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U. S. ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

**MEMORANDUM**

DATE: 9/04/02

SUBJECT: PP# 1F06313. **BAS 510 F** in/on Various Plant and Animal Commodities.
**Briefing Memo for HED's Metabolism Assessment Review Committee.**

DP Barcode: **D284952**
PC Code: 128008

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MARC/HED

## QUESTIONS FOR THE MARC

**Target Crops**

1. Does the MARC agree with the petitioner's proposal that only parent compound be regulated in primary (target) crops?

**Animal Matrices**

2. Does the MARC agree with the petitioner's proposal that only combined residues of parent and its hydroxylated metabolite M510F01 be regulated in animal matrices?

**Rotational Crops**

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3. Does the MARC agree with the petitioner's proposal that only parent compound be regulated in rotational crops?

**Residue of Concern for Dietary Risk Assessments**

4. What residue(s) in target crops, rotational crops, animal matrices, and drinking water should be included in the dietary risk assessments?

## USE INFORMATION/IDENTIFICATION OF CHEMICAL

**Proposed Tolerances**

BASF Corporation has submitted a petition for the establishment of permanent tolerances for residues of the new chemical fungicide BAS 510 F in/on various plant and animal commodities. This submission is being reviewed jointly by the US EPA and PMRA Canada.

The proposed tolerances in both countries for residues of BAS 510 F [3-pyridinecarboxamide, 2-chloro-N-(4'-chloro(1,1'-biphenyl)-2-yl)] in/on raw agricultural and processed plant commodities are as follows:

| Primary (Target) Crops | ppm |
|---|---|
| Tuberous and corm vegetables (crop group 1-C) | 0.05 |
| Bulb vegetables (crop group 3) | 3.0 |
| Leafy vegetables (crop group 4) | 11.0 |
| Legume vegetables - Beans (crop group 6) | 2.2 |
| Fruiting vegetables (crop group 8) | 1.0 |
| Stone fruit (crop group 12) | 1.7 |
| Berries (crop group 13) | 3.5 |
| Tree nuts (crop group 14) | 0.25 |
| Almond hulls | 3.0 |
| Pistachios | 0.65 |
| Grapes | 3.5 |
| Raisins | 8.5 |
| Strawberries | 1.2 |
| Peanut | 0.05 |
| Peanut meal | 0.15 |
| Peanut oil | 0.15 |
| Canola | 3.5 |

| Rotational Crops | ppm |
|---|---|
| Root vegetables (crop group 1-B) | 1.0 |
| Beet root | 1.0 |
| Leaves of root and tuber vegetables (crop group 2) | 1.0 |

| | |
|---|---|
| Head and Stem *Brassica* (crop subgroup 5-A) | 3.0 |
| Leafy *Brassica* greens (crop subgroup 5-B) | 18.0 |
| Legume vegetables - Edible Peas (crop group 6) | 2.2 |
| Foliage of legume vegetables (crop group 7) | |
| forage | 1.5 |
| hay | 2.0 |
| vines | 0.05 |
| Cucurbit vegetables (crop group 9) | 1.5 |
| Cereal grains (crop group 15) | 0.20 |
| Rice hulls | 0.5 |
| Forage, fodder and straw of cereal grains (crop group 16) | |
| forage | 2.0 |
| straw | 3.0 |
| fodder | 1.5 |
| Grass forage, fodder and hay (crop group 17) | |
| forage | 2.0 |
| hay | 8.0 |
| straw | 0.3 |
| seed | 0.2 |
| Non-grass animal feeds (crop group 18) | |
| forage | 1.0 |
| hay | 2.0 |
| seed | 0.05 |
| Mint | 30.0 |
| Cotton seed | 0.05 |
| Cotton gin by-products | 0.3 |
| Soybean seed | 0.1 |
| Soybean hulls | 0.2 |
| Flax seed | 3.5 |
| Sunflower seed | 3.5 |

The proposed tolerances for the combined residues of BAS 510 F [3-pyridinecarboxamide, 2-chloro-N-(4'-chloro(1,1'-biphenyl)-2-yl)] and its metabolite 2-chloro-N-(4'-chloro-5-hydroxy-biphenyl-2-yl)nicotinamide [aka M510F01], expressed in BAS 510 F equivalents, in animal commodities, are as follows:

| | ppm |
|---|---|
| Cow milk | 0.10 |
| Cow muscle | 0.10 |
| Cow fat | 0.30 |
| Cow meat by-products | 0.35 |
| Eggs | 0.02 |
| Poultry muscle | 0.05 |
| Poultry fat | 0.05 |

