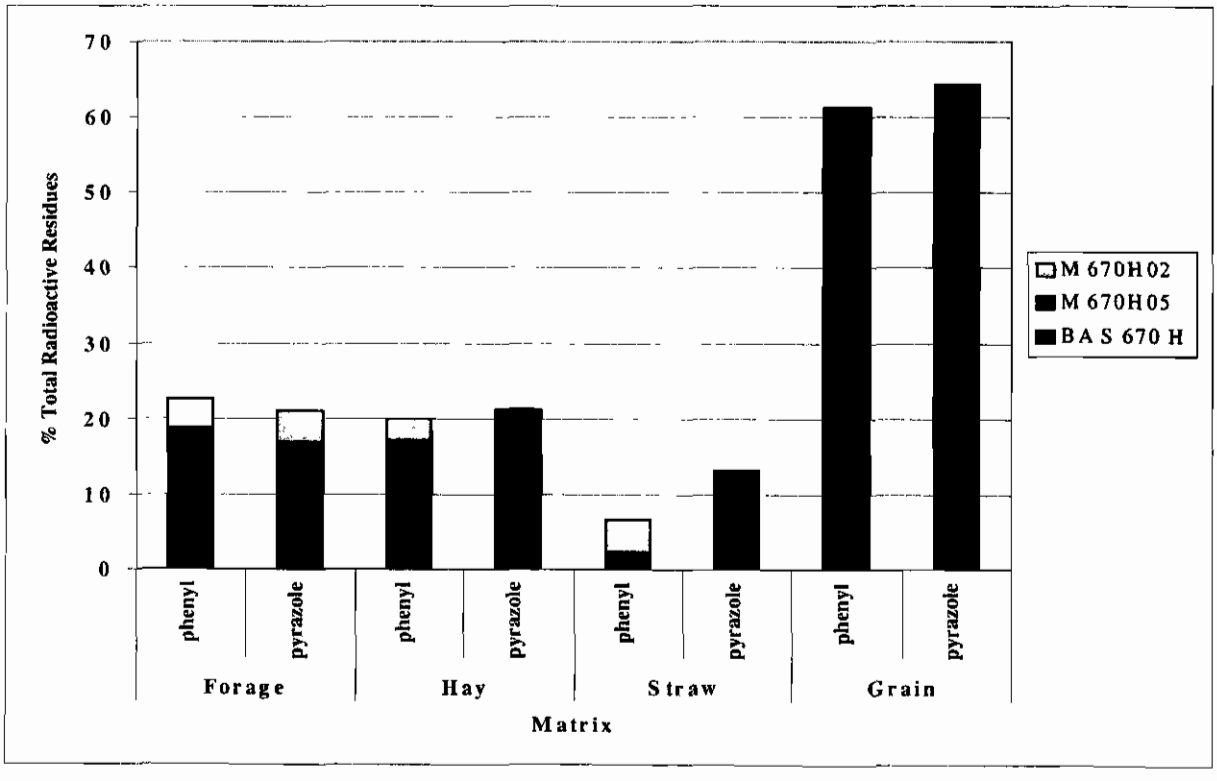


FIGURE C.2.8. Distribution of Topramezone and Minor Metabolites in Wheat Matrices at the 34 Day PBI Following One Soil Application of Radiolabelled Topramezone



C.3. Proposed Metabolic Profile

FIGURE C.3.1. Proposed Metabolic Profile of Topramezone in Rotational Radish, Wheat, Sorghum, and Mustard Greens

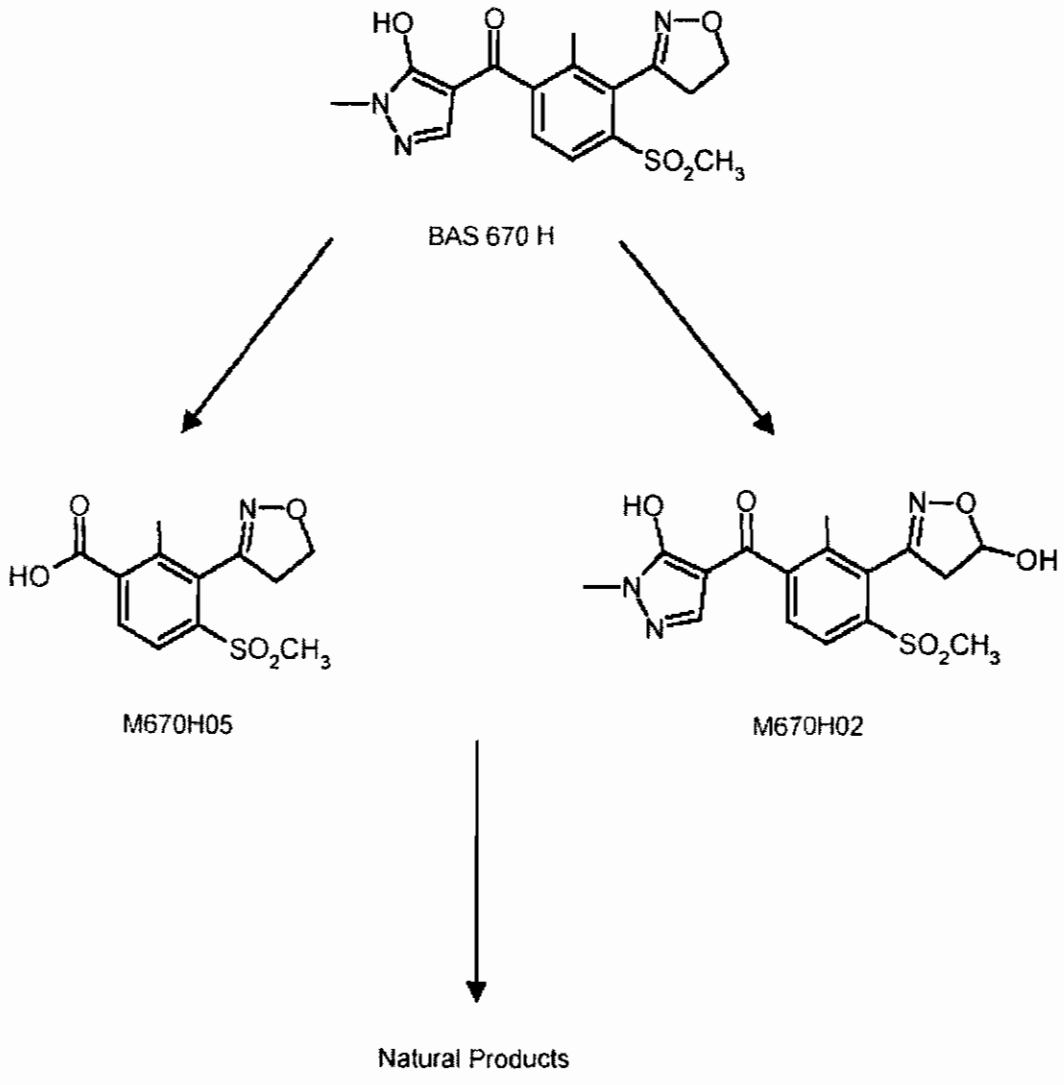
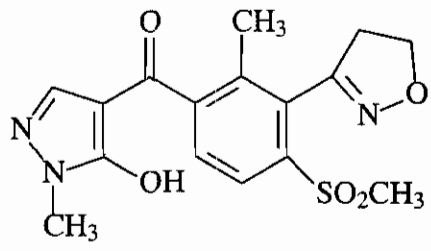
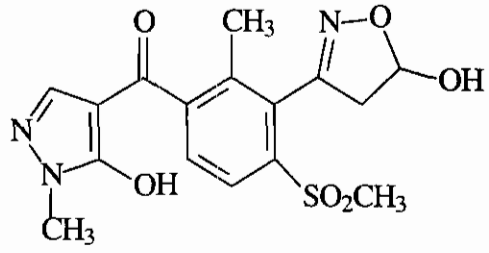
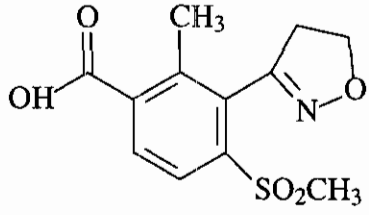


TABLE C.3.1. Identification of Compounds from the Confined Rotational Crop Study

Common name/code Figure C.3.1 ID No.	Chemical name	Chemical structure
Topramezone	[3-(4,5-dihydro-3-isoxazolyl)-2-methyl-4-(methylsulfonyl)phenyl](5-hydroxy-1-methyl-1*H*-pyrazol-4-yl)methanone	
M670H02	hydroxy metabolite	
M670H05	3-(4,5-dihydro-isoxazol-3-yl)-4-methanesulfonyl-2-methylbenzoic acid-[phenyl-U-^{14}C]	

D. CONCLUSION

Topramezone was applied to the bare soil of four plots at rates of either 0.081-0.091 kg a.i./ha (0.072-0.081 lb a.i./A) for the phenyl-label, or 0.091-0.094 kg a.i./ha (0.081-0.084 lb a.i./A) for the pyrazole-label. The rotational crops of mustard greens, radish, and wheat were to be planted at 34, 99 and 393 DAT. Sorghum replaced wheat at the 99 DAT due to climatic conditions. Mustard green and wheat samples underwent combustion/LSC, and the identification/characterization of residues from the combined MeOH/water extract was by HPLC (34 day PBI samples only). The total extractable residues in mustard green RACs were 88-91% of the TRRs (0.0034-0.0231 ppm), while the total extractable residues in wheat matrices (both labels) were 76-117% of the TRRs (0.0085-0.109 ppm). The unchanged parent topramezone was either the predominant metabolite (or the only metabolite identified) in mustard greens (31-47% of the TRRs; 0.0018-0.0079 ppm) and the majority of wheat matrices (11-64% of the TRRs; 0.0043-0.0160 ppm). M670H05 was the predominant metabolite identified in phenyl-label wheat grain (45% of the TRRs; 0.0093 ppm). Otherwise, M670H02 and M670H05 accounted for less than 11% of the TRRs in the mustard green and wheat RAC samples (≤0.0079 ppm).

Approximately 40-50% of the TRRs (0.0016-0.0126 ppm) were characterized in the mustard green samples, while 46-72% of the TRRs (0.0038-0.0828 ppm) were characterized in the wheat samples (both labels) after fractionation. Topramezone undergoes hydrolytic cleavage to form M670H05 (as found in the corn metabolism study), and hydroxylation of the isoxazole ring forms M670H02 (as found in the goat metabolism study). Further degradation of the phenyl and pyrazole rings results in incorporation into natural products.

E. REFERENCES

MRID No. 45902407. Abdel-Baky, S (2002). Validation and Accountability of BASF Method Number D0007 titled "Method for the Determination of BAS 670 H and Its Metabolite (M670H05) Residues in Plant Matrices Using LC/MS/MS." BASF Study Number: 56542. Unpublished study prepared by BASF Corporation. 93 pages.

Honeycutt, C. R. and Adler, I. L. (1975). Characterization of Bound Residues of Nitrofen in Rice and Wheat Stover. J. Agric. Food Chem. 23: 1097 – 1101.

MRID No. 45902410. Jordan, J. (2002). Storage Stability of BAS 670 H and its Cleaved Acid Metabolite M670H05 in Plant Matrices. Lab Project Number: 56750. Unpublished study prepared by BASF Corporation. 67 pages.

F. DOCUMENT TRACKING

RDI: G.F. Kramer (2/14/05), RAB1 Chemists (12/15/04), ChemSAC (3/2/05)
S. Levy:806T:CM#2:(703)305-0783:7509C:RAB1
Petition Number: 3F6568
DP #: 310772
PC Code: 123009

Evaluator

Robert Vogrig
Evaluation Officer
FREAS, PMRA

Date: March 21, 2005

Suzan Mathew
Evaluation Officer
FREAS, PMRA

Date: Mar 21/05

Peer Reviewer

Sarah Levy, MS
Chemist
RAB1, HED, EPA

Date: May 11, 2005

Approved by

Louise G. Croteau
Senior Evaluation Officer
FREAS, PMRA

Date: March 22/05

Ariff Ally, Ph.D.
Section Head
FREAS, HED, PMRA

Date: March 29, 05

G.F. Kramer, Ph.D.
Branch Senior Scientist
RAB1, HED, EPA

Date: 4.12.05

STUDY REPORTS:

MRID No. 45902412. Versoi, P. L., Ellenson, J.L. and Best, M.C. (2002). The Magnitude of BAS 670 H Residues in Corn (Field and Sweet) and Corn Grain Processed Fractions. BASF Study Number: 63882. Unpublished study prepared by BASF Agro Research. 149 pages.

EXECUTIVE SUMMARY:

BASF Agro Research submitted crop field trial data to quantify residues of topramezone and the free acid metabolite BAS 670 H 05 (also known as M670H05) for use on field and sweet corn. During the 2000 and 2001 growing seasons, a total of 24 trials were conducted on field corn encompassing Regions 1 (PA; 1 trial), 2 (NC; 1 trial), 5 (IL; 4 trials, ID; 4 trials, MN; 5 trials, MT; 2 trials, NE; 2 trials), 5B (QC; 4 trials) and 6 (OK; 1 trial). A total of 6 trials were conducted on sweet corn encompassing Regions 1 (PA; 1 trial), 3 (FL; 1 trial), 7A (AB; 1 trial), 10 (CA; 1 trial), 11 (OR; 1 trial) and 12 (OR; 1 trial). The sweet corn raw agricultural commodity (RAC) samples collected were forage, milk stage kernels plus cob with husks removed (K+CWHR), and stover. The field corn RAC samples collected were forage, milk stage K+CWHR, grain and stover. Field corn commodities harvested at the milk stage were extended to sweet corn. BAS 336 SC (soluble concentrate) herbicide was applied 2 times sequentially (1st target application at 25 g a.i./ha (0.022 lb a.i./A) and 2nd target application at 75 g a.i./ha (0.067 lb a.i./A)) as a foliar broadcast spray over the top of the corn, with a retreatment interval (RTI) of 13 to 17 days for field corn and 12 to 15 days for sweet corn. The maximum seasonal rate was 100 g a.i./ha/season (0.089 lb a.i./A/season). An adjuvant was added to the spray mixture for all applications, which contained a crop oil concentrate (COC) and an aqueous solution of urea ammonium nitrate. Field corn were harvested at pre-harvest intervals (PHIs) of 29-64 days for milk stage K+CWHR, 31-91 days for forage and 59-128 days for grain and stover. Sweet corn was harvested at PHIs of 35-49 days for K+CWHR and forage, and 57-92 days for stover.

The analytical data gathering and enforcement method (D0007) was the same for topramezone and BAS 670 H 05. Residues of topramezone were extracted from non-grain crop matrices with water, and residues in seed or grain matrices were extracted using acetonitrile (ACN):water (1:1, v:v). A 2% aliquot of the extract was removed and cleaned by partitioning with a mixture of 1 N HCl:dichloromethane (1:5, v:v) and NaCl. The extract was centrifuged (if necessary), the aqueous layer was discarded, and residues in the dichloromethane (DCM) layer were partitioned with 0.05% ammonium hydroxide (NH_4OH). The final analysis of topramezone and BAS 670 H 05 was determined by liquid chromatography (LC)-mass spectrometry (MS)/MS. The limit of quantitation (LOQ) for topramezone residues was 0.01 ppm for fresh market corn (K+CWHR) and corn grain, and 0.05 ppm for corn forage and corn stover. The limit of detection (LOD) was 0.005 ppm for fresh corn and corn grain, and 0.025 ppm for all the other sample matrices.

Storage stability data were deemed adequate as levels of topramezone and BAS 670 H 05 in field corn were evaluated concurrently with the field trials. The actual storage duration of all the RACs fell within the 18 month demonstrated storage interval.

The results from the field and sweet corn residue trials showed that at every PHI, the maximum residues for K+CWHR and grain for field corn were <0.01 ppm (LOQ - limit of quantitation), while the maximum residues for forage and stover for field and sweet corn were <0.05 ppm (LOQ). The formulation-bridging study between BAS 670 00 H (SC) and BAS 670 UAH (dry flowable - DF) produced residues < LOQ for topramezone or BAS 670 H 05. Residue decline studies indicated that residues of topramezone or BAS 670 H 05 remained below their respective LOQs with increasing PHIs. Exaggerated seasonal rates (503 g a.i./ha or 0.448 lb a.i./A)

produced residue levels below the LOQ for field corn (forage, grain and stover).

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the field trial residue data are classified as scientifically acceptable. The number and locations of the sweet corn field trials were not in accordance with OPPTS Guideline 860.1500 and Directive 98-02; Section 9. However, the field corn forage samples were collected when the grain was in the soft dough stage (a development stage that has been accepted as overlapping with the milk stage timing in sweet corn). Residue data on forage samples from ten of the fields corn sites were extended to support registration on sweet corn. Therefore, the number and location of sweet corn field trials were deemed adequate.

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document [DP# 310772] and in Canada's Regulatory Decision Document.

COMPLIANCE:

Signed and dated good laboratory practice (GLP), Quality Assurance and Data Confidentiality statements were provided. The use of three crop maintenance pesticides as seed protectants at one trial site, mixing-spraying intervals at one site, timing of interval from first to second application, personnel reporting, sample collection, timing of interval from last application to collection, equipment calibration, global positioning system (GPS) coordinates and summary reports of processing were not done in compliance with GLP. Crop sample weights collected were less than specified by study protocol in one residue decline study. Expired spiked solutions (2-3 days) and expired spiked extracts (19 days) were used in a few samples. However, the deviations listed should not have an impact on the regulatory decision or validity of the study.

A. BACKGROUND INFORMATION

BAS 670 336SC Herbicide (also known as BAS 670 00H Herbicide) is the end-use product which contains the active ingredient topramezone. It is a broad-spectrum, post-emergence herbicide used to control grassy and broadleaf weeds in corn. The herbicide is absorbed by the leaves, roots and shoots, then translocated to the growing points of the sensitive weeds. Topramezone belongs to the triketone class of chemicals, which inhibits carotenoid biosynthesis (HPPD (4-hydroxyphenylpyruvate dioxygenase) inhibitor)). This causes a strong bleaching activity on the growing zones of the shoots within 2-5 days of application. Exposure to light causes necrosis of chlorotic tissues and eventual plant death within 14 days after application.

TABLE A.1. Test Compound Nomenclature

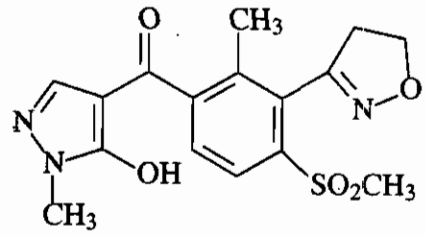
Compound	Chemical Structure
	
Common name	Topramezone
Company experimental name	BAS 670 H
IUPAC name	[3-(4,5-dihydro-1,2-oxazol-3-yl)-4-mesyl-*o*-tolyl](5-hydroxy-1-methyl-1*H*-pyrazol-4-yl) methanone
CAS name	[3-(4,5-dihydro-3-isoxazolyl)-2-methyl-4-(methylsulfonyl)phenyl](5-hydroxy-1-methyl-1*H*-pyrazol-4-yl)methanone
Molecular Formula	$C_{16}H_{17}N_3O_5S$
Molecular Mass	363.39 g/mol
CAS #	210631-68-8
End-use product/EP	Soluble Concentrate

TABLE A.2. Physicochemical Properties of Topramezone

Parameter	Value (All data came from PMRA Lab Services)
Melting point/range	220.9°C - 222.2°C
pH	2.9 (1% de-ionized water)
Density (20°C)	1.425 g/cm^3
Water solubility (20°C)	pH: 3, 5, 7, 9 g/L: 0.06, 0.98, 15, 23.4
Solvent solubility (g/100 mL at 20°C)	Acetone <1.0 Acetonitrile <1.0 Dichloromethane 2.5 - 2.9 Ethyl acetate <1.0 Methanol <1.0 N-heptane <1.0 N,N-dimethylformamide 11.4-13.3 1-octanol <1.0 Olive oil <1.0 2-propanol <1.0 Toluene <1.0
Vapor pressure at 20°C and 25°C	< 1.0 x 10^{-12} hPa
Dissociation constant (pK_a)	4.06
Octanol/water partition coefficient Log(K_{OW}) at 20°C	Buffer pH: 4, 7, 9 Log K_{ow}: – 0.81, – 1.52, – 2.34
UV/visible absorption spectrum	λ, nm: 207, 272, 300, 410 ξ, mol^{-1}cm^{-1}: 27 077, 8601, 5800, 410

B. EXPERIMENTAL DESIGN

B.1. Study Site Information

TABLE B.1.1. Trial Site Conditions

Trial Identification	Soil characteristics				Meteorological data	
	Type	%OM[1]	pH	CEC[2] meq/g	Overall rainfall range/ irrigation method	Overall T°C range
Corn, Field (2000)						
Fuquay-Varina, NC	Sandy Loam	NP[3]	NP	NP	<Normal	Normal
Dewey, IL	Clay Loam	NP	NP	NP	Normal	Normal
Hinckley, IL	Silty Clay Loam	NP	NP	NP	Normal	Normal
East Lynn, IL	Clay Loam	NP	NP	NP	Normal	Normal
Mazon, IL	Sandy Loam	NP	NP	NP	Normal	Normal
Owatonna, MN	Clay Loam	NP	NP	NP	> Normal	Normal
Warsaw, MN	Clay Loam	NP	NP	NP	>Normal	Normal
Friend, NE	Silt Loam	NP	NP	NP	< Normal/ Sprinklers	Normal
Beaver Crossing, NE	Silt Loam	NP	NP	NP	< Normal/ Sprinklers	Normal
Adel, IA	Loam	NP	NP	NP	Normal	Normal
Perry, IA	Loam	NP	NP	NP	Normal	Normal
Richland, IA	Silty Clay Loam	NP	NP	NP	Normal	Normal
Corn, Field (2001)						
Germansville, PA	Silt Loam	NP	NP	NP	< Normal/ Sprinklers & Drip Line	Normal
Ellendale, MN	Loam	NP	NP	NP	> Normal	Normal
Hartland, MN	Loam	NP	NP	NP	> Normal	Normal
Geneva, MN	Loam	NP	NP	NP	> Normal	Normal
Richland, IA	Silty Clay Loam	NP	NP	NP	> Normal	Normal
Kirksville, MO	Putnam Silty Clay Loam	NP	NP	NP	Normal	Normal
La Plata, MO	Silty Clay Loam	NP	NP	NP	Normal	Normal
Farnham, QC	Sandy Loam	NP	NP	NP	< Normal	Normal
St-Cesaire, QC	Clay Loam	NP	NP	NP	< Normal	Normal
Bedford, QC	Sandy Loam	NP	NP	NP	< Normal	Normal
St-Hugues, QC	Clay	NP	NP	NP	< Normal	Normal
Eakly, OK	Sandy Loam	NP	NP	NP	< Normal/ Sprinklers	Normal
Corn, Sweet (2000)						
Oviedo, FL	Sand	NP	NP	NP	Normal/ Flood	Normal

TABLE B.1.1. Trial Site Conditions

Trial Identification	Soil characteristics				Meteorological data	
	Type	%OM[1]	pH	CEC[2] meq/g	Overall rainfall range/ irrigation method	Overall T°C range
Corn, Sweet (2001)						
Germansville, PA	Silt Loam	NP	NP	NP	< Normal/ Drip	Normal
Medicine Hat, AB	Silt Loam	NP	NP	NP	< Normal/ Sprinklers	> Normal
Fresno, CA	Sandy Loam	NP	NP	NP	Normal/ Surface Drip	Normal
Parkdale, OR	Silt Loam	NP	NP	NP	< Normal/ Sprinklers	Normal
Hillsboro, OR	Woodbum Silt Loam	NP	NP	NP	Normal/ Sprinklers	Normal

[1] OM - Organic matter
[2] CEC - Cation exchange capacity
[3] NP - Not provided

No actual or historical data were provided by the petitioner for temperature or rainfall. Irrigation was not used unless otherwise indicated.

TABLE B.1.2. Study Use Pattern.

Location	EP[1]	Application							Tank Mix Adjuvants
		Method/Timing[2]	Vol GPA[3]		Rate[4] g a.i./ha (lb a.i./A)		RTI[5] (days)	Total Rate g a.i./ha (lb a.i./A)	
Corn, Field (2000)									
Fuquay-Varina, NC	BAS 670 336 SC	Application #1 - Sixth leaf vegetative stage. Plants 28 inches tall. Application #2 - One week pre-tassel. Plants 5 to 6 feet tall.	30.0 30.0		25 (0.022) 75 (0.067)		14	100 (0.089)	COC + UAN
Formulation Bridging			SC	DF	SC	DF		SC	DF	
Dewey, IL	BAS 670 336 SC	Application #1 - Fourth leaf vegetative stage. Plants 10 to 11 inches tall. Application #2 - Ninth leaf vegetative stage. Plants 24 to 26 inches tall.	20.1 20.5	19.5 20.0	25 (0.022) 76 (0.068)	24 (0.021) 75 (0.067)	13	101 (0.090)	99 (0.088)	OOSA & Law EL 28% UAN
Formulation Bridging			SC	DF	SC	DF		SC	DF	
Hinckley, IL	BAS 670 336 SC	Application #1 - Third leaf vegetative stage. Plants 4 to 5 inches tall. Application #2 - Sixth leaf vegetative stage. Plants 14 to 15 inches tall.	21.0 19.7	19.6 20.2	26 (0.023) 74 (0.066)	25 (0.022) 76 (0.068)	13	100 (0.089)	101 (0.090)	OOSA & Law EL 28% UAN
East Lynn, IL	BAS 670 336 SC	Application #1 - Sixth leaf vegetative stage. Plants 12 to 14 inches tall Application #2 - Ninth leaf vegetative stage Plants 28 to 30 inches tall.	19.7 20.7		25 (0.022) 77 (0.069)		13	102 (0.091)		OOSA & Law EL 28% UAN
Mazon, IL	BAS 670 336 SC	Application #1 - Fourth leaf vegetative stage. Plants 6 to 7 inches tall. Application #2 - Eighth leaf vegetative stage. Plants 17 to 18 inches tall.	20.3 19.6		25 (0.022) 74 (0.066)		14	99 (0.088)		OOSA & Law EL 28% UAN
Formulation Bridging			SC	DF	SC	DF		SC	DF	
Owatonna, MN	BAS 670 336 SC	Application #1 - Third to 4th leaf vegetative stage Plants 6 to 7 inches tall. Application #2 - Fourth to 5th leaf vegetative stage. Plants 15 inches tall	18.8 19.1	19.2 20.2	24 (0.021) 72 (0.064)	24 (0.021) 76 (0.068)	13	96 (0.085)	100 (0.089)	Cenex/LOL - COC & 28% UAN
Warsaw, MN	BAS 670 336 SC	Application #1 - Third to 4th leaf vegetative stage. Plants 7 to 9 inches tall Application #2 - Four to 5th leaf vegetative stage. Plants 9 to 16 inches tall.	20.5 19.7		26 (0.023) 74 (0.066)		13	100 (0.089)		Cenex/LOL - COC & 28% UAN

TABLE B.1.2. Study Use Pattern.

Location	EP[1]	Application										Tank Mix Adjuvants
		Method/Timing[2]	Vol GPA[3]			Rate[4] g a.i./ha (lb a.i./A)			RTI[5] (days)	Total Rate g a.i./ha (lb a.i./A)		
Friend, NE	BAS 670 336 SC	Application #1 - Fifth leaf vegetative stage. Plants 10 to 12 inches tall. Application #2 - Seventh leaf vegetative stage. Plants 28 inches tall.	19.9 20.2			25 (0.022) 76 (0.068)			15	101 (0.090)		COC & UAN
Beaver Crossing, NE	BAS 670 336 SC	Application #1 - Fifth to 6th leaf vegetative stage. Plants 17 to 19.5 inches tall. Application #2 - Sixth to 7th leaf vegetative stage. Plants 34 to 38 inches tall.	20.3 20.1			26 (0.023) 75 (0.067)			17	101 (0.090)		Wilfarm Crop Oil plus & 28% UAN
Adel, IA	BAS 670 336 SC	Application #1 - Fifth leaf vegetative stage. Plants 8 to 9 inches tall. Application #2 - Seventh to 8th leaf vegetative stage. Plants 24 inches tall.	20.0 19.6			25 (0.022) 74 (0.066)			14	99 (0.088)		COC & UAN
Perry, IA	BAS 670 336 SC	Application #1 - Sixth leaf vegetative stage. Plants 9 to 11 inches tall. Application #2 - Seventh to 8th leaf vegetative stage. Plants 24 inches tall.	20.0 20.6			25 (0.022) 77 (0.069)			14	102 (0.091)		COC & UAN
			1X	3X	5X	1X	3X	5X		1X	3X	5X	
Richland, IA	BAS 670 336 SC	Application #1 -Sixth to 7th leaf vegetative stage. Plants 19 inches tall. Application #2 -Tenth leaf vegetative stage. Plants 40 inches tall.	11.1 9.9	11.1 9.9	11.1 9.7	25 (0.022) 78 (0.070)	74 (0.066) 232 (0.207)	123 (0.11) 380 (0.339)	14	103 (0.092)	306 (0.273)	503 (0.449)	COC & UAN
Corn, Field (2001)													
Germansville, PA	BAS 670 336 SC	Application #1 -Fourth to 5th leaf vegetative stage. Plants 12 to 15 inches tall. Application #2 -Eighth to 10th leaf vegetative stage. Plants 30 inches tall.	43.7 43.65			26 (0.023) 77 (0.069)			14	103 (0.092)			Booster Plus E & UAN
Ellendale, MN	BAS 670 336 SC	Application #1 - Sixth leaf vegetative stage. Plants 12 inches tall. Application #2 - Ninth to 10th leaf vegetative stage. Plants 30 inches tall.	17.3 18.0			25 (0.022) 76 (0.068)			14	101 (0.090)			COC & UAN
Hartland, MN	BAS 670 336 SC	Application #1 - Sixth leaf vegetative stage. Plants 12 inches tall. Application #2 - Ninth to 10th leaf vegetative stage. Plants 30 inches tall.	17.3 17.85			25 (0.022) 76 (0.068)			14	101 (0.090)			COC & UAN

TABLE B.1.2. Study Use Pattern.

Location	EP[1]	Application						Tank Mix Adjuvants
		Method/Timing[2]	Vol GPA[3]	Rate[4] g a.i./ha (lb a.i./A)	RTI[5] (days)	Total Rate g a.i./ha (lb a.i./A)		
Geneva, MN	BAS 670 336 SC	Application #1 - Sixth leaf vegetative stage. Plants 12 inches tall. Application #2 - Tenth leaf vegetative stage. Plants 30 inches tall.	16.4 17.9	24 (0.021) 75 (0.067)	15	99 (0.088)		COC & UAN
Richland, IA	BAS 670 336 SC	Application #1 - Sixth leaf vegetative stage. Plants 12 to 18 inches tall. Application #2 - Tenth to 11th leaf vegetative stage. Plants 48 inches tall.	13.9 14.0	25 (0.0225) 76 (0.068)	14	101 (0.0905)		COC & UAN
Kirksville, MO	BAS 670 336 SC	Application #1 - Eighth to 9th leaf vegetative stage. Plants 22 to 24 inches tall. Application #2 - Eleventh to 12th leaf vegetative stage. Plants 50 inches tall.	18.0 17.2	24 (0.021) 76 (0.068)	13	100 (0.089)		COC & UAN
La Plata, MO	BAS 670 336 SC	Application #1 - Ninth leaf vegetative stage. Plants 28 to 30 inches tall. Application #2 - Thirteenth leaf vegetative stage. Plants 60 inches tall.	18.2 17.1	24 (0.021) 76 (0.068)	13	100 (0.089)		Wilfarm Crop Oil Plus & UAN
Formulation Bridging			SC / DF	SC / DF		SC	DF	
Farnham, QC	BAS 670 336 SC	Application #1 - Sixth to 7th leaf vegetative stage. Plants 18 to 20 inches tall. Application #2 - Tenth to 11th leaf vegetative stage. Plants 39.4 to 43.3 inches tall.	23.4 / 23.6 23.7 / 23.5	25 (0.022) / 25 (0.022) 74 (0.066) / 74 (0.066)	15	99 (0.088)	99 (0.088)	AOC & UAN
Formulation Bridging			SC	DF	SC	DF	SC	DF
St-Césaire, QC	BAS 670 336 SC	Application #1 - Sixth to 7th leaf vegetative stage. Plants 15.8 inches tall. Application #2 - Ninth to 11th leaf vegetative stage. Plants 41.3 to 43.3 inches tall.	23.8 24.6	23.9 24.1	25 (0.022) 77 (0.069)	25 (0.022) 75 (0.067)	14	102 (0.091)	100 (0.089)	AOC & UAN
Bedford, QC	BAS 670 336 SC	Application #1 - Seventh leaf vegetative stage. Plants 18 inches tall. Application #2 - Ninth to 11th leaf vegetative stage. Plants 47.2 to 51.2 inches tall.	27.3 25.2	28 (0.025) 78 (0.070)	13	106 (0.095)		AOC & UAN
St-Hugues, QC	BAS 670 336 SC	Application #1 - Sixth to 7th leaf vegetative stage. Plants 19.7 inches tall. Application #2 - Ninth to 11th leaf vegetative stage. Plants 29.5 to 33.5 inches tall.	24.9 24.8	26 (0.023) 77 (0.069)	13	103 (0.092)		AOC & UAN

TABLE B.1.2. Study Use Pattern.

Location	EP[1]	Application					Tank Mix Adjuvants
		Method/Timing[2]	Vol GPA[3]	Rate[4] g a.i./ha (lb a.i./A)	RTI[5] (days)	Total Rate g a.i./ha (lb a.i./A)	
Eakly, OK	BAS 670 336 SC	Application #1 - Fifth to 6th leaf vegetative stage. Plants 13 to 17 inches tall. Application #2 - Eight leaf vegetative stage. Plants 48 to 60 inches tall.	10.3 13.0	25 (0.022) 74 (0.066)	14	99 (0.088)	Herbimax COC & UAN
Corn, Sweet (2000)							
Oviedo, FL	BAS 670 336 SC	Application #1 - Fifty Nine days before milk stage. Plants 6 inches tall. Application #2 - Forty fifth days before milk stage. Plants 20-24 inches tall.	30.1 29.8	25 (0.022) 75 (0.067)	14	100 (0.089)	435 Soluble Oil & Douglass FLLN #674 (32-0-0)
Corn, Sweet (2001)							
Germansville, PA	BAS 670 336 SC	Application #1 - Second to 4th leaf vegetative stage. Plants 4 to 6 inches tall. Application #2 - Fourth to 6th leaf vegetative stage. Plants 12 to 18 inches tall.	43.8 43.3	26 (0.023) 76 (0.068)	12	102 (0.091)	Booster Plus E & UAN
Medicine Hat, AB	BAS 670 336 SC	Application #1 - Sixth to 7th leaf vegetative stage. Plants 13.8 inches tall. Application #2 - Beginning of anthesis. Plants 24 inches tall.	10.7 10.6	25 (0.022) 75 (0.067)	15	100 (0.089)	Surf 92 & UAN
Fresno, CA	BAS 670 336 SC	Application #1 - Second leaf vegetative stage. Plants 3 to 4 inches tall. Application #2 - Fourth to 5th leaf vegetative stage. Plants 10 to 16 inches tall	29.5 29.65	25 (0.022) 74 (0.066)	14	99 (0.088)	Britz Blend COC & UAN
Parkdale, OR	BAS 670 336 SC	Application #1 - Tassel emergence stage. Plants 36 to 48 inches tall. Application #2 - Silking stage. Plants 36 to 40 inches tall.	20.7 21.8	25 (0.022) 74 (0.066)	14	99 (0.088)	Agri-Dex (COC) & UAN

Topramezone/PC Code 123009/MET/BAS 670 H/BASF Canada Inc./BAZ
DACO 7.4.1/OPPTS 860.1500/OECD IIA 6.3.1, 6.3.2, 6.3.3 and IIIA 8.3.1, 8.3.2, 8.3.3
Crop Field Trial - Corn, field and sweet

TABLE B.1.2. Study Use Pattern.

Location	EP[1]	Application					Tank Mix Adjuvants
		Method/Timing[2]	Vol GPA[3]	Rate[4] g a.i./ha (lb a.i./A)	RTI[5] (days)	Total Rate g a.i./ha (lb a.i./A)	
Hillsboro, OR	BAS 670 336 SC	Application #1 - Ninth to 10th leaf vegetative stage. Plants 20 to 27 inches tall.	29.6	25 (0.022)	14	100 (0.089)	COC & UAN
		Application #2 - First sign of silking stage. Plants 73 inches tall	30.0	75 (0.067)			

[1] EP = End-use Product
[2] Pesticide was foliar broadcast sprayed over the top of the crop. Adjuvant rate is 1.25% v/v each. Applications were made using commercial or simulated commercial ground application equipment.
[3] Gallons per acre
[4] Two applications were made.
[5] Retreatment Interval - Days after 1st application until the day of the 2nd application.
COC - Crop oil concentrate.
UAN - Urea ammonium nitrate.
OOSA - Ortech Oil Surfactant - Adjuvant.
AOC - Assist Oil Concentrate.

TABLE B.1.3. Trial Numbers and Geographic Locations

NAFTA Growing Region	Sweet Corn			Field Corn		
	Submitted	Requested		Submitted	Requested	
	CAN/U.S.A.	CAN.	U.S.A.	CAN/U.S.A	CAN.	U.S.A
1	1		2	1		1
1A						
2			1	1		1
3	1		1			
4						
5		4	5	17	8	17
5A						
5B		2		4	4	
6				1		1
7						
7A	1	1				
8						
9						
10	1		1			
11	1		1			
12	1	1	1			
13						
14						
15						
16						
17						
18						
19						
20						
21						
Total	6	8	12	24	12	20

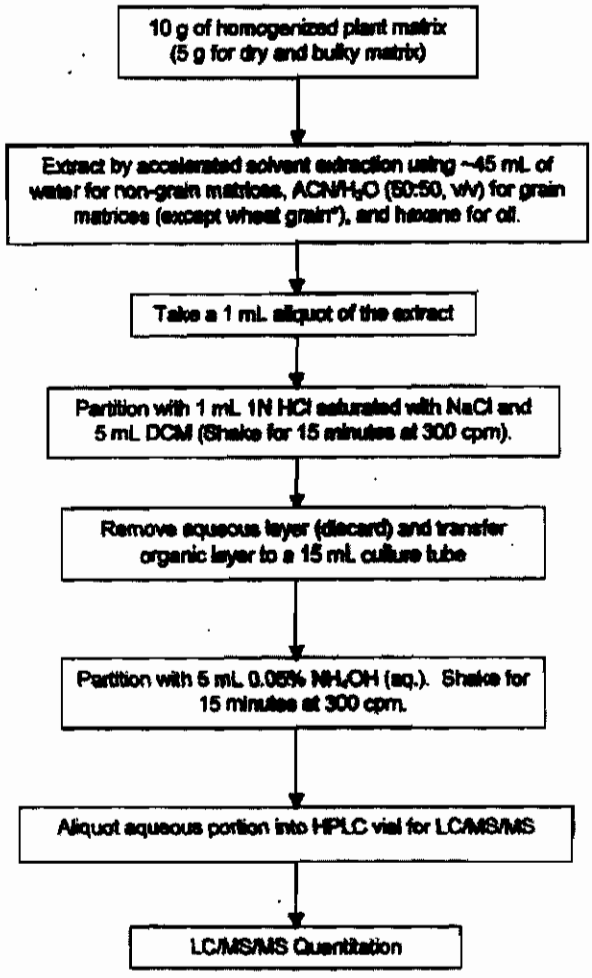
B.2. Sample Handling and Preparation

Samples were collected and placed into separate labelled sample bags. The untreated control plot was sampled prior to the treated plot(s) to avoid contamination. Samples were immediately transferred to freezers on the date of collection, except for certain samples which were overnight express-shipped at ambient temperatures on the day of collection, then delivered to BASF freezers the following day. All samples other than grain remained frozen during storage and shipment, were placed in a freezer (<-10°C) upon arrival at BASF Agro Research, and remained there prior to homogenization and analysis. The bulk grain samples were shipped frozen to the Food Protein Research & Development Center, Bryan, TX, where they were stored frozen until processing (2-3 weeks). Corn grain and meal samples were homogenized with dry ice to an appropriate consistency, while refined oil samples required no additional processing for analysis. Samples were stored frozen in plastic bags or glass jars (oil) until analysis. A Kett Infrared Moisture Determination Balance FD620 was used to determine the moisture content of certain control and treated corn samples.

B.3. Analytical Methodology

Residues of topramezone and the free acid metabolite BAS 670 H 05 were extracted from field and sweet corn commodities using the data gathering (and enforcement) analytical method D0007 (see FIGURE B.1.). Corn commodities tested included forage, "fresh market corn" or K+CWHR, stover and grain. Residues were extracted using an accelerated solvent extraction process. Homogenized plant matrices were extracted with either 45 mL water (non-grain matrices), ACN:water (1:1, v:v) (grain matrices except wheat grain), or hexane (oils). A 1 mL aliquot of the extract was removed and cleaned by partitioning with a 1 mL mixture of 1N HCl that was saturated with NaCl and 5 ml of DCM. The extract was centrifuged and residues in the DCM layer were partitioned with 5 mL of 0.05% NH_4OH. Residues in corn oil were extracted with 1N HCl and DCM saturated with NaCl. Following phase separation, the aqueous layer was discarded and residues in the organic layer were partitioned with 0.05% NH_4OH. The final chromatographic analysis of topramezone and BAS 670 H 05 (M670H05) residues in crop matrices were performed by LC-MS/MS. The LOQs for method D0007 for topramezone and BAS 670 H 05 residues were 0.01 ppm for fresh market corn (K+CWHR) and corn grain, and 0.05 ppm for corn forage and stover. The LODs were 0.005 ppm (fresh corn and corn grain) and 0.025 ppm (all other matrices). The LOD was defined as the lowest standard level injected with the analysis set with a signal to noise (S/N) ratio between 3-5. Detector response was linear in the range of 0.2 pg/μL (4 pg) to 10 pg/μL (200 pg) for topramezone (coefficient of determination, r^2, of 0.998) and BAS 670 H 05 (r^2 of 1.00). The analytical standards of topramezone (BAS 670 00 H) and BAS 670 H 05 (M670H05) were 99.9% pure. All samples (including LOQ samples) were diluted to a concentration appropriate for the standard curve.

FIGURE B.3.1. Flowchart of Analytical Method D0007 Used to Analyze Residues of Topramezone and the Free Acid Metabolite (Bas 670 H 05) in Corn (Field and Sweet) Matrices.



C. RESULTS AND DISCUSSION

A total of 24 field corn and 6 sweet corn trials were conducted during the 2000 and 2001 growing seasons. No actual or historical data were provided by the petitioner about temperature and rainfall. The temperature and rainfall (TABLE B.1.1) were in some cases above or below normal, but did not appear to have an impact on this study. Irrigation was used where needed. The field corn forage samples at most sites were collected when the grain was in the soft dough stage (a development stage considered to overlap with the milk stage timing in sweet corn). However, field corn forage was collected at the milk stage at 10 sites. Field corn grain was collected at maturity. Field corn stover samples represented mature dry stalk (targeting 15-20% moisture) after the removal of the grain and cob. Sweet corn samples were collected at the milk stage for both K+CWHR and forage. The number and location of the sweet corn field trials (TABLE B.1.3) were in some cases not in accordance with the OPPTS Guidelines 860.1500

(August 1996) or the Residue Chemistry Guidelines, June 1, 1998 (Dir98-02); Section 9 (Region 1, 2, 5, 5B -TABLE B.1.3). However, field corn commodities harvested at the milk stage were extended to sweet corn in order to meet the requirements for the number and location of sweet corn field trials.

Method validation for the active ingredient topramezone and BAS 670 H 05 was performed at various spiking levels on field corn (K+CWHR, forage, grain and stover) and sweet corn (K+CWHR and forage), and reported in TABLE C.1. Procedural recoveries were corrected for residues detected in the control samples that were analyzed simultaneously. In field and sweet corn, at the respective LOQs for forage, grain and stover, the concurrent recoveries for both the parent and the metabolite ranged from 61% to 144%. Although some individual recoveries were less than 70% or greater than 120%, the method is deemed suitable for data gathering.

Storage stability data (TABLE C.2) for topramezone was evaluated concurrently with the field trials in field corn (K+CWHR, forage, grain and stover) and sweet corn (K+CWHR, forage and stover). The actual storage duration of all the RACs fell within the 18 month demonstrated storage interval and showed no sign of dissipation.

Uncorrected residue data for field and sweet corn field trials with the parent topramezone and the free acid metabolite BAS 670 H 05 are reported in TABLE C.3. The maximum residues in K+CWHR (field and sweet corn) were <0.01 ppm (LOQ) and <0.01 ppm (LOQ) in field corn grain. The maximum residues for forage and stover in both field and sweet corn were <0.05 ppm (LOQ). A summary of the residue data following 2 applications of BAS 670 336SC is presented in TABLE C.4.1. The application of BAS 670 00 H (SC) and BAS 670 UAH (DF) produced residues < LOQ, indicating no differences between formulations. The residue decline studies in field corn (TABLE C.3) indicated that for all given PHIs, the residues of topramezone were below the respective LOQs for K+CWHR, forage, grain and stover. A trial at exaggerated seasonal rates (306 g a.i./ha or 0.273 lb a.i./A and 503 g a.i./ha or 0.448 lb a.i./A) was performed in Richland, Iowa. Data were not provided for the 306 g a.i./ha or 0.272 lb a.i./A application rate as it was concluded that marketable grain could be produced from the 503 g a.i./ha or 0.448 lb a.i./A) treated plots. Thus, the 306 g a.i./ha or 0.272 lb a.i./A application rate site was discontinued. In all cases, the residue levels of stover and grain in field corn (TABLE C.3) at the 503 g a.i./ha or 0.448 lb a.i./A application rate were at the same levels as those at the 103 g a.i./ha or 0.092 lb a.i./A application rate (LOQ). All control samples chromatograms showed no sign of interference.

TABLE C.1. Summary of Concurrent Recoveries of Topramezone from Corn (Field and Sweet).

Matrix	Spiking level (mg/kg)	Sample size (n)	Recoveries (%)		Mean ± Std Dev (%)	
			Topramezone	BAS 670 H 05	Topramezone	BAS 670 H 05
Corn, field						
K+CWHR	0.01	1	108	91	-	-
	0.10	1	97	91	-	-
Forage	0.05	4	100, 76, 65, 82	135, 81, 67, 69	80.8 ± 14.6	88.0 ± 31.9
	0.50	4	89, 78, 73, 79	95, 85, 75, 68	79.8 ± 6.7	80.8 ± 11.8
Grain	0.01	4	97, 93, 114, 82	95, 66, 93, 104	96.5 ± 13.3	89.5 ± 16.4
	0.10	4	97, 96, 99, 82	87, 84, 101, 90	93.5 ± 7.8	90.5 ± 7.4
Stover	0.05	6	66, 116, 119, 88, 99, 80	61, 102, 94, 78, 89, 78	94.7 ± 20.7	83.7 ± 14.5
	0.50	6	70, 89, 100, 91, 85, 79	65, 84, 83, 84, 84, 81	85.7 ± 10.3	80.2 ± 7.5
Corn, sweet						
K+CWHR	0.01	6	104, 129, 108, 99, 126, 115	80, 102, 91, 67, 67, 80	113.5 ± 12.1	81.2 ± 13.7
	0.02	3	89, 93, 84	66, 90, 82	88.7 ± 4.5	79.3 ± 12.2
	0.05	1	107	101	-	-
	0.10	6	141, 144, 97, 101, 123, 113	91, 99, 91, 82, 109, 86	119.8 ± 19.8	93.0 ± 9.7
	0.20	3	84, 111, 88	82, 98, 78	94.3 ± 14.6	86.0 ± 10.6
	0.50	1	101	90	-	-
Forage	0.05	7	91, 85, 84, 89, 80, 94, 78	119, 90, 70, 76, 71, 78, 69	85.9 ± 5.8	81.9 ± 17.9
	0.50	7	82, 90, 87, 99, 83, 91, 85	94, 91, 79, 93, 76, 85, 71	88.1 ± 5.8	84.1 ± 9.0

TABLE C.2. Summary of Storage Conditions.

RAC	Extract	Treatment	Storage Temp. (°C)	Maximum Actual Storage Duration (months)	Interval of Demonstrated Storage Stability (months)
Corn, field	K+CWHR	Normal	<-10	7	-
		Formulation Bridging	<-10	7	-
		Residue decline	<-10	7	-
	Forage	Normal	<-10	6	18[1]
		Formulation Bridging	<-10	7	-
		Residue decline	<-10	2	-
	Grain	Normal	<-10	7	18[1]
		Exaggerated Rate (503 g a.i./ha or 0.448 lb a.i./A)	<-10	4	-
		Formulation Bridging	<-10	5	-
		Residue decline	<-10	5	-
	Stover	Normal	<-10	8	18[1]
		Formulation Bridging	<-10	6	-
		Residue decline	<-10	6	-
Corn, sweet	K+CWHR	Normal	<-10	8	-
	Forage	Normal	<-10	8	18[1]
	Stover	Normal	<-10	8	18[1]

[1] See MRID 45902410.

TABLE C.3. Residue Data from Corn (Field and Sweet) Field Trials with Topramezone.