Poultry meat by-products ..... 0.05

**Mode of Action**

BAS 510 F (nicobifen, proposed common name) is a new foliar systemic fungicide chemically belonging to the class of oxathin fungicides, also known as carboxamide or anilide fungicides. BAS 510 F acts in the fungal cell by inhibition of mitochondrial respiration through inhibition of the succinate-ubiquinone oxidase reductase system in Complex II of the mitochondrial electron transport chain. Carboxin and flutolanil also have this mode of action. However, the spectrum of disease control of BAS 510 F is reportedly quite different. BAS 510 F inhibits spore germination, germ tube elongation, mycelial growth, and sporulation of the fungus on the leaf surface. BAS 510 F is not known to produce any metabolites that are common to any other pesticide.

**End Use Formulations**

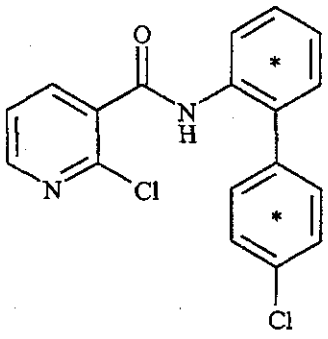
There are three end use products proposed for registration. **BAS 510 02 F Turf Fungicide** is a 70% WG (wettable granule) formulation containing BAS 510 F as sole active ingredient (ai); it is intended for use on golf course turf grass only. **BAS 510 02 F Crop Fungicide** is also a 70% WG formulation containing BAS 510 F as sole ai; it is intended for the proposed terrestrial food crop uses. **BAS 516 02 F Crop Fungicide** is a WG formulation containing 38% ai as a 2:1 mixture of BAS 510 F and BAS 500 F (pyraclostrobin); it also is intended for the proposed terrestrial food crop uses.

**Proposed Use Patterns**

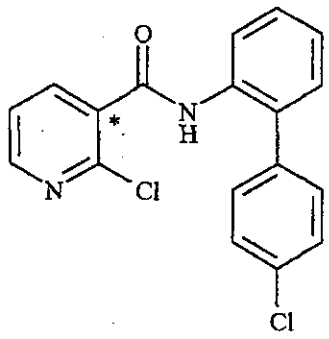
BAS 510 F is applied by ground, air, or sprinkler irrigation as a multiple application, full coverage, foliar spray with an adjuvant included in the spray mix. The application rate (0.10-0.55 lb ai/A), number of applications (2-6), between-spray interval (5-28 days), and PHI (0-30 days) vary significantly, depending on the target crop and the target disease. The maximum rate/season on the subject target crops ranges from 0.80 (canola) to1.8 (onions) lbs ai/A/year. (On golf course turf grass, the maximum rate/season is 3.0 lbs ai/A.)

**NATURE OF THE RESIDUE - PLANTS**

The petitioner has submitted plant metabolism studies for BAS 510 F in/on lettuce, beans and grapes. In addition, a confined crop rotation study was submitted. The test compound for these studies was [$^{14}$C] BAS 510 F uniformly labeled in the phenyl rings or labelled in the 3 position of the pyridine ring. The structure and position of the labels are presented below.



$^{14}$C-Diphenyl label



$^{14}$C-Pyridine label

**Grapes**

BASF Corporation has submitted a study investigating the metabolism of [$^{14}$C]BAS 510 F in grapes. Grape bunches and leaves were collected 45 days following the last of three foliar applications of [$^{14}$C]BAS 510 F at 0.713 lb ai/A/application (800 g ai/ha/application for a total of 2.13 kg ai/ha, ≈ 2X proposed gap). Grape bunches were separated into grapes and stalks. TRR (calculated by summing extractable and nonretractable residues) are presented in **Table 1** below.

Table 1. Total Radioactive Residues in Grape Commodities Following Three Foliar Applications of $^{14}$C- Labeled BAS 510 F

| Label Location | Crop Matrix | Application Rate | PHI, days | TRR, ppm | | % Mass Balance[3] |
|---|---|---|---|---|---|---|
| | | | | Combustion[1] | Calculation[2] | |
| Biphenyl label | Grapes | 3 x 0.713 lb ai/A | 45 | 1.086 | 1.181 | 108.7% |
| | Grape stalks | | 45 | Not determined | 12.356 | -- |
| | Grape leaves | | 45 | 44.451 | 43.672 | 98.2% |
| Pyridine label | Grapes | 3 x 0.713 lb ai/A | 45 | 2.281 | 2.066 | 90.6% |
| | Grape stalks | | 45 | Not determined | 19.637 | -- |
| | Grape leaves | | 45 | 60.096 | 63.359 | 105.4% |

The majority of residues (>92% TRR) in grape commodities were extracted with methanol. An additional water extraction released a small amount of radioactivity (<1% TRR). Extracts were analyzed by HPLC; identification of BAS 510 F was confirmed in grapes by LC/MS/MS. The results of the extraction and identification are presented in **Table 2** below.



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| Table 2. Summary of Characterization and Identification of Radioactive Residues in Grape Commodities Following Three Foliar Applications of Isotopically Labeled BAS 510 F at 0.713 lb ai/A/application. | | | | | | |
|---|---|---|---|---|---|---|
| Metabolite or Fraction | Grapes | | Grape stalks | | Grape leaves | |
| | %TRR | ppm | %TRR | ppm | %TRR | ppm |
| [Biphenyl-U-$^{14}$C]BAS 510 F | | | | | | |
| **BAS 510 F** | 92.7 | 1.095 | 96.4 | 11.914 | 95.6 | 41.752 |
| Water extract | 0.4 | 0.005 | -- | -- | -- | -- |
| Unknown | -- | -- | -- | -- | 2.4 | 1.049 |
| Total Identified (TI) | 92.7 | 1.095 | 96.4 | 11.914 | 95.6 | 41.752 |
| Total Characterized (TC) | 0.4 | 0.005 | -- | -- | 2.4 | 1.049 |
| Total Extractable (TE) | 93.1 | 1.1 | 96.4 | 11.914 | 98.0 | 42.801 |
| Total Bound (TB) | 6.8 | 0.081 | 3.6 | 0.442 | 2 | 0.871 |
| % Mass Balance | 99.9 | | 100.0 | | 100.0 | |
| [Pyridine-3-$^{14}$C]BAS 510 F | | | | | | |
| **BAS 510 F** | 92.2 | 1.905 | 97.5 | 19.152 | 96.1 | 60.859 |
| Water extract | 0.5 | 0.009 | -- | -- | -- | -- |
| Unknown | -- | -- | 0.1 | 0.01 | 1.8 | 1.172 |
| Total Identified (TI) | 92.2 | 1.905 | 97.5 | 19.152 | 96.1 | 60.859 |
| Total Characterized (TC) | 0.5 | 0.009 | 0.1 | 0.01 | 1.8 | 1.172 |
| Total Extractable (TE) | 92.7 | 1.914 | 97.6 | 19.162 | 97.9 | 62.031 |
| Total Bound (TB) | 7.3 | 0.15 | 2.4 | 0.475 | 2.1 | 1.328 |
| % Mass Balance | 100.0 | | 100.0 | | 100.0 | |

TC = Sum of all unidentified, extractable residues
TE = Sum of TI and TC
% Mass Balance = TE %TRR +TB % TRR. Note that the petitioner calculated TRR by summing extractable and nonretractable residues; therefore, mass balance is at or very close to 100% for all matrices. See Table 2.1 for actual mass balance based on combustion of the grape and grape leaf samples.

**No significant metabolism of BAS 510 F occurs in grapes. The unchanged parent, BAS 510 F, was the only component identified in grape commodities, accounting for 92.2-97.5% TRR** (1.095-1.905 ppm in/on grapes; 11.914-19.152 ppm in/on grape stalks; and 41.752-60.859 ppm in/on grape leaves). One unknown peak was observed in the methanol extracts of grape leaves at up to 2.4% TRR. Because of the low levels of this unknown, and the fact that grape leaves are not a major food/feed item, the petitioner did not investigate the identity of this peak. Nonextractable residues accounted for 2.0-7.3% (up to 0.15 ppm) TRR and were not further analyzed.