Trial Identification	Region	Crop/ Variety	Commodity or Matrix	Total Rate g a.i./ha (lb a.i./A)	PHI (days)	Residues (ppm)	
						Topramezone	BAS 670 H 05
Corn, Field (2000)							
Fuquay-Varina, NC	2	Pioneer 3140	K+CWHR	100 (0.089)	29	<0.01	<0.01
Dewey, IL	5	Pioneer 34B23	K+CWHR	101 SC (0.090) SC	50	<0.01 SC	<0.01 SC
			K+CWHR	99 DF (0.088) DF	50	<0.01 DF	<0.01 DF
Hinckley, IL	5	Asgrow RX 770 RR	K+CWHR	100 SC (0.089) SC	61	<0.01 SC	<0.01 SC
			K+CWHR	101 DF (0.090) DF	61	<0.01 DF	<0.01 DF
East Lynn, IL	5	Pioneer 34B23	K+CWHR	102 (0.091)	50	<0.01	<0.01
Mazon, IL	5	NK 6423	K+CWHR	99 (0.088)	50	<0.01	<0.01
Owatonna, MN	5	Dekalb DK-477	K+CWHR	96 SC (0.085) SC	56	<0.01 SC	<0.01 SC
			K+CWHR	100 DF (0.089) DF	56	<0.01 DF	<0.01 DF
Corn, Field (2001)							
Germansville, PA	1	Doebler's G642XP	K+CWHR	103 (0.092)	36	<0.01	<0.01
					42	<0.01	<0.01
					49	<0.01	<0.01
					57	<0.01	<0.01
					64	<0.01	<0.01
Farnham, QC	5B	DK 44-22 bt	K+CWHR	99 SC (0.088) SC	43	<0.01 SC	<0.01 SC
			K+CWHR	99 DF (0.088) DF	43	<0.01 DF	<0.01 DF
St-Césaire, QC	5B	42 21 RR	K+CWHR	102 SC (0.091) SC	48	<0.01 SC	<0.01 SC
			K+CWHR	100 DF (0.089) DF	48	<0.01 DF	<0.01 DF
Eakly, OK	6	NK 4242 BT	K+CWHR	99 (0.088)	40	<0.01	<0.01
Corn, Sweet (2000)							
Oviedo, FL	3	Summer Sweet - Abbott & Cobb 8102	K+CWHR	100 (0.089)	39	<0.01	<0.01
Corn, Sweet (2001)							
Germansville, PA	1	Argent	K+CWHR	102 (0.091)	49	<0.01	<0.01
Medicine Hat, AB	7A	Sheeba	K+CWHR	100 (0.089)	35	<0.01	<0.01
Fresno, CA	10	Silver Queen	K+CWHR	99 (0.088)	48	<0.01	<0.01
Parkdale, OR	11	Kandy Kris	K+CWHR	99 (0.088)	45	<0.01	<0.01
Hillsboro, OR	12	Honey & Pearls Super Sweet	K+CWHR	100 (0.089)	45	<0.01	<0.01

TABLE C.3. Residue Data from Corn (Field and Sweet) Field Trials with Topramezone.

Trial Identification	Region	Crop/ Variety	Commodity or Matrix	Total Rate g a.i./ha (lb a.i./A)	PHI (days)	Residues (ppm)	
						Topramezone	BAS 670 H 05
Corn, Field (2000)							
Fuquay-Varina, NC	2	Pioneer 3140	Forage	100 (0.089)	32	<0.05	<0.05
Dewey, IL	5	Pioneer 34B23	Forage	101 SC (0.090) SC	55	<0.05 SC	<0.05 SC
			Forage	99 DF (0.088) DF	55	<0.05 DF	<0.05 DF
Hinckley, IL	5	Asgrow RX 770 RR	Forage	100 SC (0.089) SC	64	<0.05 SC	<0.05 SC
			Forage	101 DF (0.090) DF	64	<0.05 DF	<0.05 DF
East Lynn, IL	5	Pioneer 34B23	Forage	102 (0.091)	55	<0.05	<0.05
Mazon, IL	5	NK 6423	Forage	99 (0.088)	55	<0.05	<0.05
Owatonna, MN	5	Dekalb DK-477	Forage	96 SC (0.085) SC	64	<0.05 SC	<0.05 SC
			Forage	100 DF (0.089) DF	64	<0.05 DF	<0.05 DF
Warsaw, MN	5	Ag. Venture 626	Forage	100 (0.089)	61	<0.05	<0.05
Friend, NE	5	Pioneer 33A14	Forage	101 (0.090)	45	<0.05	<0.05
Beaver Crossing, NE	5	Dekalb 589 RR	Forage	101 (0.090)	46	<0.05	<0.05
Adel, IA	5	PSA 7727	Forage	99 (0.088)	56	<0.05	<0.05
Perry, IA	5	Mycogen 2799	Forage	102 (0.091)	56	<0.05	<0.05
Richland, IA	5	Pioneer 3733	Forage	103 (0.092)	50	<0.05	<0.05
				306 (0.273)		NS	NS
				503 (0.449)		<0.05	<0.05
Corn, Field (2001)							
Germansville, PA	1	Doebler's G642XP	Forage	103 (0.092)	66	<0.05	<0.05
					72	<0.05	<0.05
					78	<0.05	<0.05
					85	<0.05	<0.05
					91	<0.05	<0.05
Ellendale, MN	5	DK C48-83	Forage	101 (0.090)	31	<0.05	<0.05
					38	<0.05	<0.05
					45	<0.05	<0.05
					52	<0.05	<0.05
					59	<0.05	<0.05
Hartland, MN	5	DK 507	Forage	101 (0.090)	45	<0.05	<0.05
Geneva, MN	5	DK C48-83	Forage	99 (0.088)	45	<0.05	<0.05

TABLE C.3. Residue Data from Corn (Field and Sweet) Field Trials with Topramezone.

Trial Identification	Region	Crop/ Variety	Commodity or Matrix	Total Rate g a.i./ha (lb a.i./A)	PHI (days)	Residues (ppm)	
						Topramezone	BAS 670 H 05
Richland, IA	5	Pioneer 34B23	Forage	101 (0.0905)	49	<0.05	<0.05
Kirksville, MO	5	Golden Harvest H-2552	Forage	100 (0.089)	60	<0.05	<0.05
La Plata, MO	5	LG 2585	Forage	100 (0.089)	60	<0.05	<0.05
Farnham, QC	5B	DK 44-22 bt	Forage Forage	99 SC (0.088) SC 99 DF (0.088) DF	50 50	<0.05 SC <0.05 DF	<0.05 SC <0.05 DF
St-Césaire, QC	5B	42 21 RR	Forage Forage	102 SC (0.091) SC 100 DF (0.089) DF	55 55	<0.05 SC <0.05 DF	<0.05 SC <0.05 DF
Bedford, QC	5B	DKC 44-41	Forage	106 (0.095)	54	<0.05	<0.05
St-Hugues, QC	5B	DK 359 RR	Forage	103 (0.092)	55	<0.05	<0.05
Eakly, OK	6	NK 4242 BT	Forage	99 (0.088)	34	<0.05	<0.05
Corn, Sweet (2000)							
Oviedo, FL	3	Summer Sweet - Abbott & Cobb 8102	Forage	100 (0.089)	39	<0.05	<0.05
Corn, Sweet (2001)							
Germansville, PA	1	Argent	Forage	102 (0.091)	49	<0.05	<0.05
Medicine Hat, AB	7A	Sheeba	Forage	100 (0.089)	35	<0.05	<0.05
Fresno, CA	10	Silver Queen	Forage	99 (0.088)	48	<0.05	<0.05
Parkdale, OR	11	Kandy Kris	Forage	99 (0.088)	45	<0.05	<0.05
Hillsboro, OR	12	Honey & Pearls Super Sweet	Forage	100 (0.089)	45	<0.05	<0.05
Corn, Field (2000)							
Fuquay-Varina, NC	2	Pioneer 3140	Grain	100 (0.089)	87	<0.01	<0.01
Dewey, IL	5	Pioneer 34B23	Grain	101 SC (0.090) SC 99 DF (0.088) DF	97 97	<0.01 SC <0.01 DF	<0.01 SC <0.01 DF
Hinckley, IL	5	Asgrow RX 770 RR	Grain	100 SC (0.089) SC 101 DF (0.090) DF	111 111	<0.01 SC <0.01 DF	<0.01 SC <0.01 DF
East Lynn, IL	5	Pioneer 34B23	Grain	102 (0.091)	97	<0.01	<0.01
Mazon, IL	5	NK 6423	Grain	99 (0.088)	102	<0.01	<0.01

TABLE C.3. Residue Data from Corn (Field and Sweet) Field Trials with Topramezone.

Trial Identification	Region	Crop/ Variety	Commodity or Matrix	Total Rate g a.i./ha (lb a.i./A)	PHI (days)	Residues (ppm)	
						Topramezone	BAS 670 H 05
Owatonna, MN	5	Dekalb DK-477	Grain	95 SC (0.085) SC 100 DF (0.089) DF	120 120	<0.01 SC <0.01 DF	<0.01 SC <0.01 DF
Warsaw, MN	5	Ag. Venture 626	Grain	100 (0.089)	117	<0.01	<0.01
Friend, NE	5	Pioneer 33A14	Grain	101 (0.090)	87	<0.01	<0.01
Beaver Crossing, NE	5	Dekalb 589 RR	Grain	101 (0.090)	88	<0.01	<0.01
Adel, IA	5	PSA 7727	Grain	99 (0.088)	104	<0.01	<0.01
Perry, IA	5	Mycogen 2799	Grain	102 (0.091)	104	<0.01	<0.01
Richland, IA	5	Pioneer 3733	Grain	103 (0.092) 306 (0.273)) 503 (0.449)	100	<0.01 NS <0.01	<0.01 NS <0.01
Corn, Field (2001)							
Germansville, PA	1	Doebler's G642XP	Grain	103 (0.092)	101 107 113 121 128	<0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01
Ellendale, MN	5	DK C48-83	Grain	101 (0.090)	84 91 98 105 112	<0.01 <0.01 <0.01 <0.01 <0.01	<0.01 <0.01 <0.01 <0.01 <0.01
Hartland, MN	5	DK 507	Grain	101 (0.090)	94	<0.01	<0.01
Geneva, MN	5	DK C48-83	Grain	99 (0.088)	93	<0.01	<0.01
Richland, IA	5	Pioneer 34B23	Grain	101 (0.0905)	90	<0.01	<0.01
Kirksville, MO	5	Golden Harvest H-2552	Grain	100 (0.089)	95	<0.01	<0.01
La Plata, MO	5	LG 2585	Grain	100 (0.089)	82	<0.01	<0.01
Farnham, QC	5B	DK 44-22 bt	Grain	99 SC (0.088) SC 99 DF (0.088) DF	93 93	<0.01 SC <0.01 DF	<0.01 SC <0.01 DF
St-Césaire, QC	5B	42 21 RR	Grain	102 SC (0.091) SC 100 DF (0.089) DF	97 97	<0.01 SC <0.01 DF	<0.01 SC <0.01 DF
Bedford, QC	5B	DKC 44-41	Grain	106 (0.095)	96	<0.01	<0.01

TABLE C.3. Residue Data from Corn (Field and Sweet) Field Trials with Topramezone.

Trial Identification	Region	Crop/ Variety	Commodity or Matrix	Total Rate g a.i./ha (lb a.i./A)	PHI (days)	Residues (ppm)	
						Topramezone	BAS 670 H 05
St-Hugues, QC	5B	DK 359 RR	Grain	103 (0.092)	97	<0.01	<0.01
Eakly, OK	6	NK 4242 BT	Grain	99 (0.088)	59	<0.01	<0.01
Corn, Field (2000)							
Fuquay-Varina, NC	2	Pioneer 3140	Stover	100 (0.089)	87	<0.05	<0.05
Dewey, IL	5	Pioneer 34B23	Stover	101 SC (0.090) SC 99 DF (0.088) DF	97 97	<0.05 SC <0.05 DF	<0.05 SC <0.05 DF
Hinckley, IL	5	Asgrow RX 770 RR	Stover	100 SC (0.089) SC 101 DF (0.090) DF	111 111	<0.05 SC <0.05 DF	<0.05 SC <0.05 DF
East Lynn, IL	5	Pioneer 34B23	Stover	102 (0.091)	97	<0.05	<0.05
Mazon, IL	5	NK 6423	Stover	99 (0.088)	102	<0.05	<0.05
Owatonna, MN	5	Dekalb DK-477	Stover	96 SC (0.085) SC 100 DF (0.089) DF	120 120	<0.05 SC <0.05 DF	<0.05 SC <0.05 DF
Warsaw, MN	5	Ag. Venture 626	Stover	100 (0.089)	117	<0.05	<0.05
Friend, NE	5	Pioneer 33A14	Stover	101 (0.090)	91	<0.05	<0.05
Beaver Crossing, NE	5	Dekalb 589 RR	Stover	101 (0.090)	88	<0.05	<0.05
Adel, IA	5	PSA 7727	Stover	99 (0.088)	104	<0.05	<0.05
Perry, IA	5	Mycogen 2799	Stover	102 (0.091)	104	<0.05	<0.05
Richland, IA	5	Pioneer 3733	Stover	103 (0.092) 306 (0.273) 503 (0.449)	100	<0.05 NS <0.05	<0.05 NS <0.05
Corn, Field (2001)							
Germansville, PA	1	Doebler's G642XP	Stover	103 (0.092)	101 107 113 121 128	<0.05 <0.05 <0.05 <0.05 <0.05	<0.05 <0.05 <0.05 <0.05 <0.05
Ellendale, MN	5	DK C48-83	Stover	101 (0.090)	84 91 98 105 112	<0.05 <0.05 <0.05 <0.05 <0.05	<0.05 <0.05 <0.05 <0.05 <0.05

TABLE C.3. Residue Data from Corn (Field and Sweet) Field Trials with Topramezone.

Trial Identification	Region	Crop/ Variety	Commodity or Matrix	Total Rate g a.i./ha (lb a.i./A)	PHI (days)	Residues (ppm)	
						Topramezone	BAS 670 H 05
Hartland, MN	5	DK 507	Stover	101 (0.090)	94	<0.05	<0.05
Geneva, MN	5	DK C48-83	Stover	99 (0.088)	93	<0.05	<0.05
Richland, IA	5	Pioneer 34B23	Stover	101 (0.0905)	90	<0.05	<0.05
Kirksville, MO	5	Golden Harvest H-2552	Stover	100 (0.089)	95	<0.05	<0.05
La Plata, MO	5	LG 2585	Stover	100 (0.089)	82	<0.05	<0.05
Farnham, QC	5B	DK 44-22 bt	Stover	99 SC (0.088) SC 99 DF (0.088) DF	93 93	<0.05 SC <0.05 DF	<0.05 SC <0.05 DF
St-Césaire, QC	5B	42 21 RR	Stover	102 SC (0.091) SC 100 DF (0.089) DF	97 97	<0.05 SC <0.05 DF	<0.05 SC <0.05 DF
Bedford, QC	5B	DKC 44-41	Stover	106 (0.095)	96	<0.05	<0.05
St-Hugues, QC	5B	DK 359 RR	Stover	103 (0.092)	97	<0.05	<0.05
Eakly, OK	6	NK 4242 BT	Stover	99 (0.088)	59	<0.05	<0.05
Corn, Sweet (2000)							
Oviedo, FL	3	Summer Sweet - Abbott & Cobb 8102	Stover	100 (0.089)	70	<0.05	<0.05
Corn, Sweet (2001)							
Germansville, PA	1	Argent	Stover	102 (0.091)	92	<0.05	<0.05
Medicine Hat, AB	7A	Sheeba	Stover	100 (0.089)	63	<0.05	<0.05
Fresno, CA	10	Silver Queen	Stover	99 (0.088)	65	<0.05	<0.05
Parkdale, OR	11	Kandy Kris	Stover	99 (0.088)	57	<0.05	<0.05
Hillsboro, OR	12	Honey & Pearls Super Sweet	Stover	100 (0.089)	66	<0.05	<0.05

Parent (Topramezone) and residues of the free acid metabolite (BAS 670 H 05) were <LOQ in/on all corn RAC samples.
SC - Replicate taken from the plot treated with an SC (BAS 670 00H) formulation of topramezone ("formulation-bridging" site).
DF - Replicate taken from the plot treated with a DF (BAS 670 UAH) formulation of topramezone ("formulation-bridging" site).
NS - Not sampled.

TABLE C.4.1. Summary of Residue Data from Corn (Field and Sweet) Field Trials with Topramezone.

Commodity	Total Applic. Rate g a.i./ha (lb a.i./A)	PHI (days)	Residue Levels (ppm)						
			n	Min.	Max.	HAFT*	Median	Mean	Std. Dev.
Topramezone									
Corn, field									
K+CWHR	99-103 (0.088-0.092)	29-64	10	<0.01	<0.01	-	-	-	-
Forage	99-106 (0.088-0.095)	31-91	19	<0.05	<0.05	-	-	-	-
Grain	99-106 (0.088-0.095)	59-128	19	<0.01	<0.01	-	-	-	-
Stover	99-106 (0.088-0.095)	59-128	19	<0.05	<0.05	-	-	-	-
Corn, sweet									
K+CWHR	99-102 (0.088-0.091)	35-49	6	<0.01	<0.01	-	-	-	-
Forage	99-102 (0.088-0.091)	35-49	6	<0.05	<0.05	-	-	-	-
Stover	99-102 (0.088-0.091)	57-92	6	<0.05	<0.05	-	-	-	-
BAS 670 H 05									
Corn, field									
K+CWHR	99-103 (0.088-0.092)	29-64	10	<0.01	<0.01	-	-	-	-
Forage	99-106 (0.088-0.095)	31-91	19	<0.05	<0.05	-	-	-	-
Grain	99-106 (0.088-0.095)	59-128	19	<0.01	<0.01	-	-	-	-
Stover	99-106 (0.088-0.095)	59-128	19	<0.05	<0.05	-	-	-	-
Corn, sweet									
K+CWHR	99-102 (0.088-0.091)	35-49	6	<0.01	<0.01	-	-	-	-
Forage	99-102 (0.088-0.091)	35-49	6	<0.05	<0.05	-	-	-	-
Stover	99-102 (0.088-0.091)	57-92	6	<0.05	<0.05	-	-	-	-

* HAFT = Highest Average Field Trial.

TABLE C.4.2. Formulation-Bridging Summary of Residue Data from Field Corn Trials with Topramezone.

Commodity	Total Applic. Rate g a.i./ha (lb a.i./A)	PHI (days)	Residue Levels (ppm)						
			n	Min.	Max.	HAFT*	Median	Mean	Std. Dev.
SC (BAS 670 00 H) formulation									
K+CWHR	96-102 (0.085-0.091)	43-61	5	<0.01	<0.01	-	-	-	-
Forage	96-102 (0.085-0.091)	50-64	5	<0.05	<0.05	-	-	-	-
Grain	96-102 (0.085-0.091)	93-120	5	<0.01	<0.01	-	-	-	-
Stover	96-102 (0.085-0.091)	93-120	5	<0.05	<0.05	-	-	-	-
DF (BAS 670 UAH) formulation									
K+CWHR	99-101 (0.088-0.090)	43-61	5	<0.01	<0.01	-	-	-	-
Forage	99-101 (0.088-0.090)	50-64	5	<0.05	<0.05	-	-	-	-
Grain	99-101 (0.088-0.090)	93-120	5	<0.01	<0.01	-	-	-	-
Stover	99-101 (0.088-0.090)	93-120	5	<0.05	<0.05	-	-	-	-

TABLE C.4.3. Formulation-Bridging Summary of Residue Data from Field Corn Trials with BAS 670 H 05.

Commodity	Total Applic. Rate g a.i./ha (lb a.i./A)	PHI (days)	Residue Levels (ppm)						
			n	Min.	Max.	HAFT*	Median	Mean	Std. Dev.
SC (BAS 670 00 H) formulation									
K+CWHR	96-102 (0.085-0.091)	43-61	5	<0.01	<0.01	-	-	-	-
Forage	96-102 (0.085-0.091)	50-64	5	<0.05	<0.05	-	-	-	-
Grain	96-102 (0.085-0.091)	93-120	5	<0.01	<0.01	-	-	-	-
Stover	96-102 (0.085-0.091)	93-120	5	<0.05	<0.05	-	-	-	-
DF (BAS 670 UAH) formulation									
K+CWHR	99-101 (0.088-0.090)	43-61	5	<0.01	<0.01	-	-	-	-
Forage	99-101 (0.088-0.090)	50-64	5	<0.05	<0.05	-	-	-	-
Grain	99-101 (0.088-0.090)	93-120	5	<0.01	<0.01	-	-	-	-
Stover	99-101 (0.088-0.090))	93-120	5	<0.05	<0.05	-	-	-	-

D. CONCLUSION

The crop field trial data on corn (field and sweet) were deemed acceptable for the determination of residues of the active ingredient topramezone and the free acid metabolite BAS 670 H 05 when using either the SC or DF foliar treatment formulations containing topramezone. The study use pattern was a maximum seasonal application rate of 100 g a.i./ha (0.089 lb a.i./A) for field corn (PHI of 29 days for K+CWHR, 31 days for forage, and 59 days for grain and stover) and for sweet corn (PHI of 35 days for K+CWHR and forage, and 57 days for stover). With these use patterns, residues of topramezone and BAS 670 H 05 from either the SC or DF formulations are not expected to exceed the LOQ (<0.01 ppm for K+CWHR and grain; <0.05 ppm for forage and stover).

E. REFERENCE

MRID No. 45902410. Jordan, J., (2002). Storage Stability of BAS 670 H and its Cleaved Acid Metabolite M670H05 in Plant Matrices. BASF Study Number: 2002/5003838, Unpublished study prepared by BASF Corporation Agro Research. 67 pages.

F. DOCUMENT TRACKING

RDI: G.F. Kramer (2/11/05), RAB1 Chemists (12/15/04), ChemSAC (3/2/05)
S. Levy:806T:CM#2:(703)305-0783:7509C:RAB1
Petition Number: 3F6568
DP #: 310772
PC Code: 123009

Template Version September 2003

Evaluator — Date: Mar 21/05
Suzan Mathew
Evaluation Officer
FREAS, HED, PMRA

Peer Reviewer — Date: 4-12-05
George F. Kramer, Ph.D.
Chemist
RAB1, HED, EPA

Approved by — Louise G. Croteau — Date: March 22/05
Louise G. Croteau
Senior Evaluation Officer
FREAS, HED, PMRA

Date: Mar 29/05
Ariff Ally, Ph.D.
Section Head
FREAS, HED, PMRA

P.V. Shah — Date: 4/18/05
P.V. Shah, Ph.D.
Branch Senior Scientist
RAB1, HED, EPA

STUDY REPORTS:

MRID No. 45902411. Malinsky, D. S. (2002). A Meat and Milk Magnitude of the Residue Study with BAS 670 H in Lactating Dairy Cows. BASF Study Number: 56760. Unpublished study prepared by BASF Agro Research. 147 p.

EXECUTIVE SUMMARY:

Topramezone (also known as BAS 670 H) was administered orally via a balling gun to 14 lactating dairy cows (*Holstein Friesian*) once daily for 29 days. Dosing was made at 0.37 mg/kg feed, 1.04 mg/kg feed and 3.57 mg/kg feed. Residues in milk and tissue samples were extracted in water, acidified with HCl and partitioned with dichloromethane (DCM). The organic phase was back-partitioned with ammonium formate, pH 10, and the aqueous phase was analysed for topramezone and the metabolite M670H02 by liquid chromatography with mass spectrometry

(LC-MS/MS). The fat in muscle samples was analysed by the Soxhlet-extraction method. Samples were stored frozen and analysed within 30 days of collection; thus, no storage stability data for livestock matrices (to determine sample stability) were required. In muscle, individual recoveries of <70% were reported for both analytes, but the mean recoveries were 71.0 and 74.5% for topramezone and M670H02, respectively. The overall recoveries for all other matrices (and all spiking levels) ranged from 75-106% (SD <20%) for topramezone, and 72-103% (SD <20%) for M670H02. Residue levels of topramezone and M670H02 in milk were below the limit of quantitation (LOQ; 0.01 ppm) for every sampling period and every treatment dose level. Since the residue levels in the 3.57 mg/kg treatment group were below the LOQ, no depuration analysis for milk was necessary. Residue levels of both analytes were also below the LOQ for fat (0.05 ppm) and muscle (0.01 ppm). Quantifiable residue levels were seen for parent topramezone in kidney and liver, but were <LOQ (0.05 ppm) for M670H02. At the 0.37 mg/kg treatment level, the residues in liver and kidney were 0.484-0.608 ppm and 0.144-0.188 ppm, respectively. At the 1.04 mg/kg treatment level, the residues in liver and kidney were 0.808-1.30 ppm and 0.197-0.208 ppm, respectively. At the 3.57 mg/kg treatment level, the residues in liver and kidney were 1.59-1.88 ppm and 0.320-0.350 ppm, respectively. Residue levels declined 2 and 7 days after the last administered dose in both liver (1.39 ppm to 0.836 ppm) and kidney (0.178 ppm to 0.113 ppm), but were still quantifiable.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the livestock feeding study 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# 310772] and in Canada's Regulatory Decision Document.

COMPLIANCE:

Signed and dated Good Laboratory Practice (GLP), Quality Assurance and Data Confidentiality statements were provided. Minor deviations from the protocol involved the exclusion of sample weight and bottle tare weight on sample labels; tissue samples were shipped together instead of separately; blood samples were analysed for different indicators than required; daily animal observations were not conducted for one cow on one day; the omission of sending a water sample to the sponsor; and milk sampling set for day 24 was delayed by one day. These deviations did not impact the study. Milk production from one cow was less than the required 12 kg throughout most of the study. This was thought to have an effect on the excretion rate of the test material in this animal. However, residues in milk were less than <0.01 ppm (LOQ) for all test cows at all treatment levels; therefore, the effect of this cow was negligible.

A. BACKGROUND INFORMATION

BAS 670 336SC Herbicide (also known as BAS 670 00H Herbicide) is the end-use product which contains the active ingredient topramezone. It is a broad-spectrum, post-emergence herbicide used to control grassy and broadleaf weeds in corn. The herbicide is absorbed by the leaves, roots and shoots, then translocated to the growing points of the sensitive weeds. Topramezone belongs to the triketone class of chemicals, which inhibits carotenoid biosynthesis (HPPD (4-hydroxyphenylpyruvate dioxygenase) inhibitor)). This causes a strong bleaching activity on the growing zones of the shoots within 2-5 days of application. Exposure to light causes necrosis of chlorotic tissues and eventual plant death within 14 days after application.

TABLE A.1. Test Compound Nomenclature

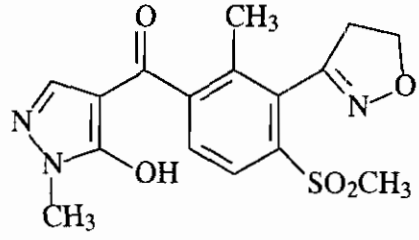
Compound	Chemical Structure 
Common name	Topramezone
Company experimental name	BAS 670 H
IUPAC name	[3-(4,5-dihydro-1.2-oxazol-3-yl)-4-mesyl-*o*-tolyl](5-hydroxy-1-methyl-1*H*-pyrazol-4-yl) methanone
CAS name	[3-(4,5-dihydro-3-isoxazolyl)-2-methyl-4-(methylsulfonyl)phenyl](5-hydroxy-1-methyl-1*H*-pyrazol-4-yl)methanone
Molecular Formula	$C_{16}H_{17}N_3O_5S$
Molecular Mass	363.39 g/mol
CAS #	210631-68-8
End-use product/EP	Soluble Concentrate

TABLE A.2. Physicochemical Properties of the Technical Grade Test Compound

Parameter	Value (all data from PMRA Lab Services)
Melting point/range	220.9°C-222.2°C
pH	2.9 (1% de-ionized water)
Density	1.425 g/cm^3
Water solubility (20°C)	pH: 3, 5, 7, 9 g/L: 0.06, 0.98, 15, 23.4
Solvent solubility (g/100 mL at 20°C)	acetone < 1 acetonitrile < 1 dichloromethane 2.5 –2.9 ethyl acetate < 1 methanol < 1 n-heptane < 1 N,N-dimethylformamide 11.4-13.3 1-octanol < 1 olive oil < 1 2-propanol < 1 toluene < 1
Vapour pressure at 20°C and 25°C	< 1 x 10^{-12} hPa
Dissociation constant (pK_a)	4.06 at 20°C
Octanol/water partition coefficient Log(K_{OW})	Buffer pH: 4, 7, 9 Log K_{ow}: – 0.81, – 1.52, – 2.34
UV/visible absorption spectrum	λ, nm: 207, 272, 300, 410 ξ, mol^{-1}cm^{-1}: 27 077, 8601, 5800, 410

B. EXPERIMENTAL DESIGN

B.1. Livestock

TABLE B.1.1. Description of Livestock Used in the Feeding Study

Species	Breed	Age	Weight at study initiation (kg)	Health status	Description of housing/holding area
Dairy Cattle (*Bos taurus*)	Holstein Friesian	5-7 yrs	484-792 kg (1068-1745 lbs)	One cow had low feed intake (anorexia), low milk production, and bloody diarrhea through most of the study period. The cow appeared to recuperate near the end of the study. All other cows were in good general condition.	Outside pens measuring 10' X 30' for days 1-28 of study period. Temperature ranged from 2-30°C (36-86°F). Humidity ranged from 2-97%. Natural lighting was used.

TABLE B.1.2. Test Animal Dietary Regime

Composition of Diet	Feed consumption (kg/day)	Water	Acclimation period	Predosing
Total mixed ration (TMR) containing: Sorghum silage - 20.97% Corn silage - 20.00% Alfalfa - 13.0% Corn - 12.00% Cotton seed - 5.00% Vitamins and minerals - 4.81% Canola pellets - 3.50% Grain screening pellets - 3.00% Dried distillers grain - 2.00% Molasses - 2.00%	*Cow 8 during the study period:* 13.2-23.1 kg/day (29-51 lbs/day) *All other cows during the study period:* 23.6-36.7 kg/day (52-81 lbs/day)	*Ad libitum* from automatic waterers	11 days	No

TABLE B.1.3. Dosing Regime.

Treatment group	Level of administered dose (mg/day)	Residue intake in diet (ppm)	Vehicle	Timing/ Duration
I II III IV	0.0 7.7 18.7 67.9	0 0.37 1.04 3.57	Gelatin capsules administered orally via a balling gun	Once daily for 29 days

TABLE B.1.4 Sample Collection				
Milk collected	Amount of milk produced during normal production	Urine, feces and cage wash collected	Interval from last dose to sacrifice	Tissues harvested and analysed
Twice daily (PM and AM) on days -1, 1, 3, 5, 7, 10, 14, 17, 21, 25, 28, 31, 34 and 36. Extra milk, cream and skim milk samples were collected from Study day 21	**Acclimation period:** *Cow 8:* 22.3-25.3 kg/day (49.1-55.8 lb/day) *All other cows:* 18.7-40.7 kg/day (41.2-89.7 lb/day) **Study period:** *Cow 8:* 6.2-11.3 kg/day (13.7-24.9 lb/day) *All other cows:* 16.0-37.2 kg/day (35.3-82.0 lb/day)	Not collected	5 hours (cows 1-12) 3 days (cow 13) 8 days (cow 14)	Fat (renal, mesenteric and peripheral), kidney, liver, muscle (thigh and loin composite), milk

B.2. Sampling Handling and Preparation

Milk samples were shipped frozen (≤-10°C) to BASF Agro Research (Research Triangle Park, NC). Samples of muscle, kidney, liver and fat were collected after animal termination. Tissue samples were homogenized with dry ice (Robot Coupe Processor) at Southwest Bio-Labs. The samples were weighed into separate labelled Ziploc storage bags, then stored frozen (≤-10°C) until shipped to BASF Agro Research. All milk and tissue samples were stored frozen at BASF until analysis.

B.3. Analytical Methodology

Analytical method D0104 was used to analyse residues of topramezone and M670H02 in the various cattle matrices (Jones, 2002). Milk and homogenized tissue samples were extracted with 100% water by mechanical shaking. Aliquots of the extracts were acidified with 1N HCl, and partitioned with DCM. A portion of the organic (DCM) layer was added to 4mM ammonium formate buffer (pH 10). The aqueous layer was then removed directly for analysis by LC-MS/MS.

The fat content from muscle samples was analysed by the Soxhlet extraction method, as described in the "Handbook of Meat Analysis" by Edward S. Koniecko (1985). The meat sample was mixed with sand and spread with a glass rod on the bottom of an aluminum dish. The rod was wiped clean with filter paper, which was then placed in the dish. The sample was dried for 2 hours at 125°C, then placed in a sealed plastic bag with desiccant. The dish was placed in a cellulose extraction thimble, and placed in the Soxhlet extraction apparatus. Petroleum ether was transferred into a pre-weighed boiling flask, then the Soxhlet extraction apparatus was assembled and placed in a water bath (50-65°C) for 4.5 hours (extraction via ether condensation). After the extraction, the ether (in an extraction flask) was placed in a rotary evaporator. After the ether

removal, the flask (and its contents) was dried for 30 minutes in a 100°C oven. The flask was then weighed.

The percent fat from the muscle sample was determined by the following calculation:

(weight of the extracted fat/weight of the fresh muscle) * 100

The limit of detection (LOD) corresponded to 4 pg/100 uL standard (0.04 ppb), which was defined as the lowest standard level injected with a signal to noise ratio (S/N) between 3-5. The LOQs were 0.01 ppm (milk, muscle and egg) and 0.05 ppm (liver, kidney and fat).

C. RESULTS AND DISCUSSION

The samples were analysed within 30 days of collection; thus, no storage stability data for livestock tissues were required. The average percent fat content of muscle ranged from 2.01-6.55% (SD <0.38%), which were reported as levels to be expected in lactating dairy cows.

The analytical method D0104 was able to produce recoveries in the acceptable range of 75-106% (SD <20%) for topramezone, and 72-103% (SD<20%) for M670H02 at all spiking levels, except for muscle. In muscle, recoveries from one of the two samples analysed (per analyte) were below 70% at the 0.01 ppm spiking level. However, this was not a concern as the mean recoveries in muscle were 71.0% (topramezone) and 74.5% (M670H02). Detection was linear in the range of 0.1-2.0 ng/mL (ppb). Samples were diluted to fit within the area of the standard curve. The coefficients of determination (r^2) were 0.9946 for topramezone and 0.9904 for M670H02. The chromatograms from the control samples of milk, liver and kidney appeared to have minor interference around the area of interest for M670H02. All other control samples for M670H02 and topramezone were free of interference. Recoveries were corrected for apparent residues in control samples, as necessary. Spiked and test samples depicted clear peaks at the areas of interest, and control samples were free of interference.

One cow (cow 8 - 1.04 mg/kg treatment group) included in the study fell ill during the 28- day study period. This manifested itself as a low feed intake, and thus low milk production in comparison to the other test cows. The animal appeared to recover by the end of the study period, thus the illness was not considered treatment related. There was concern about the data from this cow, as the low milk production may have affected the excretion rate of the test material from the animal. However, the residue levels seen in milk for both analytes were below 0.01 ppm (LOQ) for all cows, and at all feeding levels. Thus, the data from this single cow did not negatively impact the study. Since the residue levels of both analytes in milk were below the LOQ at the 3.57 mg/kg-feeding level, no depuration, skim milk or cream samples were analysed. Residue levels of topramezone and M670H02 were also below the LOQ for the muscle (<0.01 ppm) and fat (<0.05 ppm) samples from all treatment groups. Residue levels of M670H02 were also below the LOQ for liver and kidney (<0.05 ppm). This is supported by the low log K_{ow}, which indicated that sequestration of topramezone in fatty tissues was unlikely. Quantifiable

residues of topramezone were detected in liver and kidney samples at all feeding levels. Residue levels in liver rose from 0.484-0.608 ppm at the 0.37 mg/kg feeding level, to 0.808-1.296 ppm at the 1.04 mg/kg feeding level, and to 1.59-1.88 ppm at the 3.57 mg/kg feeding level. Samples taken 2 and 7 days after the last administered dose indicated residues decreased to 1.39 ppm and 0.836 ppm, respectively. Residue levels in kidney also rose from 0.144-0.188 ppm at the 0.37 mg/kg feeding level, to 0.197-0.208 ppm at the 1.04 mg/kg feeding level, and to 0.320-0.350 ppm at the 3.57 mg/kg feeding level. Samples taken 2 and 7 days after the last administered dose indicated residues again decreased to 0.178 ppm and 0.113 ppm, respectively. The residue-to-feed (R/F) ratio, the mean residues at each feeding level divided by the feeding level itself (TABLE C.4), was determined for calculating anticipated residues.

TABLE C.1. Summary of Concurrent Recoveries of Topramezone from Milk and Ruminant Tissues

Matrix	Analyte	Spike level (mg/kg)	Sample size (n)	Recoveries (%)	Mean ± std dev (CV) (%)
Milk	Topramezone	0.01	5	103, 94, 94, 89, 92	94.4 ± 5.2 (5.5)
		0.50	5	89, 92, 94, 98, 98	94.2 ± 3.9 (4.1)
	M670H02	0.01	5	103, 72, 86, 97, 91	89.8 ± 11.8 (13.2)
		0.50	5	85, 87, 92, 102, 89	91.0 ± 6.7 (7.3)
Liver	Topramezone	0.05	2	106, 97	101.5
		1	1	75	—
		2	2	88, 92	90
	M670H02	0.05	3	101, 94, 75	90.0 ± 13.5 (15.0)
		1	1	81	—
		2	2	88, 82	85
Kidney	Topramezone	0.05	2	89, 84	86.5
		1	2	92, 106	99
	M670H02	0.05	2	80, 72	76
		1	2	75, 72	73.5
Fat	Topramezone	0.05	1	98	—
		0.50	1	100	—
	M670H02	0.05	1	77	—
		0.50	1	89	—
Muscle	Topramezone	0.01	2	76, 66	71
	M670H02	0.01	2	81, 68	74.5

TABLE C.2. Summary of Storage Conditions

Matrix (RAC or Extract)	Storage Temp. (°C)	Actual Storage Duration (days)	Interval of Demonstrated Storage Stability (days or months)
Milk, muscle, fat, liver and kidney	<-10°C	<28 days	Not provided

TABLE C.3. Residue Data from Ruminant Feeding Study with Topramezone

Animal identification #	Matrix/Collection Time	Feeding Level (ppm)	Residues (ppm)	
			Topramezone	M670H02
Cow 1 (control)	Milk/Study days -1,5,14, 21	0	<0.01 for all days	<0.01 for all days
	Liver/Termination		<0.05	<0.05
	Kidney/Termination		NA	NA
	Fat/Termination		<0.05	<0.05
	Muscle/Termination		NA	NA
Cow 2 (control)	Milk/Study days -1, 7, 17, 28	0	<0.01 for all days	<0.01 for all days
	Liver/Termination		<0.05	<0.05
	Kidney/Termination		NA	NA
	Fat/Termination		<0.05	<0.05
	Muscle/Termination		<0.01	<0.01
Cow 3 (control)	Milk/Study days -1, 10, 28	0	<0.01 for all days	<0.01 for all days
	Liver/Termination		<0.05	<0.05
	Kidney/Termination		<0.05	<0.05
	Fat/Termination		NA	NA
	Muscle/Termination		NA	NA
Cow 4	Milk/Study days -1, 1, 3, 5, 7, 10	0.37	<0.01 for all days	<0.01 for all days
	Liver/Termination		0.572	<0.05
	Kidney/Termination		0.1436	<0.05
	Fat/Termination		NA	NA
	Muscle/Termination		NA	NA
Cow 5	Milk/Study days -1, 1, 3, 5, 7, 10	0.37	<0.01 for all days	<0.01 for all days
	Liver/Termination		0.484	<0.05
	Kidney/Termination		0.1468	<0.05
	Fat/Termination		NA	NA
	Muscle/Termination		NA	NA
Cow 6	Milk/Study days -1, 1, 3, 5, 7, 10	0.37	<0.01 for all days	<0.01 for all days
	Liver/Termination		0.608	<0.05
	Kidney/Termination		0.1876	<0.05
	Fat/Termination		NA	NA
	Muscle/Termination		NA	NA

Animal identification #	Matrix/Collection Time	Feeding Level (ppm)	Residues (ppm)	
			Topramezone	M670H02
Cow 7	Milk/Study days -1, 1, 3, 5, 7, 10	1.04	<0.01 for all days	<0.01 for all days
	Liver/Termination		0.808	<0.05
	Kidney/Termination		0.206	<0.05
	Fat/Termination		NA	NA
	Muscle/Termination		NA	NA
Cow 8	Milk/Study days -1, 1, 3, 5, 7, 10	1.04	<0.01 for all days	<0.01 for all days
	Liver/Termination		1.296	<0.05
	Kidney/Termination		0.1972	<0.05
	Fat/Termination		NA	NA
	Muscle/Termination		NA	NA
Cow 9	Milk/Study days -1, 1, 3, 5, 7, 10	1.04	<0.01 for all days	<0.01 for all days
	Liver/Termination		1.282	<0.05
	Kidney/Termination		0.208	<0.05
	Fat/Termination		NA	NA
	Muscle/Termination		NA	NA
Cow 10	Milk/Study days -1, 1, 3, 5, 7, 10, 14. 17, 21, 25, 28	3.57	<0.01 for all days	<0.01 for all days
	Liver/Termination		1.588	<0.05
	Kidney/Termination		0.320	<0.05
	Fat/Termination		<0.05	<0.05
	Muscle/Termination		<0.01	<0.01
Cow 11	Milk/Study days -1, 1, 3, 5, 7, 10, 14, 17, 21, 25, 28	3.57	<0.01 for all days	<0.01 for all days
	Liver/Termination		1.876	<0.05
	Kidney/Termination		0.350	<0.05
	Fat/Termination		<0.05	<0.05
	Muscle/Termination		<0.01	<0.01
Cow 12	Milk/Study days -1, 1, 3, 5, 7, 10, 14, 17, 21, 25, 28	3.57	<0.01 for all days	<0.01 for all days
	Liver/Termination		1.882	<0.05
	Kidney/Termination		0.326	<0.05
	Fat/Termination		<0.05	<0.05
	Muscle/Termination		<0.01	<0.01

Animal identification #	Matrix/Collection Time	Feeding Level (ppm)	Residues (ppm)	
			Topramezone	M670H02
Cow 13 (day 31)	Milk/Study days -1, 1, 3, 5, 7, 10, 14, 17, 21, 25, 28	3.57	<0.01 for all days	<0.01 for all days
	Liver/Termination		1.390	<0.05
	Kidney/Termination		0.178	<0.05
	Fat/Termination		<0.05	<0.05
	Muscle/Termination		NA	NA
Cow 14 (day 36)	Milk/Study days -1, 1, 3, 5, 7, 10, 14, 17, 21, 25, 28	3.57	<0.01 for all days	<0.01 for all days
	Liver/Termination		0.8360	<0.05
	Kidney/Termination		0.1134	<0.05
	Fat/Termination		NA	NA
	Muscle/Termination		NA	NA

NA - Not analysed

TABLE C.4. Summary of Residue Data from Ruminant Feeding Study with Topramezone

Matrix	Feeding Level (ppm)	Residue Levels (ppm)						
		n	Min.	Max.	Median	Mean	Std. Dev.	R/F Ratio*
Topramezone								
Milk	0.0	3	<0.01	<0.01				
	0.37	3	<0.01	<0.01	—	—	—	—
	1.04	3	<0.01	<0.01				
	3.57	5	<0.01	<0.01				
Liver	0.0	3	<0.05	<0.05	—	—	—	—
	0.37	3	0.484	0.608	0.572	0.555	0.064	1.50
	1.04	3	0.808	1.296	1.282	1.129	0.278	1.09
	3.57	3	1.588	1.882	1.876	1.782	0.168	0.499
Kidney	0.0	1	<0.05	<0.05	—	—	—	—
	0.37	3	0.1436	0.1876	0.147	0.159	0.025	0.430
	1.04	3	0.1972	0.2080	0.206	0.204	0.006	0.196
	3.57	3	0.3200	0.3500	0.326	0.332	0.016	0.0930
Fat	0.0	2	<0.05	<0.05				
	0.37	NA	NA	NA	—	—	—	—
	1.04	NA	NA	NA				
	3.57	4	<0.05	<0.05				
Muscle	0.0	1	<0.01	<0.01				
	0.37	NA	NA	NA	—	—	—	—
	1.04	NA	NA	NA				
	3.57	3	<0.01	<0.01				
M670H02								
Milk	0.0	3	<0.01	<0.01				
	0.37	3	<0.01	<0.01	—	—	—	—
	1.04	3	<0.01	<0.01				
	3.57	5	<0.01	<0.01				
Liver	0.0	3	<0.05	<0.05				
	0.37	3	<0.05	<0.05	—	—	—	—
	1.04	3	<0.05	<0.05				
	3.57	5	<0.05	<0.05				
Kidney	0.0	1	<0.05	<0.05				
	0.37	3	<0.05	<0.05	—	—	—	—
	1.04	3	<0.05	<0.05				
	3.57	5	<0.05	<0.05				
Fat	0.0	2	<0.05	<0.05				
	0.37	NA	NA	NA	—	—	—	—
	1.04	NA	NA	NA				
	3.57	4	<0.05	<0.05				
Muscle	0.0	1	<0.01	<0.01				
	0.37	NA	NA	NA	—	—	—	—
	1.04	NA	NA	NA				
	3.57	3	<0.01	<0.01				

NA - Not analysed

* Residue-to-feed ratio (mean residues divided by feeding level)

TABLE C.5. Summary of Residues of Topramezone in Tissues of Dairy Cattle from the Depuration Study

Matrix	Study Day	Animal #	Residue (ppm)
Liver	31	13	1.390
	36	14	0.8360
Kidney	31	13	0.178
	36	14	0.1134

D. CONCLUSION

Dairy cattle were treated orally with topramezone (also known as BAS 670 H) at feeding levels of 0.37 ppm, 1.04 ppm or 3.57 ppm for a period of 29 days. Procedural recoveries of parent topramezone and M670H02 were generally within the acceptable range. Residue levels of M670H02 were below the LOQ for all matrices and feeding levels. Residue levels of topramezone were below the LOQ for milk (<0.01 ppm), fat (<0.05 ppm) and muscle (<0.01 ppm) at all feeding levels. The average residues of parent topramezone at the 0.37 mg/kg, 1.04 mg/kg and 3.57 mg/kg feeding levels were 0.555, 1.13, and 1.78 ppm for liver; and 0.159, 0.204, and 0.332 ppm for kidney. Residue levels declined 2 and 7 days after the last administered dose in both liver (1.39 ppm to 0.836 ppm) and kidney (0.178 ppm to 0.113 ppm), but were still quantifiable.

E. REFERENCES

Jones, J.E., and D.S. Malinsky. (2002) "Final Report. Validation of BASF Method No. D0104: Method for Determination of BAS 670 H and Its Metabolite (M670H02) Residues in Lactating Dairy Cow Tissues, Milk and Egg using LC/MS/MS." BASF Study Number: 55913. Unpublished study prepared by BASF Corporation. 68 pages.

Koniecko, E. S. 1985. Handbook of Meat Analysis. 2nd ed. Avery Publishing Group Inc., Wayne, New Jersey.

F. DOCUMENT TRACKING

RDI: P.V. Shah (3/2/05), RAB1 Chemists (12/8/04), ChemSAC (3/2/05)
G.F. Kramer:806T:CM#2:(703)305-5079:7509C:RAB1
Petition Number: 3F6568
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Template Version September 2003

Evaluator — Date: Mar 21/05

S. Mathew

Suzan Mathew
Evaluation Officer
FREAS, PMRA

Peer Reviewer — Date: 4-12-05.

George F. Kramer, Ph.D.
Chemist
RAB1, HED, EPA

Approved by — Date: March 22/05

Louise G. Croteau
Senior Evaluation Officer
FREAS, PMRA

Date: March 30

Ariff Ally, Ph.D.
Section Head
FREAS, HED, PMRA

Date: 4/28/05

P.V. Shah, Ph.D.
Branch Senior Scientist
RAB1, HED, EPA

STUDY REPORTS:

MRID No. 45902410. Jordan, J. (November 26, 2002). Storage Stability of BAS 670 H and its Cleaved Acid Metabolite M670H05 in Plant Matrices: Lab Project Number: 56750. Unpublished study prepared by BASF Corporation. 67 pages.

EXECUTIVE SUMMARY:

Samples of corn forage, grain, straw and radish roots (spiked with parent topramezone, and the metabolite M670H05, at a level of 1.0 ppm) were stored at -20°C for a duration of 541 days.

The high-performance liquid chromatography with dual mass-selective detectors (LC-MS/MS) BASF Method No. D0007, as outlined in the analytical methodology study, was used in the

storage stability study. This method has been found to be an acceptable data gathering and enforcement method for the analysis of parent topramezone and M670H05 in corn forage, grain, straw and radish root.

Under these conditions, the decline in residues of parent and M670H05 were considered statistically insignificant ($p > 0.05$). The data indicate that residues of topramezone and the metabolite M670H05 were stable at -20°C for up to 541 days (18 months) in corn forage, grain, straw and radish roots.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the storage stability residue 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# 310772] and in Canada's Regulatory Decision Document.

COMPLIANCE:

Signed and dated Good Laboratory Practice (GLP), Quality Assurance and Data Confidentiality statements were provided. No deviations were reported.

A. BACKGROUND INFORMATION

BAS 670 336SC (soluble concentrate) Herbicide (also known as BAS 670 00H Herbicide) is the end-use product which contains the active ingredient topramezone. It is a broad-spectrum, post-emergence herbicide used to control grassy and broadleaf weeds in corn. The herbicide is absorbed by the leaves, roots and shoots, then translocated to the growing points of the sensitive weeds. Topramezone belongs to the triketone class of chemicals, which inhibits carotenoid biosynthesis (4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitor). This causes a strong bleaching activity on the growing zones of the shoots within 2-5 days of application. Exposure to light causes necrosis of chlorotic tissues and eventual plant death within 14 days after application.