## Lettuce

A study investigating the metabolism of [$^{14}$C]BAS 510 F in lettuce was submitted. Lettuce samples were collected 18 days following the last of three foliar applications of [$^{14}$C]BAS 510 F, uniformly labeled on the phenyl rings (biphenyl label) or labeled at the 3-position of the pyridine ring (pyridine label), at 0.624 lb ai/A/application (700 g ai/ha, for a total of 2.1 kg ai/ha). TRR (calculated by summing extractable and nonretractable residues) were 17.541 ppm and 17.622 ppm in/on lettuce treated with biphenyl-label [$^{14}$C]BAS 510 F and pyridine-label [$^{14}$C]BAS 510 F, respectively. Material balances, based on sample combustion, were 85.1% and 99.3% for the biphenyl and pyridine labels, respectively. These results are presented in **Table 3** below.

Table 3. Total Radioactive Residues in Lettuce Leaves Following Three Foliar Applications of Isotopically Labeled BAS 510 F.

| Label Location | Crop Matrix | Application Rate | PHI, days | TRR, ppm | | % Mass Balance[3] |
|---|---|---|---|---|---|---|
| | | | | Combustion[1] | Calculation[2] | |
| Biphenyl label | Lettuce | 3 x 0.624 lb ai/A | 18 | 20.602 | 17.541 | 85.1% |
| Pyridine label | Lettuce | 3 x 0.624 lb ai/A | 18 | 17.751 | 17.622 | 99.3% |

[1] As determined by direct combustion/L.C.
[2] Calculated by summing extractable residues and nonretractable residues. The petitioner used the calculated value for all reported results because of the high water content of lettuce.
[3] Based on sample combustion.

For both labels, 99.3% of the TRR were extracted with methanol. The extracts were analyzed by HPLC, and the results were confirmed by LC/MS/MS. The identified TRR are tabulated below in **Table 4**.

| Table 4. Summary of Characterization and Identification of Radioactive Residues in Lettuce Leaf Following Three Foliar Applications of Isotopically Labeled BAS 510 F at 0.624 lb ai/A/application. | | | | |
|---|---|---|---|---|
| Metabolite or Fraction | [Diphenyl-U-$^{14}$C]BAS 510 F | | [Pyridin-3-$^{14}$C]BAS 510 F | |
| | Lettuce (TRR = 17.541 ppm) | | Lettuce (TRR = 17.622 ppm) | |
| | %TRR | ppm | %TRR | ppm |
| **BAS 510 F** | 99.3 | 17.412 | 99.3 | 17.507 |
| Total Identified (TI) | 99.3 | 17.412 | 99.3 | 17.507 |
| Total Characterized (TC) | -- | -- | -- | -- |
| Total Extractable (TE) | 99.3 | 17.412 | 99.3 | 17.507 |
| Total Bound (TB) | 0.7 | 0.129 | 0.7 | 0.115 |
| % Mass Balance | 100 | | 100 | |

**Little, if any, metabolism of BAS 510 F occurs. The unchanged parent, BAS 510 F, was the only component identified in lettuce, accounting for 99.3% TRR** (17.412 ppm, diphenyl label, or 17.507 ppm, pyridine label). Nonextractable residues accounted for 0.7% TRR and were not further analyzed.

**Beans**

BASF Corporation has submitted a study investigating the metabolism of [$^{14}$C]BAS 510 F in beans. Samples were collected following three foliar applications of [$^{14}$C]BAS 510 F at 0.44 lb ai/A/application (500 g ai/ha, for a total of1.5 kg ai/ha/season, 1.3X the proposed label rate). TRR (calculated by summing extractable and nonretractable residues) are presented below in **Table 5**.

Table 5. Total Radioactive Residues in Bean Commodities Following Three Foliar Applications of Isotopically Labeled BAS 510 F

| Label Location | Crop Matrix | Application Rate | PHI, days | TRR, ppm | | % Mass Balance[3] |
|---|---|---|---|---|---|---|
| | | | | Combustion[1] | Calculation[2] | |
| Biphenyl label | Bean plant | 3 x 0.44 lb ai/A | 0 | 49.359 | 49.091 | 99.5% |
| | Bean forage | | 14 | 66.454 | 66.236 | 99.7% |
| | Green beans | | 14 | 0.891 | 1.027 | 115.3% |
| | Bean pods | | 14 | 0.874 | 0.903 | 103.3% |
| | Bean seeds | | 14 | 0.212 | 0.198 | 93.4% |
| | Bean straw | | 53 | 128.374 | 127.285 | 99.2% |
| | Bean dry pods | | 53 | 4.475 | 6.118 | 136.7% |
| | Bean dry seeds | | 53 | 0.183 | 0.205 | 112.0% |
| Pyridine label | Bean plant | 3 x 0.44 lb ai/A | 0 | 23.279 | 21.249 | 91.3% |
| | Bean forage | | 15 | 17.418 | 16.967 | 97.4% |
| | Green beans | | 15 | 0.099 | 0.09 | 90.9% |
| | Bean pods | | 15 | 0.124 | 0.108 | 87.1% |
| | Bean seeds | | 15 | 0.076 | 0.067 | 88.2% |
| | Bean straw | | 51 | 142.511 | 93.775 | 65.8% |
| | Bean dry pods | | 51 | 1.51 | 1.369 | 90.7% |
| | Bean dry seeds | | 51 | 0.13 | 0.126 | 96.9% |

[1] As determined by direct combustion/L.C.
[2] Calculated by summing extractable residues and nonretractable residues detected by L.C. The petitioner used the calculated value for all reported results.
[3] Based on sample combustion.

In summary, the material balances, based on sample combustion, were 87.1-136.7% for bean commodities, with the exception of pyridine-labelled bean straw, which had a material balance of 65.8%.

The majority of residues in bean commodities were extracted with methanol (47.6-99.2% TRR). An additional water extraction released a small amount of radioactivity in samples, with increasing amounts released in samples harvested at longer posttreatment intervals and more radioactivity released from pyridine-label samples than from diphenyl-label samples. Nonextractable residues of some commodities were subjected to an additional extraction with aqueous ammonia, which released small amounts of radioactivity. Extracts of all harvested bean commodities were analyzed by HPLC. Identification of BAS 510 F was confirmed in diphenyl-labelled bean straw by LC/MS/MS. The results are presented below in **Table 6.**

Table 6. Summary of Characterization and Identification of Radioactive Residues in Bean Commodities Following Three Foliar Applications of Isotopically Labelled BAS 510 F at 0.44 lb ai/A/application.

| Metabolite or Fraction | Bean plant | | Bean forage | | Green beans | | Bean pods | | Bean seeds | | Bean straw | | Bean dry pods | | Bean dry seeds | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | %TRR | ppm | %TRR | ppm | %TRR | ppm | %TRR | ppm | %TRR | ppm | %TRR | ppm | %TRR | ppm | %TRR | ppm |
| [Diphenyl-U-$^{14}$C]BAS 510 F | | | | | | | | | | | | | | | | |
| **BAS 510 F** | 99.28 | 48.724 | 98.57 | 65.269 | 97.2 | 0.999 | 96.7 | 0.872 | 87.5 | 0.173 | 95.1 | 120.97 | 94.52 | 5.788 | 72 | 0.148 |
| Bound BAS 510 F | 0.05 | 0.018 | 0.11 | 0.063 | -- | -- | -- | -- | -- | -- | 0.13 | 0.164 | 0.25 | 0.016 | -- | -- |
| Chlorophenylamino-benzene | 0.02 | 0.01 | -- | -- | 0.61 | 0.01 | -- | -- | 0.71 | 0 | 0.5 | 0.61 | -- | -- | -- | -- |
| Sugar conjugate of BAS 510 F | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | 0.352 | 0.458 | -- | -- | -- | -- |
| Water | -- | -- | -- | -- | 0.7 | 0.01 | 0.9 | 0.01 | 2.4 | 0.01 | -- | -- | -- | -- | 5.5 | 0.011 |
| Ammonia extract | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | 10.4 | 0.021 |
| Unknowns | 0.48 | 0.182 | 0.853 | 0.486 | 0.4 | 0 | 0.6 | 0.01 | 1.5 | 0 | 1.634 | 2.037 | 1.83 | 0.11 | 3 | 0.004 |
| Total Identified (TI) | 99.35 | 48.751 | 98.68 | 65.332 | 97.81 | 1.005 | 96.7 | 0.872 | 88.2 | 0.174 | 96.08 | 122.200 | 94.77 | 5.804 | 72.00 | 0.148 |
| Total Characterized (TC) | 0.48 | 0.182 | 0.85 | 0.486 | 1.1 | 0.011 | 1.5 | 0.014 | 3.9 | 0.01 | 1.63 | 2.037 | 1.83 | 0.11 | 18.9 | 0.036 |
| Total Extractable (TE) | 99.83 | 48.933 | 99.53 | 65.818 | 98.91 | 1.016 | 98.2 | 0.886 | 92.1 | 0.182 | 97.71 | 124.24 | 96.6 | 5.914 | 90.90 | 0.184 |
| Total Bound (TB) | 0.3 | 0.143 | 0.6 | 0.37 | 1.7 | 0.017 | 1.9 | 0.017 | 8.6 | 0.017 | 2.4 | 3.06 | 3.3 | 0.201 | 8.9 | 0.018 |
| % Mass Balance | 100.13 | | 100.13 | | 100.61 | | 100.1 | | 100.7 | | 100.11 | | 99.9 | | 99.8 | |
| [Pyridin-3-$^{14}$C]BAS 510 F | | | | | | | | | | | | | | | | |
| **BAS 510 F** | 98.09 | 20.86 | 98.39 | 16.695 | 78.09 | 0.071 | 87.02 | 0.095 | 64.88 | 0.043 | 93.62 | 87.78 | 79.68 | 1.09 | 36.94 | 0.047 |
| Bound BAS 510 F | 0.048 | 0.104 | 0.49 | 0.077 | -- | -- | -- | -- | -- | -- | 0.68 | 0.601 | 2.57 | 0.035 | -- | -- |
| Chloronicotinic acid | -- | -- | -- | -- | 2.8 | 0 | 2.15 | 0 | 9.97 | 0.01 | -- | -- | 1.11 | 0.015 | 1.72 | 0.002 |
| Water | -- | -- | -- | -- | 0.5 | <0.001 | 0.4 | <0.001 | 1.2 | 0 | -- | -- | -- | -- | 21.3 | 0.027 |
| Ammonia extract | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | 8.5 | 0.011 |
| Unknowns | 0.03 | 0.01 | 0.11 | 0.018 | 16.52 | 0.015 | 8.44 | 0.01 | 19.85 | 0.014 | 0.81 | 0.757 | 10.44 | 0.141 | 8.93 | 0.012 |
| Total Identified (TI) | 98.138 | 20.964 | 98.88 | 16.772 | 80.89 | 0.074 | 89.17 | 0.097 | 74.85 | 0.05 | 94.3 | 88.381 | 83.36 | 1.14 | 38.66 | 0.049 |