TABLE A.1. Test Compound Nomenclature

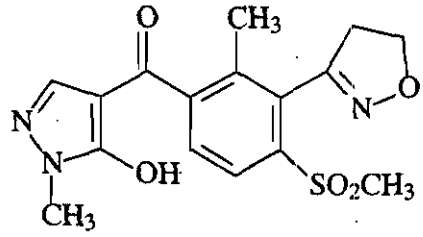
Compound	Chemical Structure 
Common name	Topramezone
Company experimental name	BAS 670 H
IUPAC name	[3-(4,5-dihydro-1,2-oxazol-3-yl)-4-mesyl-*o*-tolyl](5-hydroxy-1-methyl-1*H*-pyrazol-4-yl) methanone
CAS name	[3-(4,5-dihydro-3-isoxazolyl)-2-methyl-4-(methylsulfonyl)phenyl](5-hydroxy-1-methyl-1*H*-pyrazol-4-yl)methanone
Molecular Formula	$C_{16}H_{17}N_3O_5S$
Molecular Mass	363.39 g/mol
CAS #	210631-68-8
End-use product/EP	Soluble Concentrate

TABLE A.2. Physicochemical Properties of Topramezone

Parameter	Value (All data came from PMRA Lab Services)
Melting point/range	220.9°C - 222.2°C
pH	2.9 (1% deionized water)
Density (20°C)	1.425 g/cm^3
Water solubility (20°C)	pH: 3, 5, 7, 9 g/L: 0.06, 0.98, 15, 23.4
Solvent solubility (g/100 mL at 20°C)	Acetone <1.0 Acetonitrile <1.0 Dichloromethane 2.5 - 2.9 Ethyl acetate <1.0 Methanol <1.0 N-heptane <1.0 N,N-dimethylformamide 11.4-13.3 1-octanol <1.0 Olive oil <1.0 2-propanol <1.0 Toluene <1.0
Vapour pressure at 20°C and 25°C	< 1.0 x 10^{-12} hPa
Dissociation constant (pK_a)	4.06
Octanol/water partition coefficient Log(K_{ow}) at 20°C	Buffer pH: 4, 7, 9 Log K_{ow}: −0.81, −1.52, −2.34
UV/visible absorption spectrum	λ, nm: 207, 272, 300, 410 ξ, mol^{-1}cm^{-1}: 27 077, 8601, 5800, 410

B. EXPERIMENTAL DESIGN

B.1. Sample Handling and Preparation

Control samples from the magnitude of the residue trials were used in the storage stability study. Samples were prepared and stored in the same manner as the crop field trial. Sample aliquots of 10 g were homogenized with dry ice and stored in plastic bags. Two samples were spiked with 1.0 ppm of topramezone and 1.0 ppm of M670H05. Following the spiking procedure, the samples were sent to frozen storage at a temperature of -20°C. For every 2 spiked samples, two to 3 controls were also placed in frozen storage. Samples (2-3 controls and 2 spiked) were removed from storage and analysed at the following nominal intervals: 0, 30, 90, 180, 360, 450 and 540 days (or 0, 1, 3, 6, 12 and 18 months). The stability of topramezone and M670H05 were tested in deionized water, methanol, acetonitrile and 0.05% NH_4OH in deionized water. The storage intervals tested were 0, 1, 7, 16, 30, 62 and 90 days. Each solvent was prepared with 1.0 µg/mL topramezone and M670H05. The mixtures were either stored in the refrigerator (4°C) in the dark, or at room temperature under full light. Aliquots were diluted with 0.05% NH_4OH before analysis by LC-MS/MS.

B.2. Analytical Methodology

Storage stability samples were analysed with BASF Method No. D0007. This method quantitates residues of topramezone and M670H05 by LC-MS/MS to a limit of 0.05 ppm in corn forage, corn stover and radish root; and 0.01 ppm for corn grain. Detector response was linear in the range of 0.2 pg/µL (4 pg) to 10 pg/µL (200 pg) for topramezone (coefficient of determination, r^2, of 0.9992) and BAS 670 H 05 (r^2 of 0.9970). Note that all samples were diluted to fit within the area of the standard curve. Sample calculations were provided and were found to be acceptable.

Residues of topramezone were extracted from crop matrices with water, and residues in seed or grain matrices were extracted using acetonitrile:water (1:1, v:v). A 2% aliquot of the extract was removed and cleaned by partitioning with a mixture of 1N HCl:dichloromethane (DCM) (1:5, v:v) and NaCl. The extract was centrifuged (if necessary), the aqueous layer was discarded, and residues in the DCM layer were partitioned into 0.1% NH_4OH. The final analyses of topramezone and M670H05 were achieved by LC-MS/MS.

C. RESULTS AND DISCUSSION

The results of the solvent storage stability study (TABLE C.1.1) indicated a maximum degradation of 15.8% (topramezone) and 19.3% (M670H05) by 90 days, both occurring in the acetonitrile solvent at room temperature with light. The stability of topramezone was highest in methanol under the refrigerated and dark conditions, and in deionized water under the room temperature and light conditions. The stability of M670H05 was highest in deionized water under the refrigerated and dark conditions, and in 0.05% NH_4OH under the room temperature and light conditions. The petitioner recognized that degradation was occurring, and implemented a protocol to replace the stock solutions every 90 days, and to replace the dilutions from the stock solutions every 30 days.

The analytical method No. D0007, used for the analysis of storage stability samples, was also used in the magnitude of the residue studies. The method was validated and deemed an acceptable method for data gathering and enforcement purposes.

Control samples from the crop field trials were used in the analysis of stability of topramezone and the metabolite M670H05 in corn forage, grain, straw and radish roots (TABLE C.2). Samples were spiked with 1.0 ppm of each analyte, and stored at -20°C for up to 541 days or 18 months. During the storage time, samples were analysed at nominal intervals of 0, 30, 90, 180, 360 and 540 days. If the recoveries were out of the acceptable range of 70-120%, as in some of the day 0 samples, the data were not reported and the sample was re-analysed on day 6. In one case (day 0 forage), the recoveries were acceptable, but the stored residues were questionable. Due to the unlikelihood of degradation occurring on day 0, the data were not reported and the samples were re-analysed on day 6. The concurrent recoveries at day 180 were acceptable, however the stored samples had low recovered residues. In this case, the data were reported, but the samples were re-analysed approximately 6 days later to determine if degradation had occurred. The second analysis, at 188 days, showed that no significant degradation had occurred.

Overall, no significant degradation of either analyte was seen in any of the matrices analysed (FIGURES C.2.1-C.2.2). For both analytes, linear regression analyses (Appendix I) indicated that the residue decline was statistically insignificant in all matrices ($p > 0.05$). Therefore, both topramezone or M670H05 were considered stable in corn forage, grain, straw and radish root for up to 18 months. It should be noted that the petitioner reported that this study was ongoing to cover a storage period of 26 months.

TABLE C.1. Summary of Concurrent Recoveries of Topramezone and M670H05 from Corn Matrices (Forage, Grain and Straw) and Radish Root.

Matrix	Spike Level (mg/kg)	Storage Interval days)	Sample Size (n)	Recoveries (%)	Mean ± std dev (%)
Topramezone					
Corn, forage	1.0	0	-	-	-
		6	2	86, 92	89
		33	2	86, 94	90
		96	2	83, 88	85.5
		182	2	94, 97	95.5
		188	2	99, 96	97.5
		314	2	99, 91	95
		369	2	85, 74	79.5
		454	2	96, 95	95.5
		541	2	78, 83	80.5
Corn, grain	1.0	0	2	98, 82	90
		33	2	97, 96	96.5
		96	2	85, 95	90
		182	2	91, 90	90.5
		188	2	96, 99	97.5
		314	2	99, 102	100.5
		365	2	94, 91	92.5
		455	2	93, 95	94
		541	1	85	85
Corn, straw	1.0	0	2	-	-
		6	2	90, 87	88.5
		33	2	92, 79	85.5
		96	2	72, 83	77.5
		182	2	80, 85	82..5
		188	2	82, 86	84
		314	2	95, 89	92
		365	2	93, 81	87
		454	2	79, 84	81.5
		541	2	83, 83	83
Radish, root	1.0	0	2	102, 101	101.5
		26	2	95, 93	94
		89	2	91, 88	89.5
		175	2	106, 102	104
		181	2	102, 93	97.5
		307	2	77, 87	82
		362	2	83, 82	82.5
		448	2	89, 100	94.5
		534	2	80, 80	80

TABLE C.1. Summary of Concurrent Recoveries of Topramezone and M670H05 from Corn Matrices (Forage, Grain and Straw) and Radish Root.

Matrix	Spike level (mg/kg)	Storage Interval days)	Sample size (n)	Recoveries (%)	Mean ± std dev (%)
M670H05					
Corn, forage	1.0	0	-	-	-
		6	2	74, 76	75
		33	2	91, 84	87.5
		96	2	86, 90	88
		182	2	95, 95	95
		188	2	98, 97	97.5
		314	2	85, 80	82.5
		369	2	84, 69	76.5
		454	2	88, 96	92
		541	2	84, 81	82.5
Corn, grain	1.0	0	2	77, 73	75
		33	2	96, 91	93.5
		96	2	93, 91	92
		182	2	88, 87	87.5
		188	2	97, 99	98
		314	2	80, 87	83.5
		365	2	88, 88	88
		455	2	97, 92	94.5
		541	1	83	83
Corn, straw	1.0	0	2	-	-
		6	2	73, 80	76.5
		33	2	90, 86	88
		96	2	82, 85	83.5
		182	2	82, 88	85
		188	2	93, 90	91.5
		314	2	85, 80	82.5
		365	2	82, 78	80
		454	2	90, 84	87
		541	2	92, 83	87.5
Radish, root	1.0	0	2	85, 85	85
		26	2	86, 87	86.5
		89	2	87, 87	87
		175	2	97, 100	98.5
		181	2	100, 96	98
		307	2	66, 75	70.5
		362	2	75, 80	77.5
		448	2	91, 98	94.5
		534	2	80, 79	79.5

TABLE C.1.1. Stability of Topramezone and M670H05 Residues in Various Solvents.

Solvent	Conditions	Storage Interval days)	Topramezone* (µg/mL)	% Degradation	M670H05* (µg/mL)	% Degradation
Deionized water	4°C, Dark	0	1.008	0	1.008	0
		1	1.005	0.110	1.023	0.098
		7	0.958	0.767	0.920	0.688
		16	1.065	1.863	1.155	1.572
		30	0.970	3.288	0.953	2.948
		62	0.943	6.796	0.960	6.092
		90	0.913	9.865	0.915	8.843
	Room temperature; Light	0	1.040	0	1.045	0
		1	1.008	0.079	1.030	0.129
		7	0.960	0.555	0.930	0.900
		16	1.050	1.270	1.158	2.057
		30	0.958	2.380	0.988	3.856
		62	0.968	4.920	0.970	7.970
		90	0.943	7.141	0.925	11.569
Methanol	4°C, Dark	0	1.033	0	1.035	0
		1	0.995	0.073	1.045	0.190
		7[1]	N/A	N/A	N/A	N/A
		16	1.045	1.092	1.160	3.034
		30	0.993	2.111	0.965	5.690
		62	0.953	4.441	0.893	11.759
		90	0.943	6.479	0.915	17.069
	Room temperature; Light	0	1.050	0	1.063	0
		1	1.045	0.106	1.055	0.137
		7[1]	N/A	N/A	N/A	N/A
		16	1.083	1.700	1.180	2.187
		30	0.985	3.188	1 028	4.100
		62	0.985	6.588	0.963	8.474
		90	0.950	9.563	0.950	12.300
Acetonitrile	4°C, Dark	0	0.995	0	1.018	0
		1	1.018	0.144	1.015	0.132
		7[1]	N/A	N/A	N/A	N/A
		16	1.035	2.300	1.150	2.108
		30	0.973	4.312	1.033	3.953
		62	0.920	8.911	0.943	8.169
		90	0.895	12.935	0.895	11.858
	Room temperature; Light	0	1.033	0	1.038	0
		1	1.003	0.175	1.033	0.214
		7[1]	N/A	N/A	N/A	N/A
		16	1.040	2.806	1.143	3.429
		30	1.043	5.261	1.173	6.430
		62	0.940	10.874	0.940	13.288
		90	0.903	15.784	0.905	19.289

TABLE C.1.1. Stability of Topramezone and M670H05 Residues in Various Solvents.

Solvent	Conditions	Storage Interval days)	Topramezone* (µg/mL)	% Degradation	M670H05* (µg/mL)	% Degradation
0.05% NH_4OH	4°C, Dark	0	1.033	0	1.043	0
		1	1.003	0.120	1.023	0.137
		7	0.955	0.840	0.950	0.957
		16	1.078	1.920	1.145	2.187
		30	0.998	3.599	0.985	4.100
		62	0.958	7.438	0.950	8.473
		90	0.908	10.797	0.903	12.300
	Room temperature; Light	0	1.033	0	1.053	0
		1	1.030	0.072	1.048	0.110
		7	0.998	0.503	0.968	0.773
		16	1.085	1.150	1.165	1.767
		30	0.995	2.156	0.993	3.313
		62	0.965	4.456	0.973	6.847
		90	0.943	6.469	0.948	9.940

[1] Day 7 data point was not used due to incorrect solvent use.
* Mean concentration values

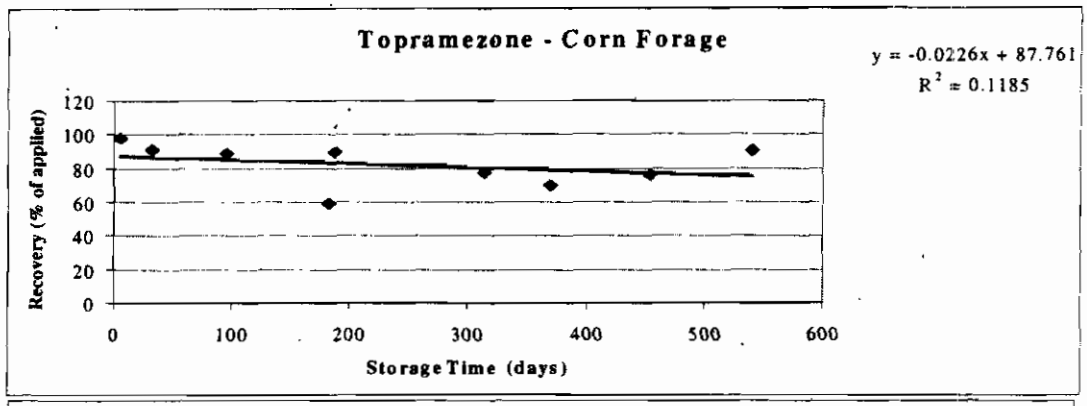
TABLE C.2. Stability of Topramezone and M670H05 Residues in Corn Matrices (Forage, Grain and Straw) and Radish Root Following Storage at -20°C.

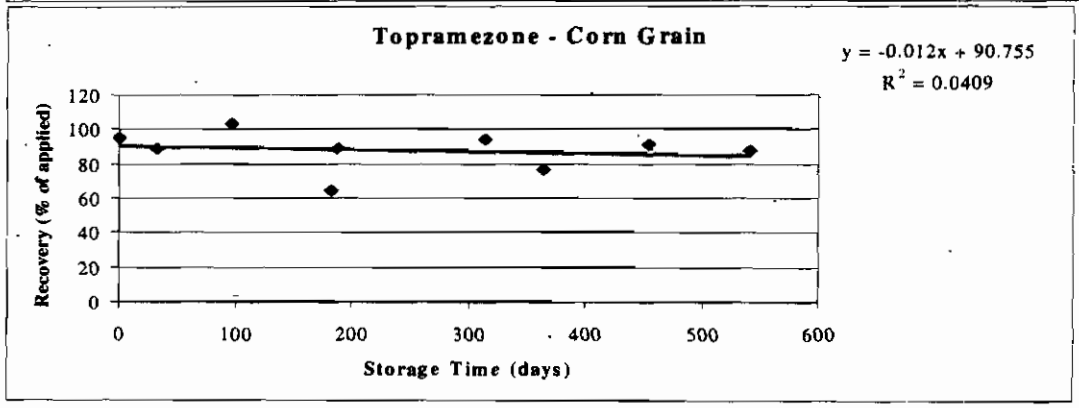
Commodity	Spike level (mg/kg)	Storage interval (days)	Recovered Residues (mg/kg)	Corrected Residues (mg/kg)	Mean Corrected Recovery (%)
Topramezone					
Corn, forage	1.0	0	-	-	-
		6	0.8790, 0.8610	0.988, 0.967	97.8
		33	0.8000, 0.8400	0.889, 0.933	91.1
		96	0.7500, 0.7738	0.877, 0.905	89.1
		182	0.5163, 0.6163	0.541, 0.645	59.3
		188	0.7430, 1.0030	0.762, 1.029	89.5
		314	0.7510, 0.7240	0.791, 0.762	77.6
		369	0.5463, 0.5650	0.687, 0.711	69.9
		454	0.7140, 0.7400	0.748, 0.775	76.1
		541	0.7250, 0.7250	0.901, 0.901	90.1
Corn, grain	1.0	0	0.8025, 0.9113	0.892, 1.013	95.2
		33	0.9090, 0.8090	0.942, 0.838	89.0
		96	0.7325, 1.1325	0.814, 1.258	103.6
		182	0.5813, 0.5938	0.642, 0.656	64.9
		188	0.8480, 0.8840	0.870, 0.907	88.8
		314	0.9540, 0.9360	0.949, 0.931	94.0
		365	0.7388, 0.6825	0.799, 0.738	76.8
		455	0.8830, 0.8300	0.939, 0.883	91.1
		541	0.7440, 0.7380	0.875, 0.868	87.2
Corn, straw	1.0	0	-	-	-
		6	0.8590, 0.8510	0.971, 0.962	96.6
		33	0.9075, 0.9413	1.061, 1.101	108.1
		96	0.7513, 0.8600	0.969, 1.110	104.0
		182	0.4238, 0.4325	0.514, 0.524	51.9
		188	0.6440, 0.5810	0.767, 0.692	72.9
		314	0.7750, 0.7540	0.842, 0.820	83.1
		365	0.6125, 0.6475	0.704, 0.744	72.4
		454	0.7710, 0.7700	0.946, 0.945	94.5
		541	0.7440, 0.6940	0.896, 0.836	86.6
Radish, root	1.0	0	0.8690, 0.8080	0.856, 0.796	82.6
		26	1.0930, 0.9160	1.163, 0.975	106.9
		89	0.8350, 0.8613	0.933, 0.962	94.8
		175	0.5888, 0.5838	0.566, 0.561	56.4
		181	0.9030, 0.8980	0.926, 0.921	92.4
		307	0.7250, 0.7140	0.884, 0.871	87.7
		362	0.6613, 0.6675	0.802, 0.809	80.5
		448	0.8240, 0.7560	0.872, 0.800	83.6
		534	0.6630, 0.7130	0.829, 0.891	86.0

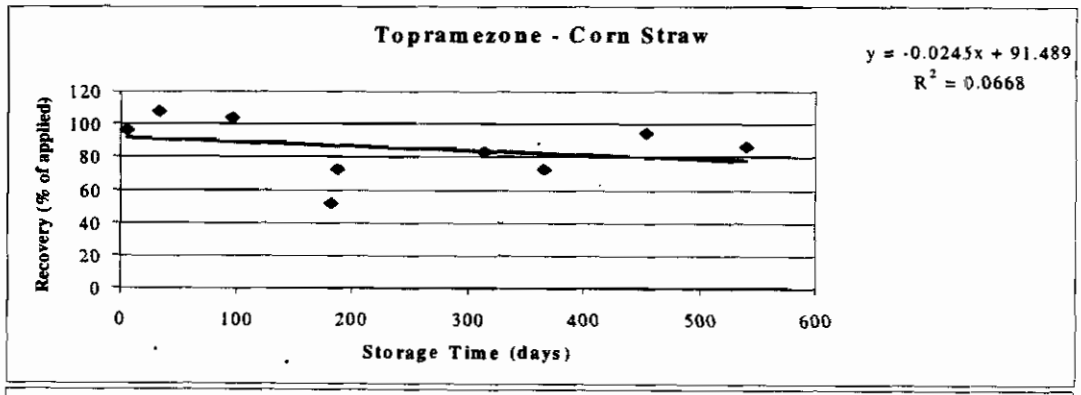
TABLE C.2. Stability of Topramezone and M670H05 Residues in Corn Matrices (Forage, Grain and Straw) and Radish Root Following Storage at -20°C.

Commodity	Spike level (mg/kg)	Storage interval (days)	Recovered residues (mg/kg)	Corrected Residues (mg/kg)	Mean Corrected Recovery (%)
M670H05					
Corn, forage	1.0	0	-	-	-
		6	0.7550, 0.7910	1.007, 1.055	103.1
		33	0.8263, 0.8938	0.944, 1.022	98.3
		96	0.7213, 0.7325	0.820, 0.832	82.6
		182	0.4680, 0.5800	0.493, 0.611	55.2
		188	0.7940, 0.9140	0.814, 0.937	87.6
		314	0.5790, 0.5700	0.702, 0.691	69.6
		369	0.4700, 0.4788	0.614, 0.626	62.0
		454	0.6910, 0.6910	0.751, 0.751	75.1
		541	0.7500, 0.7000	0.909, 0.849	87.9
Corn, grain	1.0	0	0.6688, 0.7913	0.892, 1.055	97.3
		33	0.8860, 0.7550	0.948, 0.808	87.8
		96	0.6675, 0.8375	0.726, 0.910	81.8
		182	0.5400, 0.4813	0.617, 0.550	58.4
		188	0.8840, 0.8910	0.902, 0.909	90.6
		314	0.6910, 0.6930	0.828, 0.830	82.9
		365	0.5713, 0.5400	0.649, 0.614	63.1
		455	0.8610, 0.8280	0.911, 0.876	89.4
		541	0.6810, 0.6750	0.821, 0.813	81.7
Corn, straw	1.0	0	-	-	-
		6	0.7750, 0.7840	1.013, 1.025	101.9
		33	0.9013, 0.8713	1.024, 0.990	100.7
		96	0.6925, 0.7875	0.829, 0.943	88.6
		182	0.4463, 0.4075	0.525, 0.479	50.2
		188	0.7180, 0.6180	0.785, 0.675	73.0
		314	0.5850, 0.5830	0.709, 0.707	70.8
		365	0.4538, 0.5200	0.567, 0.650	60.9
		454	0.8040, 0.7780	0.924, 0.894	90.9
		541	0.6750, 0.7310	0.771, 0.835	80.3
Radish, root	1.0	0	0.8000, 0.7630	0.941, 0.898	91.9
		26	1.0160, 0.8590	1.175, 0.993	108.4
		89	0.7225, 0.7475	0.831, 0.859	84.5
		175	0.5313, 0.5038	0.539, 0.512	52.5
		181	0.7990, 0.7680	0.815, 0.784	79.9
		307	0.5360, 0.5510	0.760, 0.782	77.1
		362	0.5113, 0.5113	0.660, 0.660	66.0
		448	0.7790, 0.8140	0.824, 0.861	84.3
		534	0.6750, 0.6180	0.849, 0.777	81.3

FIGURE C.2.1 Regression Lines for Topramezone Storage Stability in Various Matrices







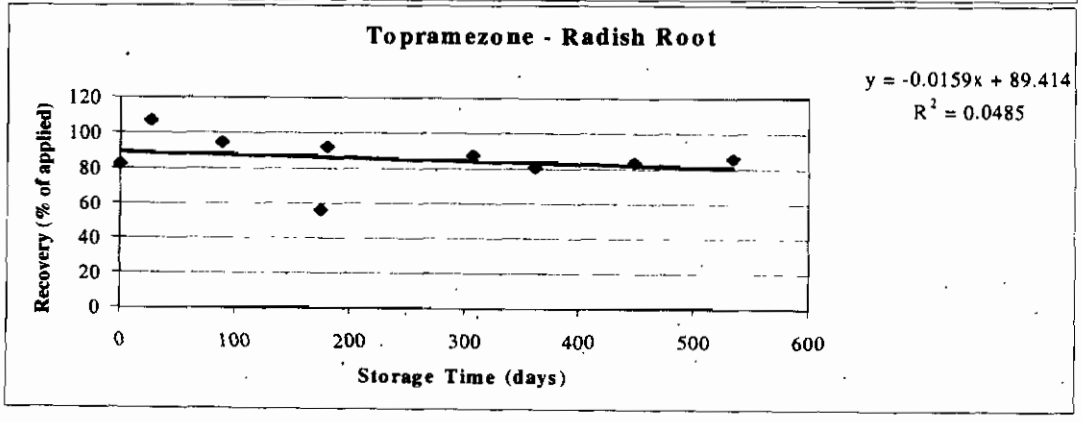
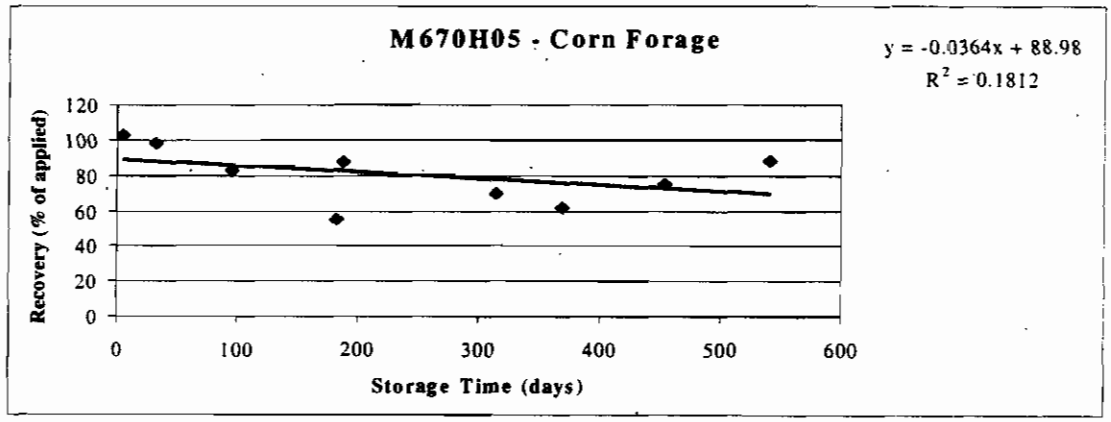
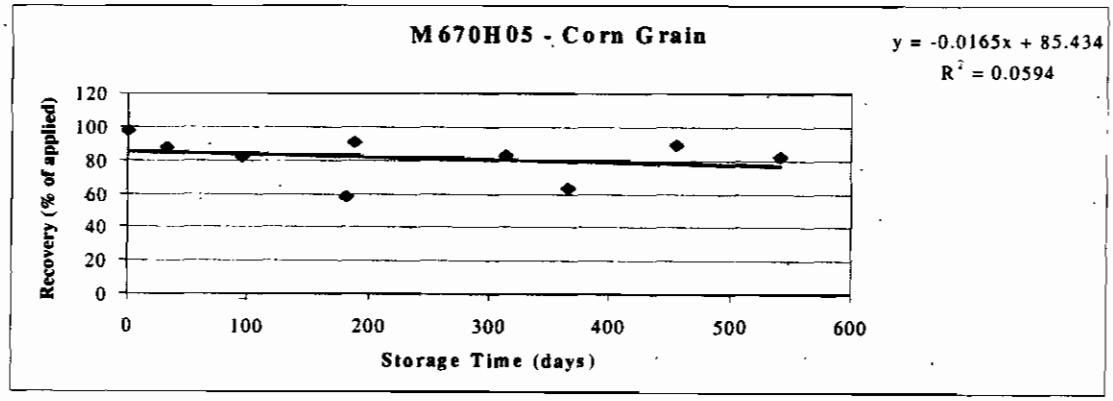
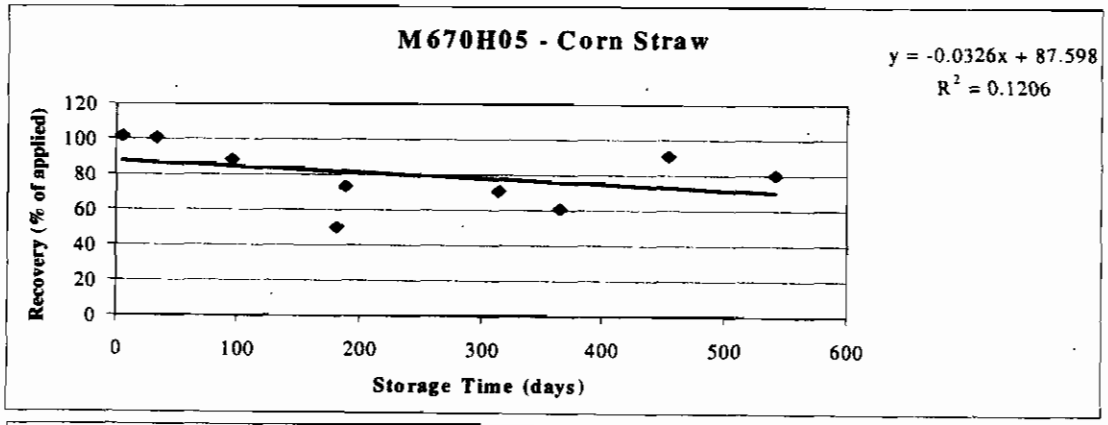
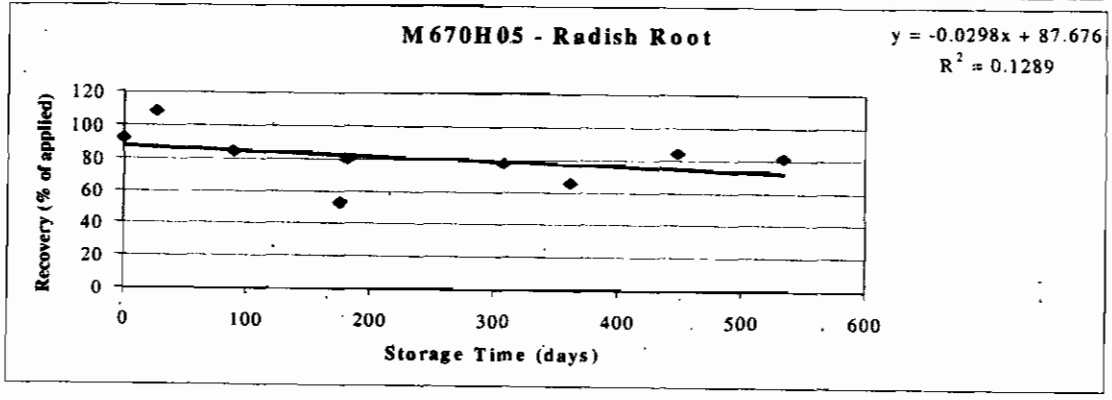


FIGURE C.2.2 Regression Lines for M670H05 Storage Stability in Various Matrices









D. CONCLUSION

The overall decline of residues (topramezone and M670H05) in frozen storage was considered statistically insignificant ($P > 0.05$). Thus, the residues of topramezone and the metabolite M670H05 were considered to be stable in corn forage, grain, straw and radish root in frozen storage up to a demonstrated interval of 541 days or 18 months.

E. REFERENCES

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Jones, J. E. (2002). Determination of the Stability of BAS 670 H and M670H05 in Various Solvents. BASF Study Number: 56755. Unpublished study prepared by BASF Corporation Agricultural Products Center. 48 pages.

F. DOCUMENT TRACKING

RDI: P.V. Shah (3/2/05), RAB1 Chemists (12/8/04), ChemSAC (3/2/05)
G.F. Kramer:806T:CM#2:(703)305-5079:7509C:RAB1
Petition Number: 3F6568
DP #: 310772
PC Code: 123009

Template Version September 2003

Appendix I - Regression Analyses

SUMMARY OUTPUT - BAS 670 H Corn Forage

Regression Statistics	
Multiple R	0.3442138
R Square	0.1184832
Adjusted R Square	-0.007448
Standard Error	12.39446
Observations	9

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	144.5370942	144.5371	0.940858	0.3643708
Residual	7	1075.358461	153.6226		
Total	8	1219.895556			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	87.760697	7.001521248	12.53452	4.74E-06	71.20474226	104.316652
X Variable 1	-0.022605	0.023304436	-0.96998	0.364371	-0.07771099	0.0325014

SUMMARY OUTPUT - BAS 670 H Corn Grain

Regression Statistics	
Multiple R	0.2023412
R Square	0.040942
Adjusted R Square	-0.0960663
Standard Error	11.713157
Observations	9

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	40.99868123	40.99868	0.298828	0.601599768
Residual	7	960.3864003	137.1981		
Total	8	1001.385082			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	90.755437	6.581618367	13.78923	2.49E-06	75.19239338	106.3184799
X Variable 1	-0.0119906	0.021934666	-0.54665	0.6016	-0.06385784	0.039876577

SUMMARY OUTPUT - BAS 670 H Corn Straw

Regression Statistics	
Multiple R	0.2585258
R Square	0.0668356
Adjusted R Square	-0.066474
Standard Error	18.341295
Observations	9

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	168.658286	168.6583	0.5013577	0.501793659
Residual	7	2354.821714	336.4031		
Total	8	2523.48			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	91.48905	10.36037687	8.830668	4.826E-05	66.99066935	115.9874311
X Variable 1	-0.024461	0.034546811	-0.70807	0.5017937	-0.1061516	0.057228741

SUMMARY OUTPUT - BAS 670 H Radish Root

Regression Statistics	
Multiple R	0.22030637
R Square	0.0485349
Adjusted R Square	-0.0873887
Standard Error	14.1869839
Observations	9

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	71.86864362	71.86864	0.357075	0.56895362
Residual	7	1408.893579	201.2705		
Total	8	1480.762222			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	89.4141109	7.869293943	11.36241	9.16E-06	70.8062009	108.02202
X Variable 1	-0.0159411	0.026677088	-0.59756	0.568954	-0.07902234	0.0471402

SUMMARY OUTPUT - M670H05 Corn Forage

Regression Statistics	
Multiple R	0.4256388
R Square	0.1811684
Adjusted R Square	0.0641925
Standard Error	15.547401
Observations	9

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	374.3703964	374.3704	1.548766	0.25336754
Residual	7	1692.051826	241.7217		
Total	8	2066.422222			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	88.979707	8.782590088	10.13137	1.96E-05	68.2121966	109.747218
X Variable 1	-0.03638	0.029232691	-1.24449	0.253368	-0.1055042	0.03274436

SUMMARY OUTPUT - M670H05 Corn Grain

Regression Statistics	
Multiple R	0.2437864
R Square	0.0594318
Adjusted R Square	-0.0749351
Standard Error	13.261108
Observations	9

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	77.78327871	77.78328	0.44231	0.527306052
Residual	7	1230.998944	175.857		
Total	8	1308.782222			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	85.433933	7.451411292	11.46547	8.63E-06	67.81415757	103.0537081
X Variable 1	-0.0165158	0.024833439	-0.66506	0.527306	-0.07523753	0.04220589

SUMMARY OUTPUT - M670H05 Corn Straw

Regression Statistics	
Multiple R	0.3473223
R Square	0.1206328
Adjusted R Square	-0.004991
Standard Error	17.674314
Observations	9

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	299.9703277	299.9703	0.9602695	0.35976472
Residual	7	2186.669672	312.3814		
Total	8	2486.64			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	87.598268	9.983621982	8.774197	5.031E-05	63.99076974	111.2057653
X Variable 1	-0.032622	0.033290517	-0.97993	0.3597647	-0.111342	0.046097017

SUMMARY OUTPUT - M670H05 Radish Root

Regression Statistics	
Multiple R	0.35898885
R Square	0.12887299
Adjusted R Square	0.00442628
Standard Error	15.5595282
Observations	9

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	250.7098062	250.7098	1.035568	0.34273009
Residual	7	1694.692416	242.0989		
Total	8	1945.402222			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	87.6755519	8.630622397	10.15866	1.93E-05	67.2673874	108.08372
X Variable 1	-0.0297738	0.029258009	-1.01763	0.34273	-0.09895793	0.0394104

Evaluators

for Kimberley Meadows
Evaluation Officer
FREAS, PMRA

Date: Mar 21/05

Suzan Mathew
Evaluation Officer
FREAS, PMRA

Date: Mar 21/05

Peer Reviewer

Sarah Levy, M.S.
Chemist
RAB1, HED, EPA

Date: May 11, 2005

Approved by

Louise G. Croteau
Senior Evaluation Officer
FREAS, PMRA

Date: March 22/05

Ariff Alli, Ph.D.
Section Head
FREAS, HED, PMRA

Date: March 29/05

George F. Kramer, Ph.D.
Chemist
RAB1, HED, EPA

Date: 4-12-05

STUDY REPORTS:

MRID No. 45902409. Fomenko, John (2002) Study title: PAM I Multiresidue Testing for BAS 670 H In Plant and Animal Matrix. BASF Study Number: 56745. Unpublished study prepared by BASF Corporation. 47 pages.

EXECUTIVE SUMMARY:

Topramezone (also known as BAS 670 H) was analyzed by Maxim Technologies Inc. (Middleport, NY) according to the U.S. Food and Drug Administration (FDA) Multiresidue Methodologies Test guidelines in the Pesticide Analytical Method (PAM), Vol. 1, Third edition (January 1994) as amended by study protocol. Protocols A and B were not required as topramezone did not possess either an N-methylcarbamate structure, carboxylic acid, or phenolic moieties. Using Protocol C, injections of topramezone did not yield adequate responses to any of the DG modules. Since Protocol C failed to produce an adequate response, topramezone was not analyzed by Protocols D, E, and F. Protocol G was not required as topramezone did not possess a substituted urea structure.

Topramezone was not adequately recovered using any of the multiresidue methods.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the multiresidue 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# 310772] and in Canada's Regulatory Decision Document.

COMPLIANCE:

Signed and dated good laboratory practice (GLP), Quality Assurance, and Data Confidentiality statements were provided. The following deviations were reported with respect to GLP compliance:

The reference standards used for the Protocol C GC evaluations in this study (such as chlorpyrifos, ethion, p,p'-DDT, phosalone, and permethrin) were purchased commercially and were not characterized according to GLP standards. It should be noted that GLP-characterized versions of these standards were not available commercially. The reference standard materials used, however, were believed to be of the highest quality available. Also, any and all information provided by the supplier relating to these materials, including a certificate of analysis (which includes purity, lot number, expiration date, and method of characterization), was maintained in the study records.

This deviation does not impact the validity of the study.

A. BACKGROUND INFORMATION

BAS 670 336SC (soluble concentrate) Herbicide (also known as BAS 670 00H Herbicide) is the end-use product which contains the active ingredient topramezone. It is a broad-spectrum, post-emergence herbicide used to control grassy and broadleaf weeds in corn. The herbicide is absorbed by the leaves, roots and shoots, then translocated to the growing points of the sensitive weeds. Topramezone belongs to the triketone class of chemicals, which inhibits carotenoid biosynthesis (HPPD (4-hydroxyphenylpyruvate dioxygenase) inhibitor). This causes a strong bleaching activity on the growing zones of the shoots within 2-5 days of application. Exposure to light causes necrosis of chlorotic tissues and eventual plant death within 14 days after application.

TABLE A.1. Test Compound Nomenclature

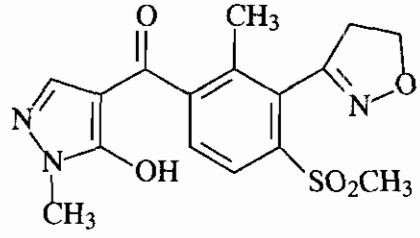
Compound	Chemical Structure
	
Common name	Topramezone
Company experimental name	BAS 670 H
IUPAC name	[3-(4,5-dihydro-1,2-oxazol-3-yl)-4-mesyl-*o*-tolyl](5-hydroxy-1-methyl-1*H*-pyrazol-4-yl) methanone
CAS name	[3-(4,5-dihydro-3-isoxazolyl)-2-methyl-4-(methylsulfonyl)phenyl](5-hydroxy-1-methyl-1*H*-pyrazol-4-yl)methanone
Molecular Formula	$C_{16}H_{17}N_3O_5S$
Molecular Mass	363.39 g/mol
CAS #	210631-68-8
End-use product/EP	Soluble Concentrate

TABLE A.2. Physicochemical Properties of Topramezone

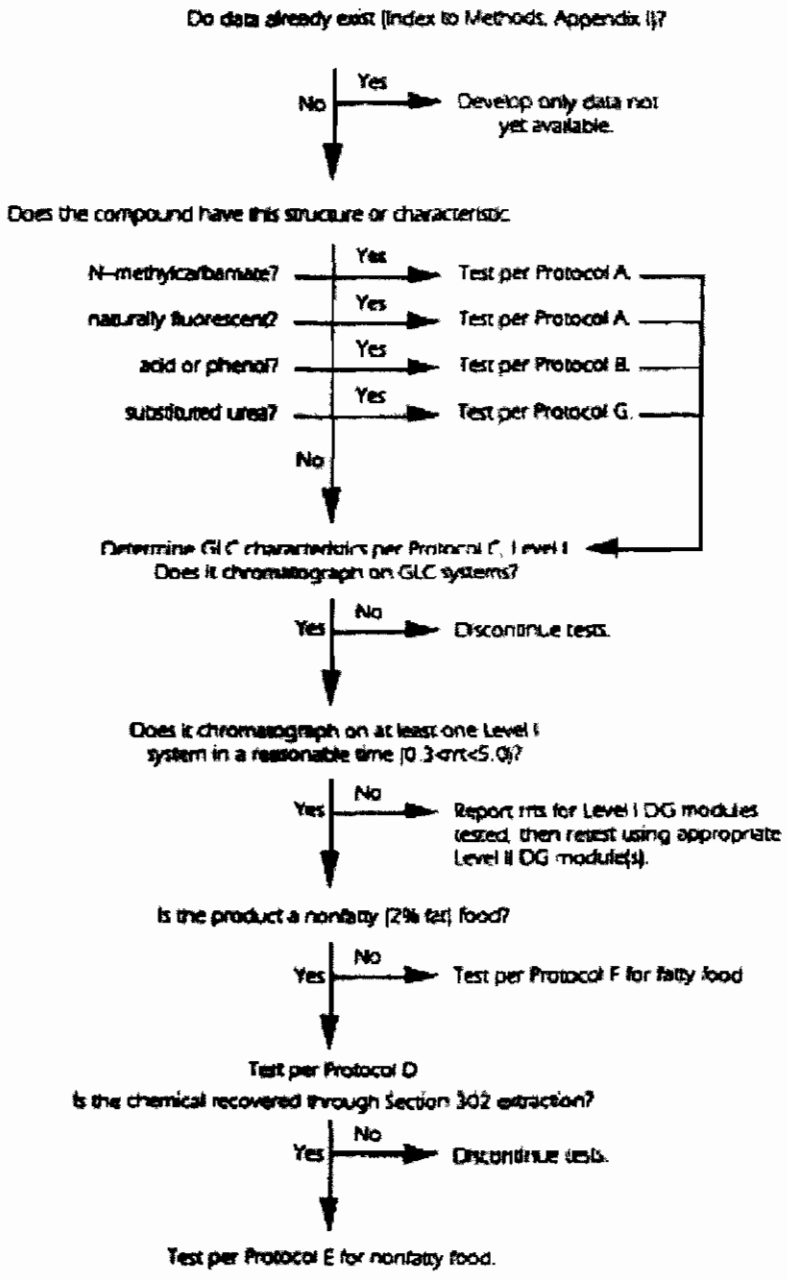
Parameter	Value (All data came from PMRA Lab Services)
Melting point/range	220.9°C - 222.2°C
pH	2.9 (1% de-ionized water)
Density (20°C)	1.425 g/cm^3
Water solubility (20°C)	pH: 3, 5, 7, 9 g/L: 0.06, 0.98, 15, 23.4
Solvent solubility (g/100 mL at 20°C)	Acetone <1.0 Acetonitrile <1.0 Dichloromethane 2.5 - 2.9 Ethyl acetate <1.0 Methanol <1.0 N-heptane <1.0 N,N-dimethylformamide 11.4-13.3 1-octanol <1.0 Olive oil <1.0 2-propanol <1.0 Toluene <1.0
Vapor pressure at 20°C and 25°C	< 1.0 x 10^{-12} hPa
Dissociation constant (pK_a)	4.06
Octanol/water partition coefficient Log(K_{OW}) at 20°C	Buffer pH: 4, 7, 9 Log K_{ow}: – 0.81, – 1.52, – 2.34
UV/visible absorption spectrum	λ, nm: 207, 272, 300, 410 ξ, mol^{-1}cm^{-1}: 27 077, 8601, 5800, 410

B. MATERIALS AND METHODS

Topramezone was analyzed using the FDA Multiresidue Methodologies Test guidelines in PAM Vol. 1, Third edition (January 1994) as amended by study protocol.

- Testing with Protocol A was not conducted because topramezone did not possess an N-methylcarbamate structure.
- Testing with Protocol B was not conducted because topramezone did not possess a carboxylic acid or phenolic moiety.
- Testing with Protocol C was conducted since it met the appropriate conditions.
- Testing of topramezone with Protocols D, E, and F was not conducted because the compound did not produce a sufficient response in Protocol C to justify testing with the matrix under these protocols.
- Protocol G was not used because topramezone did not possess a substituted urea structure.

Figure B.1. Decision Tree for Multiresidue Method (MRM) Testing



C. RESULTS AND DISCUSSION

TABLE C.1. Results of Multiresidue Methods Testing with Topramezone

PAM I Protocol	Results	Comments
A	N/A	Not required as topramezone did not possess an N-methylcarbamate structure.
B	N/A	Not required as topramezone did not possess a carboxylic acid or phenolic moiety.
C	The detectors used were an electron-capture detector (ECD), a nitrogen-phosphorus detector (NPD) and a flame photometric detector in the sulfur mode (FPD-S). The columns used were DB-1, DB-17 and Rtx-225. Topramezone was evaluated using the DG 1 (DB-1/ECD), DG 10 (DB-1/ECD), DG 13 (DB-17/ECD), DG 18 (Rtx-225/ECD), DG 5 (DB-1/NPD), DG 17 (DB-17/NPD), and DG 15 (DB-17/FPD-S) modules. Topramezone eluted from the DB-1 and DB-17 columns, but not the Rtx-225 column under standard GC conditions. Since topramezone produced a relative retention time value greater than 5.0 on the DB-1 and DB-17 columns, it was evaluated at high temperatures (on the DG 10 module). The response of topramezone was fair on the ECD, but was poor on both the NPD and the FPD-S. Insufficient responses from the DG modules were reported.	
D	N/A	Not required as topramezone did not produce a sufficient response in Protocol C to justify testing with the matrix under this protocol.
E	N/A	
F	N/A	
G	N/A	Not required as topramezone did not possess a substituted urea structure.

N/A= Not applicable

D. CONCLUSION

Topramezone was not adequately recovered using any of the multiresidue methods.

E. REFERENCES

None.

F. DOCUMENT TRACKING

RDI: G.F. Kramer (2/11/05), RAB1 Chemists (12/15/04), ChemSAC (3/2/05)
S. Levy:806T:CM#2:(703)305-0783:7509C:RAB1
Petition Number: 3F6568
DP #: 310772
PC Code: 123009

Template Version September 2003

Evaluators

for Stephanie Wille — Date: Mar 21/05
Evaluation Officer
FREAS, PMRA

Suzan Mathew — Date: Mar 21/05
Evaluation Officer
FREAS, PMRA

Peer Reviewer

Sarah Levy — Date: May 11, 2005
Sarah Levy, M.S.
Chemist
RAB1, HED, EPA

Approved by

Louise G. Croteau — Date: March 22/05
Louise G. Croteau
Senior Evaluation Officer
FREAS, PMRA

Ariff Alley — Date: Mar 24/05
Ariff Alley, Ph.D.
Section Head
FREAS, PMRA

George F. Kramer — Date: 4-12-05
George F. Kramer, Ph.D.
Chemist
RAB1, HED, EPA

STUDY REPORTS:

MRID No. 45902406. Jones, J.E., and D.S. Malinsky. (2002) "Final Report. Validation of BASF Method No. D0104: Method for Determination of BAS 670 H and Its Metabolite (M670H02) Residues in Lactating Dairy Cow Tissues, Milk and Egg using LC/MS/MS." BASF Study Number: 55913. Unpublished study prepared by BASF Corporation. 68 pages.

MRID No. 45902405. Nejad, H. (2002) "Final Report. Independent Laboratory Validation of BASF Method No. D0104: Method for Determination of BAS 670 H and Its Metabolite (M670H02) Residues in Lactating Dairy Cow Tissues, Milk and Egg using LC/MS/MS." BASF Study Number: 56757. Unpublished study prepared by BASF Corporation. 53 pages.

EXECUTIVE SUMMARY:

BASF has developed a method for determining residues of topramezone and the metabolite M670H02 in livestock tissues, milk, and eggs (method number D0104). Residues were extracted in water, acidified with hydrochloric acid (HCl) and partitioned with dichloromethane (DCM). The organic phase was back-partitioned with ammonium formate, pH 10, and the aqueous phase was analysed by liquid chromatography (LC)-mass spectrometry (MS)/MS. The limit of detection (LOD) was estimated at 8 pg/100 µL (0.08 ppb) injected on high-performance liquid chromatography (HPLC); the limits of quantitation (LOQ) were 0.01 ppm for milk, muscle, and egg, and 0.05 ppm for liver, kidney, and fat. In the data-gathering method study, samples were spiked with both analytes at the LOQ and at 0.50 ppm. Recoveries were corrected by the petitioner for apparent residues of topramezone and M670H02 in the control samples. In general, recoveries of topramezone and M670H02 were within the acceptable range. The overall recoveries for topramezone and M670H02 ranged from 72-102% (SD <20%), and 62-118% (SD <20%), respectively, for all spiking levels and all matrices. The detector response was linear between 0.10 and 2.0 pg/µL (10 and 200 pg injected). No residues were detected in any control samples (<8 pg injected). The method was successfully validated in milk and liver by an independent laboratory, indicating good reproducibility and reliability. Satisfactory radiovalidation data on goat liver were submitted. No interference testing or confirmatory method was proposed as the detector used was highly specific.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the livestock residue analytical method 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# 310772] and in Canada's Regulatory Decision Document.

The following deviation was noted:

- The same individuals were involved with the homogenization of samples for both the data-gathering method study and the independent laboratory validation (ILV), as the ILV was conducted in the same lab as the data-gathering method study. Also, the use of different equipment for the ILV study cannot be confirmed.

These deviations are not expected to impact the validity of the study.

COMPLIANCE:

Signed and dated good laboratory practice (GLP), Quality Assurance and Data Confidentiality statements were provided for both studies. The data-gathering method study was conducted under GLP with no deviations stated. The radiovalidation study was conducted using non-GLP method development.

A. BACKGROUND INFORMATION

BAS 670 336SC (soluble concentrate) Herbicide (also known as BAS 670 00H Herbicide) is the end-use product which contains the active ingredient topramezone. It is a broad-spectrum, post-emergence herbicide used to control grassy and broadleaf weeds in corn. The herbicide is absorbed by the leaves, roots and shoots, then translocated to the growing points of the sensitive weeds. Topramezone belongs to the triketone class of chemicals, which inhibits carotenoid biosynthesis (HPPD (4-hydroxyphenylpyruvate dioxygenase) inhibitor). This causes a strong bleaching activity on the growing zones of the shoots within 2-5 days of application. Exposure to light causes necrosis of chlorotic tissues and eventual plant death within 14 days after application.

TABLE A.1. Test Compound Nomenclature

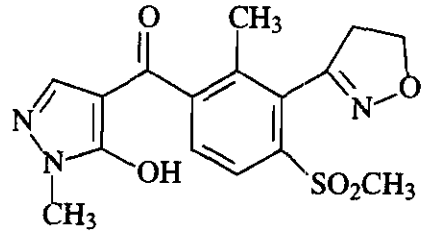
Compound	Chemical Structure 
Common name	Topramezone
Company experimental name	BAS 670 H
IUPAC name	[3-(4,5-dihydro-1,2-oxazol-3-yl)-4-mesyl-*o*-tolyl](5-hydroxy-1-methyl-1*H*-pyrazol-4-yl) methanone
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TABLE A.2. Physicochemical Properties of Topramezone

Parameter	Value
Melting point/range	220.9°C - 222.2°C
pH	2.9 (1% de-ionized water)
Density (20°C)	1.425 g/cm^3
Water solubility (20°C)	pH: 3 — 0.06 g/L 5 — 0.98 7 — 15 9 — 23.4
Solvent solubility (g/100 mL at 20°C)	Acetone <1.0 Acetonitrile <1.0 Dichloromethane 2.5 - 2.9 Ethyl acetate <1.0 Methanol <1.0 N-heptane <1.0 N,N-dimethylformamide 11.4-13.3 1-octanol <1.0 Olive oil <1.0 2-propanol <1.0 Toluene <1.0
Vapor pressure at 20°C and 25°C	< 1.0 x 10^{-12} hPa
Dissociation constant (pK$_a$)	4.06
Octanol/water partition coefficient Log(K$_{OW}$) at 20°C	Buffer pH: 4 — Log K$_{ow}$ −0.81 7 — −1.52 9 — −2.34
UV/visible absorption spectrum	λ, nm: 207 — ξ, mol^{-1}cm^{-1} 27 077 272 — 8601 300 — 5800 410 — 410

All data came from PMRA Lab Services.

B. MATERIALS AND METHODS

B.1. Data-Gathering Method

B.1.1. Principle of the Method:

Tissue samples were chopped by hand, refrozen, then passed through a meat grinder. Samples were stored frozen at -15°C until analysis. Aliquots were weighed into bottles and spiked with a mixed standard solution in water (topramezone + M670H02). Residues were extracted from milk, egg, and homogenized tissue samples in water using mechanical shaking for 30 minutes. A small aliquot of the extract was removed and centrifuged for 5 minutes, then added to 1N HCl and DCM, vortexed briefly, and centrifuged for 5 minutes. A portion of the organic (DCM) layer was added to 4mM ammonium formate buffer (pH 10), vortexed briefly, and centrifuged for 5

minutes. The aqueous layer of control or low concentration samples could be removed directly for LC-MS/MS analysis. The aqueous layer of higher concentration samples was removed and diluted in 4 mM ammonium formate (pH 10) for HPLC, if necessary. A flowchart of the method is depicted in FIGURE B.1.

Suggested primary HPLC instrument conditions were on a Phenomenex Luna C18 column with a mobile gradient of 4 mM ammonium formate in water or in methanol (MeOH). Alternatively, a Discovery HS-C18 column with a mobile gradient of acetonitrile (ACN) and 4mM aqueous ammonium formate could be used. The final procedure stated that the method of ionization was by turbo ion spray in the negative mode, but the method validation study was conducted using atmospheric pressure chemical ionization (APCI) in the positive mode. The following transitions were monitored: for topramezone, 362.1 to 334 or 318.1 m/z; for M670H02, 378.1 to 235.8 or 173.8 m/z. The suggested operating conditions for LC-MS/MS may be modified if necessary.

Figure B.1. Flow chart of Method

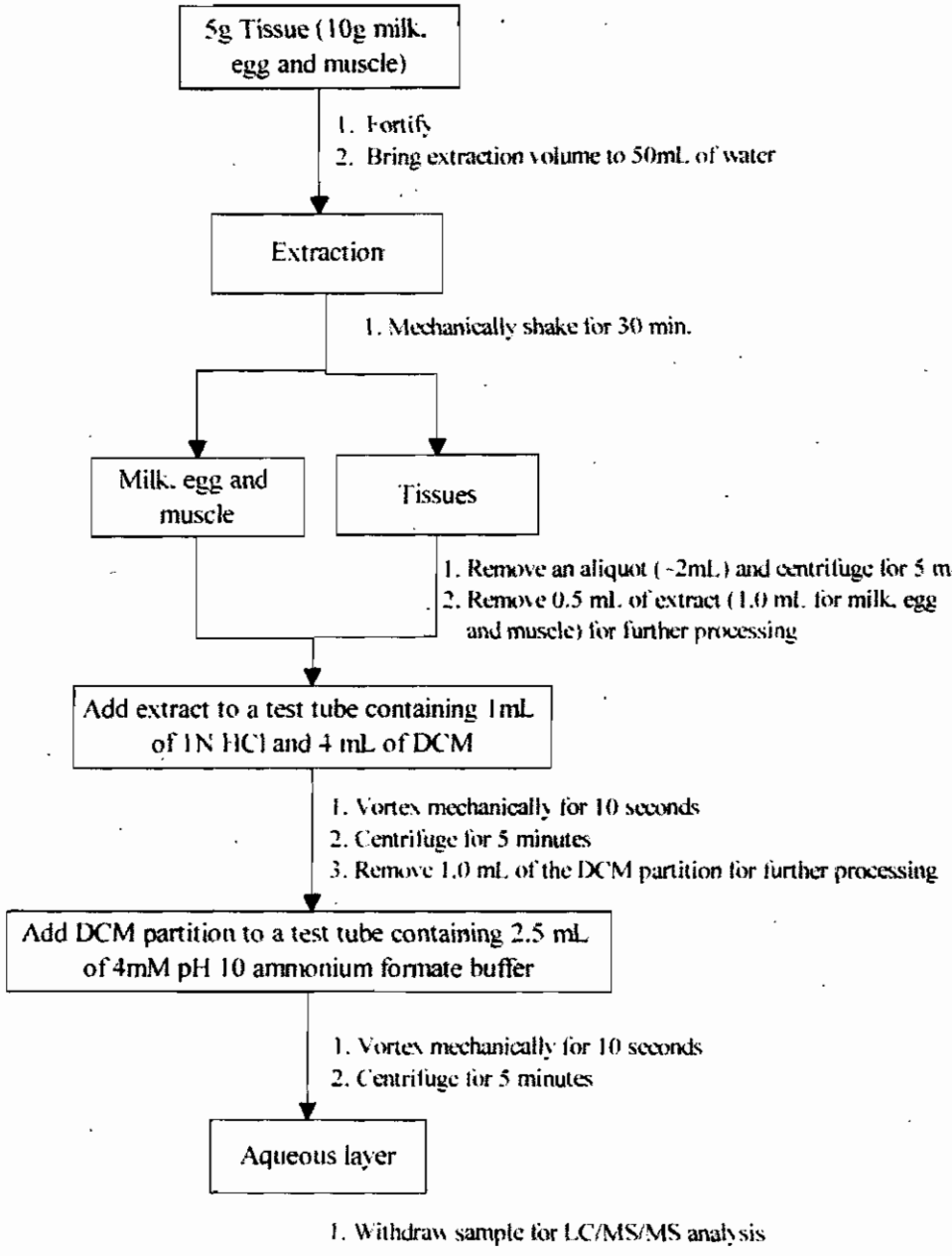

TABLE B.1.1. Summary Parameters for the Analytical Method Used for the Quantitation of Topramezone Residues in Bovine Tissues, Milk and Eggs.	
Method ID	D0104
Analytes	Topramezone and M670H02
Extraction solvent/technique	Water with mechanical shaking
Cleanup strategies	Acidified with HCl; partitioned with DCM; organic phase back-partitioned into pH 10 aqueous ammonium formate (aqueous layer for analysis).
Instrument/Detector	LC-MS/MS - Suggested conditions: PE Sciex API 3000 Biomolecular Mass Analyzer Primary HPLC: Phenomenex Luna C_{18} column, mobile gradient of 4 mM ammonium formate in water or in MeOH Alternative HPLC: Discovery HS-C_{18} column, mobile gradient of ACN and 4mM aqueous ammonium formate Ionization: turbo ion spray in the negative mode Transitions monitored: Topramezone - 362.1 to 334 or 318.1 m/z; M670H02 - 378.1 to 235.8 or 173.8 m/z
Standardization method	External standards of Topramezone and M670H02 in a working range of 0.1-2.0 pg/µL (10 to 200 pg injected). Samples were diluted to fit within the area of the standard curve. Least squares linear fit using peak area for calibration curves (peak area may be used if there is interference).
Stability of std solutions	Standard stock solutions (topramezone or M670H02) should be prepared in ACN fresh every 3 months and stored refrigerated. Mixed standard solutions diluted in HPLC buffer (4mM aqueous ammonium formate, pH 10) or in fortification solution (water) should be prepared fresh every month. Standard solution was stable in LC-MS/MS standard solution of water:4mM ammonium formate for up to one month. The spiking standards of topramezone and M670H02 were stable in a spiking solution of water for up to one month.
Retention times	Primary conditions: Topramezone ~4.2 min; M670H02 ~3.5 min Alternate conditions: Topramezone ~3.4 min; M670H02 ~2.9 min

B.2. Enforcement Method

The enforcement method is the same as the data-gathering method.

C. RESULTS AND DISCUSSION

C.1. Data-Gathering Method

TABLE C.1.1. Recovery Results from Method Validation of Livestock Tissues, Milk, and Eggs using the Data-Gathering Analytical Method D0104. HPLC Standards were Prepared in 4mM Aqueous Ammonium Formate, pH 10.

Matrix	Spiking Level (mg/kg)	Recoveries Obtained (%)	Mean Recovery ± SD (CV) (%)
Topramezone			
Milk	0.01	92, 90, 100, 100, 96	95.6 ± 4.6 (4.8)
	0.50	94, 98, 91, 94, 95	94.4 ± 2.5 (2.7)
Fat	0.05	92, 101, 90, 90, 96	93.8 ± 4.7 (5.0)
	0.50	90, 93, 80, 83, 85	86.2 ± 5.3 (6.1)
Liver	0.05	83, 81, 86, 90, 82	84.4 ± 3.6 (4.3)
	0.50	91, 86, 76, 84, 97	86.8 ± 7.9 (9.0)
Muscle	0.01	90, 83, 81, 72, 79	81.0 ± 6.5 (8.0)
	0.50	84, 79, 88, 88, 86	85.0 ± 3.7 (4.4)
Kidney	0.05	86, 99, 91, 102, 95	94.6 ± 6.3 (6.7)
	0.50	88, 92, 88, 77, 86	86.2 ± 5.6 (6.5)
Egg	0.01	99, 93, 101, 102, 95	98.0 ± 3.9 (4.0)
	0.50	94, 73, 97, 84, 79	85.4 ± 10.1 (11.8)
M670H02			
Milk	0.01	82, 84, 80, 96, 87	85.8 ± 6.3 (7.3)
	0.50	97, 97, 95, 89, 97	95.0 ± 3.5 (3.6)
Fat	0.05	78, 90, 100, 89, 110	93.4 ± 12.1 (13.0)
	0.50	102, 94, 95, 90, 82	92.6 ± 7.3 (7.9)
Liver	0.05	87, 86, 86, 103, 87	89.8 ± 7.4 (8.2)
	0.50	100, 77, 88, 90, 100	91.0 ± 9.6 (10.5)
Muscle	0.01	66, 74, 69, 62, 69	68.0 ± 4.4 (6.5)
	0.50	74, 69, 80, 71, 78	74.4 ± 4.6 (6.2)
Kidney	0.05	108, 110, 108, 118, 70	102.8 ± 18.8 (18.3)
	0.50	90, 90, 90, 84, 93	89.4 ± 3.3 (3.7)
Egg	0.01	89, 83, 98, 86, 89	89.0 ± 5.6 (6.3)
	0.50	64, 78, 87, 89, 72	78.0 ± 10.4 (13.4)

A data-gathering method study was conducted using bovine tissues (liver, kidney, muscle, and fat), bovine whole milk, and chicken eggs. Samples were spiked at the proposed LOQ (0.01 ppm for milk, muscle, and egg; 0.05 ppm for liver, kidney, and fat), and at 0.50 ppm. The results are shown in Table C.1.1. No confirmatory method was proposed and no interference study was conducted. However, the method is highly specific to the analytes of interest as only limited transitions are monitored by the MS/MS. The LOD was defined as being <80% of the lowest

standard, or 8 pg/100 µL (0.08 ppb) of analyte injected on HPLC. Low levels of topramezone residues were detected in the control samples of milk, liver, kidney, fat and egg; while low levels of M670H02 residues were detected in the control samples of milk and muscle. Thus, the petitioner corrected the recoveries found in these matrices as necessary. The spiked sample chromatograms indicated a single defined, symmetrical analyte peak around the area of interest.

All the individual recoveries of topramezone were in the range of 72-102%, which was within the acceptable range (70 - 120%). The standard deviations were <20% for each matrix and spiking level as well (TABLE C.1.1). Individual recoveries of M670H02 from milk, liver, kidney and fat were all within the acceptable range at 70-118%. Although one egg sample spiked at 0.50 ppm had a low recovery (64%), the mean recovery of egg spiked at 0.50 ppm was 78.0 ± 10.4%. The relative standard deviations were <20% for each of these matrices and each spiking level. In muscle, 4 out of 5 samples spiked at 0.01 ppm had recoveries of less than 70% (62 - 69%), giving a mean recovery of 68.0 ± 4.4%. Since the standard deviation (4.4) and coefficient of variation (6.5) were low, the recoveries for M670H02 in muscle at the 0.01 ppm spiking level were considered acceptable. Therefore, the proposed LOQ of 0.01 ppm was validated for muscle, milk and egg; and the proposed LOQ of 0.05 ppm was validated for liver, kidney and fat.

A radiovalidation study (non-GLP method development) was conducted using a radiolabelled liver sample from the goat metabolism study by the analytical method D0104. The liver samples extracted with either methanol, water or pH 7 buffer provided extractabilities of 97%, 111% and 95%, respectively. This result indicated that the extraction of liver with water (as in method D0104) was as effective as extraction with methanol (from the goat metabolism study). Liver was chosen as it is the most difficult matrix to extract residues. Since egg samples had extremely low residue levels (<0.0005 ppm), no radiovalidation was conducted on this matrix. The extraction efficiencies of liver with either methanol (88.86-89.03%), water (84.99-85.08%) or pH 7 buffer (85.23-85.27%) were similar, again indicating that water was as effective as either methanol or buffer in extracting residues from livestock matrices. These data satisfy the radiovalidation requirements for the data-gathering/enforcement method.

TABLE C.1.2. Characteristics for the Data-Gathering Analytical Method Used for the Quantitation of Topramezone Residues in Livestock Tissues, Milk, and Eggs.	
Analytes	Topramezone and M670H02
Equipment ID	LC-MS/MS - Conditions used for Method Validation: PE Sciex API 3000 Biomolecular Mass Analyzer Discovery HS-C_{18} column, mobile gradient of ACN and 4mM aqueous ammonium formate + 0.1% formic acid (alternative conditions) APCI in the positive mode Transitions monitored: Topramezone - 362.1 to 334 or 318.1 m/z; M670H02 - 378.1 to 235.8 or 173.8 m/z
LOQ	Milk, muscle, and eggs: 0.01 ppm Fat, liver, and kidney: 0.05 ppm
LOD	Estimated at 80% of lowest standard or 8 pg/100µL injected on HPLC (0.08 ppb).
Accuracy/Precision	Topramezone: Recoveries from all matrices ranged from 72 - 102% (TABLE C.1.1). Indicates acceptable accuracy/precision in the range of spiking levels (0.01 and 0.50 ppm for milk, muscle, and eggs; 0.05 and 0.50 ppm for fat, liver, and kidney).
	M670H02: Recoveries from all matrices ranged from 62 - 118% (TABLE C.1.1). Individual recoveries of <70% in muscle spiked at 0.01 ppm (62%, 66%, 2 x 69%) and at 0.50 ppm (69%), and in egg spiked at 0.50 ppm (64%). Indicates acceptable accuracy/precision in the range of spiking levels (0.01 and 0.50 ppm for muscle, milk and egg; 0.05 and 0.50 ppm for fat, liver, and kidney).
Reliability of the Method/[ILV]	An ILV was conducted to verify the reliability of method No. D0104 for the determination of topramezone residues in livestock matrices. The values obtained are indicative that the method is reliable at the method LOQ and at 10-fold the LOQ.
Linearity	The detector response was linear for both analytes (coefficient of determination, $r^2 \geq 0.98$) within the range of 0.1-2.0 pg/µL (10 to 200 pg absolute concentration) injected on HPLC.
Specificity	The control chromatograms generally have no peaks above the chromatographic background and the spiked sample chromatograms contain only the analyte peak of interest. Peaks were well defined and symmetrical. There appeared to be no carryover to the following chromatograms.

C.2. Enforcement Method

The enforcement method is the same as the data-gathering method.

C.3. Independent Laboratory Validation (ILV)

An ILV of the method was conducted using the primary conditions described in the method procedure by the same laboratory that developed the data-gathering method. While the supervisors and analysts were different for the ILV, the individuals involved with the sample homogenization were the same. As well, there was no indication of whether different equipment was used while conducting the ILV. Communication occurred with both the method supervisor (concerning higher unacceptable residues and errors in LC-MS/MS conditions), and a senior scientist (concerning acceptability of higher recoveries for EU registration). The only modification to the procedure was to clarify that the ionization method should be turbo ion spray in the negative mode for both the LC-MS/MS primary and alternate instruments. This change was included in the final technical procedure. The matrices tested were bovine milk and liver.

Liver was chosen as it was the most difficult matrix to analyse. Samples were spiked with topramezone and M670H02 at the LOQ (0.01 ppm for milk and 0.05 ppm for liver) and at 10-fold the LOQ (0.10 ppm for milk and 0.50 ppm for liver). The LOD was defined as the lowest standard level injected with the sets, or 10 pg injected on column. All individual recoveries were within the acceptable range of 70 - 120%, and relative standard deviations were <20% for each matrix and spiking level (TABLE C.3.1). No interference around the area of interest was observed in the control sample chromatograms, indicating adequate specificity of the method. Recoveries were corrected for residues detected in the control samples, as applicable. The spiked chromatograms again indicated a single analyte peak around the area of interest. The method has been successfully validated for milk and liver, which can be extended to all other livestock matrices.

The method took one analyst 3 hours to conduct the extraction and cleanup of a set of 13 samples for each matrix. The LC-MS/MS injections were conducted overnight.

TABLE C.3.1. Recovery Results Obtained by an Independent Laboratory Validation (ILV) of the Enforcement Method for the Determination of Topramezone in Livestock Matrices.

Matrix	Spiking Level (µg/g)	Recoveries Obtained (%)	Mean Recovery ± SD (CV) (%)
Topramezone			
Milk	0.01	97.5, 93.5, 96, 100, 93	96.0 ± 2.9 (3.0)
	0.1	95, 87.5, 93.5, 92.5, 93	92.3 ± 2.8 (3.1)
Liver	0.05	88.8, 82.4, 84.4, 86, 80.8	84.5 ± 3.1 (3.7)
	0.50	91.2, 89.6, 85.6, 91.6, 98	91.2 ± 4.5 (4.9)
M670H02			
Milk	0.01	83, 86, 83, 90, 83.5	85.1 ± 3.0 (3.5)
	0.1	89, 83.5, 79.5, 81.5, 86.5	84.0 ± 3.8 (4.5)
Liver	0.05	90, 87.2, 92.4, 91.6, 82.4	88.7 ± 4.1 (4.6)
	0.50	93.2, 82.4, 98.8, 86.8, 95.2	91.3 ± 6.6 (7.2)

D. CONCLUSION

The data-gathering/enforcement method D0104 for residues of topramezone and the metabolite M670H02 in livestock tissues, milk, and egg has been validated by the petitioner for all matrices. Satisfactory radiovalidation data were submitted for goat liver. An independent laboratory (using cow liver and milk) successfully validated the method.

E. REFERENCES

MRID No. 45902402. Van Cott, Andrew and Lam, Wing W. (2000). Final Report: Nature of Residues of [^{14}C]-BAS 670 H in Lactating Goats. BASF Study Number 55911. Unpublished study prepared by BASF Agro Research. 247 p.

F. DOCUMENT TRACKING

RDI: G.F. Kramer (2/11/05), RAB1 Chemists (12/15/04), ChemSAC (3/2/05)
S. Levy:806T:CM#2:(703)305-0783:7509C:RAB1
Petition Number: 3F6568
DP #: 310772
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Evaluators

for Kimberley Meadows
Evaluation Officer
FREAS, PMRA

Date: Mar 21/05

Suzan Mathew
Evaluation Officer
FREAS, PMRA

Date: Mar 21/05

Peer Reviewer

Sarah Levy, M.S.
Chemist
RAB1, HED, EPA

Date: 4/15/05

Approved by

Louise G. Croteau
Senior Evaluation Officer
FREAS, PMRA

Date: March 22/05

Ariff Ally, Ph.D.
Section Head
FREAS, PMRA

Date: March 29/05

George F. Kramer, Ph.D.
Chemist
RAB1, HED, EPA

Date: 4-12-05

STUDY REPORTS:

MRID No. 45902407. Abdel-Baky, S (November 4, 2002). Validation and Accountability of BASF Method Number D0007 titled " Method for the Determination of BAS 670 H and Its Metabolite (M670H05) Residues in Plant Matrices Using LC/MS/MS:" Lab Project Number: 56542. Unpublished study prepared by BASF Corporation. 93 pages.

MRID No. 45902404 Lehmann, A. & Mackenroth, C. (November 20, 2002). Independent Lab Validation of the Analytical Method D0007: Method for the Determination of BAS 670 H and its

Metabolite M670H05 in Plant Matrices. Lab Project Number: 135238. Unpublished study prepared by BASF Corporation. 50 pages.

EXECUTIVE SUMMARY:

BASF Corporation proposed liquid chromatography (LC)-mass spectrometry (MS)/MS method No. D0007 for data gathering and enforcement purposes for residues of topramezone and the free acid metabolite M670H05 in/on plant commodities.

Residues of topramezone and M670H05 were extracted from the matrix (excluding wheat grain, corn starch, and oil) using an Accelerated Solvent Extractor (ASE) with water for non-grain matrices, or acetonitrile (ACN):water (1:1, v:v) for grain matrices. Extraction by mechanical shaking, using the same solvents as the ASE, was also performed. Aliquots of the extract were subjected to acid and base partitioning prior to analysis with LC-MS/MS. The samples of wheat grain, corn starch, and oil were extracted, then partitioned with 1N hydrochloric acid (HCl) saturated with sodium chloride (NaCl) and dichloromethane (DCM). Basic partitioning by ammonium hydroxide (NH_4OH) occurred prior to analysis with LC-MS/MS.

Data gathering method recoveries of topramezone extracted by ASE were within the acceptable range in samples spiked at the limit of quantitation (LOQ; 0.01 ppm for kernel + cob with husk removed (K+CWHR) and corn grain; 0.05 ppm for corn forage, corn stover and radish root), and at 10-fold the LOQ (0.10 and 0.50 ppm). Recoveries of M670H05 extracted by ASE were also generally within the acceptable range, except for slightly lower recoveries in two of five corn grain samples spiked at the LOQ. Several recoveries of topramezone and M670H05 extracted by mechanical shaking were lower than 70%, particularly in samples of corn matrices spiked at the LOQ. An independent laboratory validation (ILV) was conducted using maceration by polytron homogenizer, followed by micro-partitioning. The ILV was successfully validated for corn grain, lettuce, oilseed rape seed, and apple. Acceptable radiovalidation data were submitted for corn forage and stover. It should be noted that on one occasion the percent recoveries for radish root were corrected by the petitioner for apparent residues of M670H05 in the control sample.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the plant analytical method 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# 310772] and in Canada's Regulatory Decision Document.

COMPLIANCE:

Signed and dated good laboratory practice (GLP), Quality Assurance, and Data Confidentiality statements were provided.

A. BACKGROUND INFORMATION

BAS 670 336SC (soluble concentrate) Herbicide (also known as BAS 670 00H Herbicide) is the end-use product which contains the active ingredient topramezone. It is a broad-spectrum, post-emergence herbicide used to control grassy and broadleaf weeds in corn. The herbicide is absorbed by the leaves, roots and shoots, then translocated to the growing points of the sensitive weeds. Topramezone belongs to the triketone class of chemicals, which inhibits carotenoid biosynthesis (HPPD (4-hydroxyphenylpyruvate dioxygenase) inhibitor). This causes a strong bleaching activity on the growing zones of the shoots within 2-5 days of application. Exposure to light causes necrosis of chlorotic tissues and eventual plant death within 14 days after application.

TABLE A.1. Test Compound Nomenclature

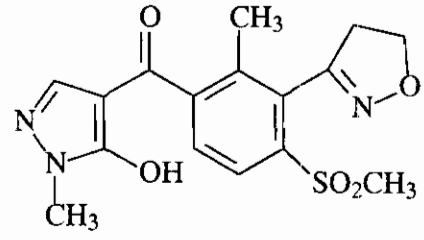
Compound	Chemical Structure 
Common name	Topramezone
Company experimental name	BAS 670 H
IUPAC name	[3-(4,5-dihydro-1,2-oxazol-3-yl)-4-mesyl-*o*-tolyl](5-hydroxy-1-methyl-1*H*-pyrazol-4-yl) methanone
CAS name	[3-(4,5-dihydro-3-isoxazolyl)-2-methyl-4-(methylsulfonyl)phenyl](5-hydroxy-1-methyl-1*H*-pyrazol-4-yl)methanone
Molecular Formula	$C_{16}H_{17}N_3O_5S$
Molecular Mass	363.39 g/mol
CAS #	210631-68-8
End-use product/EP	Soluble Concentrate

TABLE A.2. Physicochemical Properties of Topramezone

Parameter	Value (All data came from PMRA Lab Services)
Melting point/range	220.9°C - 222.2°C
pH	2.9 (1% de-ionized water)
Density (20°C)	1.425 g/cm^3
Water solubility (20°C)	pH: 3, 5, 7, 9 g/L: 0.06, 0.98, 15, 23.4
Solvent solubility (g/100 mL at 20°C)	Acetone <1.0 Acetonitrile <1.0 Dichloromethane 2.5 - 2.9 Ethyl acetate <1.0 Methanol <1.0 N-heptane <1.0 N,N-dimethylformamide 11.4-13.3 1-octanol <1.0 Olive oil <1.0 2-propanol <1.0 Toluene <1.0
Vapor pressure at 20°C and 25°C	< 1.0 x 10^{-12} hPa
Dissociation constant (pK$_a$)	4.06
Octanol/water partition coefficient Log(K$_{OW}$) at 20°C	Buffer pH: 4, 7, 9 Log K$_{ow}$: – 0.81, – 1.52, – 2.34
UV/visible absorption spectrum	λ, nm: 207, 272, 300, 410 ξ, mol^{-1}cm^{-1}: 27 077, 8601, 5800, 410

B. MATERIALS AND METHODS

B.1. Data-Gathering Method

B.1.1. Principle of the Method:

Grain was homogenized with dry ice in a mill. Forage and hay samples were cut with a mechanical chopper before homogenization with dry ice. Samples were stored frozen at -15°C until analysis.

Aliquots were weighed into bottles, then spiked with a mixed standard solution of topramezone and M670H05. Non-grain matrices were extracted with water for ASE, while grain and seed matrices were extracted with 1:1 ACN:water (except wheat grain and corn starch) for ASE.

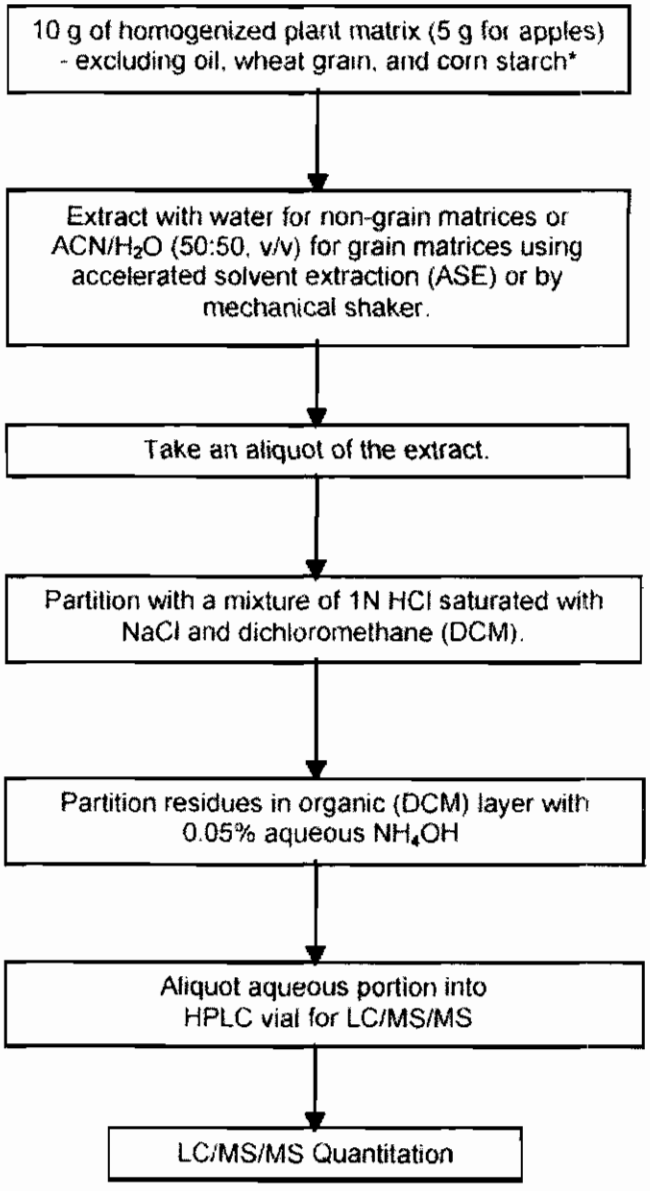
Distilled water was added to the resulting extracts before micro-partitioning. Matrices (except oil, wheat grain, and corn starch) were also extracted by mechanical shaking with the same solvents as ASE. The resulting extracts were then centrifuged for 5 minutes before micro-partitioning.

Micro-partitioning of extracts from ASE or shaker extraction of all matrices (other than oil, wheat grain, and corn starch) involved the addition of HCl saturated with NaCl and DCM to an aliquot of the sample. The extracts were vortexed for one minute, then samples were centrifuged using a centrivap concentrator with no heat (or swinging bucket rotor) for 5 minutes. A portion of the DCM layer was removed before the addition of 0.05% NH_4OH. Samples were vortexed and centrifuged again before undergoing analysis by LC-MS/MS.

The oil matrix was extracted by adding 1N HCl saturated with NaCl and DCM to the sample before mechanical shaking for 15 minutes. The extract was centrifuged, the DCM layer was separated, and the aqueous layer was discarded. An aliquot of 0.05% NH_4OH was added, the sample was shaken again, then centrifuged if necessary before analysis by LC-MS/MS. The extraction of wheat grain and corn starch matrices were similar to that of oil, except for the addition of more solvent (HCl/NaCl/DCM) to the sample (2 mL instead of 1 mL) before mechanical shaking. The addition of a few drops of 28-30% NH_4OH was used as necessary to adjust the pH of the oil, wheat grain, and corn starch samples above pH 7 before analysis by LC-MS/MS.

Analytical Method D0007 was suitable for measuring residues of topramezone and the metabolite M670H05 with the following LOQ: 0.01 ppm (corn grain, corn oil, wheat grain, and corn starch), 0.02 ppm (kernel + cob with husk removed), and 0.05 ppm (all other matrices). The limit of detection (LOD) was reported to be 0.10 pg/µL (0.10 ppb). A flowchart of the data gathering method is depicted in FIGURE B.1. A flowchart of the final technical procedure for Method D0007 is depicted in FIGURE B.2.

FIGURE B.1 Flow Chart of Data Gathering Method



*Wheat grain, oil, and corn starch samples are extracted by shaking with acidified DCM saturated with NaCl, and following phase separation, residues in the organic layer are partitioned into 0.05% NH4OH.

FIGURE B.2 Flow Chart of Analytical Method D0007 (Final Technical Procedure)

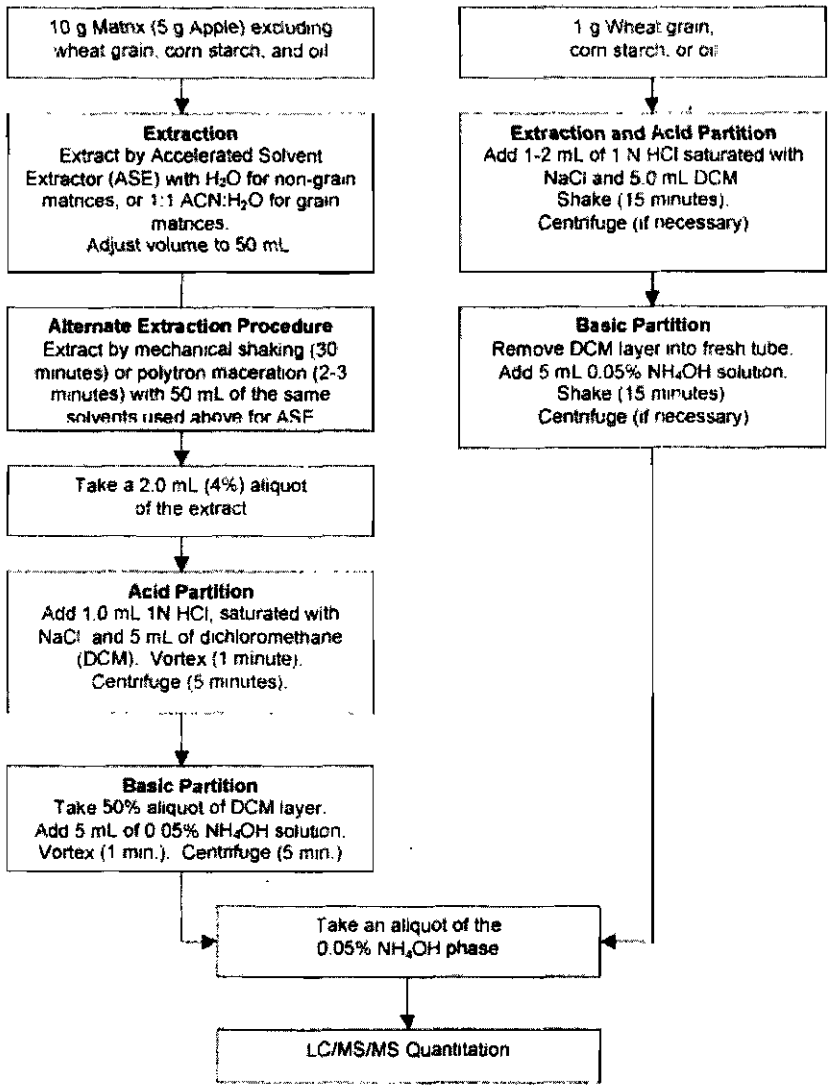


TABLE B.1.1. Summary Parameters for the Analytical Method used for the Quantitation of Topramezone and M670H05 Residues in Apple, Radish Root, and Corn Forage, Fresh Corn, Stover, Grain.	
Method ID	D0007
Analyte(s)	Topramezone and M670H05
Extraction solvent/technique	Extract with water for non-grain matrices or ACN:water (1:1, v:v) for grain matrices using ASE or by mechanical shaker.
Cleanup strategies	Partitioning with a mixture of 1N HCl saturated with NaCl and DCM.
Instrument/Detector	LC-MS/MS - suggested conditions: PE Sciex API 300 Biomolecular Mass Analyzer Column: Columbus C18 100mm X 2mm 5µ Injection: 20 µL (or higher) Mobile phase: Eluent A: 4.0 mM NH_4CO_2H in water Eluent B: 4.0 mM NH_4CO_2H in methanol Expected retention times: Topramezone - 3.1 minutes; M670H05 - 1.4 minutes Negative ionization mode Transitions: Topramezone: 362 → 334 or 362 → 318 M670H05: 282 → 238 or 282 → 78.55
Standardization method	The standard curve is obtained by direct injection of the mixed topramezone and M670H05 standards into LC-MS/MS in the range of 0.10 ng/mL to 1 0 ng/mL. In a given injection run, the same volume is used for all samples and standards. Typical standard amounts injected on column (20 µL) range as follows: 2, 4. 8 and 20 pg. Standards were diluted to fit within the area of the standard curve.
Stability of std solutions	Standard solutions were kept refrigerated. A study concerning the storage stability of standard solutions (Reference 1) found no decomposition of solutions made in 0.05% NH_4OH (aq.) when stored in the refrigerator for 90 days. BASF recommended that stock solutions (1 mg/mL in MeOH) be made fresh every three months. Dilutions of stock solutions should be refrigerated and used no longer than one month.
Retention times	Topramezone: 3.1 minutes; M670H05: 1.4 minutes

B.2. Enforcement Method

The enforcement method being proposed is the same as the data gathering method No. D0007.

C. RESULTS AND DISCUSSION

C.1. Data-Gathering Method

Adequate data from the data-gathering method was submitted for LC-MS/MS method No. D0007 for topramezone and M670H05 in apple, radish root, fresh corn (K+CWHR), corn forage, corn stover, and corn grain. The results are summarized in TABLE C.1.1. Recoveries of topramezone from the ASE were 78-131% at the LOQ (0.01 ppm for K+CWHR and corn grain; 0.05 ppm for corn forage, corn stover and radish root), and 77-105% at the 10-fold LOQ (0.01 ppm and 0.05 ppm). Two of five recoveries of M670H05 at the LOQ were below 70% in corn

grain (64 and 68%). However the mean recovery for that matrix was 75%, with a standard deviation of less than 20% (9.3%). All other recoveries of M670H05 at the LOQ were in the acceptable range of 72-105%. The recoveries of M670H05 at the 10-fold LOQ were also within the acceptable range at 73-100%. Several recoveries of topramezone and M670H05 extracted by mechanical shaking were lower than 70%, particularly in the corn matrices (forage, stover and grain). At the LOQ, recoveries were in the acceptable range for apple fruit (both analytes) and corn grain (M670H05 alone). In corn forage, two of five recoveries for each analyte were below 70% (66-68%), resulting in mean recoveries of 76.0 ± 7.8% (topramezone) and 72.8 ± 6.5% (M670H05). In corn stover, four of 10 recoveries for each analyte were below 70% (58-69%), resulting in mean recoveries of 74.7 ± 13.4% (topramezone) and (M670H05). In corn grain, two of 10 recoveries of topramezone were below 70% (59-64%), resulting in a mean recovery of 83.6 ± 14.6%. At 10-fold the LOQ, the recoveries in apple fruit, corn forage, and corn grain were in the acceptable range. In corn stover, only one recovery out of 10 was below 70% for M670H05 (69%), resulting in a mean recovery of 80.8 ± 9.2%. Six of 10 recoveries of topramezone in corn stover were below 70% (63-67%). While the mean recovery was low (67.8%), the standard deviation was small (4.4%). It appeared that extraction by mechanical shaking was less effective in recovering residues of both analytes compared to ASE, particularly in samples spiked at the LOQ.

It should be noted that the ILV for method D0007 was conducted by macerating the samples by polytron homogenizer without ASE or mechanical shaking, followed by micro-partitioning. No confirmatory method was proposed. However, the method was highly specific to the analytes of interest as only limited transitions were monitored by the MS/MS. The LOD was reported to be 0.10 pg/µL (0.10 ppb). Recovery values were corrected for residues in control samples in only one instance, for residues of M670H05 in radish root. Except for one radish root chromatogram, no control chromatograms had peaks above the chromatographic background. The spiked sample chromatograms contained only the analyte peak of interest. Peaks were well defined and symmetrical. There appeared to be no carryover to the following chromatograms.

A radiovalidation study was conducted concurrently with the method validation study. Radiolabelled samples (corn forage and stover) from the metabolism study were extracted by mechanical shaking and analyzed using method No. D0007. The results from the radiovalidation study are reported in TABLE C.1.2. The TRRs determined from the metabolism study for topramezone treated forage and stover were 0.22 ppm and 0.3 ppm, respectively. In comparison, the results using the data gathering method D0007 were similar in that average TRRs for topramezone treated forage and stover were 0.24 ppm and 0.29 ppm, respectively. The TRRs determined from the metabolism study for M670H05 treated forage and stover were 0.03 ppm and 0.05 ppm, respectively. These were again comparable to the average TRRs for M670H05 in treated forage and stover (0.04 ppm and 0.05 ppm, respectively) using data gathering method D0007. These data satisfied the radiovalidation requirements for the data gathering/enforcement method.

TABLE C.1.1. Recovery Results from Method Validation of Apple, Radish Root, Corn Forage, K+CWHR, Stover, and Grain using the Data-Gathering Analytical Method. Standards were Prepared in 0.05% NH_4OH (aq.)

Matrix	Spiking Level (mg/kg)	Recoveries Obtained (%) [1]		Mean Recovery ± SD (CV) (%)	
		Topramezone	M670H05	Topramezone	M670H05
ASE					
K+CWHR [2]	0.01	104, 105, 124, 100, 96 (96-124)	72, 99, 79, 76, 91 (72-99)	105.8 ± 10.8 (10.2)	83.4 ± 11.2 (13.5)
	0.10	90, 77, 93, 85, 90 (77-93)	82, 79, 84, 73, 86 (73-86)	87.0 ± 6.3 (7.2)	80.8 ± 5.1 (6.3)
Corn, grain	0.01	87, 131, 103, 130, 101 (87-131)	73, 86, 69, 83, 64 (64-86)	110.4 ± 19.4 (17.5)	75.0 ± 9.3 (12.4)
	0.10	105, 98, 94, 105, 102 (94-105)	100, 86, 85, 96, 91 (85-100)	100.8 ± 4.8 (4.7)	91.6 ± 6.4 (7.0)
Corn, forage	0.05	99, 105, 98, 100, 108 (98-108)	90, 95, 99, 105, 84 (84-105)	102.0 ± 4.3 (4.2)	94.6 ± 8.1 (8.5)
	0.50	91, 91, 95, 97, 95 (91-97)	93, 92, 99, 99, 97 (92-99)	93.8 ± 2.7 (2.9)	96.0 ± 3.3 (3.5)
Corn, stover	0.05	83, 90, 82, 86, 89 (82-90)	74, 84, 74, 83, 84 (74-84)	86.0 ± 3.5 (4.1)	79.8 ± 5.3 (6.7)
	0.50	80, 84, 79, 82, 79 (79-84)	89, 93, 86, 93, 82 (82-93)	80.8 ± 2.2 (2.7)	88.6 ± 4.7 (5.3)
Radish, root	0.05	90, 87, 95, 83, 79 (79-85)	95, 86, 86, 78, 78 (78-95)	86.8 ± 6.2 (7.1)	84.6 ± 7.1 (8.3)
	0.50	97, 97, 97, 92, 82 (82-97)	99, 82, 77, 76, 83 (76-99)	93.0 ± 6.5 (7.0)	83.4 ± 9.2 (11.1)
Mechanical Shaking					
Apple fruit	0.05	107, 94, 92, 101, 90 (90-107)	102, 101, 102, 101, 102 (101-102)	96.8 ± 7.0 (7.3)	101.6 ± 0.5 (0.5)
	0.50	105, 111, 89, 98, 111 (89-111)	95, 93, 94, 92, 102 (92-102)	102.8 ± 9.4 (9.1)	95.2 ± 4.0 (4.2)
Corn, forage	0.05	83, 82, 67, 68, 80 (67-83)	80, 75, 66, 66, 77 (66-80)	76.0 ± 7.8 (10.3)	72.8 ± 6.5 (8.9)
	0.50	81, 81, 74, 76, 77 (74-81)	87, 86, 75, 83, 81 (75-87)	77.8 ± 3.1 (4.0)	82.4 ± 4.8 (5.6)

Mechanical Shaking					
Corn, stover	0.05	67, 72, 74, 62, 67, 73, 110, 81, 69, 72 (62-110)	68, 73, 58, 64, 69, 86, 101, 91, 87, 88 (58-101)	74.7 ± 13.4 (17.9)	78.5 ± 13.9 (17.7)
	0.50	64, 64, 65, 63, 67, 65, 70, 75, 71, 74 (63-75)	72, 73, 79, 69, 73, 84, 83, 87, 97, 91 (69-97)	67.8 ± 4.4 (6.5)	80.8 ± 9.2 (11.4)
Corn, grain	0.01	59, 64, 86, 71, 83, 96, 98, 90, 102, 87 (59-102)	96, 91, 103, 98, 112, 103, 110, 109, 105, 109 (91-112)	83.6 ± 14.6 (17.4)	103.6 ± 6.8 (6.6)
	0.10	90, 88, 94, 95, 96, 89, 90, 91, 74, 85 (74-96)	82, 77, 82, 86, 86, 95, 94, 99, 91, 95 (77-99)	89.2 ± 6.3 (7.1)	88.7 ± 7.1 (8.1)

[1] Range of recoveries are in parentheses
[2] K+CWHR = kernel + cob with husk removed, or "fresh corn"

TABLE C.1.2. Extraction Efficiency of the Enforcement Analytical Method using Radiolabelled Samples from the Corn Metabolism Study.

Matrix	Metabolism Study[1]		Radiovalidation Study[2]	
	% TRR	ppm	% TRRs (average)	ppm (average)
Topramezone				
Treated forage	42.14	0.22	43.09, 42.3, 52.79 (46.06)	0.22, 0.22, 0.27, (0.24)
Treated stover	39.41	0.30	37.7, 36.06[3] (36.88)	0.29, 0.28 (0.29)
M670H05				
Treated forage	6.28	0.03	6.64, 7.25, 8.69 (7.53)	0.03, 0.04, 0.05 (0.04)
Treated stover	6.92	0.05	5.25, 6.21[3] (5.73)	0.04, 0.05 (0.05)

[1] From metabolism study BASF Study No. 98129. TRRs were 0.512 and 0.758 ppm in the forage and stover RAC samples, respectively.
[2] The average result for the individual extractions is shown in brackets.
[3] Avg of duplicate analyses of the same sample extract.

TABLE C.1.3. Characteristics for the Data-Gathering Analytical Method used for the Quantitation of Topramezone and M670H05 Residues in Apple, Radish Root, Corn Forage, K+CWHR, Stover, and Grain.	
Analyte	Topramezone and M670H05
Equipment ID	PE Sciex API 300 Biomolecular Mass Analyzer
LOQ	0.01 ppm for corn grain and K+CWHR 0.05 ppm for radish root, corn forage, corn stover and apple fruit
LOD	0.10 ppb
Accuracy/Precision	**Topramezone:** Individual recoveries ranged from 77-131% using ASE for K+CWHR, corn grain, corn forage, corn stover and radish root at spiking levels of the LOQ and 10-fold LOQ. Indicates acceptable accuracy/precision in the range of spiking levels (0.05 and 0.50 ppm for corn forage, corn stover, radish root; 0.01 and 0.10 ppm for K+CWHR and corn grain). Individual recoveries ranged from 59-111% using extraction by mechanical shaking for apple fruit, corn grain, corn forage, and corn stover, at spiking levels of the LOQ and 10-fold LOQ. Indicates acceptable accuracy/precision in the range of spiking levels (0.05 and 0.50 ppm for corn forage, corn stover, apple fruit; 0.01 and 0.10 ppm for corn grain).
	M670H05: Individual recoveries ranged from 64-105% using ASE for K+CWHR, corn grain, corn forage, corn stover, and radish root at spiking levels of the LOQ and 10-fold LOQ. Indicates acceptable accuracy/precision in the range of spiking levels (0.05 and 0.50 ppm for corn forage, corn stover, radish root; 0.01 and 0.10 ppm for K+CWHR and corn grain). Individual recoveries ranged from 58-112% using extraction by mechanical shaking for apple fruit, corn grain, corn forage, and corn stover at spiking levels of the LOQ and 10-fold LOQ. Indicates acceptable accuracy/precision in the range of spiking levels (0.05 and 0.50 ppm for corn forage, corn stover, apple fruit; 0.01 and 0.10 ppm for corn grain).
Reliability of the Method/ [ILV]	An ILV was conducted to verify the reliability of method No. D0007 for the determination of topramezone residues in corn grain, lettuce, oilseed rape, and apple. The samples were macerated by polytron homogenizer without ASE or mechanical shaking, followed by micro-partitioning. The values obtained are indicative that method No. D0007 is reliable using ASE or maceration by polytron homogenizer, but not by mechanical shaking.
Linearity	The detector response was linear for both topramezone and M670H05 (coefficient of determination, r^2=0.99) within the range of 0.10 to 1.0 pg/μL (or ppb).
Specificity	The control chromatograms generally have no peaks above the chromatographic background and the spiked sample chromatograms contain only the analyte peak of interest. Peaks were well defined and symmetrical. There appeared to be no carryover to the following chromatograms.

C.2. Enforcement Method

The enforcement method being proposed is the same as data-gathering method No. D0007.

C.3. Independent Laboratory Validation (ILV)

An ILV study was conducted for Method No. D0007 by the Crop Protection division of BASF in Germany. Although the ILV was conducted by a division of BASF Corporation, the analyses were conducted by a separate lab and, therefore, are acceptable. Untreated corn grain, lettuce, oilseed rape seed, and apple samples were spiked with topramezone and the metabolite M670H05 at both the LOQ and at 10-fold the LOQ: 0.01 ppm and 0.10 ppm (corn grain), 0.05 ppm and 0.50 ppm (lettuce, oilseed rape, and apple). Instead of undergoing ASE (the primary extraction technique from the data-gathering method), the samples were macerated by polytron homogenizer, followed by micro-partitioning. Recoveries were corrected for matrix interference of the appropriate unspiked samples. Other than a single recovery of topramezone in corn grain spiked at the LOQ (68.5%), all other recoveries of topramezone and M670H05 were within the acceptable range for each matrix and spiking level (TABLE C.3.1). Mean recoveries obtained during the ILV study analyses were between 74.2% and 110.1% (SD <20%) for topramezone, and between 76% and 86.5% (SD <20%) for M670H05. Although the primary extraction technique (ASE) was not utilized in the ILV, the recoveries at the LOQ and 10-fold the LOQ were in the acceptable range, with low standard deviations and coefficient of variations.

The ILV was conducted according to Method D0007, except for the following deviations: (i) The mobile phase gradient program was changed. (ii) The sample sizes changed to 25 g of corn grain and 10 g of other material. The difference was due to the difference in LOQs, and that the analysis of non-homogenized material is usual for enforcement labs. (iii) The sample material was macerated by polytron homogenizer instead of shaken, for the use of non/less - homogenized sample material. (iv) The pH value after the last partition step was checked for each sample. (v) Instrument recovery was checked with each analytical series, as recoveries of topramezone could range from 110-120%, while recoveries of M670H05 could range between 50-60%. (vi) The inclusion of additional mass transitions for topramezone and M670H05 for confirmatory purposes, as the EU enforcement labs require a second transition or another method for confirmation in case of positive findings (residue levels above the maximum residue limit (MRL) value). These deviations were included in the final technical procedure.