Table 6. Summary of Characterization and Identification of Radioactive Residues in Bean Commodities Following Three Foliar Applications of Isotopically Labelled BAS 510 F at 0.44 lb ai/A/application.

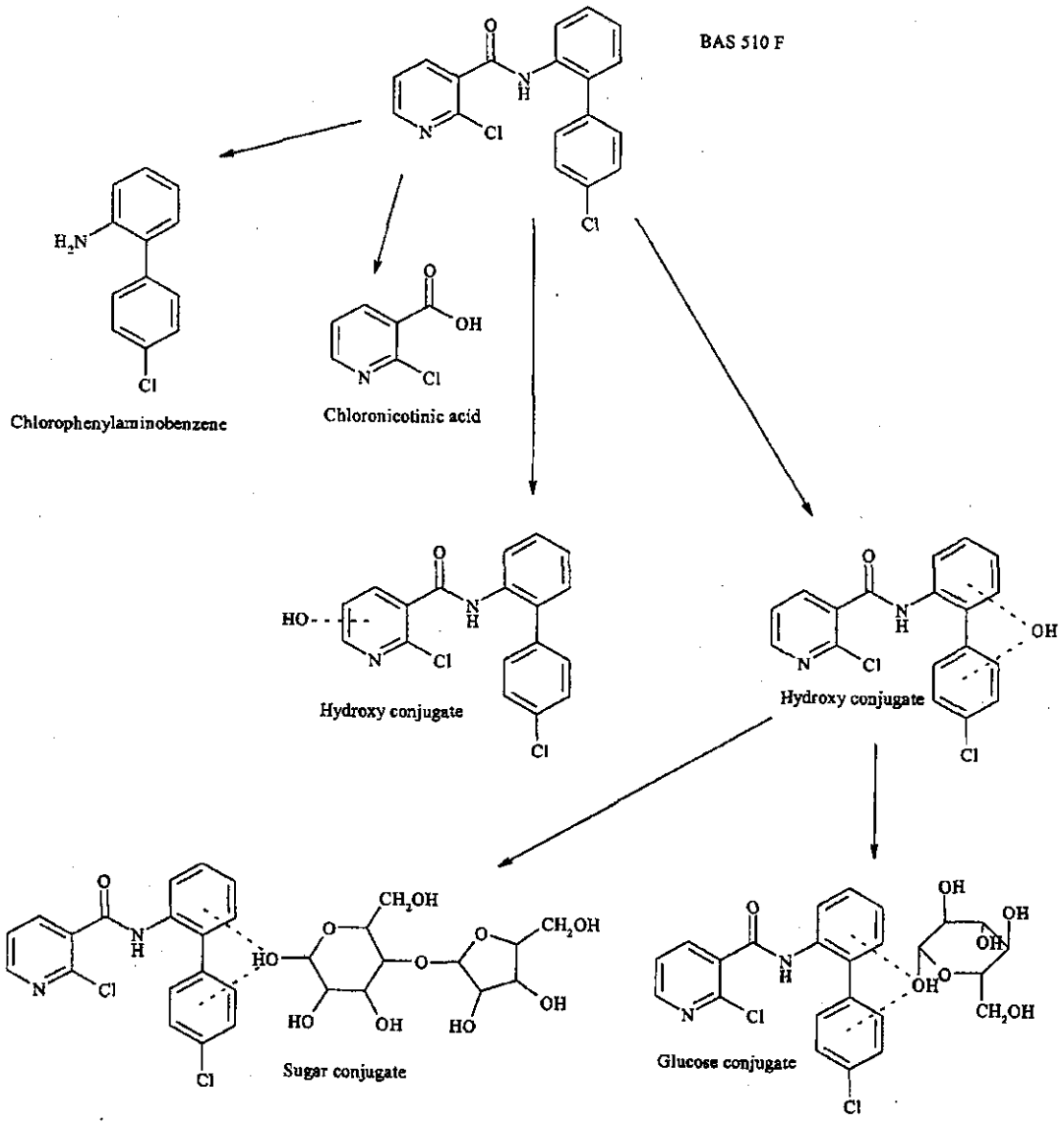
| Metabolite or Fraction | Bean plant | | Bean forage | | Green beans | | Bean pods | | Bean seeds | | Bean straw | | Bean dry pods | | Bean dry seeds | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | %TRR | ppm | %TRR | ppm | %TRR | ppm | %TRR | ppm | %TRR | ppm | %TRR | ppm | %TRR | ppm | %TRR | ppm |
| Total Characterized (TC) | 0.03 | 0.01 | 0.11 | 0.018 | 17.02 | 0.016 | 8.84 | 0.01 | 21.05 | 0.015 | 0.81 | 0.757 | 10.44 | 0.141 | 38.73 | 0.05 |
| Total Extractable (TE) | 98.168 | 20.971 | 98.99 | 16.79 | 97.91 | 0.09 | 98.01 | 0.106 | 95.9 | 0.065 | 95.11 | 89.138 | 93.8 | 1.281 | 77.39 | 0.099 |
| Total Bound (TB) | 0.7 | 0.145 | 0.6 | 0.108 | 2.1 | 0 | 2 | 0 | 4.1 | 0 | 4 | 3.794 | 5.9 | 0.081 | 13.5 | 0.017 |
| % Mass Balance | 98.868 | | 99.59 | | 100.01 | | 100.01 | | 100 | | 99.11 | | 99.7 | | 90.89 | |

Bound BAS 510 F = BAS 510 F found in ammonia extract
TC = Sum of all unidentified, extractable residues
TE = Sum of TI and TC
% Mass Balance = TE %TRR +TB % TRR. Note that the petitioner calculated TRR by summing extractable and nonextractable residues; therefore, %Mass Balance is at or very close to 100% for all matrices. See Table 2.1 for actual mass balance based on combustion of the samples.
[1] Identified in the hexane phase following liquid/liquid partitioning of the methanol extract.
[2] Identified in the water phase following liquid/liquid partitioning of the methanol extract.
[3] Analysis using a different column indicated the presence of chlorophenylaminobenzene in this fraction at 0.02% TRR.
[4] The petitioner used LC/MS/MS analyses to identify three of the unknowns as a glucose conjugate of BAS 510 F and two hydroxy conjugates of BAS 510 F; however, no quantitative data were provided for these metabolites.