A potential problem was reported with respect to incomplete separation of the aqueous and organic layers. If longer centrifugation is not successful in separating the two phases, care should be taken that the thin layer of residual solids is not taken up with the DCM phase. It was also noted that low or high recoveries could be due to suppression or enhancement caused by matrix effects. An injection of a control spike should be used to determine LC-MS/MS performance.

The time required to run the analysis of a set (21 samples, 2 recoveries and 1 control) was approximately 8 hours, which included LC-MS/MS analysis and calculation times.

TABLE C.3.1. Recovery Results Obtained by an Independent Laboratory Validation (ILV) of the Enforcement Method for the Determination of Topramezone and M670H5 in Corn Grain, Lettuce, Oilseed Rape, and Apple.

Matrix	Spiking Level (µg/g)	Recoveries Obtained (%) [1]		Mean Recovery ± SD (CV) (%)	
		Topramezone	M670H05	Topramezone	M670H05
Corn grain	0.01	68.5, 77, 73.8, 74.3, 77.2 (68.5-77.2)	83.6, 86.9, 92, 85.4, 84.6 (83.6-92.0)	74.2 ± 3.5% (4.7%)	86.5 ± 3.3% (3.8%)
	0.10	95.1, 87.1, 82.7, 81.7, 83.1 (81.7-95.1)	78.2, 85.5, 75.5, 75.6, 77.1 (75.5-85.5)	85.9 ± 5.5% (6.4%)	78.4 ± 4.2% (5.3%)
Lettuce	0.05	83.1, 81.5, 89.8, 88.3, 92 (81.5-92.0)	73.6, 73.5, 77.8, 77.6, 77.7 (73.5-77.8)	86.9 ± 4.5% (5.2%)	76.0 ± 2.3% (3.0%)
	0.50	91.2, 80.1, 88.3, 89.5, 90.3 (80.1-91.2)	82.6, 82.3, 82.8, 83.6, 84.1 (82.3-84.1)	87.9 ± 4.5% (5.1%)	83.1 ± 0.7% (0.9%)
Oilseed rape	0.05	102.1, 105.8, 108.4, 116, 118.1 (102.1-118.1)	81.5, 82.9, 76.9, 82.1, 78.4 (76.9-82.9)	110.1 ± 6.8% (6.2%)	80.3 ± 2.6% (3.2%)
	0.50	73.5, 75.5, 80.2, 78.8, 75.7 (73.5-80.2)	83.5, 85, 87.2, 88.1, 89.3 (83.5-89.3)	76.7 ± 2.7% (3.5%)	86.6 ± 2.3% (2.7%)
Apple	0.05	84.5, 93.2, 87.4, 78.3, 86.6 (78.3-93.2)	74.8, 75.3, 70, 74.4, 72 (70.0-75.3)	86..0 ± 5.4% (6.3%)	73.3 ± 2.3% (3.1%)
	0.50	92.5, 84.5, 91.2, 92, 87.2 (84.5-92.5)	83, 75.9, 81.7, 73.1, 79.3 (73.1-83.0)	89.5 ± 3.5% (3.9%)	78.6 ± 4.1% (5.2%)

[1] Range of recoveries are in parentheses

D. CONCLUSION

Method validation data have been submitted for LC-MS/MS data-gathering/enforcement method No. D0007. The recoveries obtained using the proposed method for data gathering and enforcement appear to be adequate for topramezone and the free acid M670H05. Acceptable radiovalidation data have been submitted for corn forage and stover.

A successful ILV for LC-MS/MS method No. D0007 has been completed using samples of untreated corn grain, lettuce, oilseed rape seed, and apple. Several deviations and recommendations to increase efficiency were applied during the study and were included in the final technical procedure. The use of the primary extraction technique (ASE) or maceration by polytron homogenizer produced acceptable recoveries, while extraction by mechanical shaking did not. It is thus recommended that mechanical shaking not be used for enforcement purposes.

E. REFERENCES

MRID No. 45902401. Ellenson, James L. (2002) Final Report: Metabolism of [^{14}C]-BAS 670 H in corn. BASF Study Number 98129. Unpublished study prepared by BASF Agro Research. 208 pages.

F. DOCUMENT TRACKING

RDI: G.F. Kramer (2/11/05), RAB1 Chemists (12/15/04), ChemSAC (3/2/05)
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Evaluators

Susha Zachariah — Date: March 21, 2005
Susha Zachariah
Evaluation Officer
FREAS, HED, PMRA

Suzan Mathew — Date: Mar 21/05
Suzan Mathew
Evaluation Officer
FREAS, HED, PMRA

Peer Reviewer

George F. Kramer — Date: 4/12.05
George F. Kramer, Ph.D.
Chemist
RAB1, HED, EPA

Approved by

Louise G. Croteau — Date: March 22/05
Louise G. Croteau
Senior Evaluation Officer
FREAS, HED, PMRA

Ariff Ally — Date: Mar 29/05
Ariff Ally, Ph.D.
Section Head
FREAS, HED, PMRA

P.V. Shah — Date: 2/18/05
P.V. Shah, Ph.D.
Branch Senior Scientist
RAB1, HED, EPA

STUDY REPORTS:

MRID No. 45902403. Anders, S. and Lam, W.W. (2000) Final Report: Nature of Residues of ^{14}C-BAS 670 H in Laying Hens. BASF Study No. 55910. Unpublished study prepared by BASF Corporation. 174 pages.

EXECUTIVE SUMMARY:

BASF Corporation has conducted a hen metabolism study with [pyrazole-4-^{14}C] (4.65 MBq/mg specific radioactivity; >98% radiochemical purity) topramezone and [phenyl-U-^{14}C] (5.76 MBq/mg specific radioactivity; >98% radiochemical purity) topramezone. Topramezone (also known as BAS 670 H) was orally administered to 10 Leghorn laying hens for each label at feeding levels of 12.3 mg/kg (pyrazole label) or 13.4 mg/kg (phenyl label) for 10 consecutive days. Eggs were collected twice daily, excreta was collected once daily, and cage rinse samples were collected on study day 0 and after termination. Tissue samples (liver, muscle, fat and GI tract/contents) were harvested upon termination, 21 - 23 hours after the final dose. The collected samples were assayed for total radioactive residues (TRRs) by combustion and liquid-scintillation counting (LSC). Samples were subjected to solvent extraction to recover the maximum TRRs for further chromatographic characterization and elucidation of the nature of residues. Metabolite identification/characterization of the matrices was achieved by high-performance liquid chromatography (HPLC) and co-chromatography and/or comparison of retention times of reference standards or isolated metabolites. All livestock matrices were analysed within 4 months of sampling. Therefore, no storage stability data (for the determination of sample integrity) were required for this study.

The total applied dose recovery was 94.1% for the pyrazole-label, of which 92.9% was found in excreta, 0.70% in the GI tract/contents (0.796 ppm) and 0.29% in cage rinse. The dose recovery in egg, muscle and fat was <0.005 ppm each (0.0% of the TRRs), while virtually all the recoveries in edible tissues were in the liver (0.22% of the TRRs; 0.739 ppm). For the phenyl-label, the total applied dose recovery was 93.1%, of which 91.4% was in excreta, 0.29% in cage rinse, and 1.07% in the GI tract/contents (1.318 ppm). The dose recovery in fat, muscle and egg were each ≤0.004 ppm each (0.0% of the TRRs), while the vast majority of residues in edible tissues were found in the liver (1.680 ppm).

A total of 88.6-93.4% of the TRRs (0.656-1.568 ppm) were extracted with methanol (phenyl-label) or methanol/water (pyrazole-label) from radiolabelled liver samples. Of the 80.8-90.8% of the TRRs identified in both labels (0.597-1.525 ppm), the parent topramezone accounted for the majority of residues (58.5-64.4% of the TRRs; 0.475-0.982 ppm). The hydroxy metabolite M670H02 accounted for 16.4-29.9% of the TRRs in both labels (0.122-0.503 ppm), while the desmethyl hydroxy metabolite M670H04 was only detected in the phenyl-label sample (2.4% of the TRRs; 0.04 ppm). Residues characterized as a series of minor peaks/regions comprised 2.6-7.8% of the TRRs (0.043-0.059 ppm). The unextractable residues (post-extraction solids, PES) accounted for 3.7-7.9% of the TRRs (0.059-0.062 ppm), for an overall accountability ranging from 96.8-97.0%.

A total of 86.0-88.5% of the TRRs were extracted with acetone:phosphate buffer from both the pyrazole-label and phenyl-label excreta samples. The parent topramezone was the only metabolite identified in the pyrazole label excreta (64.0% of the TRRs). Of the 70.1% of the TRRs identified in the phenyl-label excreta, 59.5% of the TRRs were identified as the parent topramezone, while M670H02 accounted for 10.6% of the TRRs. Residues characterized as a

series of minor peaks/regions comprised 15.9-24.5% of the TRRs. The unextractable residues (PES) accounted for 3.4-6.3% of the TRRs, for an overall accountability ranging from 89.4-94.8%.

A total of 32.9-42.0% of the TRRs (0.0006-0.0007 ppm) were extracted with acetonitrile and water from both the day 7 and day 9 phenyl-label egg samples. Of the 23.2-31.7% of the TRRs identified (0.0004-0.0006 ppm), the parent topramezone accounted for 6.75-13.8% of the TRRs (0.0001-0.0003 ppm). M670H02 accounted for 11.6-11.9% of the TRRs (0.0002 ppm each) for both samples, while the acid metabolite M670H05 accounted for 4.6-6.3% of the TRRs (0.0001 ppm each). Residues characterized as a series of minor peaks/regions comprised 9.65-10.2% of the TRRs (0.0001-0.0002 ppm). The unextractable residues (PES) accounted for 21.1-23.0% of the TRRs (0.0004 ppm each), for overall accountabilities of 70% each.

The metabolic pathway of topramezone in hens proceeded from the hydroxylation at the 4-position of the isoxazole ring to form the hydroxy metabolite M670H02. Further N-demethylation of M670H02 occurred and formed the desmethyl hydroxy metabolite M670H04. M670H04 was only detected in the phenyl labelled liver. The pyrazole ring of topramezone could also be cleaved to form the acid metabolite M670H05.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the field trial residue data are classified as scientifically acceptable. No second spectroscopic method was used to confirm identification data in this study.

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document [DP# 310772] and in Canada's Regulatory Decision Document.

COMPLIANCE:

Signed and dated Good Laboratory Practice (GLP), Quality Assurance and Data Confidentiality statements were provided. Deviations from the protocol included the following:

- Phenyl-dosed hens were not weighed 48 h prior to initial dosing.
- The relative humidity in the study room was not reported for the day of necropsy.
- The animals were in a shorter light period (14-h light/10-h dark) for the first 2 days after receipt versus the rest of the study period (18-h light/6-h dark).
- A larger subsample of eggs was taken after homogenization on each day.
- Hens were to be chosen based on egg production, then selectively randomized. Pyrazole-dosed hens and control hens were chosen based on both egg production and dietary feed intake (to allow for consistent feed intakes within a group). Phenyl-dosed hens were selected based on egg production but were not randomized.
- Combustion efficiency was not calculated (99.27%) until after the combustion of pyrazole-dosed and control hen excreta.

These deviations are not expected to impact the validity of the study.

A. BACKGROUND INFORMATION

BAS 670 336SC Herbicide (also known as BAS 670 00H Herbicide) is the end-use product which contains the active ingredient topramezone. It is a broad-spectrum, post-emergence herbicide used to control grassy and broadleaf weeds in corn. The herbicide is absorbed by the leaves, roots and shoots, then translocated to the growing points of the sensitive weeds. Topramezone belongs to the triketone class of chemicals, which inhibits carotenoid biosynthesis (4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitor). This causes a strong bleaching activity on the growing zones of the shoots within 2-5 days of application. Exposure to light causes necrosis of chlorotic tissues and eventual plant death within 14 days after application.

TABLE A.1. Test Compound Nomenclature

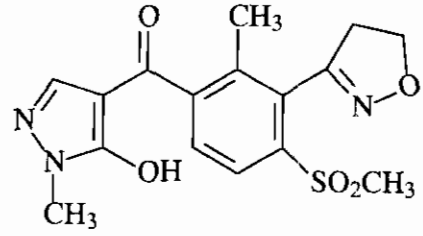
Compound	Chemical Structure 
Common name	Topramezone
Company experimental name	BAS 670 H
IUPAC name	[3-(4,5-dihydro-1,2-oxazol-3-yl)-4-mesyl-*o*-tolyl](5-hydroxy-1-methyl-1*H*-pyrazol-4-yl) methanone
CAS name	[3-(4,5-dihydro-3-isoxazolyl)-2-methyl-4-(methylsulfonyl)phenyl](5-hydroxy-1-methyl-1*H*-pyrazol-4-yl)methanone
Molecular Formula	$C_{16}H_{17}N_3O_5S$
Molecular Mass	363.39 g/mol
CAS #	210631-68-8
End-use product/EP	Soluble Concentrate

TABLE A.2. Physicochemical Properties of Topramezone	
Parameter	**Value**
Melting point/range	220.9°C - 222.2°C
pH	2.9 (1% de-ionized water)
Density (20°C)	1.425 g/cm^3
Water solubility (20°C)	pH: 3, 5, 7, 9 g/L: 0.06, 0.98, 15, 23.4
Solvent solubility (g/100 mL at 20°C)	Acetone <1.0 Acetonitrile <1.0 Dichloromethane 2.5 - 2.9 Ethyl acetate <1.0 Methanol <1.0 N-heptane <1.0 N,N-dimethylformamide 11.4-13.3 1-octanol <1.0 Olive oil <1.0 2-propanol <1.0 Toluene <1.0
Vapour pressure at 20°C and 25°C	< 1.0 x 10^{-12} hPa
Dissociation constant (pK_a)	4.06
Octanol/water partition coefficient Log(K_{OW}) at 20°C	Buffer pH: 4, 7, 9 Log K_{ow}: – 0.81, – 1.52, – 2.34
UV/visible absorption spectrum	λ, nm: 207, 272, 300, 410 ξ, mol^{-1}cm^{-1}: 27 077, 8601, 5800, 410

All data came from PMRA Lab Services.

B. EXPERIMENTAL DESIGN

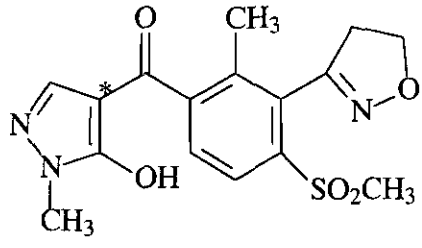
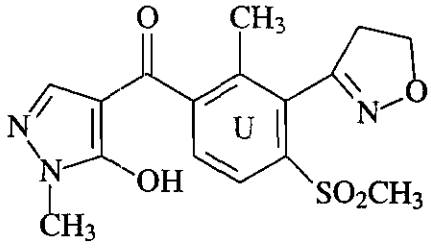
B.1. Livestock

TABLE B.1.1. General Test Animal Information					
Species	Breed	Age	Weight at study initiation (kg)	Health Status	Description of housing/holding area
Laying Hen *Gallus gallus*	Leghorn	67 weeks	1.36-1.73	Normal health	Housed in individual metabolism cages, at 18-24°C (65-76°F), 44-74% relative humidity, 18 hours light/day.

TABLE B.1.2. Test Animal Dietary Regime				
Composition of Diet	Feed consumption (g/day)	Water	Acclimation period	Predosing
Purina ® Layena Crumbles provided *ad libitum* for ^{14}C-pyrazole and control group EverLay 2000 provided *ad libitum* for ^{14}C-phenyl group	pyrazole group: 106 phenyl group: 109	*ad libitum*	15-16 days	None

TABLE B.1.3. Test Animal Dosing Regime			
Treatment Type	Feeding Level (ppm test material in food)	Vehicle	Timing/Duration
Oral	12.3 (pyrazole label) 13.4 (phenyl label)	gelatin capsule	Once a day (following AM egg collection) for 10 consecutive days

B.2. Test Materials

TABLE B.2.1. Test Material Characteristics		
Chemical structure		
Radiolabel position	Pyrazole	Phenyl
Lot No.	706-1013	714-1026
Purity	>98% (radio-HPLC)	>98% (radio-HPLC)
Specific activity (Bq)*	5.76 MBq/mg	4.65 MBq/mg

*Bq = disintegrations per second

B.3. Sampling Information

TABLE B.3.1. Sample Collection Information

Eggs collected	Amount of eggs produced during normal production	Excreta and cage wash collected	Interval from last dose to sacrifice	Tissues harvested and analysed
twice daily (PM and AM)	Not reported during normal production. During the 10 day dosing period: 1) control group: 0.81 ± 0.16 eggs per hen per day. 2) pyrazole group: 0.9 ± 0.09 eggs per hen per day. 3) phenyl group: 0.48 ± 0.25 eggs per hen per day.	Excreta collected from study day 0 to termination. Cage wash collected on Study Day 0 and after the termination for each hen following the excreta collection.	21- 23 hours	liver, muscle (thigh and breast), fat (visceral and fat adhering to the skin and muscle), gastrointestinal (GI) tract and contents collected at animal termination

B.4. Identification/ Characterization of Residues

B.4.1. Sample Handling and Preparation

At necropsy, the individual tissue sample weights were recorded and the samples were then pooled by tissue and by group (^{14}C-pyrazole, ^{14}C-phenyl, and control) and placed into labelled Ziploc® storage bags. Eggs, excreta, cage washes and tissues were stored frozen at ≤-15°C before, during and after shipment to BASF, where the samples were stored until needed for preparation and analysis. Eggs, excreta and tissue samples were homogenized and subsamples were combusted to determine TRRs. Samples were combusted using a Packard Model 307 oxidizer. The evolved $^{14}CO_2$ was trapped in Carbo-Sorb® E$^+$ and combined with Permafluor® E$^+$. All combusted samples were counted by LSC. Samples with TRRs <0.01 mg/kg from both labels did not undergo further chromatographic characterization. An attempt was made to analyse the low level Days 7 and 9 ^{14}C-phenyl-labelled egg samples.

Eggs

The eggs were homogenized using a Tekmar Tissumizer® following the PM and AM collections each day. A subsample of each homogenate was removed and five aliquots from each were weighed onto Combusto-Cones™ with pads contained in labelled liquid scintillation vials and weights were recorded. The aliquots were allowed to air-dry prior to combustion analysis. Remaining bulk samples were divided into three approximately equal portions and stored frozen until further analysis.

The day-7 egg sample was extracted using three portions of acetonitrile, then centrifuged, and the resulting supernatants were measured and radioassayed. The first acetonitrile extract was concentrated and filtered prior to HPLC analysis. The acetonitrile extracted egg sample was further extracted using two portions of water, then centrifuged and the resulting supernatants were measured and radioassayed. The extracted solids were combusted for recovery.

The day-9 egg sample was extracted using three portions of acetonitrile, which were combined

and concentrated. The concentrate was cleaned up using a C-18 solid-phase extraction (SPE) method. The sample was eluted using for solvent systems in the following sequence: 1% formic acid (100%), 1% formic acid:methanol (75:25), 1% formic acid:methanol (50:50), and methanol (100%). The 1% formic acid:methanol (75:25) and 1% formic acid:methanol (50:50) fractions were collected, combined and concentrated for HPLC analysis.

Excreta

Pooled excreta samples (by group) were thoroughly blended with water using Waring Blendor®. A subsample of each blended sample was removed and five aliquots were weighed onto Combusto-Cones™ with pads contained in labelled liquid scintillation vials and the weights were recorded. The aliquots were allowed to air-dry prior to combustion analysis. Remaining bulk samples were divided into three approximately equal portions and stored frozen until further analysis.

Similar work-up schemes were used for both the ^{14}C-phenyl group and the ^{14}C-pyrazole group. Excreta was extracted using three portions of a (1:1) mixture of acetone:phosphate buffer (pH 7.0). The resulting extracts were centrifuged and the volumes of supernatants were measured, combined and radioassayed. An aliquot of this combined supernatant was concentrated to dryness and re-dissolved in water. This resulting concentrate was filtered prior to HPLC analysis. The PES were combusted for recovery calculation.

Cage Rinse

Cage rinse specimens were collected from each animal on Study Day 0 and after the termination for each animal following the excreta collection. Each cage was rinsed with a 20% methanol:water solution. The cage rinse samples were collected and then pooled by group. Five aliquots of each day's pooled cage rinse samples were weighed directly into labelled liquid-scintillation vials, combined with liquid scintillation cocktail and analysed by LSC.

Tissue Samples (liver, fat, muscle and GI tract/contents)

Pooled liver, fat, muscle and GI tract/content samples were processed first through a Hobart Grinder® and then homogenized using a Brinkmann Polytron homogenizer®. After processing, five aliquots of each homogenate were weighed onto Combusto-Cones™ with pads contained in labelled scintillation vials. The weights were recorded and then air-dried prior to combustion analysis. Remaining bulk samples were divided into one subsample. The remaining sample was divided into three approximately equal portions before being stored frozen until further analysis.

Liver

For Pyrazole-label:

Liver was extracted using four portions of methanol. The resulting extracts were centrifuged, diluted, combined and radioassayed. An aliquot of this combined methanol extract was concentrated to dryness and was re-dissolved in acidified water (pH 1). This acidic aqueous extract was partitioned with three portions of ethyl acetate, which were combined and then diluted. The ethyl acetate fractions and the diluted extracted aqueous acid fractions were radioassayed. The ethyl acetate fraction was back extracted with three portions of ammonium

hydroxide solution (pH 11). The combined ammonium extracts and the diluted extracted ethyl acetate were radioassayed. A composite sample containing the aqueous acid and the ammonium extracts were made, then concentrated and analysed using HPLC. The PES were combusted for recovery calculation.

Following methanol extraction, the liver was further extracted using two portions of water. The resulting water extracts were centrifuged, diluted, combined and radioassayed. The combined water extract was concentrated to dryness and re-dissolved in water before HPLC analysis.

For Phenyl-label:
Liver was extracted using four portions of methanol. The resulting extracts were centrifuged, diluted, combined and radioassayed. An aliquot of this combined methanol extract was concentrated to dryness and re-dissolved in acidified water (pH 1). This resulting concentrate was filtered prior to HPLC analysis. The PES were combusted for recovery calculation.

B.4.2. Analytical Methodology

TRRs of each matrix were determined by combustion, followed by LSC. Based upon the LSC results, samples with TRRs > 0.01 mg/kg from both labels were subjected to solvent extraction for further chromatographic characterization and elucidation of the nature of residues.

HPLC methods used in this study are summarized in TABLE B.4.2.1.

TABLE B.4.2.1. Summary of HPLC Methods

HPLC Method Number	Column	Eluents		Flow Rate [mL/min]	Gradients		
		A	B		Time [min]	%A	%B
1 (CLMBSS TD)	Columbus	10mM Phosphate Buffer; pH 7.00	MeOH	1	0-20 21-26 28-35	95-20 5 95	5-80 95 5
2 (PRP-1-5)	PRP-1	10mM Phosphate Buffer; pH 7.00	MeoH	1	0-50 51-56 58-65	100-60 5 95-100	0-40 95 5-0
3 (PRP-1-10)	PRP-1	10mM Phosphate Buffer; pH 7.00	MeoH	1.5	0-20 21-26 28-35	95-20 5 95	5-80 95 5
4 (98129-03)	Columbus	Water with 0.2% Formic Acid	ACN with 0.2% Formic Acid	1	0-2 3-20 30-40 45-47	98 85 70-2 2-98	2 15 30-98 98-2

The identification of the metabolite residues was via co-chromatography and/or comparison of retention times of reference standards or isolated metabolites. The following standards/metabolites were used for identification: diluted ^{14}C-topramezone dosing solution,

hydroxy metabolite (M670H02) isolated from goat urine, cleaved acid reference standard (M670H05) and the desmethyl hydroxy metabolite (M670H04) isolated from wheat cell culture. The metabolite standards for M670H02 and M670H04 were initially identified using LC-MS and/or nuclear magnetic resonance (NMR) techniques.

The limit of quantitation (LOQ) was defined as two times the background radioactivity of a matrix divided by the specific activity. The LOQ for ^{14}C pyrazole labelled matrices was determined to be 0.0009 ppm for excreta, 0.0011 ppm for egg, 0.0013 ppm for muscle, 0.002 ppm for fat and 0.0015 ppm for liver. The LOQ for ^{14}C-phenyl-labelled matrices was determined to be 0.0015 ppm for excreta, 0.0011 ppm for egg, 0.0015 ppm for muscle, 0.0031 ppm for fat and 0.0016 ppm for liver.

C. RESULTS AND DISCUSSION

All livestock matrices were analysed within 4 months of sampling (Table C.1). Therefore, no storage stability data were required for this study.

The residues in this study were identified by only 1 analytical technique (HPLC) with no confirmatory spectroscopic data. The residues were identified by the comparison of retention times to known standards using HPLC chromatography with 2 different column phases - a polymer-based reverse-phase column (PRP-1, HPLC method 3) and a silica-based reverse-phase column (Columbus C18, HPLC method 1). Each column used methanol and 10 mM phosphate buffer (pH 7) as eluants. Although the 2 methods had different flow rates (1.5 mL/min for PRP-1, and 1.0 mL/min for the Columbus C-18), they both had the same mobile phase and gradient program. Both methods were able to identify all of the metabolites.

Samples from all matrices were combusted to determine the TRRs. Samples with TRRs > 0.01 mg/kg from both labels were subjected to solvent extraction to recover the maximum TRRs for further chromatographic characterization and elucidation of the nature of residues. Although the residue level found in phenyl-labelled egg samples were very low (< 0.01 mg/kg), representative egg samples from days 7 and 9 were characterized. Residue levels in tissues that were below 0.01 ppm were not subjected to solvent extraction for further chromatographic characterization. An attempt was made to analyse the low level days 7 and 9 ^{14}C-phenyl-labelled egg samples.

Extracted matrices (liver, excreta and egg) were analysed by two different HPLC methods that each employed different columns (Table B.4.2.1). Pyrazole-label liver and excreta were analysed by HPLC methods 1 and 2. Phenyl-label liver, excreta and egg (day 7) were analysed by HPLC methods 1 and 3. The phenyl-label day 9 egg sample was analysed by only HPLC method 1. Both methods 1 and 3 were able to identify the parent and all the metabolites (see Table C.3.1), while method 2 identified only the parent and the hydroxy metabolite (M670H02). The results from method 1 are listed in the summary tables (Tables C.2.3.1 and C.2.3.2) because the retention times for the hydroxy and desmethyl hydroxy metabolites were more distinct than with method 3.

TRRs in eggs, tissue and excreta are reported in TABLE C.2.1 and FIGURE C.2.2. In hens treated with pyrazole-label topramezone, 94.09% of the TRRs were recovered. Excreta was the major route of elimination accounting for 92.9% of the administered dose (total over the 10 day period). Cage rinses contained 0.29% of the administered dose and 0.70% of the administered dose was in the GI tract/contents (0.796 ppm). The highest level of radioactive residues found in edible tissues were in the liver (0.22% of the TRRs; 0.739 ppm). Residues in fat (0.003 ppm), muscle (0.001 ppm) and eggs (≤0.005 ppm per day) were negligible.

For the phenyl label, 93.1% of the TRRs were recovered. Again, majority of the radioactivity was found in the excreta (91.4%) and the cage rinse accounted for 0.29% of the administered dose. Approximately 1.07% of the administered dose was recovered in the GI tract/content. The highest level of radioactive residues found in edible tissues were again in the liver (1.680 ppm), followed by negligible levels in fat (0.004 ppm), muscle (0.001 ppm) and eggs (≤0.002 ppm per day).

Excreta: The distribution of radioactivity for both labels is reported in TABLE C.2.2.1 and FIGURE C.2.1. For the pyrazole label (Day-1 sample), the TRRs extracted using an acetone/phosphate buffer were 88.5% over the 10 day sampling period. The parent topramezone was the only residue identified, accounting for 64.0% of the TRRs (HPLC method 1). Residues accounting for a total of 24.5% of the TRRs were characterized as a series of minor peaks/regions, and 6.3% of the TRRs remained unextractable in the PES. The accountability was determined to be 94.8% for pyrazole-label excreta (TABLE C.2.3.1).

For the phenyl-label (Day-5 sample), the TRRs extracted using an acetone/phosphate buffer were 86.0% over the 10 day sampling period. The predominant residue identified was the parent topramezone (59.5% of the TRRs; HPLC method 1). The hydroxy metabolite M670H02 was present at 10.6% of the TRRs (HPLC method 1). Residues accounting for a total of 15.9% of the TRRs were characterized as a series of minor peaks/regions. Approximately 3.4% of the TRRs were unextractable in the PES. The accountability was determined to be 89.4% for the phenyl-label excreta (TABLE C.2.3.2).

Liver: For the pyrazole-label, the extractability of the liver using methanol was 89.4% of the TRRs (0.66 ppm), with an additional 1.7% of the TRRs (0.013 ppm) from the ethyl acetate extract (TABLE C.2.2.2). The predominant residues from the acid/base composite extracts were identified as topramezone (62.2% of the TRRs; 0.459 ppm) and M670H02 (15.8% of the TRRs; 0.117 ppm). Further extractions using water allowed for an additional 4.3% of the TRRs (0.032 ppm). HPLC analysis (method 1) of the water extract also identified topramezone (2.2% of the TRRs; 0.016 ppm) and M670H02 (0.6% of the TRRs; 0.005 ppm). Approximately 6.3% of the TRRs (0.048 ppm) were characterized as a series of minor peaks/regions from the acid/base extract, and an additional 1.5% of the TRRs were characterized from the water extract (0.011 ppm). Approximately 7.9% of the TRRs (0.059 ppm) remained unextractable in the PES. The accountability was determined to be 96.8% for pyrazole-label liver (TABLE C.2.3.1).

For the phenyl-label, the extractability of the liver using methanol was 93.4% of the TRRs (1.568

ppm) (TABLE C.2.2.3). Topramezone was the major residue present at 58.5% of the TRRs (0.982 ppm). The metabolites M670H02 and M670H04 accounted for 29.9% of the TRRs (0.503 ppm) and 2.4% of the TRRs (0.040 ppm), respectively (HPLC method 1). Approximately 2.6% of the TRRs (0.043 ppm) were characterized as a series of minor peaks/regions, while 3.7% of the TRRs (0.062 ppm) were unextractable. The accountability was determined to be 97.0% for phenyl-label liver (TABLE C.2.3.2).

Egg: Approximately 42.0% of the TRRs (0.0008 ppm) were extracted with acetonitrile from the day 7 phenyl-label egg sample (TABLE C.2.2.4). Subsequent extractions using water resulted in an additional 16.91% of the TRRs (0.0003 ppm). Using HPLC method 1, the residues identified were topramezone (13.8% of the TRRs; 0.0003 ppm), M670H02 (11.6% of the TRRs; 0.0002 ppm) and the cleaved acid metabolite M670H05 (6.3% of the TRRs; 0.0001 ppm). Approximately 10.2% of the TRRs (0.0001 ppm) were characterized as several minor peaks/regions. Unextractable residues accounted for 21.1% of the TRRs (0.0004 ppm), for an overall accountability of 70% (TABLE C.2.3.2).

Approximately 59.8% of the TRRs (0.0011 ppm) were extracted with acetonitrile from the day 9 egg sample (TABLE C.2.2.4). Subsequent extractions using water resulted in an additional 19.9% of the TRRs (0.0004 ppm). For the day 9 sample (HPLC method 1), the major metabolite identified was M670H02 (11.9% of the TRRs; 0.0002 ppm), followed by topramezone (6.75% of the TRRs; 0.0001 ppm) and M670H05 (4.6% of the TRRs; 0.0001 ppm). Approximately 9.65% of the TRRs (0.0002 ppm) were characterized as several minor peaks/regions. Unextractable residues accounted for 23.04% of the TRRs (0.0004 ppm), for an overall accountability of 70% (TABLE C.2.3.2). Approximately 32.9% of the 59.81% of the TRRs were accounted for through the identification or characterization of residues from the acetonitrile extract. This left 26.9% of the TRRs unaccounted for, which may have been due to that since the overall TRRs were extremely low to begin with (0.002 ppm). Thus, any loss would have an impact on the results. Also, the unextractable residues of both the day 7 and day 9 samples were above 10% of the TRRs, but the absolute values of both were again very low (0.004 ppm each), so no further extraction/fractionation of residues was necessary.

As per FIGURES C.2.1 and C.2.2, the overall TRRs were similar in both labels for excreta, cage rinse, muscle, fat and eggs. However, a greater percentage of TRRs were attributed to the phenyl-label liver and GI tract/contents than the corresponding pyrazole-label matrices. This may have been the result of a more efficient breakdown of the pyrazole ring into natural products. As per FIGURE C.2.3, topramezone was the predominant metabolite identified in both radiolabelled matrices, except for the phenyl-label day 9 egg sample. This result indicated that the chemical was not extensively metabolized in the body.

It should be noted that the egg production in the phenyl-label group during the 10-day dosing period was lower than either the control or pyrazole-label groups by nearly one-half (TABLE B.3.1). No explanation was provided by the petitioner for the finding, but this may have indicated a label-specific physiological effect.

C.1. Storage Stability

TABLE C.1. Summary of Storage Conditions

Matrix	Storage Temp.(°C)	Actual Storage Duration (months)	Interval of Demonstrated Storage Stability
^{14}C Pyrazole label			
Liver	≤ -15 °C	3.4	not conducted
Excreta (Day 1-10)	≤ -15 °C	0.8 - 2.7	
^{14}C Phenyl label			
Liver	≤ -15 °C	2.1	not conducted
Egg (Day 7 and Day 9)	≤ -15 °C	3.6 - 3.9	
Excreta (Day 1-10)	≤ -15 °C	2.0 - 2.2	

Note: Storage intervals for muscle and fat from both pyrazole and phenyl labels and for egg from pyrazole label were not given since the TRRs were below 0.01 mg/kg and therefore not further analysed. Samples were analysed within 3 - 27 days of extraction.

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

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

Matrix	^{14}C-Pyrazole Label		^{14}C-Phenyl Label	
	ppm[1]	% Administered Dose	ppm[1]	% Administered Dose
Excreta Day 1	—	8.16	—	8.09
Day 2	—	7.96	—	9.71
Day 3	—	8.37	—	8.87
Day 4	—	9.74	—	9.50
Day 5	—	9.19	—	9.27
Day 6	—	9.24	—	9.95
Day 7	—	9.13	—	9.04
Day 8	—	9.52	—	8.87
Day 9	—	10.30	—	8.49
Day 10	—	11.27	—	9.62
Muscle	0.001	0.002	0.001	0.001
Fat	0.003	0.0008	0.004	0.0007
Liver	0.739	0.22	1.68	0.34
Eggs Day 1	0	0.00002	0	0.00003
Day 2	0	0.0001	0	0.00009
Day 3	0.001	0.0004	0.001	0.0002
Day 4	0.002	0.0009	0.001	0.0003
Day 5	0.003	0.0009	0.001	0.0004
Day 6	0.004	0.002	0.001	0.0003
Day 7	0.004	0.001	0.002	0.0004
Day 8	0.004	0.0009	0.002	0.0006
Day 9	0.005	0.001	0.002	0.0006
Day 10	0.005	0.003	0.002	0.0007
GI Tract and Contents	0.796	0.7	1.318	1.07
Cage Rinse	—	0.29	—	0.29
Total Recovery	—	94.09	—	93.11

[1] ppm values were not reported for excreta

TABLE C.2.2.1 Distribution of the Parent and the Metabolites in Excreta when Dosed with ^{14}C-Pyrazole or ^{14}C-Phenyl-label Topramezone

Metabolite Fraction	^{14}C-Pyrazole (Day 1)		^{14}C-Phenyl (Day 5)	
	%TRR	ppm [1]	%TRR	ppm [1]
Concentrated Acetone:Phosphate buffer extract	88.5	—	86	—
HPLC method 1				
Topramezone	64.0	—	59.5	—
M670H02	--	—	10.6	—
Pyrazole: 13 unknown peaks/regions, each <8.16% of the TRRs	24.5	—	15.9	—
Phenyl: 11 unknown peaks/regions, each <3.89% of the TRRs				
HPLC method 2				
Topramezone	60.6	—	NA	—
43 unknown peaks/regions, each <5.2% of the TRRs	27.8	—	NA	—
HPLC method 3				
Topramezone	NA	—	62.2	—
M670H02	NA	—	12.6	—
10 unknown peaks/regions, each <3.42% of the TRRs	NA	—	11.2	—
Unextractable (PES)	6.3	—	3.4	—

NA- Not analysed

[1] ppm values were not reported

TABLE C.2.2.2 Distribution of the Parent and the Metabolites in Liver when Dosed with ^{14}C-Pyrazole-label Topramezone

Metabolite Fraction	^{14}C-Pyrazole (TRR = 0.739 ppm)	
	%TRR	ppm
Methanol Extract	89.4	0.66
Acid/Base Composite Extract I	84.5	0.624
HPLC method 1		
Topramezone	62.2	0.459
M670H02	15.8	0.117
4 unknown peaks/regions, each <0.013 ppm	6.3	0.048
Acid/Base Composite Extract II	85.7	0.633
HPLC method 2		
Topramezone	63.4	0.468
M670H02	17	0.125
16 unknown peaks/regions, each <0.009 ppm	5.3	0.039
Ethyl Acetate Extract	1.7	0.013
Water Extract	4.3	0.032
HPLC method 1		
Topramezone	2.2	0.016
M670H02	0.6	0.005
30 unknown peaks/regions, each <0.010 ppm	1.5	0.011
HPLC method 2		
Topramezone	3.6	0.027
M670H02	0.7	0.005
Unextractable (PES)	7.9	0.059

TABLE C.2.2.3 Distribution of the Parent and the Metabolites in Liver when Dosed with ^{14}C-Phenyl-label Topramezone

Metabolite Fraction	^{14}C-Phenyl (TRR = 1.680 ppm)	
	%TRR	ppm
Combined/Concentrated Methanol Extract	93.4	1.568
HPLC method 1		
Topramezone	58.5	0.982
M670H02	29.9	0.503
M670H04	2.4	0.040
6 unknown peaks/regions, each <0.010 ppm)	2.6	0.043
HPLC method 3		
Topramezone	60.8	1.021
M670H02	29.4	0.494
5 unknown peaks/regions, each <0.018 ppm)	3.1	0.052
Unextractable (PES)	3.7	0.062

TABLE C.2.2.4 Distribution of the Parent and the Metabolites in Egg when Dosed with ^{14}C-Phenyl-label Topramezone

Metabolite Fraction	Day 7 (TRR = 0.002 ppm)		Day 9 (TRR = 0.002 ppm)	
	%TRR	ppm	%TRR	ppm
Concentrated Acetonitrile Extract	42.0	0.0008	59.8	0.0011
HPLC method 1				
Topramezone	13.8	0.0003	6.8	0.0001
M670H02	11.6	0.0002	11.9	0.0002
M670H05	6.3	0.0001	4.6	0.0001
10 unknown peaks/regions, each <0.0001 ppm)	10.2 [1]	0.0001	9.6	0.0002
HPLC method 3				
Topramezone	8.91	0.0002	NA	—
M670H02	11.7	0.0002	NA	—
M670H05	7.1	0.0001	NA	—
3 Unknowns	14.3	0.0003	NA	—
10 unknown peaks/regions, each <0.0002 ppm)				
Water Extract	16.9	0.0003	19.9	0.0004
Unextractable (PES)	21.1	0.0004	23.0	0.0004

NA- Not analysed

Table C.2.3.1 Summary of Characterization and Identification of Radioactive Residues in Livestock Matrices Following Application of Pyrazole-label Topramezone at 12.3 mg/kg using HPLC Method 1.

Compound	Excreta (Day 1) [1]		Liver (TRR = 0.739 ppm)	
	% TRR	ppm	% TRR	ppm
Total identified	64.0	—	80.8	0.597
Topramezone	64.0	—	64.4	0.475
M670H02	—	—	16.4	0.122
M670H04	—	—	—	—
M670H05	—	—	—	—
Total characterized	24.5	—	7.8	0.059
Total extractable	88.5	—	88.6	0.656
Unextractable (PES)	6.3	—	7.9	0.059
Accountability	94.8%		96.8% [2]	

[1] ppm values were not reported for excreta

[2] Accountability = (Total extractable (ppm) + Total unextractable (ppm))/(TRRs from combustion analysis (ppm)) * 100.

Table C.2.3.2 Summary of Characterization and Identification of Radioactive Residues in Livestock Matrices Following Application of Phenyl-label Topramezone at 13.4 mg/kg using HPLC Method 1.

Compound	Excreta (Day 5) [1]		Liver (TRR = 1.680 ppm)		Egg (Day 7) (TRR = 0.002 ppm)		Egg (Day 9) (TRR = 0.002 ppm)	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
Total identified	70.1	—	90.8	1.525	31.7	0.0006	23.2	0.0004
Topramezone	59.5	—	58.5	0.982	13.8	0.0003	6.8	0.0001
M670H02	10.6	—	29.9	0.503	11.6	0.0002	11.9	0.0002
M670H04	—	—	2.4	0.04	—	—	—	—
M670H05	—	—	—	—	6.3	0.0001	4.6	0.0001
Total characterized	15.9	—	2.6	0.043	27.2	0.0004	29.6	0.0006
Total extractable	86.0	—	93.4	1.568	58.9	0.001	52.8	0.001
Unextractable (PES)	3.4	—	3.7	0.062	21.9	0.0004	23.0	0.0004
Accountability	89.4%		97.0% [2]		70.0% [2]		70.0% [2]	

[1] ppm values were not reported for excreta

[2] Accountability = (Total extractable (ppm) + Total unextractable (ppm))/(TRRs from combustion analysis (ppm)) * 100.

FIGURE C.2.1. Distribution of Radioactivity in Excreta and Cage Rinse of Laying Hens Following Administration of Radiolabelled Topramezone

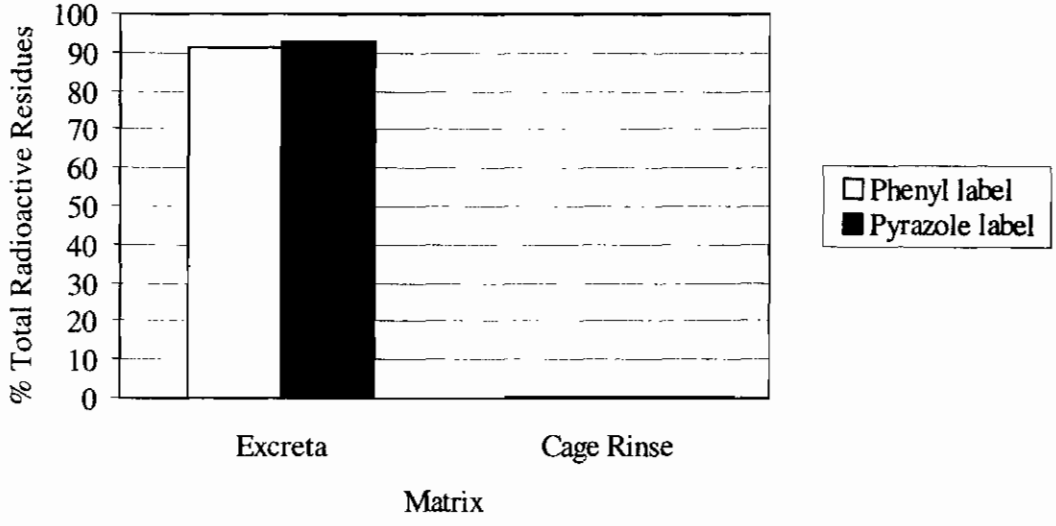


FIGURE C.2.2. Distribution of Radioactivity in the Muscle, Fat, Liver, GI Tract/Contents and Egg (All Samples) of Laying Hens Following Administration of Radiolabelled Topramezone

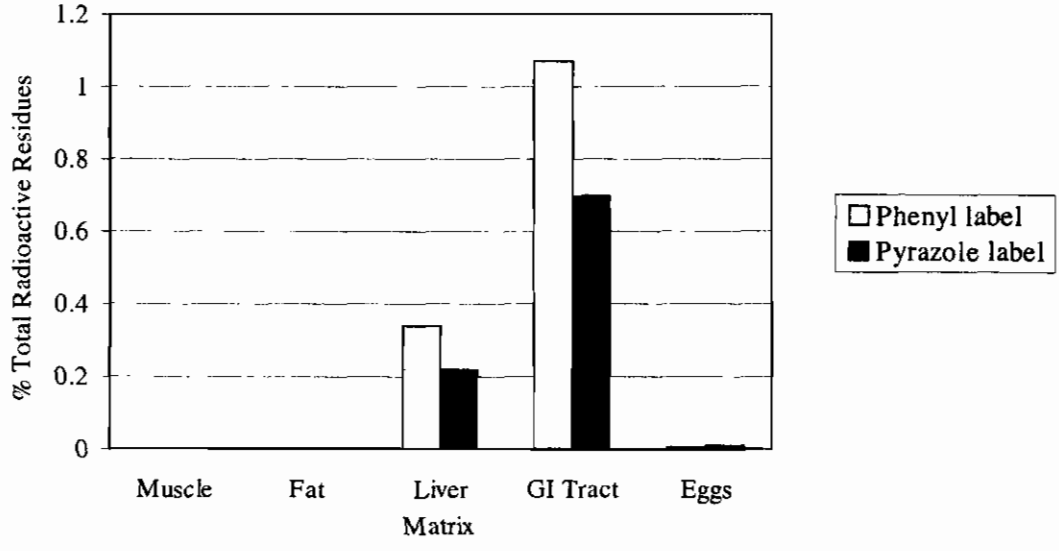
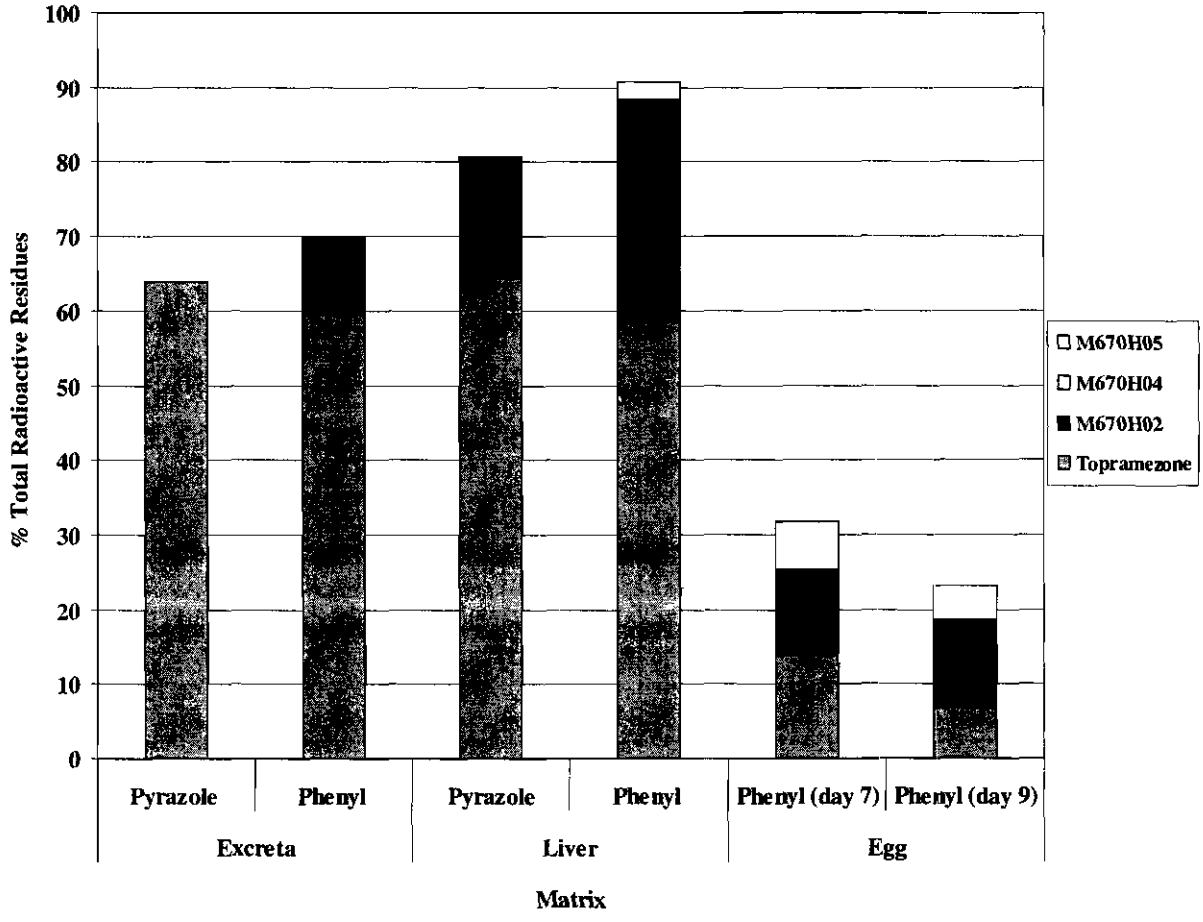


FIGURE C.2.3. Distribution of Parent (Topramezone) and Metabolites in Laying Hen Excreta, Liver and Egg (Days 7 and 9) Following Dosing with Radiolabelled Topramezone Using HPLC Method 1.



C.3. Proposed Metabolic Profile

The metabolic pathway of topramezone in hen proceeds from the hydroxylation at the 4-position of the isoxazole ring to form the hydroxy metabolite (M670H02). Further N-demethylation of the hydroxy metabolite (M670H02) occurred and formed the desmethyl hydroxy metabolite (M670H04). M670H04 was only detected in the phenyl-labelled liver. The pyrazole ring of topramezone can also be cleaved to form the cleaved acid metabolite (M670H05).

FIGURE C.3.1. Proposed Metabolic Profile of Topramezone in Laying Hen

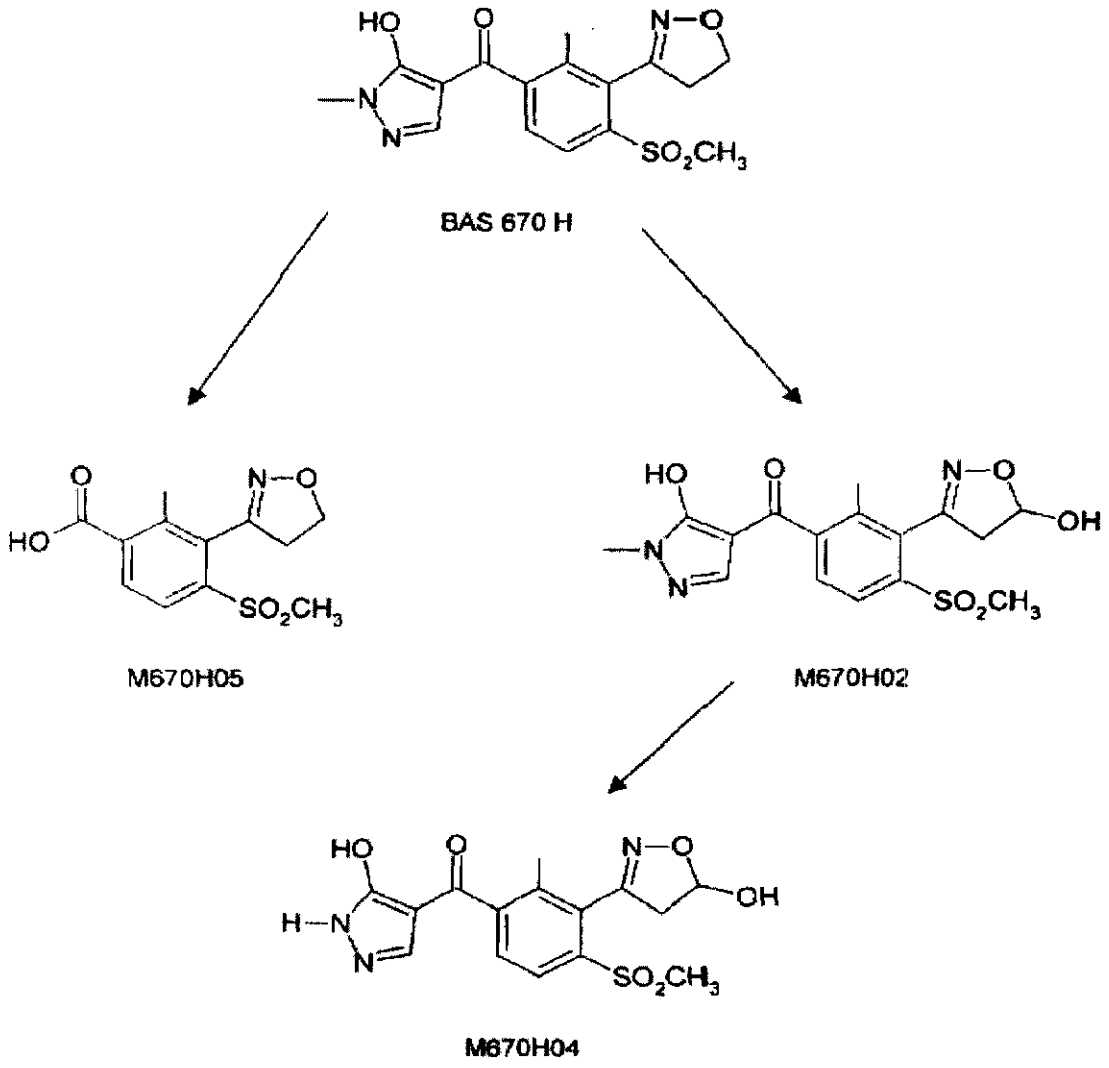
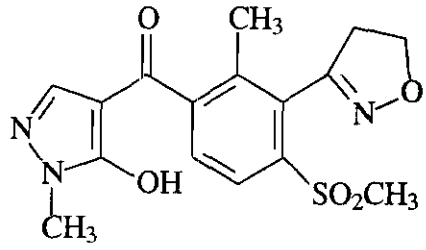
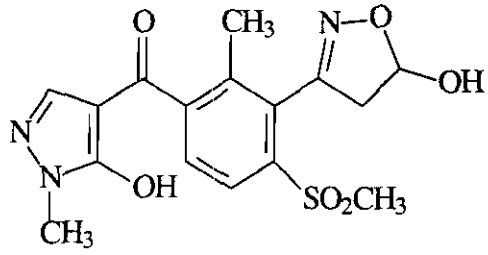
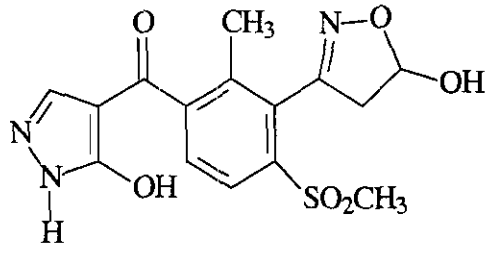
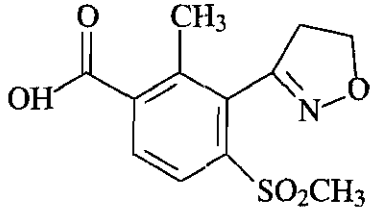


TABLE C.3.1. Identification of Compounds from Metabolism Study		
Common name/code	Chemical name	Chemical structure
Topramezone	[3-(4,5-dihydro-3-isoxazolyl)-2-methyl-4-(methylsulfonyl)phenyl](5-hydroxy-1-methyl-1*H*-pyrazol-4-yl)methanone	
M670H02	hydroxy metabolite	
M670H04	N-desmethyl hydroxy metabolite	
M670H05	cleaved acid: 3-(4,5-dihydro-isoxazole-3-yl)-4-methanesulfonyl-2-methyl-benzoic acid	

D. CONCLUSION

Laying hens were orally dosed for 10 consecutive days with either [pyrazole-4-^{14}C] or [phenyl-U-^{14}C] topramezone (BAS 670 H) at dose levels of 12.3 mg/kg and 13.4 mg/kg, respectively. Topramezone was not extensively metabolized, as the unchanged parent was the predominant residue in all matrices other than phenyl-label day-9 egg (13.8-64.4% of the TRRs; 0.0003-0.982 ppm). M670H02 was the major metabolite in all matrices other than pyrazole-label excreta

(10.6-29.9% of the TRRs; 0.0002-0.503 ppm). The metabolites M670H04 and M670H05 were identified in less than 7% of the TRRs (≤0.040 ppm). Approximately 2.6-24.5% of the TRRs were characterized in the radiolabelled matrices (0.0001-0.059 ppm). The metabolism of topramezone in laying hens occurs by the hydroxylation of the isoxazole ring to form the hydroxy metabolite M670H02. This metabolite can undergo further N-demethylation to form the desmethyl hydroxy metabolite M670H04. The cleavage of the pyrazole ring from the parent results in the formation of the acid metabolite M670H05.

E. REFERENCES

MRID No. 45902402. Van Cott, Andrew and Lam, Wing W. (2000). Final Report: Nature of Residues of [^{14}C]-BAS 670 H in Lactating Goats. BASF Study Number 55911. Unpublished study prepared by BASF Agro Research. 247 p.

F. DOCUMENT TRACKING

RDI: P.V. Shah (3/2/05), RAB1 Chemists (12/8/04), ChemSAC (3/2/05)
G.F. Kramer:806T:CM#2:(703)305-5079:7509C:RAB1
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Evaluators

Suzan Mathew
Evaluation Officer
FREAS, HED, PMRA

Date: Mar 21/05

for Stephanie Wille
Evaluation Officer
FREAS, HED, PMRA

Date: Mar 21/05

Peer Reviewer

George F. Kramer, Ph.D.
Chemist
RAB1, HED, EPA

Date: 4-12-05

Approved by

Louise G. Croteau
Senior Evaluation Officer
FREAS, HED, PMRA

Date: March 22/05

Ariff Alli, Ph.D.
Section Head
FREAS, HED, PMRA

Date: March 29/05

P.V. Shah, Ph.D.
Branch Senior Scientist
RAB1, HED, EPA

Date: 4/18/05

STUDY REPORTS:

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EXECUTIVE SUMMARY:

BASF Corporation has conducted a goat metabolism study with [phenyl-U-^{14}C] topramezone (5.76 MBq/mg specific radioactivity; >98% radiochemical purity) and [pyrazole-4-^{14}C] topramezone (4.65 MBq/mg specific radioactivity; >97% radiochemical purity). Topramezone (also known as BAS 670 H) was administered to two lactating dairy goats (Nubian and Alpine cross) per label at feeding levels of 9.9 mg/kg (phenyl label) and 11.2 mg/kg (pyrazole label) by a single daily oral administration for 5 days. Milk was collected twice daily over the treatment period, and excreta was pooled and collected once daily. Tissue samples (muscle, bile, kidney, liver, fat and GI tract) were harvested upon termination, 21-23 hours after the final dose administration. The samples were assayed for total radioactive residues (TRRs) by combustion and liquid-scintillation counting (LSC). Extracts were characterized by thin-layer chromatography (TLC) and identified by high-performance liquid chromatography (HPLC). Confirmation of residue identification was performed by liquid chromatography with mass spectrometry (LC-MS/MS). Nuclear magnetic resonance (NMR) analysis elucidated the structures of the metabolites found in the goat urine samples. All samples other than milk were stored frozen and analysed within 4 months of collection; thus, no storage stability study was conducted. Milk was stored frozen and analysed within 11 months of collection, but no storage stability study was conducted due to low radioactivity levels. The limits of quantitation (LOQs) for both the pyrazole-label and phenyl-label samples were 0.0004 ppm each for urine and milk; and 0.001 ppm each for feces, muscle, liver and kidney. The LOQs for fat were 0.0028 ppm in pyrazole-label samples, and 0.002 ppm in phenyl-label samples.

The total applied dose recoveries of topramezone were 92.5% in pyrazole-label samples, of which 44.5% was found in the urine, 38.3% in the feces, 0.29% in the cage rinse (1.18 ppm), 2.67-5.27% in the GI tissue and contents (0.780-1.16 ppm), and negligible levels in bile (0.137 ppm). The dose recoveries in the edible tissues were predominantly in the liver at 1.28% of the administered dose (1.891 ppm), followed by kidney at 0.04% (0.282 ppm). The dose recoveries in fat, muscle and milk were <0.01% each (≤0.007 ppm). The total applied dose recoveries of topramezone were 81.9% in phenyl-label samples, of which 33.3% was found in the urine, 34.7% in the feces, 0.49% in the cage rinse (0.986 ppm), 1.20-10.2% in the GI tissue and contents (0.322-1.57 ppm), and negligible levels in bile (0.059 ppm). The dose recoveries in the edible tissues were again predominantly in the liver (1.96% of the administered dose; 2.18 ppm), followed by kidney (0.05% of the administered dose; 0.352 ppm). The dose recoveries in fat, muscle and milk were negligible at ≤0.002 ppm each.

A total of 87.7-90.8% of the TRRs (1.66-1.98 ppm) were extracted with methanol/ethyl acetate (EtOAc) from the pyrazole-label and phenyl-label liver samples. Of the 82.1-85.5% of the TRRs identified (1.55-1.86 ppm), the parent topramezone accounted for 51.8-83.3% of the TRRs (0.9799-1.817 ppm). The hydroxy metabolite M670H02 accounted for 29.6% of the TRRs (0.560 ppm) in the pyrazole-label sample, but was a minor metabolite in the phenyl-label sample (2.2% of the TRRs; 0.048 ppm). The cyano metabolite M670H01 was only identified in the pyrazole-label sample, at 0.67% of the TRRs (0.013 ppm). Approximately 5.34-5.6% of the TRRs (0.1057-0.1168 ppm) were characterized as fractions from the extracts. Approximately

3.16-3.73% of the TRRs were unextractable (0.069-0.071 ppm), for an overall accountability ranging from 91.4-94.0 %.

A total of 87.8-92.1% of the TRRs (0.259-0.309 ppm) were extracted with methanol/EtOAc from the pyrazole-label and phenyl-label kidney samples. Of the 84.0-90.1% of the TRRs identified (0.2538-0.2955 ppm), the parent topramezone accounted for the vast majority of residues, at 79.5-83.2% of the TRRs (0.224-0.293 ppm). M670H02 was identified as 9.95% of the TRRs (0.028 ppm) in the pyrazole-label sample, but accounted for only 0.41% of the TRRs (0.0014 ppm) in the phenyl-label sample. M670H01 was identified in both labels, at 0.35-0.68% of the TRRs (0.0012-0.0019 ppm). Approximately 1.96-3.88% of the TRRs (0.0056-0.0137 ppm) were characterized as either a single minor peak, or as fractions from the extracts. A total of 2.45-6.71% of the TRRs were unextractable (0.0086-0.0189 ppm), for an overall accountability ranging from 90.3-98.7%.

Only samples of pyrazole-label milk were analysed. Approximately 34.5% of the TRRs (0.002 ppm) were extractable with methanol/water and acetone. Of the 27.6% of the TRRs identified (0.00189 ppm), the parent topramezone accounted for 25.3% of the TRRs (0.0018 ppm). M670H02 and M670H01 were minor metabolites at 0.88-1.18% of the TRRs (0.00006-0.00008 ppm). Approximately 0.81% of the TRRs (0.00006 ppm) were characterized as a series of minor peaks, while 6.03% of the TRRs (0.0004 ppm) were found in the acetone extract. No residues were unextractable in the post-extraction solids (PES). The overall accountability was 33.6%, due to the impact of procedural loss on a sample with low residue levels.

One hundred percent of the TRRs were extractable in both the pyrazole-label and phenyl-label urine samples. Of the 99.5% of the TRRs identified in the pyrazole-label sample, the parent topramezone was the predominant metabolite (90.0% of the TRRs). M670H02 (6.88% of the TRRs) and M670H01 (2.6% of the TRRs) were considered minor metabolites. The parent topramezone was the only metabolite identified in the phenyl-label sample as 96.75% of the TRRs. Approximately 0.48-3.25% of the TRRs were characterized as a single minor peak in both labels. The accountability was 100% for both labels.

A total of 64.8-83.0% of the TRRs were extracted with methanol/water from both the pyrazole-label and phenyl-label feces samples. The only metabolite identified in both labels was the parent topramezone, at 59.9-72.9% of the TRRs. Approximately 4.9-10.1% of the TRRs were characterized as a series of minor peaks. Approximately 15.5-22.2% of the TRRs were unextractable in the PES, for an overall accountability ranging from 87.0-98.5%.

The metabolic profiles of topramezone in goats were similar between the pyrazole and phenyl treatment groups. The proposed metabolic pathway proceeded from the hydroxylation of the parent compound topramezone at the 4-position of the isoxazole ring to form M670H02. The isoxazole ring was then cleaved to form the cyano metabolite (M670H01). The linkage between the phenyl and pyrazole rings remained intact in all of the metabolites identified. The unmetabolized parent was the predominant metabolite in tissues and excreta, indicating limited metabolism and degradation.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the goat 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# 310772] and in Canada's Regulatory Decision Document.

COMPLIANCE:

Signed and dated Good Laboratory Practice (GLP), Quality Assurance and Data Confidentiality statements were provided. Deviations from the protocol included the following:

- Analysis of bile samples were not conducted.
- Pyrazole-label milk, urine and feces samples were pooled late; cage washes were not pooled prior to weighing.
- The order of tissue sample collection upon animal termination differed from the protocol.
- One control kidney aliquot (out of 5) was 229 mg instead of 500 mg.
- Pyrazole-treated animals and control animals were weighed an extra time.
- A change in specific activity of the radiolabelled substances from approximately 100,000 dpm/µg to approximately 150,000 dpm/µg was reported.
- An extra cage wash sampling was reported for pyrazole treated and control animals.
- Sample shipment documentation was not always recorded.
- Sample ID numbering was incorrect and had to be resolved.
- Routine water analyses were not conducted according to EPA FIFRA GLP standards
- The amount of diluted ^{14}C-pyrazole dosing solution spiked in the control samples for combustion recovery was not recorded in the raw data. The LSC results indicate that appropriate amounts of the spiking solution were used in the control samples.

These deviations do not impact the validity of the study.

A. BACKGROUND INFORMATION

BAS 670 336SC (soluble concentrate) Herbicide (also known as BAS 670 00H Herbicide) is the end-use product which contains the active ingredient topramezone. It is a broad-spectrum, post-emergence herbicide used to control grassy and broadleaf weeds in corn. The herbicide is absorbed by the leaves, roots and shoots, then translocated to the growing points of the sensitive weeds. Topramezone belongs to the triketone class of chemicals, which inhibits carotenoid biosynthesis (4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitor). This causes a strong bleaching activity on the growing zones of the shoots within 2-5 days of application. Exposure to light causes necrosis of chlorotic tissues and eventual plant death within 14 days after application.

TABLE A.1. Test Compound Nomenclature

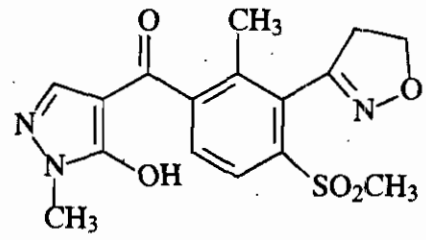
Compound	Chemical Structure 
Common name	Topramezone
Company experimental name	BAS 670 H
IUPAC name	[3-(4,5-dihydro-1,2-oxazol-3-yl)-4-mesyl-*o*-tolyl](5-hydroxy-1-methyl-1*H*-pyrazol-4-yl) methanone
CAS name	[3-(4,5-dihydro-3-isoxazolyl)-2-methyl-4-(methylsulfonyl)phenyl](5-hydroxy-1-methyl-1*H*-pyrazol-4-yl)methanone
Molecular Formula	$C_{16}H_{17}N_3O_5S$
Molecular Mass	363.39 g/mol
CAS #	210631-68-8
End-use product/EP	Soluble Concentrate

TABLE A.2. Physicochemical Properties of Topramezone

Parameter	Value
Melting point/range	220.9°C - 222.2°C
pH	2.9 (1% de-ionized water)
Density (20°C)	1.425 g/cm^3
Water solubility (20°C)	pH: 3, 5, 7, 9 g/L: 0.06, 0.98, 15, 23.4
Solvent solubility (g/100 mL at 20°C)	Acetone <1.0 Acetonitrile <1.0 Dichloromethane 2.5 - 2.9 Ethyl acetate <1.0 Methanol <1.0 N-heptane <1.0 N,N-dimethylformamide 11.4-13.3 1-octanol <1.0 Olive oil <1.0 2-propanol <1.0 Toluene <1.0
Vapour pressure at 20°C and 25°C	< 1.0 x 10^{-12} hPa
Dissociation constant (pK$_a$)	4.06
Octanol/water partition coefficient Log(K_{OW}) at 20°C	Buffer pH: 4, 7, 9 Log K_{ow}: – 0.81, – 1.52, – 2.34
UV/visible absorption spectrum	λ, nm: 207, 272, 300, 410 ξ, mol^{-1}cm^{-1}: 27 077, 8601, 5800, 410

All data came from PMRA Lab Services.

B. EXPERIMENTAL DESIGN

B.1. Livestock

TABLE B.1.1. General Test Animal Information

Species	Breed	Age	Weight at study initiation (kg)	Health Status	Description of housing/holding area
Lactating goat	Nubian and Alpine cross (pyrazole); Alpine cross (phenyl)	2 to 3 years old	32.65 - 46.71 kg (pyrazole); 33.56 - 52.15 kg (phenyl)	Healthy	Metabolism cages in temperature controlled study rooms

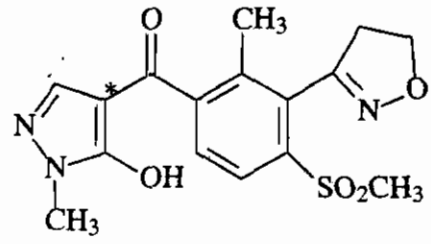
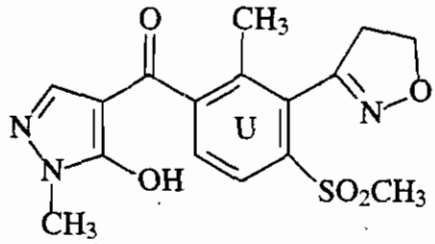
TABLE B.1.2. Test Animal Dietary Regime

Composition of Diet	Feed consumption (kg/day)	Water	Acclimation period	Predosing
Total mixed ration and Purina goat chow *ad libitum* (pyrazole); Purina goat chow and alfalfa hay *ad libitum* (phenyl)	1.74-2.80	*ad libitum*	15 days (pyrazole); 12 days (phenyl)	No

TABLE B.1.3. Test Animal Dosing Regime

Treatment Type	Feeding Level (ppm test material in food)	Vehicle	Timing/Duration
Oral	11.2 mg/kg (pyrazole); 9.9 mg/kg (phenyl)	Gelatin capsule via balling gun	Once daily for 5 consecutive days

B.2. Test Materials

TABLE B.2.1. Test Material Characteristics		
Chemical structure		
Radiolabel position	Pyrazole	Phenyl
Batch No.	706-1013	714-1026
Purity	> 98% (HPLC)	> 98% (HPLC)
Specific activity (Bq)*	5.76 MBq/mg	4.65 MBq/mg

*Bq = disintegrations per second

B.3. Sampling Information

TABLE B.3.1. Sample Collection Information			
Milk collected	Urine, feces and cage wash collected	Interval from last dose to sacrifice	Tissues harvested and analysed
Twice daily (AM and PM)	Urine and feces collected and pooled daily; cage wash collected and pooled at Study Day 0 and before termination.	21-23 hours	urine, feces, liver, kidney, fat, milk, GI tissue, bile, muscle

B.4. Identification/ Characterization of Residues

B.4.1. Sample Handling and Preparation

Milk was collected twice daily (A.M. and P.M.) throughout the 5 day treatment period. Samples of urine, feces and cage wash were collected once daily throughout the same period and pooled by group. Samples of liver, kidney, fat, GI tissue, muscle and bile were harvested upon termination, 21-23 hours after the last administration. The samples were frozen at -15°C, then were shipped on dry ice to BASF, where they remained frozen until analysis.

The TRRs in liquid samples were determined by direct LSC. Solid samples were combusted prior to HPLC. Urine samples were analysed by HPLC without cleanup. Other samples underwent solvent extraction for further cleanup.

Samples of liver, kidney and feces for both labels were extracted with either MeOH (liver) or

MeOH/water (kidney - 1:1, v/v; feces - 6:4, v/v), mechanically shaken for 30 minutes, then centrifuged/ filtered. This procedure was repeated up to 4 times, then the extracts were combined and assayed using LSC. Liver and kidney sample extracts were cleaned up by a rotary evaporator to remove the methanol. The sample was dissolved in water, acidified by HCl (to pH 1), partitioned with ethyl acetate, and back extracted with 0.5 N ammonium solution (to pH 11). The basic aqueous extract was re-acidified with HCl (to pH 1) before partitioning with ethyl acetate. The ethyl acetate fractions were combined, concentrated to dryness and then dissolved in phosphate buffer for HPLC analysis. For feces, the methanol:water extract was concentrated, filtered and analysed using HPLC methods without further clean up. Urine samples directly underwent analysis by HPLC without clean up. Cage rinse samples were rinsed with a 20% methanol/water solution.

Milk was extracted by acetone before analysis by TLC. The resulting solids were further extracted with MeOH/water (1:1, v/v), concentrated to dryness, and purified by flash chromatography before analysis by HPLC or C-18 solid-phase extraction (SPE) clean up. The resulting SPE fraction was then analysed using HPLC.

B.4.2. Analytical Methodology

Eight different HPLC methods and 4 different TLC methods were used in this study. They are summarized in TABLES B.4.2.1 and B.4.2.2, respectively. TLC analyses of standards and extracts were conducted on pre-coated silica gel plates (Baker F254). TLC method 1, involving EtOAc:MeOH:NH_4OH (65:35:5 v/v/v) was used for extract characterization. Full-scan MS and product-ion MS/MS were obtained with electrospray ionization on a Finnigan LCQ (Finnigan-MAT, San Jose, CA; BASF# 1083), interfaced to a Michrom MAGIC 2002 HPLC (Michrom BioResources, Auburn, CA; BASF# 1199) and a ThermoSeparations AS3000 autosampler (ThermoSeparations, San Jose, CA; BASF# 192). Deuterated methanol-d4 was used to dissolve the metabolites prior to analysis. 1-D and/or 2-D spectra were acquired on a Varian INOVA 600 MHz NMR spectrometer. A radiochemical purity check was performed on the dosing solution (both labels) prior to and after the dosing of the goats. The pre- and post-dose purity was 100% for both the phenyl-label and pyrazole-label solutions.

Residues of the parent topramezone, a hydroxy metabolite (M670H02), a cyano metabolite (M670H01) and a minor unidentified peak/region were isolated from goat urine for LC-MS and/or NMR analysis. Work-up schemes for metabolite isolation in urine are depicted in FIGURES B.4.1-B.4.5.

Urine was acidified to pH 1 by HCl, and partitioned with 3 portions of ethyl acetate. The extracts were combined, concentrated and partitioned with water (to pH 11). A portion of the aqueous fraction was purified by HPLC method 5. Fractions at 20-21 minutes were collected, combined and concentrated before cleanup with TLC method 1. The metabolite isolated from TLC was purified by HPLC method 1, then again by HPLC method 2, and a third time by HPLC method 1 again. Fractions at 11.2-12.4 minutes were combined, concentrated and dissolved in 0.05 N HCl, then partitioned with ethyl acetate. The extracts were combined, concentrated and analysed by

both HPLC and NMR.

The LOQ was defined as two times the background radioactivity of a matrix divided by the specific activity. In both the pyrazole-label and phenyl-label samples, the LOQs were 0.0004 ppm for urine and milk; and 0.001 ppm for feces, muscle, liver and kidney. The LOQs for fat were 0.0028 ppm in pyrazole-label samples and 0.002 ppm in phenyl-label samples.

TABLE B.4.2.1. Summary of HPLC Methods

HPLC Method No.	Column	Mobile Phase A	Mobile Phase B	Flow Rate (mL/min)
1 (Clmbsstd)	Phenomenex Columbus C_{18} 4.6 x 250 mm	10 mM pH 7 Phosphate Buffer	Methanol	1
2 (PRP1670B)	Hamilton PRP1 4.1 x 250 mm	10 mM pH 7 Phosphate Buffer	Methanol	1.5
3 (670prep1)	Phenomenex Columbus C_{18} 10.0 x 250 mm	10 mM pH 7 Phosphate Buffer	Methanol	3
4 (Col670A)	Phenomenex Columbus C_{18} 4.6 x 250 mm	10 mM pH 7 Phosphate Buffer	Methanol	1
5 (670prep2)	Phenomenex Columbus C_{18} 10.0 x 250 mm	10 mM pH 7 Phosphate Buffer	Methanol	3
6 (670AVC1)	Phenomenex Columbus C_{18} 4.6 x 250 mm	10 mM pH 7 Phosphate Buffer	Methanol	1
7 (670prep1)	Phenomenex Columbus C_{18} 10.0 x 250 mm	10 mM pH 7 Phosphate Buffer	Methanol	3
8 (670Form1)	Phenomenex Columbus C_{18} 4.6 x 250 mm	0.2% Formic Acid	CH_3CN	1

TABLE B.4.2.2. Summary of TLC Methods

TLC Method No.	Solvent System
1	EtOAc:MeOH:NH_4OH (65:35:5 v/v/v)
2	EtOAc:MeOH:Acetic Acid (90:9:1 v/v/v)
3	EtOAc:MeOH:Acetic Acid:Water (55:35:5:5 v/v/v/v)
4	EtOAc:MeOH:NH_4OH (60:35:5 v/v/v)

FIGURE B.4.1. Isolation of M670H02 for LC/MS from Goat Urine (Day 2) When Dosed Daily with ^{14}C-Pyrazole label Topramezone for 5 Days at 11.2 mg/kg Feed.

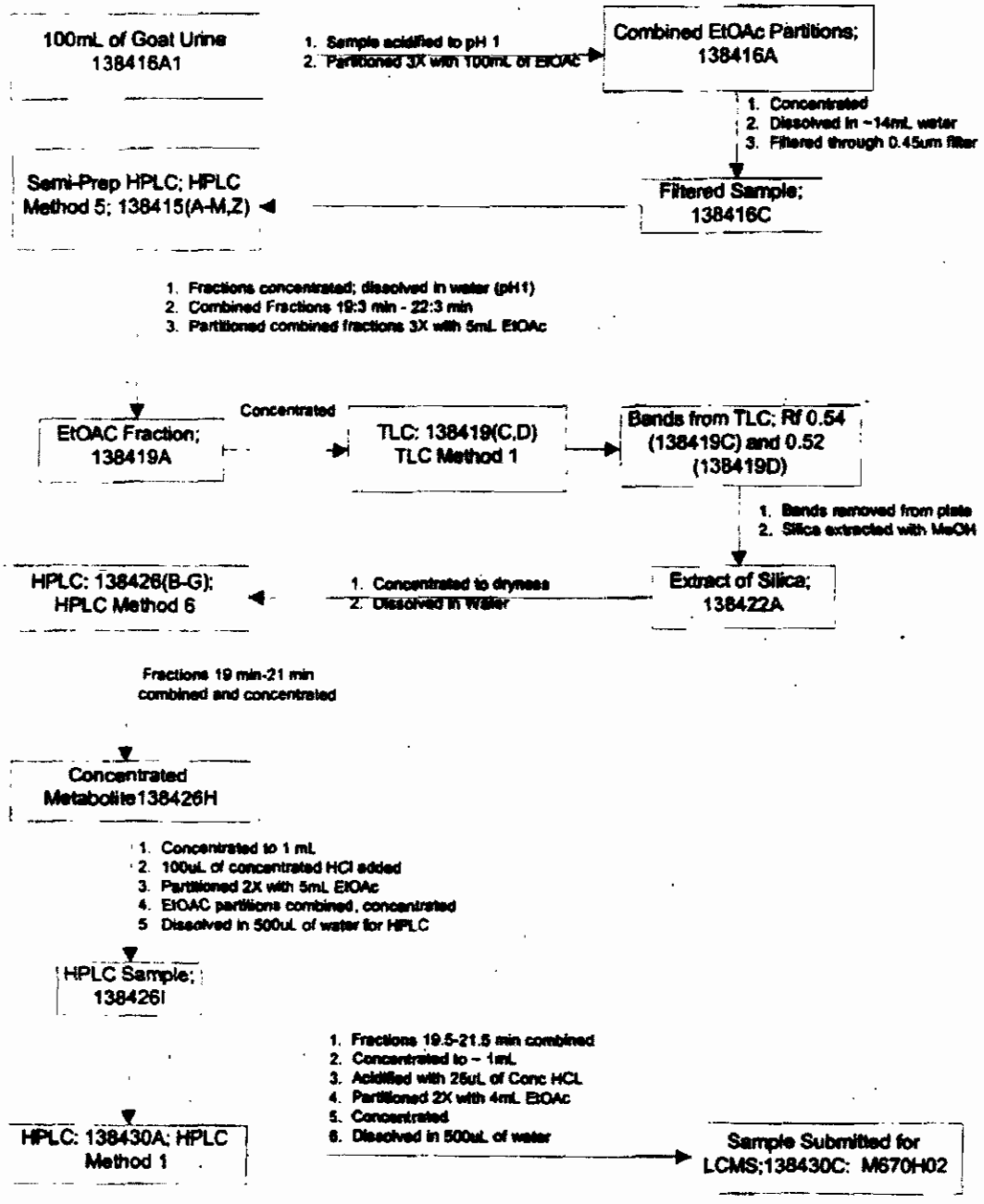


FIGURE B.4.2. Isolation of M670H02 for NMR Analysis from Goat Urine (Day 3) When Dosed Daily with ^{14}C-Pyrazole label Topramezone for 5 Days at 11.2 mg/kg Feed.

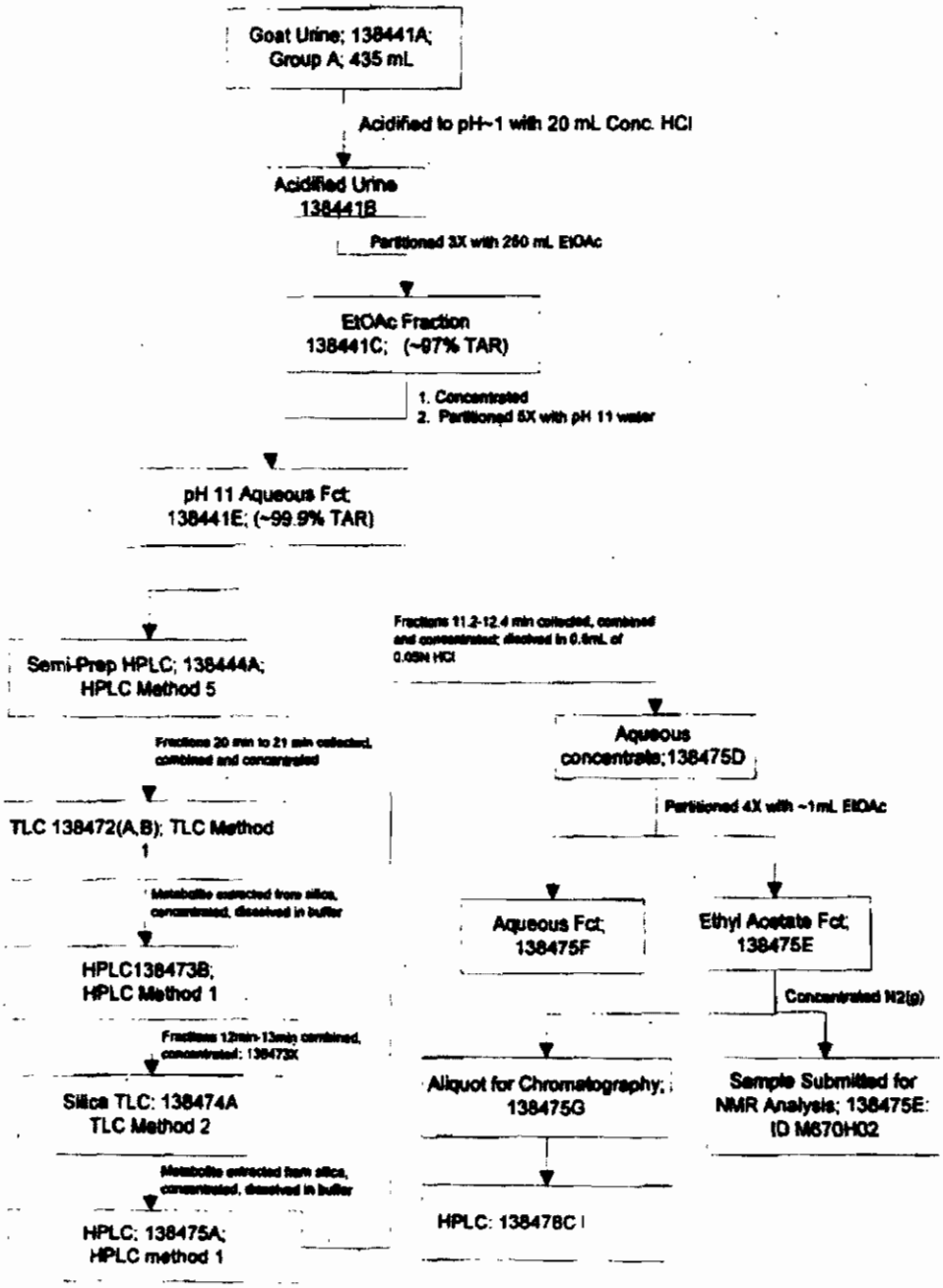


FIGURE B.4.3. Isolation of M670H01 and Topramezone for LC/MS from Goat Urine (Day 2) When Dosed Daily with ^{14}C-Pyrazole label Topramezone for 5 Days at 11.2 mg/kg Feed.

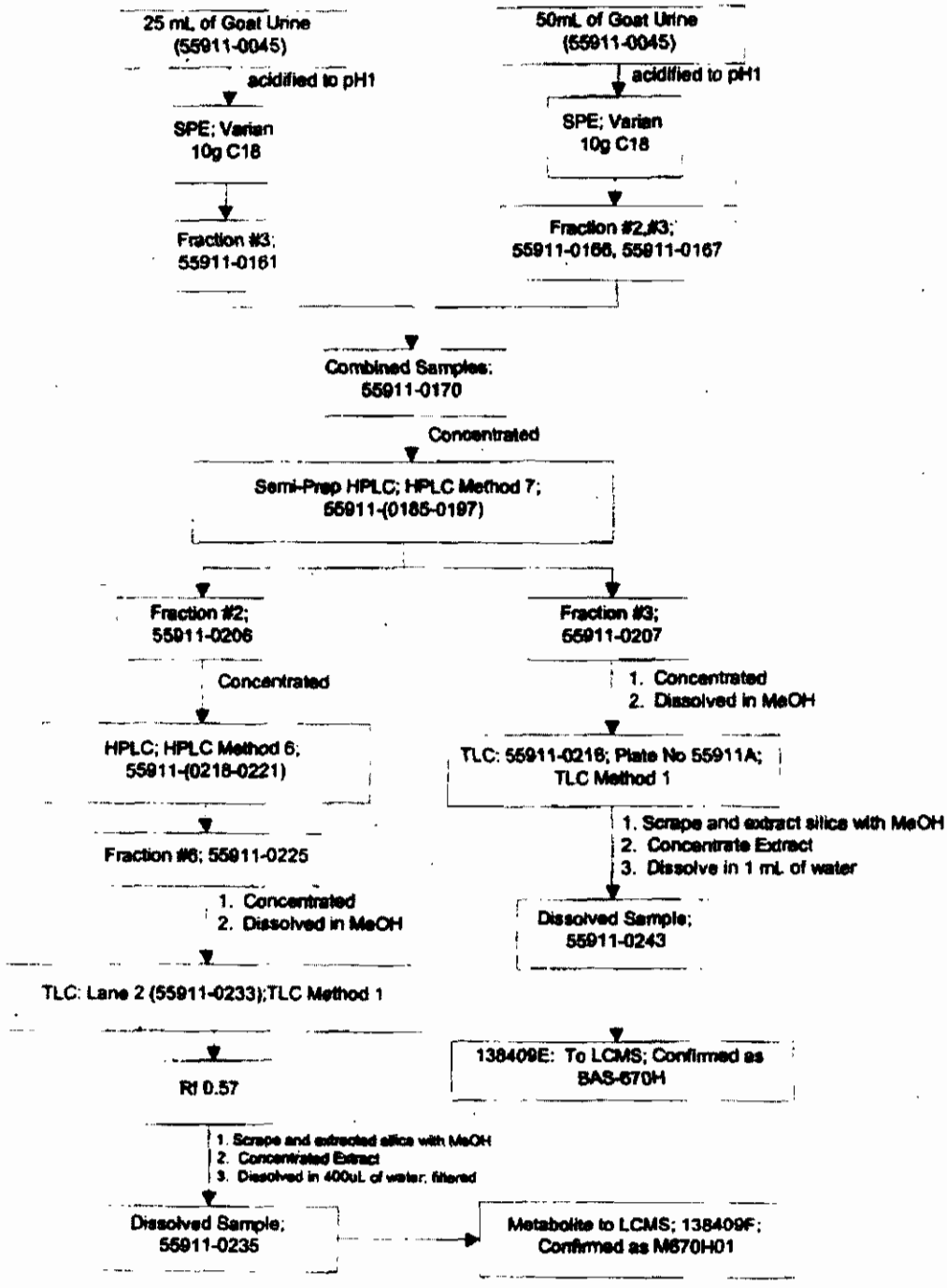


FIGURE B.4.4. Isolation of M670H01 for NMR Analysis from Goat Urine (Day 3) When Dosed Daily with ^{14}C-Pyrazole label Topramezone for 5 Days at 11.2 mg/kg Feed

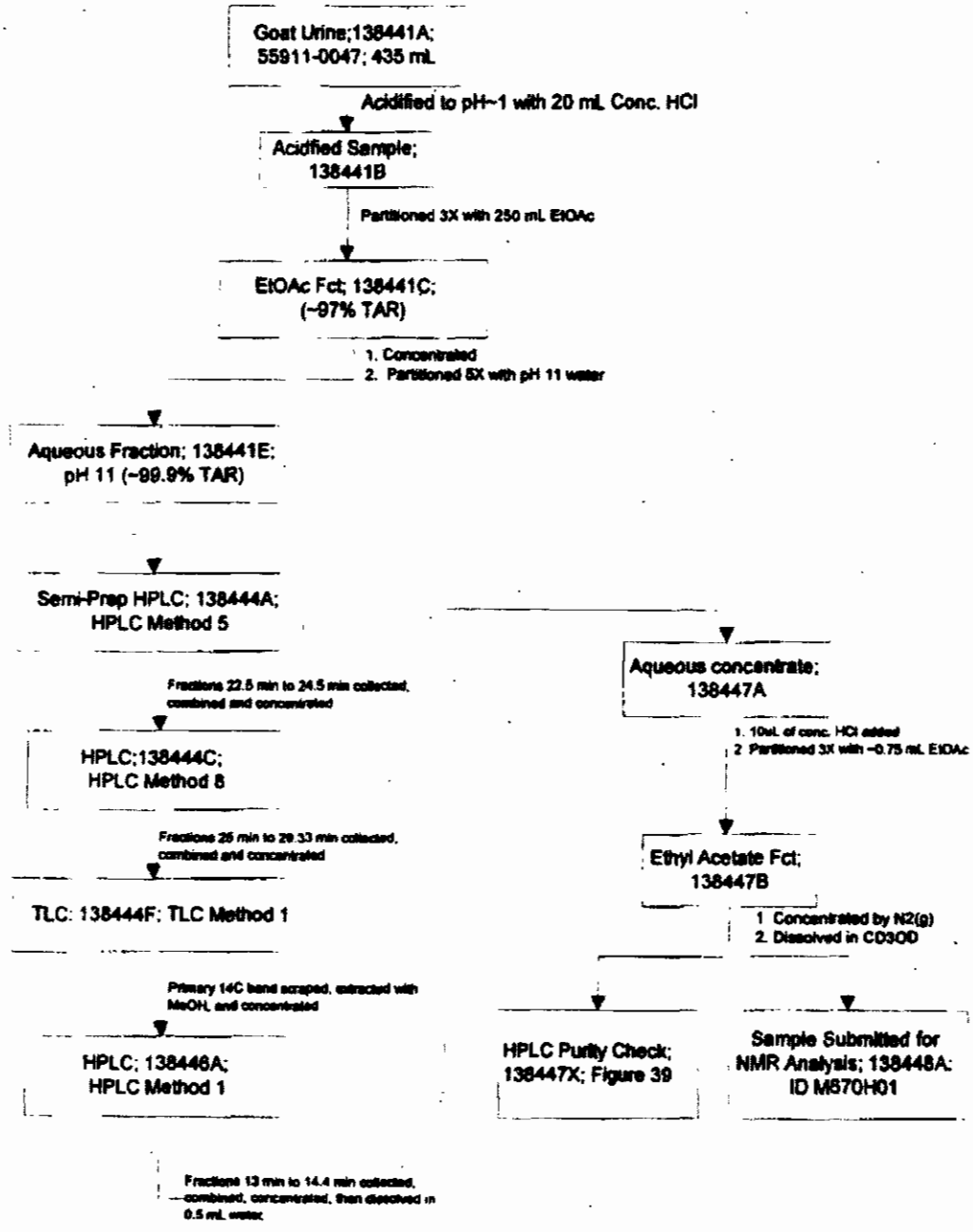
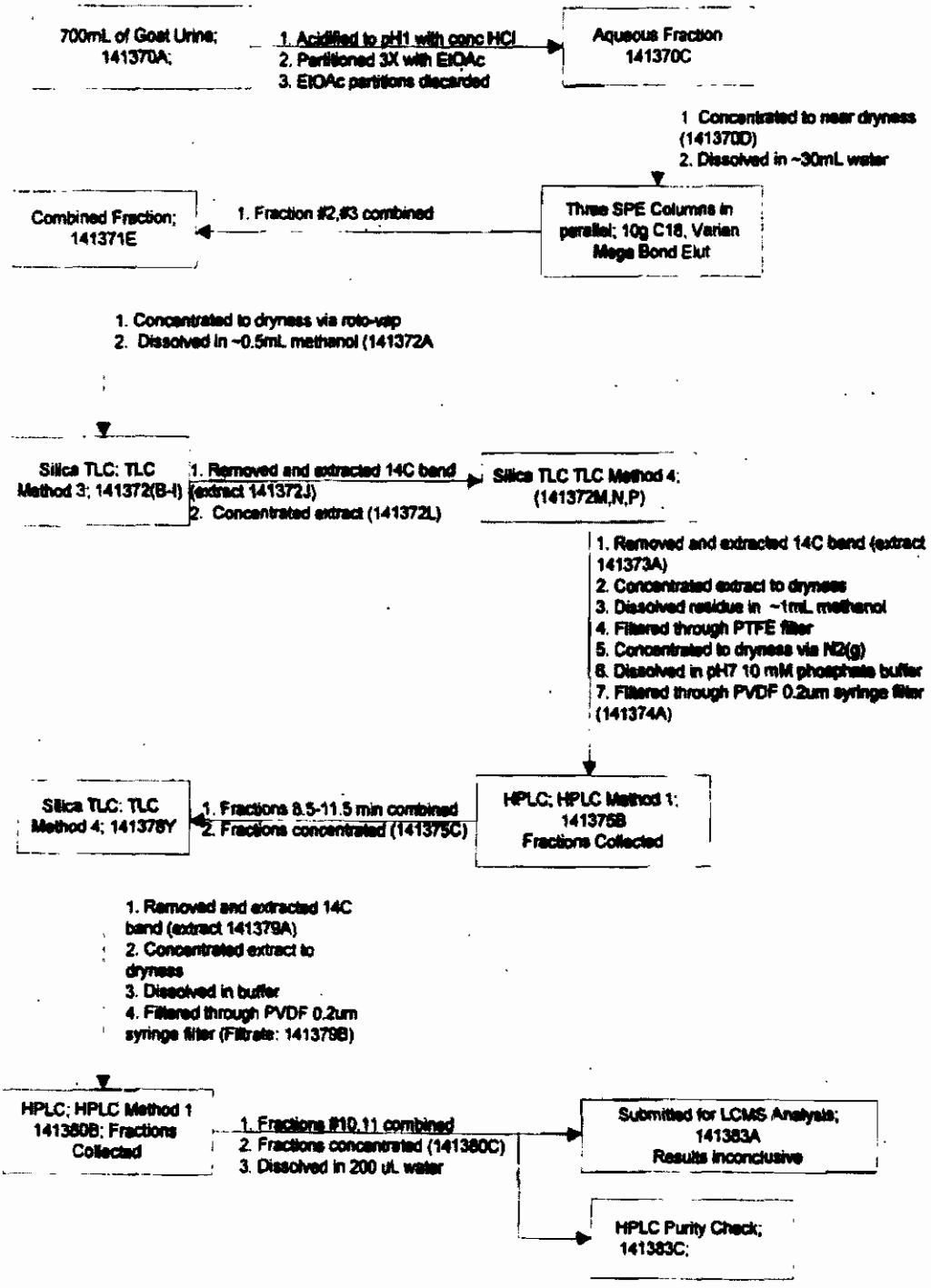


FIGURE B.4.5. Isolation of Unknown Metabolite from Goat Urine (Day 4) When Dosed Daily with ^{14}C-Phenyl label Topramezone for 5 Days at 9.9 mg/kg Feed



C. RESULTS AND DISCUSSION

All analyses (except milk) were performed within 4 months of sampling, so no storage stability data were collected for the animal tissue matrices. The day-5 pyrazole milk sample was analysed at 10.2 months after collection, resulting in residue levels well below 0.01 mg/kg. Since the radioactivity level was so low, no storage stability data for milk was gathered.

Each matrix extract (except for milk) was analysed by 2 different HPLC methods (TABLE B.4.2.1). Milk was analysed by HPLC method 1 alone. Urine, liver, kidney and phenyl-label feces were analysed by methods 1 and 2. Pyrazole-label feces were analysed by methods 2 and 3. The summary tables list the results of HPLC method 1 for all matrices, except for pyrazole-label feces (HPLC method 3).

The overall TRRs were determined by combustion/LSC. Overall recoveries of radioactivity were 92.46% of the administered dose in pyrazole-label samples (TABLE C.2.1). The highest dose recoveries were in the urine (a total of 44.5% of the administered dose over the 5 day dosing period) and feces (a total of 38.3% of the administered dose over the 5 day period). Recoveries in cage rinse were 0.29% of the administered dose (1.182 ppm), while the recoveries in bile were less than 0.01% (0.137 ppm). The recoveries in the GI tract and contents (batches 1 and 2) were 2.67-5.27% of the administered dose (0.780-1.158 ppm). The highest recoveries in the edible tissues were in the liver (1.28% of the administered dose; 1.891 ppm), followed by kidney (0.04% of the administered dose; 0.282 ppm) and milk (≤0.01% of the administered dose; ≤0.007 ppm). Recoveries in fat and muscle were below 0.01% of the administered dose (≤0.007 ppm); thus, no further analyses of these tissues were necessary.

The overall recoveries of radioactivity were 81.9% of the administered dose in phenyl-label samples (TABLE C.2.1). The highest recoveries in this label were also in the urine (a total of 33.3% of the administered dose over the 5-day dosing period) and feces (a total of 34.7% of the administered dose over the 5 day period). Cage rinse recoveries were reported at 0.49% of the administered dose (0.986 ppm), while the recoveries in bile were less than 0.01% (0.059 ppm). Recoveries in the GI tract tissues were 1.20% of the administered dose (0.322 ppm), while residues in the GI contents were 10.2% of the administered dose (1.567 ppm). The residues in edible tissues were highest in the liver (1.96% of the administered dose; 2.182 ppm) and kidney (0.05% of the administered dose; 0.352 ppm). No discernible residues were found in the samples of muscle, fat and phenyl-label milk (<0.003 ppm each); thus, no further analyses of these tissues were necessary. Approximately 18% of the administered dose was not accounted for in the phenyl-label samples. The majority of the loss may be from the phenyl-label urine sample, which is approximately 11% lower than the pyrazole-label urine sample. There were no differences reported in how the 2 radiolabelled urine samples were analysed. The reason for the loss is uncertain.

Liver: Approximately 92.3% of the TRRs (1.7453 ppm) were found in the methanol extract of pyrazole-label samples, which indicated adequate method extraction (TABLE C.2.2.1). From the methanol/EtOAc extract (82.1% of the TRRs, 1.553 ppm), the predominant metabolite identified

was topramezone (51.8% of the TRRs; 0.9799 ppm). The hydroxy metabolite M670H02 was identified at 29.6% of the TRRs (0.5603 ppm) in pyrazole-label samples, while the cyano metabolite M670H01 was identified as 0.67% of the TRRs (0.0127 ppm). Aqueous or EtOAc fractions from the methanol (or methanol/EtOAc) extracts comprised the 5.6% of the TRRs that were characterized (0.1057 ppm). Unextractable residues in the PES accounted for 3.73% of the TRRs (0.0705 ppm). Since the unextractable residues contributed to <10% of the TRRs, no further extraction procedures were conducted. The accountability was 91.4% (TABLE C.2.3.1).

Approximately 92.7% of the TRRs in phenyl-label samples were also found in the methanol extract (2.0221 ppm), which again indicated adequate method extraction (TABLE C.2.2.2). From the methanol/EtOAc extract (85.5% of the TRRs; 1.8648 ppm), the predominant metabolite identified was topramezone (83.3% of the TRRs; 1.817 ppm). M670H02 was identified as only 2.2% of the TRRs (0.048 ppm), while M670H01 was not identified at all in the phenyl-label. Aqueous or EtOAc fractions from the methanol (or methanol/EtOAc) extracts comprised the 5.34% of the TRRs that were characterized (0.1168 ppm). Unextractable residues in the PES accounted for 3.16% of the TRRs (0.0690 ppm), so no further extraction procedures were conducted. The accountability was 94.0% (TABLE C.2.3.2).

Kidney: The methanol extract contained 94.9% of the TRRs in samples from the pyrazole-label sample (0.2673 ppm) (TABLE C.2.2.1). From the methanol/EtOAc extract (91.4% of the TRRs; 0.2575 ppm), the predominant metabolite identified was topramezone at 79.5% of the TRRs (0.2239 ppm). M670H02 was identified as 9.95% of the TRRs (0.028 ppm), while M670H01 was identified as 0.68% of the TRRs (0.0019 ppm). Approximately 1.3% of the TRRs (0.0037 ppm) were characterized from HPLC analysis as a single peak/region, and another 0.66% of the TRRs (0.0019 ppm) were characterized as part of the aqueous fraction from the methanol extract. Unextractable residues in the PES accounted for 6.71% of the TRRs (0.0189 ppm). Since the unextractable residues contributed to <10% of the TRRs, no further extraction procedures were conducted. The accountability was 98.7% (TABLE C.2.3.1).

The methanol extract again contained 97.5% of the TRRs (0.3432 ppm) in phenyl-label samples (TABLE C.2.2.2). From the methanol/EtOAc extract (85.4% of the TRRs; 0.3006 ppm), the predominant metabolite identified was parent topramezone at 83.2% of the TRRs (0.2929 ppm). M670H01 and M670H02 were considered minor metabolites at only 0.35% (0.0012 ppm) and 0.41% of the TRRs (0.0014 ppm), respectively. Approximately 1.44% of the TRRs were characterized (0.0051 ppm) from HPLC analysis as a single peak/region, and another 2.44% of the TRRs (0.0086 ppm) were characterized as aqueous fractions from the methanol (or methanol/EtOAc) extracts. Unextractable residues in the PES accounted for 2.45% of the TRRs (0.0086 ppm), so no further extraction procedures were conducted. The accountability was 90.3% (TABLE C.2.3.2).

Milk: Only the analysis of the pyrazole-label sample was conducted. The TRRs from the combined acetone and methanol/water extracts were 58.6% (0.0041 ppm), as per TABLE C.2.2.3. Topramezone was the predominant metabolite identified in fractions 9, 10 and 11 as a total of 25.3 % of the TRRs (0.0018 ppm). M670H02 and M670H01 were identified in <1.2% of

the TRRs (<0.00008 ppm) from fractions 9, 10 and 11. In addition, 0.81% of the TRRs (0.00006 ppm) were characterized as several minor peaks/regions from fractions 10 and 11. No unextractable residues were detected in this matrix. Accountability was low at 33.6% (TABLE C.2.3.1). Approximately 28.4% of the 52.6% of the TRRs from the methanol/water extract were identified or characterized. This left 24.2% of the TRRs unaccounted for. It should also be noted that almost half of the TRRs were lost after the first extraction with methanol/water. This may have been the result of analysing a sample with extremely low residue levels (0.007 ppm), where any loss would have an impact on the results.

Feces: A majority of the TRRs were found in the methanol/water extract of the pyrazole-label samples (83.0% of the TRRs), as per TABLE C.2.2.4. The only metabolite identified was the parent topramezone at 72.9% of the TRRs. The remaining 10.1% of the TRRs were characterized as several minor peaks/regions. Unextractable residues accounted for 15.5% of the TRRs, for an overall accountability of 98.5% (TABLE C.2.3.1).

The majority of the TRRs were also found in the methanol/water extract of the phenyl-label samples as 64.8% of the TRRs (TABLE C.2.2.4). The only metabolite identified was the parent topramezone at 59.9% of the TRRs. The remaining 4.91% of the TRRs were characterized as several minor peaks/regions. Unextractable residues accounted for 22.2% of the TRRs, for an overall accountability of 87.0% (TABLE C.2.3.2).

Although the PES accounted for more than 10% of the TRRs in the pyrazole-label and phenyl-label feces samples, further extraction or fractionation was not conducted. However, the characterization of these samples are not required, but facilitate in the characterization of low level residues present in the tissues.

Urine: 100% of the TRRs were extractable in the pyrazole-label urine samples, thus no residues were considered unextractable (TABLE C.2.2.5), and the accountability was 100% (TABLE C.2.3.1). Topramezone was the major metabolite identified in the day 5 pyrazole-label samples at 90.0% of the TRRs. Residues of M670H02 (6.88% of the TRRs) and M670H01 (2.6% of the TRRs) were identified as minor metabolites, while the remaining 0.48% of the TRRs were characterized as a single minor peak.

100% of the TRRs were also extractable in the phenyl-label urine samples (TABLE C.2.2.5), so again no residues were considered unextractable, and the accountability was 100% (TABLE C.2.3.2). Topramezone was the only metabolite identified in the day 5 phenyl-label samples, at 96.8% of the TRRs. The remaining 3.25% of the TRRs were characterized as a single minor peak.

NMR analysis was conducted to confirm the presence of M670H02 and M670H01 in the urine. The UV_{254} chromatograms indicated the presence of peaks with the same retention times as M670H02 and M670H01 from the ^{14}C-chromatograms, thus confirming the identity of the metabolites. A minor peak (>3.25% of the TRRs) with a retention time of approximately 8.3 minutes was also analysed by NMR for identification purposes. The results, however, were

inconclusive.

HPLC method 1 was able to identify the parent and the metabolites M670H02, M670H01 and M670H05. HPLC method 2 could identify all metabolites except for M670H05, while HPLC method 3 could only identify the parent. As per TABLES C.2.2.6 and C.2.2.7, the residue levels of parent topramezone and M670H02 were similar between HPLC methods 1 and 2 in samples from both labels. Very low residue levels of M670H01 were not always detected by HPLC method 2. As per TABLE C.2.2.8, TLC analysis of the crude methanol extract detected similar residue levels of parent topramezone as HPLC method 1, but was not able to identify low residue levels of M670H02 and M670H01. Since HPLC method 1 was able to identify all the metabolites, the identified residues from this method were listed in the summary tables (except for pyrazole-label feces, which were identified by HPLC method 3).

As per FIGURES C.2.1 and C.2.2, the percentage of TRRs attributed to the urine and feces samples were markedly higher than in other matrices for both labels, indicating the compound was rapidly excreted from the body. As per FIGURE C.2.3, topramezone was the predominant metabolite identified in both radiolabelled matrices. This result indicated that the chemical was not extensively metabolized in the body.

The accountability demonstrated in this study was less than 100%, but it must be noted that procedural loss was observed throughout the study.

The metabolite profiles were similar between the pyrazole and phenyl treatment groups. The proposed metabolic pathway involved the hydroxylation of the isoxazole ring at the 4th position to form the hydroxy metabolite M670H02. The isoxazole ring was then cleaved off, leaving behind a triple C-N bond to form the cyano metabolite M670H01. The linkage between the phenyl and pyrazole rings in all the metabolites remained intact.

C.1. Storage Stability

TABLE C.1. Summary of Storage Conditions

Matrix	Storage Temp.(°C)	Actual Storage Duration (days or months)	Interval of Demonstrated Storage Stability (months)
Liver, Kidney, Feces, Urine	<-15°C	≤3.6	Not provided
Milk	<-15°C	≤10.2	Not provided

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

TABLE C.2.1. Total Radioactive Residues (TRRs) in Milk, Tissue and Excreta

Matrix	Collection Timing	Pyrazole	Phenyl
		ppm (% Admin. Dose)	ppm (% Admin. Dose)
Urine	Daily (pooled by group) until termination	Day 1: (7.07%) Day 2: (8.04%) Day 3: (6.99%) Day 4: (12.0%) Day 5: (10.4%) (Total - 44.5%)	Day 1: (4.20%) Day 2: (7.18%) Day 3: (6.47%) Day 4: (7.61%) Day 5: (7.80%) (Total - 33.3%)
Feces	Daily (pooled by group) until termination	Day 1: (2.41%) Day 2: (8.83%) Day 3: (8.04%) Day 4: (9.85%) Day 5: (9.20%) (Total - 38.3%)	Day 1: (2.01%) Day 2: (5.06%) Day 3: (8.66%) Day 4: (7.44%) Day 5: (11.55%) (Total - 34.7%)
Milk	Twice daily to termination	0.005-0.007 (0.007-0.01%)	0.000-0.002 (0.0003-0.003%)
Bile	At termination	0.137 (0.003%)	0.059 (0.002%)
Muscle	At termination	0.001 (0.001%)	0.001 (0.0011%)
Fat	At termination	0.007 (0.006%)	0.002 (0.0014%)
Liver	At termination	1.891 (1.28%)	2.182 (1.96%)
Kidney	At termination	0.282 (0.04%)	0.352 (0.05%)
GI tract (batch 1 / tissue)	At termination	0.780 (5.27%)	0.322 (1.20%)
GI tract (batch 2 / contents)	At termination	1.158 (2.67%)	1.567 (10.2%)
Cage Rinse	Daily (pooled by group) until termination	1.182 (0.29%)	0.986 (0.49%)
% of Administered Dose		92.5	81.9

TABLE C.2.2.1 Distribution of the Parent and the Metabolites in Goat Kidney and Liver when Dosed with ^{14}C-Pyrazole label Topramezone (Analysis by HPLC Method 1)

Metabolite Fraction	Kidney 0.282 ppm		Liver 1.891 ppm	
	%TRR	ppm	%TRR	ppm
Methanol Extract	94.9	0.2673	92.3	1.7453
Methanol/EtOAc Extract	91.4	0.2575	82.1	1.553
Topramezone	79.5	0.2239	51.8	0.9799
M670H02	9.95	0.028	29.6	0.5603
M670H01	0.68	0.0019	0.67	0.0127
Unknown (kidney: 1 peak/region at 18.1 min Rt)	1.3	0.0037	—	—
Aqueous Fraction from methanol extract	0.66	0.0019	3.78	0.0714
EtOAc Fraction from Methanol/EtOAc extract	—	—	0.03	0.0005
Aqueous Fraction from Methanol/EtOAc extract	—	—	1.79	0.0338
Unextractable (PES)	6.71	0.0189	3.73	0.0705

TABLE C.2.2.2 Distribution of the Parent and the Metabolites in Livestock Liver and Kidney when Dosed with ^{14}C-Phenyl-label Topramezone (Analysis by HPLC Method 1)

Metabolite Fraction	Kidney 0.352 ppm		Liver 2.182 ppm	
	%TRR	ppm	%TRR	ppm
Methanol extract	97.5	0.3432	92.7	2.0221
Methanol/EtOAc Extract	85.4	0.3006	85.5	1.8648
Topramezone	83.2	0.2929	83.3	1.817
M670H02	0.41	0.0014	2.2	0.048
M670H01	0.35	0.0012	—	—
Unknown (kidney: 1 peak/region at 18.7 min Rt)	1.44	0.0051	—	—
Aqueous Fraction from methanol extract	1.9	0.0067	3.24	0.0708
EtOAc Fraction from Methanol/EtOAc extract	—	—	0.18	0.004
Aqueous Fraction from Methanol/EtOAc extract	0.54	0.0019	1.92	0.042
Unextractable (PES)	2.45	0.0086	3.16	0.069

TABLE C.2.2.3 Distribution of the Parent and the Metabolites in Goat Milk when Dosed with ^{14}C-Pyrazole label Topramezone (Analysis by HPLC Method 1)

Metabolite Fraction	Milk 0.007 ppm	
	%TRR	ppm
Acetone extract	6.03	0.0004
Methanol/water extract	52.6	0.0037
Fraction 9 (from MeOH/water extract)	21.4	0.0015
Concentrated/filtered sample for HPLC	18.6	0.0013
Topramezone	16.5	0.0012
M670H02	0.68	0.00005
M670H01	0.63	0.00004
Unknown from fraction 9	—	—
Fraction 10 (from MeOH/water extract)	7.59	0.0005
Fraction 11 (from MeOH/water extract)	7.71	0.0005
Combined Fraction (10 +11)	10.4	0.0007
Concentrated/filtered sample for HPLC	9.58	0.0006
Topramezone	8.83	0.00055
M670H02	0.5	0.00003
M670H01	0.25	0.00002
Unknown from fractions 10+11 (6 peaks/regions, each <0.3% of the TRRs)	0.81	0.00006
Unextractable (PES)	—	—

TABLE C.2.2.4 Distribution of the Parent and the Metabolites in Day 5 Goat Feces when Dosed with either ^{14}C-Pyrazole or ^{14}C-Phenyl label Topramezone (Analyses by HPLC Methods 3 and 1)

Metabolite Fraction	Pyrazole HPLC method 3		Phenyl HPLC method 1	
	%TRR	ppm[1]	%TRR	ppm[1]
Methanol/water extract	83.0	—	64.8	—
Topramezone	72.9	—	59.9	—
M670H02	—	—	—	—
M670H01	—	—	—	—
Unknown: (pyrazole: 7 peaks/regions, each < 2.28% of the TRRs. phenyl: 5 peaks/regions, each < 1.46% of the TRRs)	10.1	—	4.91	—
Unextractable (PES)	15.5	—	22.2	—

[1] ppm values were not reported.

TABLE C.2.2.5 Distribution of the Parent and the Metabolites in Day 5 Goat Urine when Dosed with Either ^{14}C-Pyrazole or ^{14}C-Phenyl label Topramezone (Analysis by HPLC Method 1)

Metabolite Fraction	Pyrazole		Phenyl	
	%TRR	ppm [1]	%TRR	ppm [1]
Extract	100	—	100	—
Topramezone	90.0	—	96.8	—
M670H02	6.88	—	—	—
M670H01	2.6	—	—	—
Unknown (pyrazole: 1 peak/region at 8.3 min Rt phenyl: 1 peak/region at 8.3 min Rt)	0.48	—	3.25	—

[1] ppm values were not reported.

TABLE C.2.2.6 Distribution of the Parent and the Metabolites in Goat Matrices when Dosed with ^{14}C-Pyrazole label Topramezone (Analysis by HPLC Method 2)

Metabolite Fraction	Urine HPLC method 2		Feces HPLC method 2		Liver HPLC method 2		Kidney HPLC method 2	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
Extract	100	—	83.0	—	82.1	1.553	91.4	0.2575
Topramezone	89.7	—	77.9	—	51.0	0.9641	81.6	0.2299
M670H02	7.43	—	—	—	31.1	0.5889	9.81	0.0276
M670H01	2.9	—	—	—	—	—	—	—
Unknown (feces: 2 peaks/regions at 9.7 and 10.5 min Rt)	—	—	5.04	—	—	—	—	—

TABLE C.2.2.7 Distribution of the Parent and the Metabolites in Goat Matrices when Dosed with ^{14}C-Phenyl label Topramezone (Analysis by HPLC Method 2)

Metabolite Fraction	Urine		Feces		Liver		Kidney	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
Extract	100	—	64.8	—	85.5	1 8648	85.4	0.3006
Topramezone	96.9	—	62.9	—	83.5	1.822	82.8	0.2915
M670H02	—	—	—	—	1.96	0.043	0.53	0.0019
M670H01	—	—	—	—	—	—	0.15	0.0005
Unknown (Urine: 1 peak/region at 4.3 min Rt) (Feces: 3 peaks/regions, each < 0.84% of the TRRs) (Kidney: 1 peak/region at 16.2 min Rt)	3.13	—	1.91	—	—	—	1.90	0.0067

TABLE C.2.2.8 Distribution of the Parent and the Metabolites in Goat Liver and Kidney when Dosed with Either ^{14}C-Pyrazole or ^{14}C-Phenyl label Topramezone (Analysis by TLC Method 1)

Metabolite Fraction	Liver - Pyrazole		Liver - Phenyl		Kidney - Pyrazole		Kidney - Phenyl	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
Methanol Extract	92.3	1.7453	92.7	2.0221	94.9	0.2673	97.5	0.3432
Topramezone	53.2	1.0053	89.4	1.951	89.6	0.2523	96.0	0.3381
M670H02	36.4	0.6876	2.87	0.063	5.31	0.015	—	—
M670H01	—	—	—	—	—	—	—	—
Unknown (Pyrazole liver: 2 peaks/regions, each < 0.0332 ppm) (Phenyl kidney: 4 peaks/regions, each < 0.0017 ppm)	2.7	0.0507	—	—	—	—	1.47	0.0051

Table C.2.3.1 Summary of Characterization and Identification of Radioactive Residues in Livestock Matrices Following Application of Pyrazole-label Topramezone at 11.2 mg/kg.[1]

Compound	Urine		Feces		Kidney 0.282 ppm		Liver 1.891 ppm		Milk[2] 0.007 ppm	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
Total identified	99.5	—	72.86	—	90.1	0.2538	82.1	1.553	27.6	0.0019
Topramezone	90.0	—	72.86	—	79.5	0.2239	51.8	0.9799	25.3	0.0018
M670H02	6.88	—	—	—	9.95	0.028	29.6	0.5603	1.18	0.00008
M670H01	2.6	—	—	—	0.68	0.0019	0.67	0.013	0.88	0.00006
Total characterized	0.48	—	10.1	—	1.96	0.0056	5.6	0.1057	6.84	0.00046
Total extractable	100	—	83.0	—	92.1	0.2594	87.7	1.659	34.5	0.002
Unextractable (PES)	0	—	15.5	—	6.71	0.0189	3.73	0.071	—	—
Accountability[3]	100%		98.5%		98.7%		91.4%		33.6%	

1 Identified residues quantitated by HPLC method 1, except for feces (HPLC method 3)
2 Identified metabolite residue %TRRs based on sum of fractions 9, 10 and 11.
3 Accountability = (Total extractable + Total unextractable)/(TRRs from combustion analysis) * 100

Table C.2.3.2 Summary of Characterization and Identification of Radioactive Residues in Livestock Matrices Following Application of Phenyl-label Topramezone at 9.9 mg/kg[1]

Compound	Urine		Feces		Kidney 0.352 ppm		Liver 2.182 ppm	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
Total identified	96.8	—	59.9	—	84.0	0.2955	85.5	1.865
Topramezone	96.8	—	59.9	—	83.2	0.2929	83.3	1.817
M670H02	—	—	—	—	0.41	0.0014	2.2	0.048
M670H01	—	—	—	—	0.35	0.0012	—	—
Total characterized	3.25	—	4.9	—	3.88	0.0137	5.34	0.1168
Total extractable	100	—	64.8	—	87.8	0.3092	90.8	1.982
Unextractable (PES)	0	—	22.2	—	2.45	0.0086	3.16	0.069
Accountability[2]	100%		87.0%		90.3%		94.0%	

1 Identified residues quantitated by HPLC method 1

2 Accountability = (Total extractable + Total unextractable)/(TRRs from combustion analysis) * 100.

FIGURE C.2.1. Distribution of Radioactivity in Excreta of Lactating Goats Following Administration of Radiolabelled Topramezone

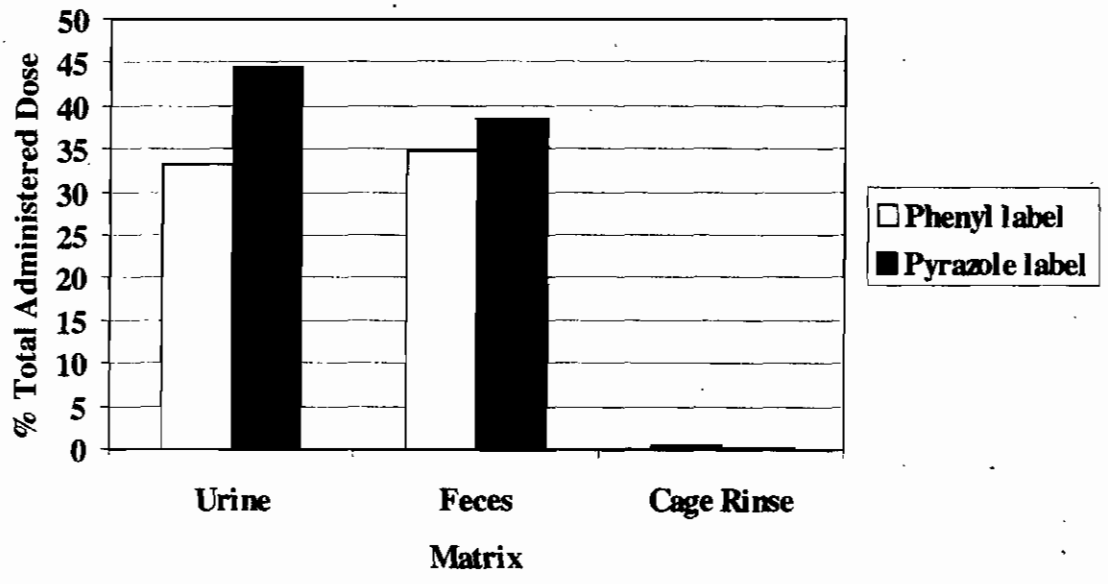


FIGURE C.2.2. Distribution of Radioactivity in the GI tract (2 batches), Liver, Kidney, Milk and Fat of Lactating Goats Following Administration of Radiolabelled Topramezone

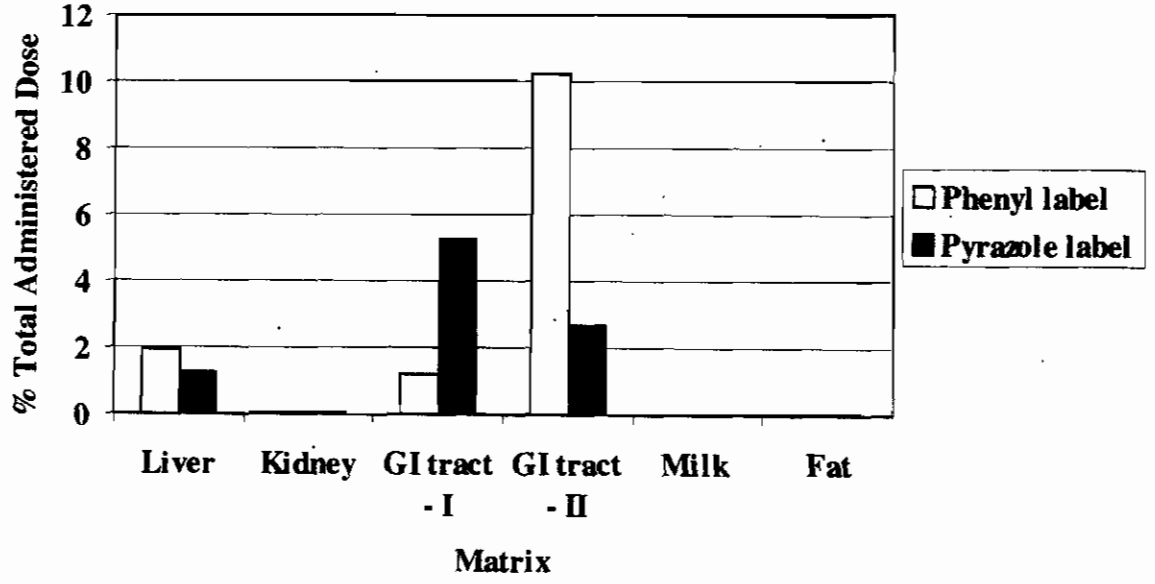
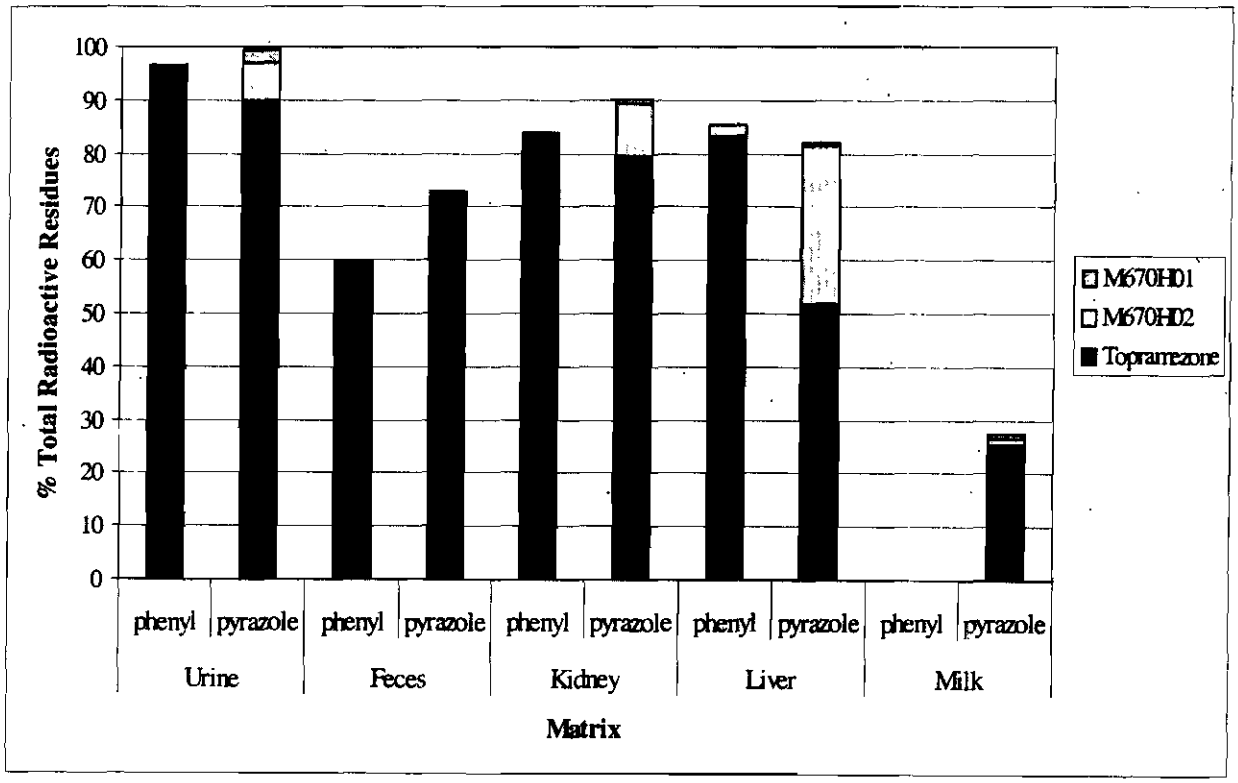
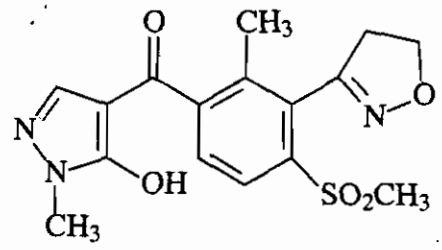


FIGURE C.2.3. Distribution of Topramezone and Minor Metabolites in Goat Matrices Following Dosing with Radiolabelled Topramezone

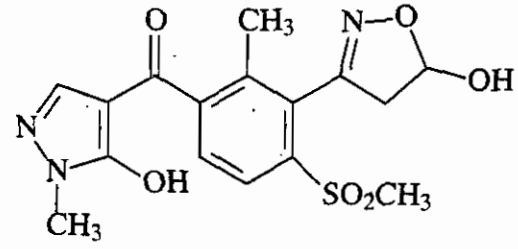


C.3. Proposed Metabolic Profile

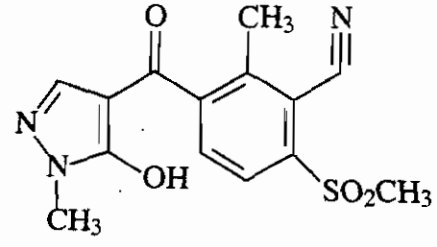
FIGURE C.3.1. Proposed Metabolic Profile of Topramezone in the Lactating Goat



BAS 670 H

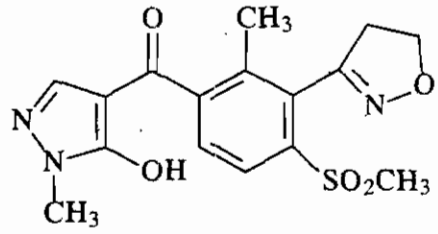
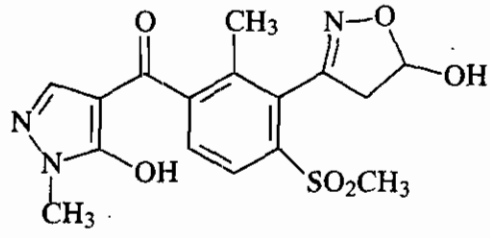
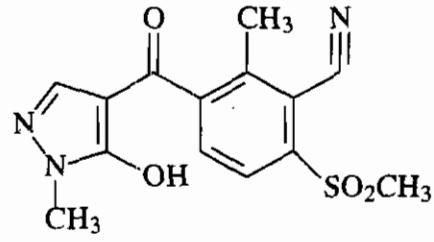


M670H02



M670H01

TABLE C.3.1. Identification of Compounds from Metabolism Study

Common name/code	Chemical name	Chemical structure
Topramezone	[3-(4,5-dihydro-3-isoxazolyl)-2-methyl-4-(methylsulfonyl)phenyl](5-hydroxy-1-methyl-1*H*-pyrazol-4-yl)methanone	
M670H02	hydroxy metabolite	
M670H01	cyano metabolite	

D. CONCLUSION

Topramezone was administered orally once daily to goats at feeding levels of either 9.9 mg/kg (phenyl) or 11.2 mg/kg (pyrazole) for 5 days. Recoveries of topramezone were high in both the pyrazole- and phenyl-label extracts (82-92% of the administered dose). Topramezone is mainly excreted via the urine and feces (68-83%), followed by recoveries up to 11% in the GI tract/contents. Up to 2% of the administered dose was recovered in the liver, followed by kidney (0.04-0.05%) and pyrazole-label milk (≤0.01%); with virtually no residues in muscle, fat and phenyl-label milk. The predominant metabolite identified in meat, excreta and milk was the parent compound topramezone (from 25.32% of the TRRs in pyrazole-label milk to 96.8% of the TRRs in phenyl-label urine). The hydroxy metabolite M670H02 was a major metabolite identified in pyrazole-label liver (29.6% of the TRRs), but was a minor metabolite along with M670H01 in the other matrices. The metabolic breakdown of topramezone involves the parent compound undergoing hydroxylation of the isoxazole ring to form M670H02. Further degradation results in the cleavage of the isoxazole ring, leaving only a cyano-bond as part of the cyano metabolite M670H01.

E. REFERENCES

None.

F. DOCUMENT TRACKING

RDI: P.V. Shah (3/2/05), RAB1 Chemists (12/8/04), ChemSAC (3/2/05)
G.F. Kramer:806T:CM#2:(703)305-5079:7509C:RAB1
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Evaluators

Suzan Mathew — Date: Mar 21/05
Evaluation Officer
FREAS, HED, PMRA

for Stephanie Wille — Date: Mar 21/05
Evaluation Officer
FREAS, HED, PMRA

Peer Reviewer

George F. Kramer, Ph.D. — Date: 4-12-05
Chemist
RAB1, HED, EPA

Approved by

Louise G. Croteau — Date: March 22/05
Senior Evaluation Officer
FREAS, HED, PMRA

Ariff Ally, Ph.D. — Date: Mar 22/05
Section Head
FREAS, HED, PMRA

P.V. Shah, Ph.D. — Date: 4/18/05
Branch Senior Scientist
RAB1, HED, EPA

STUDY REPORTS:

MRID No. 45902401. Ellenson, James L. (2002). Final Report: Metabolism of [^{14}C]-BAS 670 H in Corn. BASF Study Number 98129. Unpublished study prepared by BASF Agro Research. 208 p.

EXECUTIVE SUMMARY:

BASF Agro Research conducted a corn metabolism study with [phenyl-U-^{14}C] (5.76 MBq/mg specific radioactivity; >98% radiochemical purity) topramezone and [pyrazole-4-^{14}C] (4.65 MBq/mg specific radioactivity; >98% radiochemical purity) topramezone. Topramezone (also known as BAS 670 H) was applied to corn plants at the 3-5 leaf growth stage (GS 13-15) at rates of 0.148 kg a.i./ha (0.132 lb a.i./A) for the pyrazole label, or 0.146 kg a.i./ha (0.130 lb a.i./A) for the phenyl label as a single foliar application. The plants were grown in pots within a climate-controlled growth chamber (phytotron), which was set to simulate the North American growing season (15-20°C temperature range and 37-47% humidity at time of application). Immature plant samples were collected at 1, 9, 15 or 16, and 29 or 30 days after treatment (DAT). The corn forage samples were collected at the late dough stage (59-60 DAT), while stover and grain were harvested at maturity (77 DAT). Samples were analysed within 5.1 months of harvest; thus, no storage stability data (to determine if sample integrity was maintained) were collected or are necessary for this study.

The overall total radioactive residues (TRRs) in treated corn samples were determined by both combustion/liquid-scintillation counting (LSC) and by calculation (the sum of the radioactive residues in the extracts and the remaining residues in the post-extraction solids (PES)). Chromatographic determinations of radioactivity were performed by high-performance liquid chromatography (HPLC), with mass spectrometry (MS) providing confirmation. Extraction of radioactivity was conducted by accelerated solvent extraction (ASE). Major metabolites (>10% of the TRRs) were identified by co-chromatography and MS, while minor metabolites (<10% of the TRRs) were identified by co-chromatography alone.

Fractionation procedures of the PES involved extraction with aqueous ammonia solution, acidification with H_3PO_4, extraction with dimethylsulfoxide (DMSO) and water and ethanol, refluxing with NaOH, acidification with HCl, and drying of the precipitate. The calculated limits of quantitation (LOQs) for phenyl-label corn forage, stover and grain were 0.00122 ppm, 0.000738 ppm and 0.000674 ppm, respectively. The calculated LOQs for pyrazole-label corn forage, stover and grain were 0.000491 ppm, 0.000298 ppm and 0.000272 ppm, respectively.

Calculated TRRs in immature corn samples treated with [phenyl-U-^{14}C] label topramezone declined from 7.64 ppm at 1 DAT to 0.468 ppm at 30 DAT. The overall TRRs in forage (59-60 DAT), stover and grain (77 DAT) were 0.534 ppm, 0.730 ppm, and 0.107 ppm, respectively. The TRRs in immature whole corn samples treated with [pyrazole-4-^{14}C] label topramezone also declined, from 7.68 ppm at 1 DAT to 0.544 ppm at 29 DAT. The overall TRRs in forage, stover and grain were lower in pyrazole-label samples at 0.294 ppm, 0.213 ppm and 0.032 ppm, respectively.

A total of 74.9-81.7% of the TRRs (0.351-6.117 ppm) of phenyl-label immature corn plants were aqueous extractable. Of the 63.9-75.8% of the TRRs identified (0.299-5.117 ppm), the parent topramezone accounted for the majority of residues (55.7-69.9% of the TRRs; 0.264-4.255 ppm). The desmethyl metabolite M670H03 and the acid metabolite M670H05 were considered minor

metabolites (<10% of the TRRs each; ≤0.717 ppm). Residues characterized as a series of minor peaks/regions comprised 5.9-13.0% of the TRRs (0.052-1.00 ppm). The unextractable residues (PES) accounted for 9.9-16.5% of the TRRs (0.077-0.755 ppm), for an overall accountability ranging from 89.9-93.5%.

A total of 40.1-84.1% of the TRRs (0.218-6.46 ppm) of pyrazole-label immature corn plants were aqueous extractable. Of the 35.2-84.1% of the TRRs identified (0.192-6.458 ppm), the parent topramezone was the predominant metabolite at 33.2-78.7% of the TRRs (0.181-6.05 ppm). The only other metabolite identified was M670H03 at <6% of the TRRs (≤0.412 ppm). Residues characterized as a series of minor peaks/regions comprised 4.9-11.4% of the TRRs (0.026-0.137 ppm). The unextractable residues (PES) accounted for 5.8-33.1% of the TRRs (0.18-0.475 ppm), for an overall accountability ranging from 71.3-89.9%.

A total of 78.8-82.8% of the TRRs (0.420-0.605 ppm) of phenyl-label forage and stover samples were aqueous extractable, while 83.1% of the TRRs of grain (0.089 ppm) were extractable by ACN:water. Of the 51.2-56.3% of the TRRs identified in forage and stover (0.273-0.412 ppm), the parent topramezone accounted for the majority of residues (40.4-40.9% of the TRRs; 0.216-0.299 ppm). The metabolites M670H03, M670H05 and the methyl ketone metabolite M670H08 each comprised <11% of the TRRs (≤0.075 ppm). Only 5.5% of the TRRs were identified in grain (0.006 ppm), and there were more residues of M670H05 (3.4% of the TRRs; 0.004 ppm) than parent (2.1% of the TRRs; 0.002 ppm) identified. After exhaustive extraction/fractionation procedures, a total of 26.5-27.6% of the TRRs were characterized (0.147-0.193 ppm) in forage and stover. In grain, the majority of the residues were characterized (77.6% of the TRRs, 0.083 ppm). The final unextractable residues in all matrices after fractionation accounted for 2.4-5.4% of the TRRs (0.005-0.029 ppm), for an overall accountability ranging from 84.1-87.9%.

A total of 65.3-83.3% of the TRRs (0.178-0.193 ppm) were aqueous extractable from pyrazole-label forage and stover samples, while 77.3% of the TRRs were extracted with ACN:water from grain (0.025 ppm). Of the 23.6-28.4% of the TRRs identified in forage and stover (0.061-0.07 ppm), the parent topramezone accounted for the majority of residues (16.9-19.6% of the TRRs; 0.042-0.05 ppm). Each of the metabolites M670H03 and M670H08 comprised <5% of the TRRs (≤0.011 ppm). Only 2.5% of the TRRs were identified as topramezone in grain (0.001 ppm), with no metabolites identified. After exhaustive extraction/fractionation procedures, a total of 41.7-54.9% of the TRRs were characterized (0.117-0.123 ppm) in forage and stover. In grain, the majority of the residues were characterized (74.8% of the TRRs, 0.024 ppm). The final unextractable residues in all matrices after fractionation accounted for 2.7-22.6% of the TRRs (0.006-0.066 ppm), for an overall accountability ranging from 86.4-97.2%.

Hydrolysis of grain PES indicated that radioactivity was incorporated into glucose. Saponification of oil indicated that radioactivity was also incorporated into the fatty acid constituents.

The metabolism of topramezone in corn involved the hydrolytic cleavage of the parent to form the acid metabolite M670H05. Further hydrolysis and cleavage resulted in the formation of

M670H08, while desmethylation resulted in M670H03. After cleavage, the pyrazole ring appeared to undergo complete catabolism and reincorporation within the carbon backbone of natural products such as starch, soluble polysaccharides and fatty acids. The phenyl ring portion of the molecule also underwent degradation, resulting in the incorporation of radioactivity into natural products.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the corn 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# 310772] and in Canada's Regulatory Decision Document.

COMPLIANCE:

Signed and dated Good Laboratory Practice (GLP), Quality Assurance and a No Data Confidentiality statement were provided. Also, there were protocol changes resulting from repeating the pyrazole-label portion of the study due to radioactive contamination of the original samples. Neither issue would negatively impact the validity of the study.

A. BACKGROUND INFORMATION

BAS 670 336SC (soluble concentrate) Herbicide (also known as BAS 670 00H Herbicide) is the end-use product which contains the active ingredient topramezone. It is a broad-spectrum, post-emergence herbicide used to control grassy and broadleaf weeds in corn. The herbicide is absorbed by the leaves, roots and shoots, then translocated to the growing points of the sensitive weeds. Topramezone belongs to the triketone class of chemicals, which inhibits carotenoid biosynthesis (4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitor). This causes a strong bleaching activity on the growing zones of the shoots within 2-5 days of application. Exposure to light causes necrosis of chlorotic tissues and eventual plant death within 14 days after application.

TABLE A.1. Test Compound Nomenclature

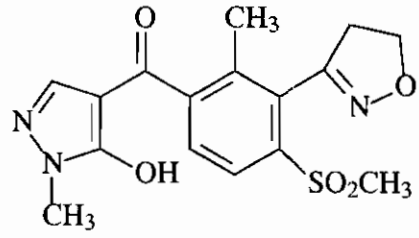
Compound	Chemical Structure 
Common name	Topramezone
Company experimental name	BAS 670 H
IUPAC name	[3-(4,5-dihydro-1,2-oxazol-3-yl)-4-mesyl-*o*-tolyl](5-hydroxy-1-methyl-1*H*-pyrazol-4-yl) methanone
CAS name	[3-(4,5-dihydro-3-isoxazolyl)-2-methyl-4-(methylsulfonyl)phenyl](5-hydroxy-1-methyl-1*H*-pyrazol-4-yl)methanone
Molecular Formula	$C_{16}H_{17}N_3O_5S$
Molecular Mass	363.39 g/mol
CAS #	210631-68-8
End-use product/EP	Soluble Concentrate

TABLE A.2. Physicochemical Properties of Topramezone

Parameter	Value
Melting point/range	220.9°C - 222.2°C
pH	2.9 (1% deionized water)
Density (20°C)	1.425 g/cm^3
Water solubility (20°C)	pH: 3, 5, 7, 9 g/L: 0.06, 0.98, 15, 23.4
Solvent solubility (g/100 mL at 20°C)	Acetone <1.0 Acetonitrile <1.0 Dichloromethane 2.5 - 2.9 Ethyl acetate <1.0 Methanol <1.0 N-heptane <1.0 N,N-dimethylformamide 11.4-13.3 1-octanol <1.0 Olive oil <1.0 2-propanol <1.0 Toluene <1.0
Vapour pressure at 20°C and 25°C	< 1.0 x 10^{-12} hPa
Dissociation constant (pK_a)	4.06
Octanol/water partition coefficient Log(K_{OW}) at 20°C	Buffer pH: 4, 7, 9 Log K_{ow}: – 0.81, – 1.52, – 2.34
UV/visible absorption spectrum	λ, nm: 207, 272, 300, 410 ξ, mol^{-1}cm^{-1}: 27 077, 8601, 5800, 410

All data came from PMRA Lab Services.

B. EXPERIMENTAL DESIGN

B.1. Test Site and Crop Information

TABLE B.1.1. Test Site Information

Testing Environment	Soil characteristics			
	Type	%OM [1]	pH	CEC [2]
Pots placed in controlled environment growth chamber (phytotron)	*Lehmiger* Loamy Sand	Not provided	6.5 ($CaCl_2$)	7.1

1 OM - Organic matter
2 CEC - Cation exchange capacity

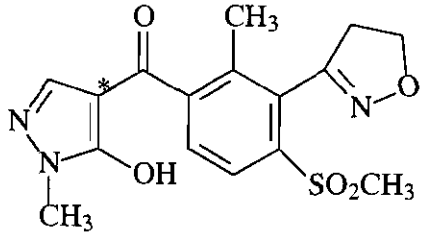
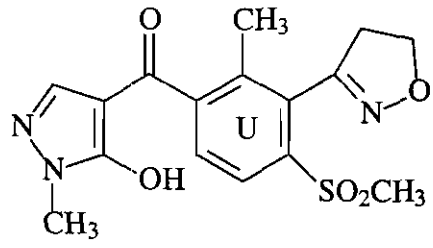
Plants were tended and fertilized under normal agricultural practices, with irrigation provided as needed through an automated droplet system.

TABLE B.1.2. Crop Information

Crop/crop group	Variety	Growth stage at application	Growth stage at harvest	Harvested Commodity	Harvesting procedure
Corn (crop group 15)	*Merlin*	3-5 leaf (GS 13-15)	forage - late dough stover and grain - mature	Forage, stover and grain	Not reported

B.2. Test Materials

TABLE B.2.1. Test Material Characteristics

Chemical structure		
Radiolabel position	Pyrazole	Phenyl
Lot No.	706-1013	714-1026
Purity	98% (radio-HPLC)	98% (radio-HPLC)
Specific activity (Bq)*	5.76 MBq/mg	4.65 MBq/mg

* Bq = disintegrations per second

B.3. Study Use Pattern

TABLE B.3.1. Use Pattern Information	
Chemical name	Topramezone
Application method	Track sprayer
Application rate	pyrazole: 0.1484 kg a.i./ha (0.132 lb a.i./A) phenyl: 0.1464 kg a.i./ha (0.130 lb a.i./A)
Number of applications	one
Timing of applications	at 3-5 leaf growth stage
Pre-harvest interval (PHI)	59-60 days for forage; 77 days for grain and stover

B.4. Identification/ Characterization of Residues

B.4.1. Sample Handling and Preparation

All plant samples were cut by hand approximately 5 cm (2.0 inches) above the soil surface. Sample parts were subdivided and each part was shipped on different dates to reduce the risk of sample loss. The samples were stored frozen (-18°C) before and during shipment to BASF Agro Research, where they were stored frozen except during preparation and analysis.

All samples were ground into a fine powder under liquid nitrogen (Brinkmann Polytron homogenizer®). Solid samples were weighed into paper cones (Packard Systems automated sample oxidizer) or porcelain vessels (J. Harvey manual sample oxidizer) for combustion. Evolved $^{14}CO_2$ from the solid samples was trapped in absorption cocktails (Harvey Cocktail or Carbosorb™). Liquid samples were mixed with scintillation cocktail (Permafluor) before counting by LSC.

Automated extraction of radioactivity in the plant material was performed by ASE, where samples were extracted three times with water (50-100°C). Liquid extraction involved mechanical shaking at room temperature using the same solvent system and extraction steps as ASE. Because of mechanical fouling problems observed with the grain matrix on the ASE instrument at elevated temperatures, the grain matrix had to be extracted with hexane three times, followed by a triple extraction with ACN:H_2O (50:50, v:v). All extracts were concentrated, filtered, chemically hydrolysed with 1N HCl, then analysed by HPLC.

Radioactivity in the PES of the forage, stover and grain samples were fractionated sequentially into different classes of natural products using schemes based on the procedure of Honeycutt and Adler (1975). This involved extraction with 1% aqueous NH_4OH; acidification with 2N H_3PO_4 (protein fraction); extraction with DMSO and water (9:1, v:v); addition of ethanol to the DMSO/water extract (starch fraction); further extraction of solids by refluxing with a 10% NaOH solution (cellulose fraction) to become base hydrolysate; acidification to pH 1 with concentrated HCl (polysaccharide fraction); then the drying of the remaining precipitate (lignin). All subsample supernatants and hydrolysates from each step were shaken and filtered before being

analysed by LSC.

B.4.2. Analytical Methodology

Chromatographic determinations were performed with a Hewlett Packard (series 1050) HPLC, with radioactivity being monitored by an IN/US Model 2B BRAM, equipped with either a solid - (Li-doped glass as the scintillant) or liquid (ULTIMA-FLOW™: column eluant, 3:1) phase flow-through cell. The HPLC methods used in this study are listed below.

TABLE B.4.2. HPLC Methods

HPLC Method No.	Column	Eluents		Flow Rate (mL/min)	Gradient		
		A	B		Time[1]	%A	%B
9812901, BAS670H, COLMB55T, SYNERGI1 [2]	Phenomenex Columbus (250 x 4.6 mm, 5µ)	25mM TRIS (pH 7)	MeOH	1	0 1 12 35 55 60 61	95 95 80 80 5 5 95	5 5 20 20 95 95 5
OIL_FA1 [3]	Nucleosil C18 (250 x 10 mm, 5µ)	Isopropyl alcohol	ACN with 0.1% formic acid	3	0 7 25 30	60 60 100 60	40 40 0 40
GLUCPAC1	Hamilton PRP-1 Semi-Prep (250 x 10 mm, 5µ)	0.1% aqueous formic acid	ACN with 0.1% formic acid	3	0 20 35 37	60 5 5 60	40 95 95 40
GLUCPAC6	Hamilton PRP-1 Semi-Prep (250 x 10 mm, 5µ)	0.1% aqueous formic acid	ACN with 0.1% formic acid	3	0 15 16 20 25 30	60 31.5 3 3 60 60	40 68.5 97 97 40 40
POLYLC02	PolyLC polyhdroxymethyl A Semi-Prep (250 x 10 mm, 5µ)	H_2O	ACN	4	0 20 35 37 40	20 95 95 20 20	80 5 5 80 80

[1] Time in minutes.
[2] Same as COLMB55T except using Phenomenex Synergi column (250 x 4.6 mm, 5µ).
[3] Used for analysis of corn oil and saponified corn oil.

Mass spectrometry was performed on a PE-Sciex API 3000 mass spectrometer (PE-Sciex, Toronto, Canada).

Molecular-weight fractionation was performed on the pyrazole-label 15 DAT immature plant samples and pyrazole-label stover samples. The soluble aqueous extract was passed through a 1K molecular weight cutoff filter, where species <1Kdal went into a filtrate, while species >1Kdal were retained above the filter. The samples were then centrifuged overnight.

Saponification of pyrazole-label and phenyl-label corn oil (from the corn grain samples) was carried out by the addition of H_2O and 10 N NaOH (60°C) to the hexane extracted oil. The product was cooled, dissolved in H_2O, then HCl was added to convert the sodium salts to free

fatty acids. The fatty acids were then partitioned with hexane. The resulting organic hexane layer was removed and the partitioning was repeated. The two hexane layers were combined, then examined by HPLC and LSC. Profiles of the resulting ultraviolet (UV) absorption and ^{14}C activity were compared to the UV profiles of authentic corn oil and three of the major fatty acids that constitute corn oil (oleic, linoleic and palmitic acids).

The starch fraction produced in the natural product fractionation of the grain PES was tested for the incorporation of ^{14}C into glucose using the procedure of Nadeau *et al.*, (1991). To convert glucose molecules into glucose pentaacetate molecules, the sample was brought to dryness, and then redissolved in pyridine and acetic anhydride (incubation of the reaction mixture at 50°C for 2-4 hours). The reaction product was then taken to dryness again, redissolved in chromatographic elution buffer and analysed by HPLC methods PolyLCO2 and GLUCPAC6 (TABLE B.4.2). In addition, an aliquot of the same sample was spiked with ^{14}C-glucose and acetylated under the same conditions to verify that the conversion to glucose pentaacetate was complete.

Identification of major metabolites (parent and the metabolite M670H05) was based upon both co-chromatography with known reference compounds and mass spectrometry. Minor metabolites (e.g. M670H03) were based on co-chromatography only. Co-injection experiments were conducted to confirm the presence of the parent and the metabolites by HPLC method COLMB55T (TABLE B.4.2).

The LOQs were based on twice the background level of activity (in the range of 40 dpm), the total amount of sample being measured, the specific activity of the test substance and the sample weight. Values obtained from blank combustion were used for calculations involving the background level of radioactivity of samples. The LOQs for phenyl-label corn forage, stover and grain were 0.001219 ppm, 0.000738 ppm and 0.000674 ppm, respectively. The LOQs for pyrazole-label corn forage, stover and grain were 0.000491 ppm, 0.000298 ppm and 0.000272 ppm, respectively.

FIGURE B.4.1. Flowchart of Phenyl-label Grain PES Hydrolysis

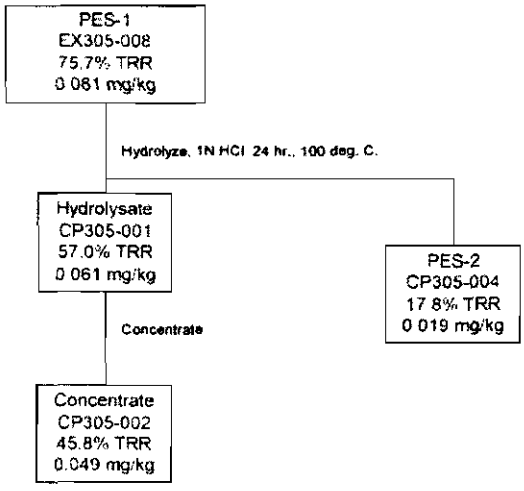


FIGURE B.4.2. Flowchart of Saponification of Phenyl-label Corn Oil

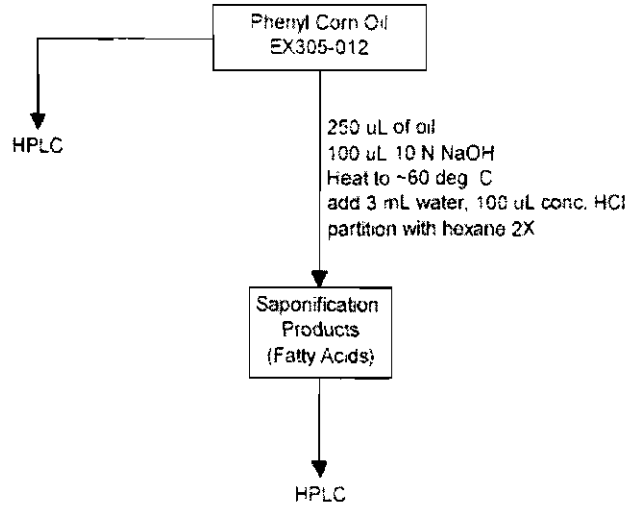
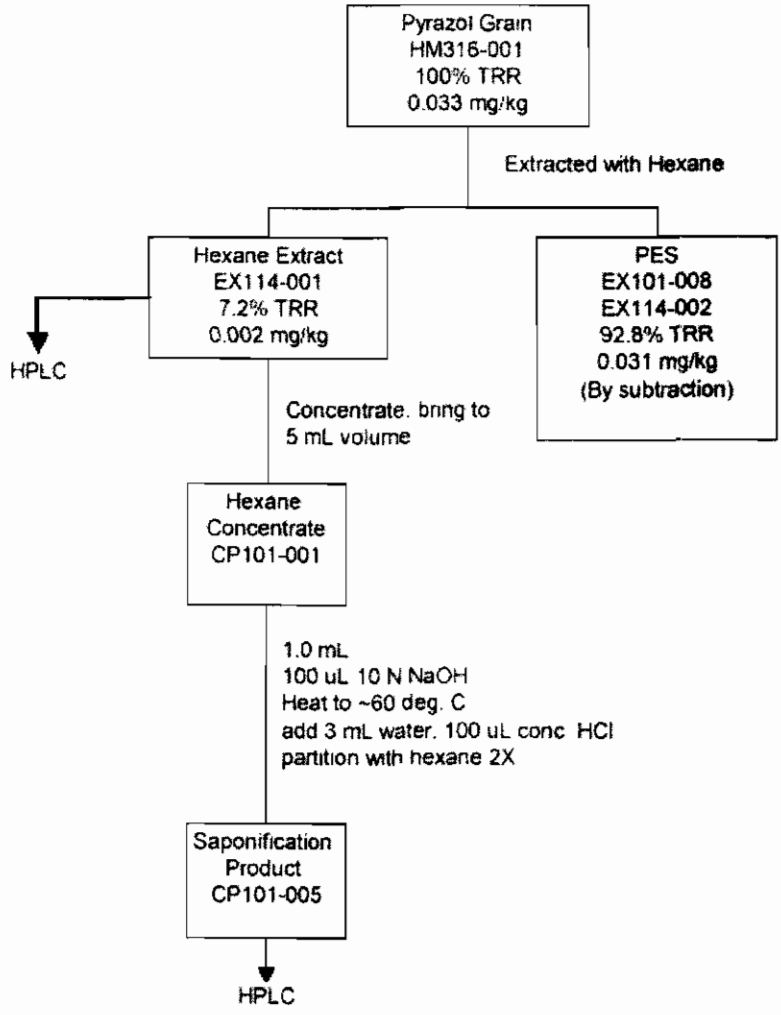


FIGURE B.4.3. Flowchart of Saponification of Pyrazole-label Corn Oil



C. RESULTS AND DISCUSSION

The petitioner provided overall TRRs determined by both combustion/LSC and by calculation (the sum of total extractable residues and total unextractable residues). The TRRs were similar among the phenyl- and pyrazole-label corn samples (immature and RAC) (TABLE C.2.1). Calculated TRRs in phenyl-label corn samples (146.4 g a.i./ha) were 7.645 ppm at 1 DAT; 2.089 ppm at 9 DAT; 0.827 ppm at 16 DAT; 0.468 ppm at 30 DAT; 0.534 ppm for forage; 0.730 ppm for stover; and 0.107 ppm for grain (FIGURES C.2.1 and C.2.2). TRRs by combustion/LSC in phenyl-label corn samples were 8.181 ppm at 1 DAT; 2.166 ppm at 9 DAT; 0.850 ppm at 16 DAT; 0.526 ppm at 30 DAT; 0.512 ppm for forage; 0.758 ppm for stover; and 0.108 ppm for grain. Calculated TRRs in pyrazole-label corn samples (148.4 g a.i./ha) were 7.681 ppm 1 DAT; 2.130 ppm at 9 DAT; 1.163 ppm at 15 DAT; 0.544 ppm at 29 DAT; 0.294 ppm for forage; 0.213 ppm for stover; and 0.032 ppm for grain. TRRs by combustion/LSC in pyrazole-label corn samples were 6.496 ppm at 1 DAT; 2.192 ppm at 9 DAT; 1.246 ppm at 15 DAT; 0.574 ppm at 29 DAT; 0.326 ppm for forage; 0.264 ppm for stover; and 0.033 ppm for grain. Larger samples were available for the purpose of calculating TRRs in comparison to the smaller (and less homogenous) samples available for combustion. Therefore, the TRRs in the rest of this study are based on calculated TRRs.

As per FIGURE C.2.1, the overall TRRs were similar for both labels in immature corn plants. However, a far greater percentage of TRRs were attributed to the phenyl-label samples compared to the pyrazole-label in the RAC matrices (FIGURE C.2.2). This may have resulted from the more efficient breakdown of the pyrazole ring into natural products. Residues in both the phenyl- and pyrazole-label immature plant samples decreased with increasing PHI, indicating that either chemical degradation or an increase in plant mass had occurred.

As reported in TABLE C.2.2.1, the majority of phenyl-label residues in the immature corn samples were in the aqueous extract (83.5-90.1% of the TRRs; 0.391-6.89 ppm). The predominant metabolite identified in all the immature samples was the parent topramezone (55.7-69.9% of the TRRs; 0.264-4.255 ppm), while the metabolites M670H03 and M670H05 each comprised less than 10% of the TRRs (≤0.717 ppm). Approximately 5.9-13.0% of the TRRs were characterized as a series of minor polar peaks/regions (0.052-1.00 ppm). The unextractable residues accounted for 9.9-16.5% of the TRRs (0.077-0.755 ppm), for an accountability ranging from 89.9-93.5% (TABLE C.2.3.1). Although the PES accounted for more than 10% of the TRRs in the 9, 16 and 30 DAT samples, further extraction or fractionation was not conducted. However, the collection of these immature samples are not required, but facilitate in the identification/characterization of residues present in the RACs.

The majority of pyrazole-label residues in the immature corn samples (TABLE C.2.2.2) were also in the aqueous extract (66.9-94.2% of the TRRs; 0.364-7.233 ppm). The predominant metabolite identified in all the immature samples was again the parent topramezone (33.2-78.7% of the TRRs; 0.181-6.046 ppm). Unlike the phenyl-label, the only other metabolite identified in the pyrazole label was M670H03 at less than 6% of the TRRs (≤0.412 ppm). Approximately 4.9-11.4% of the TRRs were characterized in the 9, 15 and 29 DAT samples as a series of minor

polar peaks/regions (0.026-0.137 ppm). The unextractable residues accounted for 5.8-33.1% of the TRRs (0.18-0.475 ppm). The accountability ranged from 71.3-89.9% (Table C.2.3.2). Once again further extraction/fractionation of the PES was not conducted, even though it accounted for more than 10% of the TRRs in the 9, 15 and 29 DAT samples. It should be noted again that the collection of these immature samples are not required, but facilitate in the identification/characterization of residues present in the RACs. The pyrazole-label 15 DAT immature plant samples were analysed further by molecular weight fractionation to characterize residues. Approximately 21% of the soluble extract was >1 Kdal (above the filter), while 66% of the soluble extract was <1 Kdal (in the filtrate). The parent topramezone was determined to constitute the majority of residues <1 Kdal.

Radiochromatographic analysis of both the phenyl- and pyrazole-label immature aqueous extracts indicated a peak at 30 minutes at all DAT (1, 9, 15-16, 29-30). Co-injection experiments indicated this peak to be the parent topramezone. As well, 2 bands of residues at 40-55 minutes and 5-15 minutes were seen in samples with a higher DAT. A peak at 20 minutes was seen in the early immature plants, but appeared to diminish over time. In phenyl-label immature samples, a peak was also seen at 15 minutes. The co-injection experiment indicated this peak to be the metabolite M670H05. Radiochromatographic analyses also indicated the presence of a peak at approximately 48 minutes that wasn't seen in the aqueous extracts. This was determined by co-injection to be the acid metabolite M670H08.

The majority of phenyl-label residues in the forage and stover samples (TABLE C.2.2.3) were in the concentrated aqueous extract (65.3-67.6% of the TRRs; 0.361-0.477 ppm), with fewer residues in the hydrolysed aqueous extract (38.8-59.5% of the TRRs; 0.207-0.434 ppm). The parent topramezone was the predominant metabolite identified at 40.4-40.9% of the TRRs (0.216-0.299 ppm), while the metabolites M670H03 and M670H05 each comprised less than 11% of the TRRs (≤0.075 ppm). The metabolite M670H08, found only in the hydrolysed aqueous extract, comprised 1.2-2.9% of the TRRs (0.006-0.021 ppm). Approximately 9.2-10.7% of the TRRs were characterized as a series of minor polar peaks/regions (0.057-0.067 ppm). The unextractable residues accounted for 19.7-22.3% of the TRRs (0.119-0.144 ppm). The PES were subjected to fractionation procedures to further characterize residues. The fractionation procedures were exhaustive, and resulted in the further characterization of 16.9-17.3% of the TRRs (0.09-0.126 ppm) in the phenyl-label forage and stover. The majority of residues consisted of soluble polysaccharides from the NaOH supernatant (8.1-10.9% of the TRRs; 0.058-0.059 ppm), with proteins, lignins and cellulose each comprising ≤3.0% of the TRRs (≤0.016 ppm). The remaining bound residues were only 2.4-5.4% of the TRRs (0.018-0.029 ppm) after fractionation, for an overall accountability ranging from 84.1-85.3% (TABLE C.2.3.3).

The pyrazole-label residues in the forage and stover samples (TABLE C.2.2.4) appeared to be similarly split between the concentrated aqueous extract (33.3-45.5% of the TRRs; 0.097-0.098 ppm), and the hydrolysed aqueous extract (30.3-31.6% of the TRRs; 0.067-0.089 ppm) in comparison to the phenyl-label. The parent topramezone was again the predominant metabolite identified at 16.9-19.6% of the TRRs (0.042-0.05 ppm), while the metabolites M670H03 and M670H08 each comprised less than 5% of the TRRs (≤0.011 ppm). Unlike the phenyl-label,

M670H05 was not identified in these samples. Approximately 13.6-19.6% of the TRRs were characterized as a series of minor polar peaks/regions (0.040-0.042 ppm). The unextractable residues accounted for 38.0-50.7% of the TRRs (0.081-0.149 ppm). A further 28.1-35.3% of the TRRs (0.075-0.083 ppm) from the PES subjected to fractionation procedures were characterized. Once again, the majority of residues consisted of soluble polysaccharides from the NaOH supernatant (11.2-21.6% of the TRRs; 0.033-0.046 ppm), with starch, lignins and cellulose each comprising <5% of the TRRs (≤0.012 ppm). The remaining bound residues were 2.7-22.6% of the TRRs (0.006-0.066 ppm) after fractionation, for an overall accountability ranging from 86.4-88.1% (TABLE C.2.3.4).

The phenyl- and pyrazole-label residues in grain samples (TABLE C.2.2.5 and C.2.2.6) were similar in that the majority of residues were in the PES (75.7-87.5% of the TRRs; 0.028-0.081 ppm). Fewer residues were in the ACN:water extracts (10.3-12.1% of the TRRs; 0.003-0.013 ppm). Only the pyrazole-label had residues in the hydrolysed extract (4.4% of the TRRs; 0.0014 ppm). The parent topramezone was identified at 2.1-2.5% of the TRRs (0.0008-0.002 ppm), while the metabolite M670H05 was identified only in the phenyl-label samples at 3.4% of the TRRs (0.004 ppm). Approximately 6.1-6.6% of the TRRs were characterized as a series of minor polar peaks/regions (0.0021-0.007 ppm). Further extraction/fractionation procedures were able to characterize 68.7-71.0% of the TRRs for phenyl- and pyrazole-label grain (0.022-0.076 ppm). The majority of residues characterized consisted of starch from the DMSO + EtOH supernatant precipitate (31.3-57.1% of the TRRs, 0.010-0.061 ppm). There were more residues characterized from the pyrazole-label sample (21.9% of the TRRs; 0.007 ppm) compared to the phenyl-label (5.6% of the TRRs; 0.006 ppm). Proteins, lignins and cellulose each comprised <4% of the TRRs (≤0.002 ppm) in both labelled grain matrices. The final bound residues were 4.7% of the TRRs (0.005 ppm) for the phenyl-label, and 18.8% of the TRRs (0.006 ppm) for the pyrazole-label after fractionation. The overall accountability ranged from 87.9-97.2% (TABLES C.2.3.3 and C.2.3.4).

As per FIGURE C.2.3, the parent topramezone was the predominant metabolite identified in all immature plant samples, regardless of the radiolabel position. Because the structure of M670H05 involved the pyrazole ring being cleaved off, the metabolite was not radiochemically visible in the pyrazole-label samples. Except for phenyl-label grain, the parent topramezone was the predominant metabolite in RAC matrices with both labels (FIGURE C.2.4). Since only the extracts in the mature RAC samples underwent hydrolysis, residues of M670H08 could only be identified in the mature RAC matrices and were not reported in the immature plant samples.

Radiochromatographic analyses of pyrazole- and phenyl-label forage and stover, and phenyl-label grain aqueous extracts indicated peaks for topramezone (both labels) and M670H05 (in phenyl-label matrices only). Peaks at approximately 21 minutes were seen in the RAC matrices, except for pyrazole-label stover and phenyl-label grain. This was determined by co-injection to be the metabolite M670H03. Radiochromatographic analyses of forage and stover acid hydrolysed extracts also indicated the presence of a peak at approximately 48 minutes that wasn't seen in the aqueous extracts. This was determined by co-injection to be the acid metabolite M670H08.

More PES (before fractionation) was in pyrazole-label forage and stover samples compared to the phenyl label, primarily due to increased amounts of soluble polysaccharides in the pyrazole label. After fractionation, the final unextractable residue levels were low in these matrices (<6% of the TRRs), except in pyrazole-label forage. A lesser proportion of residues characterized as soluble polysaccharides was noted in this matrix. The petitioner suggested that a metabolite common to both the phenyl and pyrazole structures was a precursor to carbohydrate formation, but that the pathway leading to incorporation into polypeptides differs. This may have been the result of the catabolism of the pyrazole ring. The cleavage of the parent at the carbonyl and pyrazole structures may generate an unstable intermediate that is rapidly broken down into components that are reincorporated into the structures of various natural products.

The hydrolysis of phenyl-label corn grain PES with HCl is depicted in FIGURE B.4.1. The saponification schemes for phenyl-and pyrazole-label corn oil are depicted in FIGURES B.4.2 and B.4.3, respectively. The peaks depicted in HPLC-UV chromatograms from both pyrazole- and phenyl-label saponified oil samples were indistinguishable from peaks from a sample of saponified commercial corn oil. Since saponification of the oil by hexane broke down the triglycerides into glycerine and fatty acid salts, the petitioner indicated that this meant the radioactivity was incorporated into the fatty acid constituents of the oil. The hydrolysis of corn PES was conducted to determine if radioactivity was incorporated into the glucose subunits of grain starch. Chromatograms of the PES samples indicated the presence of peaks with the same retention time as glucose. Pyrazole-label grain PES was not hydrolysed as extensively, but the petitioner reports similar results to that of the phenyl-label grain PES. Since the acylation of glucose with acetic anhydride forms glucose pentaacetate, the PES sample was acylated to determine the presence of radioactivity in glucose. The results depicted the presence of chromatographic species similar to glucose pentaacetate. Both results indicated that the radioactivity appeared to be incorporated into the glucose subunits of the PES. After catabolism, both the phenyl and pyrazole rings appear to have the ability to be reincorporated into the carbohydrate class of natural products.

The accountability demonstrated in this study was less than 100%, but it must be noted that procedural loss was observed throughout the study.

The degradation of topramezone begins with the cleavage of the pyrazole ring (by hydrolysis) from the parent to form the acid metabolite M670H05. Hydrolysis and cleavage of the pyrazole group also occurs in the formation of M670H08. Desmethylation in the phenyl ring results in the formation of M670H03. On its own, the pyrazole moiety appears to be completely catabolized, then reincorporated into the carbon backbone of natural products such as starch, soluble polysaccharides and fatty acids. The phenyl ring eventually undergoes degradation as well, before being reincorporated into natural products.

C.1. Storage Stability

TABLE C.1. Summary of Storage Conditions			
Matrix (RAC)	Storage Temp. (°C)	Actual Study Duration (months)	Interval of Demonstrated Storage Stability (months)
Corn Forage, Stover and Grain	<-18°C	≤5.1	18

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

TABLE C.2.1. Total Radioactive Residues (TRRs) in Corn Matrices

Matrix	Timing and Applic. No.	PHI (days)	Phenyl label ppm		Pyrazole label ppm	
			A	B	A	B
Whole plant	one application at 3-5 leaf stage; 148.4 g a.i./ha (pyrazole); 146.4 g a.i./ha (phenyl)	1	7.645	8.181	7.681	6.496
Whole plant	one application at 3-5 leaf stage; 148.4 g a.i./ha (pyrazole); 146.4 g a.i./ha (phenyl)	9	2.089	2.166	2.13	2.192
Whole plant	one application at 3-5 leaf stage; 148.4 g a.i./ha (pyrazole); 146.4 g a.i./ha (phenyl)	15-16	0.827	0.85	1.163	1.246
Whole plant	one application at 3-5 leaf stage; 148.4 g a.i./ha (pyrazole); 146.4 g a.i./ha (phenyl)	29-30	0.468	0.526	0.544	0.574
Forage	one application at 3-5 leaf stage; 148.4 g a.i./ha (pyrazole); 146.4 g a.i./ha (phenyl)	59-60	0.534	0.512	0.294	0.326
Stover	one application at 3-5 leaf stage; 148.4 g a.i./ha (pyrazole); 146.4 g a.i./ha (phenyl)	77	0.73	0.758	0.213	0.264
Grain	one application at 3-5 leaf stage; 148.4 g a.i./ha (pyrazole); 146.4 g a.i./ha (phenyl)	77	0.107	0.108	0.032	0.033

[A] Calculated TRRs

[B] TRRs based on combustion/LSC

TABLE C.2.2.1 Distribution of the Parent and the Metabolites in Immature Corn Plants when Dosed with ^{14}C-Phenyl Topramezone

Metabolite Fraction	1 DAT TRR= 7.645 ppm		9 DAT TRR= 2.089 ppm		16 DAT TRR= 0.827 ppm		30 DAT TRR= 0.468 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
Aqueous extract	90.1	6.89	88.7	1.853	84.5	0.699	83.5	0.391
Concentrated Aqueous Extract	88.7	6.78	84.6	1.767	80.2	0.663	81.2	0.38
Topramezone	55.7	4.255	69.9	1.46	58.3	0.482	56.4	0.264
M670H03	9.4	0.717	3.2	0.066	4.6	0.038	3.4	0.016
M670H05	1.9	0.145	2.7	0.057	4.2	0.035	4.1	0.019
1 DAT: 7 peaks/regions, each at ≤0.265 ppm **9 DAT:** 4 peaks/regions, each at ≤0.092 ppm **16 DAT:** 5 peaks/regions, each at ≤0.058 ppm **30 DAT:** 5 peaks/regions, each at ≤0.027 ppm	13	1	5.9	0.123	10.9	0.09	11	0.052
Acid Hydrolysate	73.8	5.644	62.1	1.298	29.7	0.246	N/A	N/A
Unextractable (PES)	9.9	0.755	11.3	0.236	15.5	0.128	16.5	0.077

N/A = not available

TABLE C.2.2.2 Distribution of the Parent and the Metabolites in Immature Corn Plants when Dosed with ^{14}C-Pyrazole Topramezone

Metabolite Fraction	1 DAT TRR = 7.681 ppm		9 DAT TRR = 2.130 ppm		15 DAT TRR = 1.163 ppm		29 DAT TRR = 0.544 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
Aqueous extract	94.2	7.233	77.7	1.655	77.8	0.905	66.9	0.364
Conc./Filt. Aqueous Extract	91.3	7.009	73	1.555	60.6	0.705	45.2	0.246
Topramezone	78.7	6.046	47.7	1.017	33.3	0.387	33.2	0.181
M670H03	5.4	0.412	4.4	0.093	4.4	0.051	2	0.011
9 DAT: 7 peaks/regions, each at ≤0.033 ppm **15 DAT:** 12 peaks/regions, each at ≤0.027 ppm **29 DAT:** 2 peaks/regions, each at ≤0.015 ppm	—	—	6.5	0.137	11.4	0.133	4.9	0.026
Unextractable (PES)	5.8	0.448	22.3	0.475	22.2	0.258	33.1	0.18

TABLE C.2.2.3 Distribution of the Parent and the Metabolites in Corn RAC Forage and Stover when Dosed with ^{14}C-Phenyl Topramezone

Metabolite Fraction	Corn, forage TRR = 0.534 ppm		Corn, stover TRR = 0.730 ppm	
	%TRR	ppm	%TRR	ppm
Concentrated Aqueous Extract	67.6	0.361	65.3	0.477
Topramezone	40.4	0.216	40.9	0.299
M670H03	3	0.016	0.8	0.006
M670H05	6	0.032	7.2	0.052
Forage: 8 peaks/regions, each at ≤0.013 ppm Stover: 10 peaks/regions, each at ≤0.017 ppm	10.7	0.057	9.2	0.067
Hydrolysed aqueous extract	38.8	0.207	59.5	0.434
Topramezone	24.7	0.132	39.8	0.29
M670H03	1.9	0.01	2.3	0.017
M670H05	6.6	0.035	10.2	0.075
M670H08	1.2	0.006	2.9	0.021
Forage: 5 peaks/regions, each at ≤0.004 ppm Stover: 1 peak/region at 0.001 ppm	2.7	0.014	0.1	0.001
Unextractable (PES)	22.3	0.119	19.7	0.144
Ammonia Supernatant	1.3	0.007	4.8	0.035
Ammonia Pellet (protein)	0.4	0.002	—	—
DMSO Extract	0.7	0.004	1.1	0.008
NaOH Supernatant (soluble polysaccharides)	10.9	0.058	8.1	0.059
Cellulose	3	0.016	1.2	0.009
Precipitate (Lignin)	0.6	0.003	2.1	0.015
Final Unextractable (PES)[1]	5.4	0.029	2.4	0.018

[1] Residues remaining after exhaustive extractions.

TABLE C.2.2.4 Distribution of the Parent and the Metabolites in Corn RAC Forage and Stover when Dosed with ^{14}C-Pyrazole Topramezone

Metabolite Fraction	Corn, forage TRR = 0.294 ppm		Corn, stover TRR = 0.213 ppm	
	%TRR	ppm	%TRR	ppm
Concentrated aqueous extract	33.3	0.098	45.5	0.097
Topramezone	10.5	0.031	19.6	0.042
M670H03	1.1	0.003	—	—
Forage: 12 peaks/regions, each at ≤0.006 ppm Stover: 11 peaks/regions, each at ≤0.011 ppm	13.6	0.04	19.6	0.042
Hydrolysed aqueous extract	30.3	0.089	31.6	0.067
Topramezone	16.9	0.05	15	0.032
M670H03	3.1	0.009	4.2	0.009
M670H08	3.6	0.011	4.6	0.01
Forage and stover: 4 peaks/regions, each at ≤0.004 ppm	3.4	0.011	5.3	0.012
Unextractable (PES)	50.7	0.149	38	0.081
Ammonia Supernatant	8.8	0.026	8	0.017
DMSO + EtOH Supernatant	1.7	0.005	1.9	0.004
DMSO precipitate (Starch)	0.3	0.001	0.5	<0.001
NaOH Supernatant (soluble polysaccharides)	11.2	0.033	21.6	0.046
Precipitate (Lignin)	4.1	0.012	0.5	0.001
Cellulose	2	0.006	2.8	0.006
Final Unextractable (PES)[1]	22.6	0.066	2.7	0.006

[1] Residues remaining after exhaustive extractions.

TABLE C.2.2.5 Distribution of the Parent and the Metabolites in Corn RAC Grain when Dosed with ^{14}C-Phenyl Topramezone

Metabolite Fraction	Corn, grain TRR = 0.107 ppm	
	%TRR	ppm
Concentrated ACN:H_2O Extract	12.1	0.013
BAS 670 H	2.1	0.002
M670H05	3.4	0.004
4 peaks/regions, each at ≤0.005 ppm	6 6	0.007
Unextractable (PES)	75.7	0.081
Ammonia Supernatant	0 9	0.001
Ammonia Pellet (protein)	0 9	0.001
DMSO + EtOH Supernatant	3 7	0.004
Precipitate (Starch)	57.1	0.061
NaOH Supernatant (Soluble Polysaccharides)	5.6	0.006
Precipitate (Lignin)	0.9	<0.001
Cellulose	1.9	0.002
Final Unextractable (PES)[1]	4.7	0.005

[1] Residues remaining after exhaustive extractions.

TABLE C.2.2.6 Distribution of the Parent and the Metabolites in Distribution of the Parent and the Metabolites in Corn RAC Grain when Dosed with ^{14}C-Pyrazole Topramezone

Metabolite Fraction	Corn, grain TRR = 0.032 ppm	
	%TRR	ppm
Concentrated ACN:H_2O Extract	10.3	0.003
Topramezone	2.5	0.0008
6 peaks/regions, each at ≤0.0011 ppm	6.1	0.0021
Hydrolysed Concentrated Extract	4.4	0.0014
BAS 670 H	1.4	0.0004
2 peaks/regions, each at ≤0.002 ppm	1.2	0.0003
Unextractable (PES)	87.5	0.028
Ammonia Supernatant	3.1	0.001
Ammonia Pellet (protein)	3.1	0.001
DMSO + EtOH Supernatant	3.1	0.001
Precipitate (Starch)	31.3	0.01
NaOH Supernatant (Soluble Polysaccharides)	21 9	0.007
Precipitate (Lignin)	3.1	0.001
Cellulose	3.1	0.001
Final Unextractable (PES)[1]	18.8	0.006

[1] Residues remaining after exhaustive extractions.

Table C.2.3.1 Summary of Characterization and Identification of Radioactive Residues in Immature Corn Plants Following Application of Phenyl-label Topramezone

Compound	1 DAT TRR = 7.645 ppm		9 DAT TRR = 2.089 ppm		16 DAT TRR = 0.827 ppm		30 DAT TRR = 0.468 ppm	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
Total identified	66.9	5.117	75.8	1.583	67.1	0.555	63.9	0.299
Topramezone	55.7	4.255	69.9	1.46	58.3	0.482	56.4	0.264
M670H03	9.4	0.717	3.2	0.066	4.6	0.038	3.4	0.016
M670H05	1.9	0.145	2.7	0.057	4.2	0.035	4.1	0.019
Total characterized	13.1	1.001	5.9	0.123	10.9	0.09	11.0	0.052
Total extractable	80.0	6.117	81.7	1.706	78	0.645	74.9	0.351
Unextractable (PES)	9.9	0.755	11.3	0.236	15.5	0.128	16.5	0.077
Accountability[1]	89.9%		93.0%		93.5%		91.5%	

[1] Accountability = (Total extractable + Total unextractable)/(calculated TRRs) * 100.

Table C.2.3.2 Summary of Characterization and Identification of Radioactive Residues in Immature Corn Plants Following Application of Pyrazole-label Topramezone

Compound	1 DAT TRR = 7.681 ppm		9 DAT TRR = 2.130 ppm		15 DAT TRR = 1.163 ppm		29 DAT TRR = 0.544 ppm	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
Total identified	84.1	6.458	52.1	1.11	37.7	0.438	35.2	0.192
Topramezone	78.7	6.046	47.7	1.017	33.3	0.387	33.2	0.181
M670H03	5.4	0.412	4.4	0.093	4.4	0.051	2.0	0.011
Total characterized	—	—	6.5	0.137	11.4	0.133	4.9	0.026
Total extractable	84.1	6.458	58.6	1.247	49.1	0.571	40.1	0.218
Unextractable (PES)	5.8	0.448	22.3	0.475	22.2	0.258	33.1	0.18
Accountability[1]	89.9%		80.8%		71.3%		73.2%	

[1] Accountability = (Total extractable + Total unextractable)/(calculated TRRs) * 100.

Table C.2.3.3 Summary of Characterization and Identification of Radioactive Residues in Corn RAC Following Application of Phenyl-label Topramezone

Compound	Corn, forage TRR = 0.534 ppm		Corn, stover TRR = 0.730 ppm		Corn, grain TRR = 0.107 ppm	
	% TRR	ppm	% TRR	ppm	% TRR	ppm
Total identified [1]	51.2	0.273	56.3	0.412	5.5	0.006
Topramezone	40.4	0.216	40.9	0.299	2.1	0.002
M670H03	3.0	0.016	2.3	0 017	—	—
M670H05	6.6	0.035	10.2	0.075	3.4	0.004
M670H08	1.2	0.006	2.9	0.021	—	—
Total characterized [2]	27.6	0.147	26.5	0.193	77.6	0.083
Final Extractable	78.8	0.42	82.8	0.605	83.1	0.089
Final Unextractable (PES) [3]	5.4	0.029	2.4	0.018	4.7	0.005
Accountability [4]	84.1%		85.3%		87.9%	

[1] If the metabolite was detected in the concentrated aqueous extract, and subsequently in the hydrolysed aqueous extract, the highest concentration value for the metabolite was used in determining the total identified.

[2] If characterized residues were detected in the concentrated aqueous extract , as well as in the hydrolysed aqueous extract, the highest concentration value for characterized residues was used in determining the total characterized.

[3] Residues remaining after exhaustive extractions.

[4] Accountability = (Final extractable + Final unextractable)/(calculated TRRs) * 100.

Table C.2.3.4 Summary of Characterization and Identification of Radioactive Residues in Corn RAC Following Application of Pyrazole-label Topramezone

Compound	Corn, forage TRR = 0.294 ppm		Corn, stover TRR = 0.213 ppm		Corn, grain TRR = 0.032 ppm	
	% TRR	ppm	% TRR	ppm	% TRR	ppm
Total identified [1]	23.6	0.07	28.4	0.061	2.5	0.001
Topramezone	16.9	0.05	19.6	0.042	2.5	0.001
M670H03	3.1	0.009	4.2	0.009	—	—
M670H05	—	—	—	—	—	—
M670H08	3.6	0.011	4.6	0 01	—	—
Total characterized [2]	41.7	0.123	54.9	0.117	74.8	0.0241
Final Extractable	65.3	0.193	83.3	0.178	77.3	0.0251
Final Unextractable (PES) [3]	22.6	0.066	2.7	0.006	18.8	0.006
Accountability [4]	88.1%		86.4%		97.2%	

[1] If the metabolite was detected in the concentrated aqueous extract, and subsequently in the hydrolysed aqueous extract, the highest concentration value for the metabolite was used in determining the total identified.

[2] If characterized residues were detected in the concentrated aqueous extract, as well as in the hydrolysed aqueous extract, the highest concentration value for characterized residues was used in determining the total characterized.

[3] Residues remaining after exhaustive extractions.

[4] Accountability = (Final extractable + Final unextractable)/(calculated TRRs) * 100.

FIGURE C.2.1. Calculated TRRs in Immature Plants Treated at the 3- to 5-Leaf Stage With Radiolabelled Topramezone

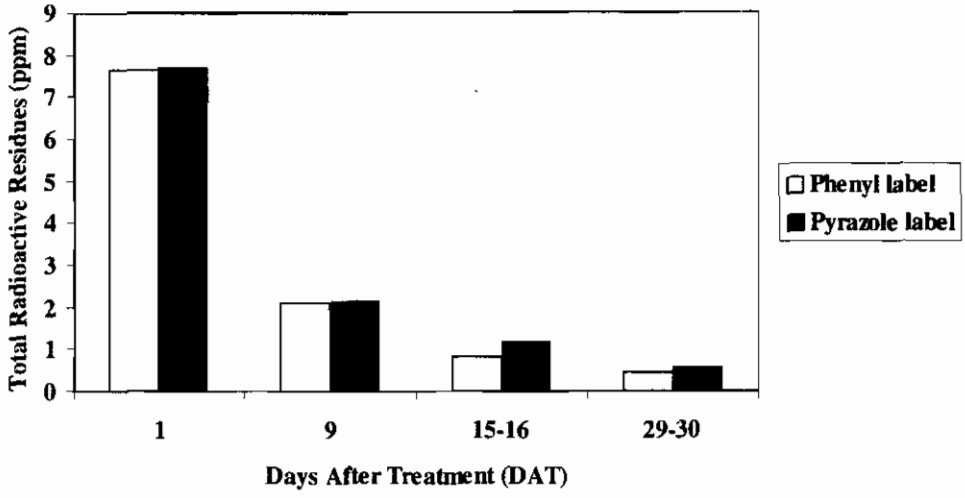


FIGURE C.2.2. Calculated TRRs in Corn RAC Fractions Treated at the 3- to 5-Leaf Stage With Radiolabelled Topramezone

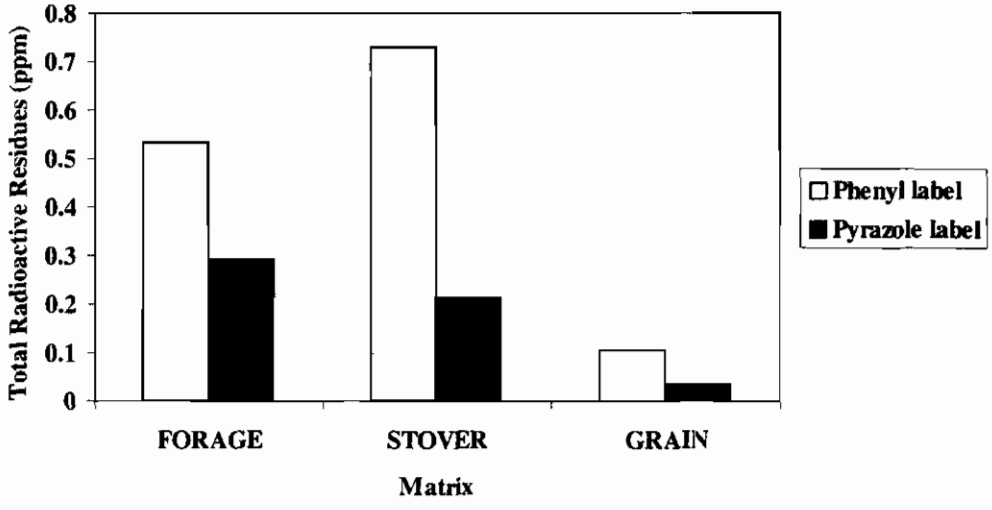
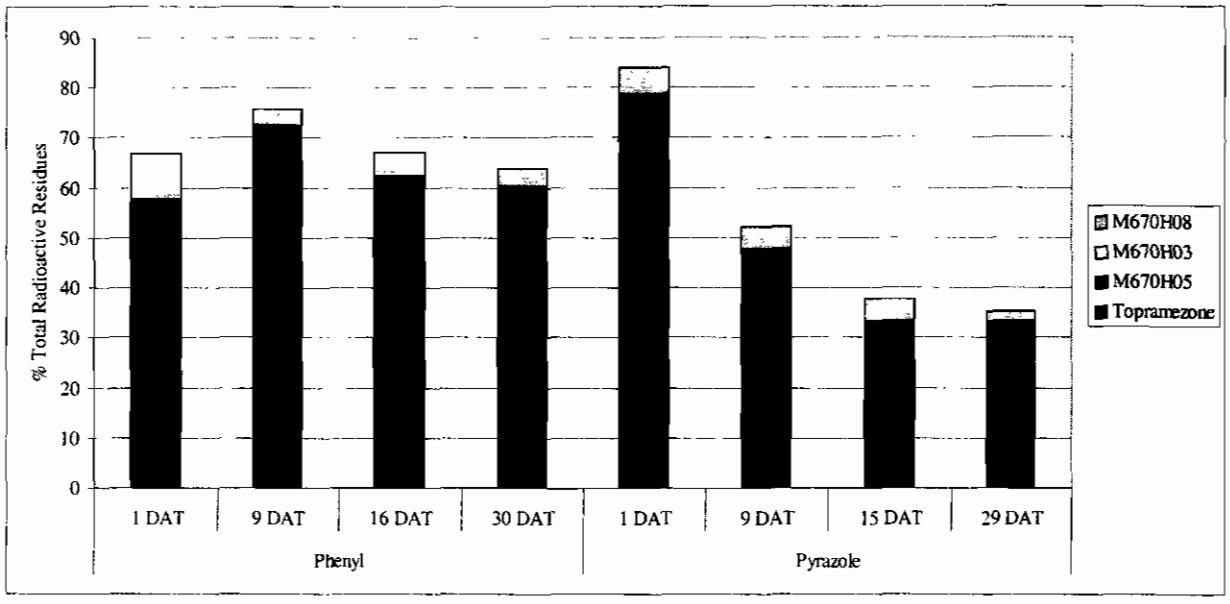
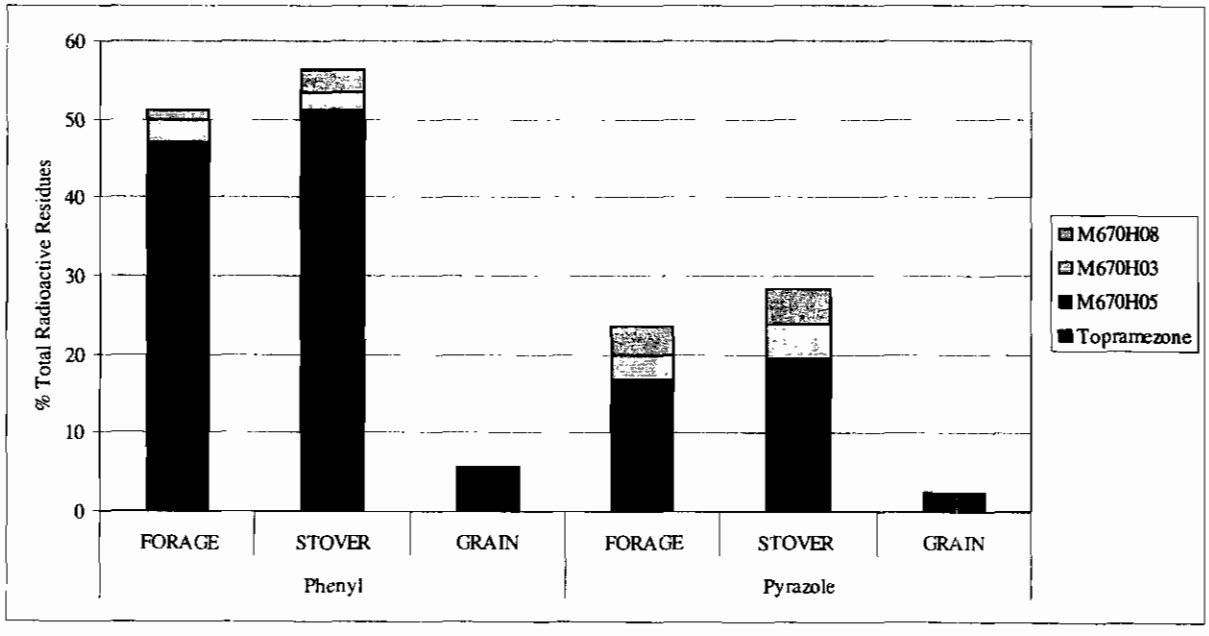


FIGURE C.2.3. Distribution of Topramezone and Minor Metabolites in Immature Corn Plants Following One Application of Radiolabelled Topramezone*



*Based on calculated TRRs

FIGURE C.2.4. Distribution of Topramezone and Minor Metabolites in Corn RAC Fractions Following One Application of Radiolabelled Topramezone*



*Based on calculated TRRs

C.3. Proposed Metabolic Profile

FIGURE C.3.1. Proposed Metabolic Profile of Topramezone in Corn

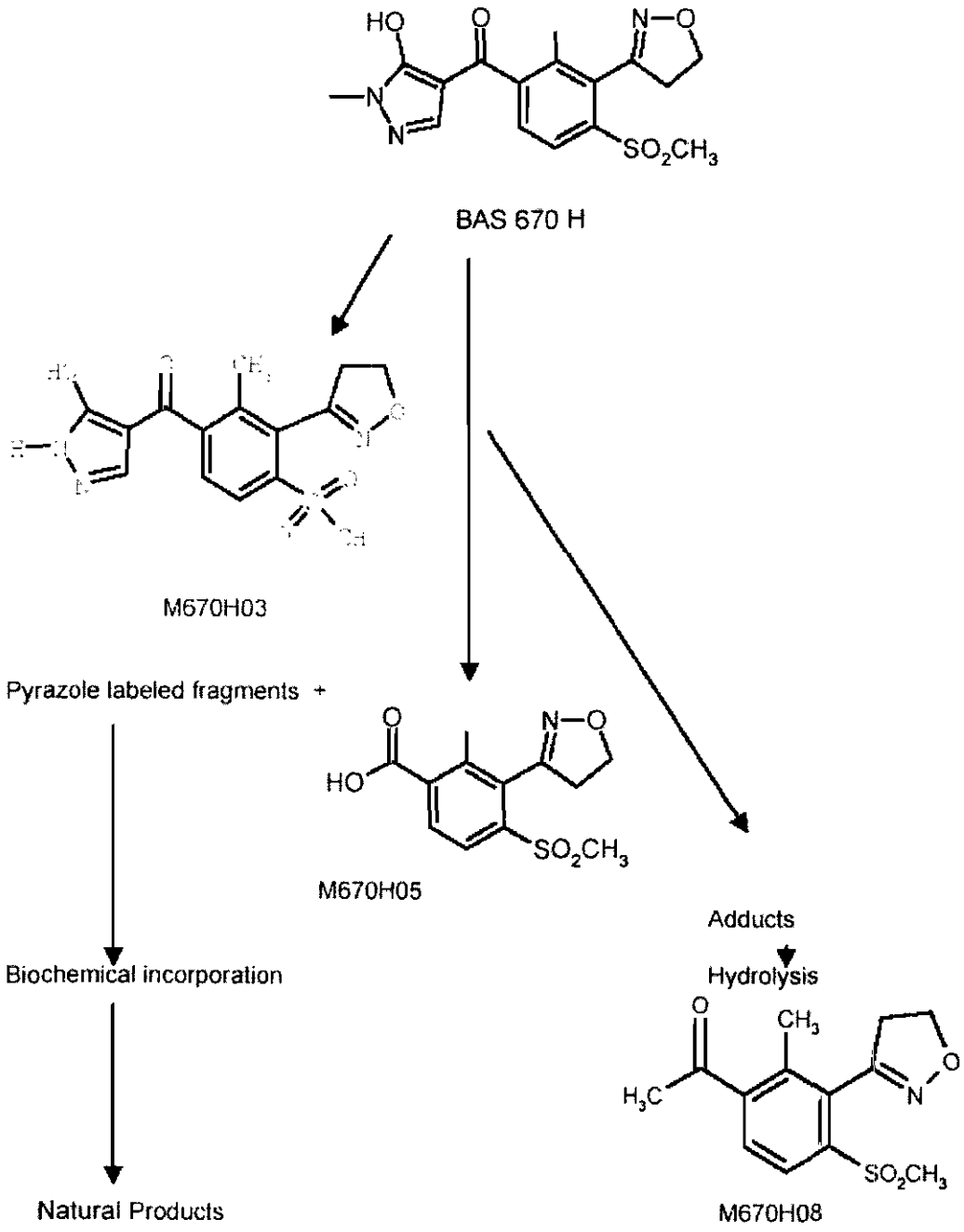
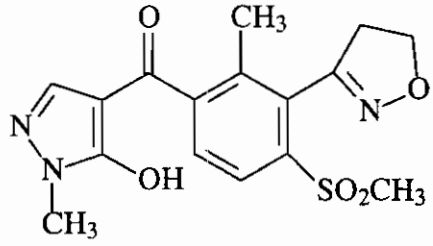
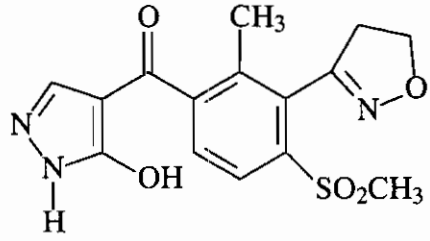
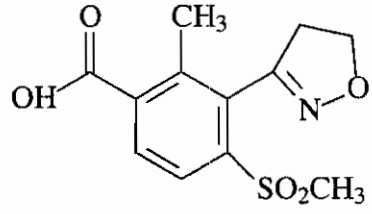
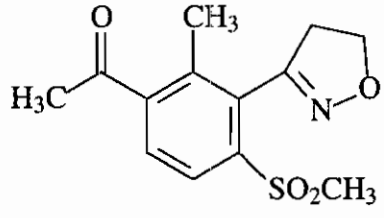


TABLE C.3.1. Identification of Compounds from Metabolism Study

Common name/code Figure C.3.1 ID No.	Chemical name	Chemical structure
Topramezone	[3-(4,5-dihydro-3-isoxazolyl)-2-methyl-4-(methylsulfonyl)phenyl](5-hydroxy-1-methyl-1*H*-pyrazol-4-yl)methanone	
M670H03	Des-N-methyl	
M670H05	3-(4,5-dihydro-isoxazol-3-yl)-4-methanesulfonyl-2-methylbenzoic acid-[phenyl-U-^{14}C]	
M670H08	Methyl Ketone	

D. CONCLUSION

Topramezone was applied to corn at the 3-5 leaf stage as a single foliar application at 0.146 (phenyl)-0.148 (pyrazole) kg a.i./ha (0.130-0.132 lb a.i./A). Total extractable residues in phenyl- and pyrazole-label corn RACs ranged from 65.3-83.3% of the TRRs (0.0251-0.605 ppm), while final bound residues ranged from 2.4-22.6% (0.005-0.066 ppm). The unchanged parent compound topramezone was the predominant residue identified in both phenyl- and pyrazole-label forage and stover (16.9-40.9% of the TRRs; 0.042-0.299 ppm), but was a minor metabolite in grain (<2.5% of the TRRs; <0.002 ppm). M670H05, M670H03 and M670H08 were identified at <10.2% of the TRRs (<0.075 ppm) in both labelled RAC matrices. Approximately 26.5-

77.6% of the TRRs were characterized in both labelled RAC matrices (0.0241-0.193 ppm). Topramezone underwent hydrolytic cleavage to form the acid metabolite M670H05 and M670H08, while desmethylation formed M670H03. The phenyl and pyrazole rings were catabolized, then reincorporated into natural products.

E. REFERENCES

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Jordan, J. (2002). Storage Stability of BAS 670 H and its Cleaved Acid Metabolite M670H05 in Plant Matrices: Lab Project Number: 56750. Unpublished study prepared by BASF Corporation. 67 pages.

Nadeau, R.G., Howe, R.K., Burnett, T. J., and Lange, B. D. (1991). Characterization of ^{14}C Residues in the Grain of Rice Plants Grown in Soil Treated with [phenyl-^{14}C-]-2-(Diphenylmethoxyacetic Acid Methyl Ester). J. Agric. Food Chem. 39, 2285-2289.

F. DOCUMENT TRACKING

RDI: P.V. Shah (3/2/05), RAB1 Chemists (12/8/04), ChemSAC (3/2/05)
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PC Code:	**123009**
HED File Code	**11500 Petition Files Chemistry**
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