**The unchanged parent, BAS 510 F, was the major component identified in all bean commodities**, accounting for 36.94% TRR (0.049 ppm; pyridine label) and 72.0% TRR (0.148 ppm; diphenyl label) in/on bean dry seeds to 98.09% (20.860 ppm; pyridine label) and 99.28% TRR (48.724 ppm; diphenyl label) in/on bean plants (0-day PHI). Additional bound BAS 510 F (<3% TRR) was released by ammonia extraction of nonextractable residues. Chlorophenyl-aminobenzene and chloronicotinic acid were identified in small amounts, 0.02-0.7% TRR (0.001-0.610 ppm) and 1.11-9.97% TRR (0.002-0.015 ppm), respectively. A sugar conjugate of the parent was identified in bean straw at 0.35% TRR; in addition, a glucose conjugate and two hydroxy conjugates of BAS 510 F were identified in bean straw (although quantitative data for these metabolites were not provided, they were not present in amounts greater than 0.61% TRR each). Nonextractable residues accounted for <9% TRR in all bean commodities, except bean dry seed, which had nonextractable residues of 13.5% TRR (0.017 ppm).

Based on the submitted study, the petitioner has proposed that BAS 510 F is metabolized slowly in beans, as metabolites were only seen at low concentrations. Some cleavage of the parent molecule occurred, as demonstrated by the presence of the cleavage products chloronicotinic acid and chlorophenylaminobenzene. The proposed metabolic pathway is presented in **Figure 1**.

Figure 1. Proposed metabolic pathway of BAS 510 F in beans.



## NATURE OF THE RESIDUE - ANIMALS

The petitioner has submitted metabolism studies in lactating goats and laying hens to illustrate the metabolism of BAS 510 F in animals. In these experiments, the petitioner has only used BAS 510 F labelled in the phenyl rings. The results therefore only elucidate the metabolism of the diphenyl ring portion of the molecule. As there was no apparent cleavage of the parent in these studies, and only a very small amount of cleavage product were observed in plant material, the lack of information from experiments carried out with the molecule labelled in the pyridine moiety is not critical.

### Lactating Goat

Two lactating goats were orally dosed with [$^{14}$C]BAS 510 F, uniformly labeled in the diphenyl rings, at an average of 32.43 ppm in the diet once daily for 5 consecutive days. Milk was collected twice daily. The goats were sacrificed 23 hours following the last dose, and samples of kidney, liver, muscle, and fat were collected. The distribution of the dose is presented in **Table 7** below.

Table 7. Total Radioactive Residues in Lactating Goats Following Oral Dosing with [Diphenyl-U-$^{14}$C]BAS 510 F at 32.43 ppm for 5 Consecutive Days.

| Matrix | Treatment Day | TRR (ppm) | | |
|---|---|---|---|---|
| | | Goat 1 | Goat 2 | Pooled |
| Milk | Day 1, afternoon | 0.03 | 0.138 | 0.037, 0.039 |
| | Day 1, morning | 0.024 | 0.024 | |
| | Day 2, afternoon | 0.053 | 0.118 | |
| | Day 2, morning | 0.012 | 0.028 | |
| | Day 3, afternoon | 0.045 | 0.112 | |
| | Day 3, morning | 0.01 | 0.026 | |
| | Day 4, afternoon | 0.031 | 0.111 | |
| | Day 4, morning | 0.011 | 0.023 | |
| | Day 5, afternoon | 0.042 | 0.109 | |
| | Day 5, morning | 0.011 | 0.028 | |
| Liver | 23 hours after last dose | -- | -- | 2.593 |
| Kidney | | -- | -- | 0.27 |
| Muscle | | -- | -- | 0.012 |
| Fat | | -- | -- | 0.036 |
| Urine | Days 1-5 | 23.68% of dose | 44.63% of dose | -- |
| Feces | | 64.25% of dose | 46.37% of dose | -- |
| Cage wash | 23 hours after last dose | 0.90% of dose | 2.44% of dose | -- |
| Subtotal, excreta | Entire study | 88.83% of dose | 93.44% of dose | -- |

The total radioactive residues (TRR) were 0.010-0.138 ppm in milk collected throughout the dosing period, 0.270 ppm in kidney, 2.593 ppm in liver, 0.036 ppm in fat, and 0.012 ppm in muscle. Residues were highest in liver and lowest in muscle. Radioactivity in milk was highest after dosing and lower by the next milking; however, residues appeared to plateau by the second or third dosing day. Radioactivity in urine, feces, and cage wash collected throughout the dosing period and at sacrifice accounted for 23.7-44.6%, 46.4-64.3%, and 0.9-2.4%, respectively, of the applied radioactivity.

The components of the TRR were separated and identified by multiple separation techniques with various detection systems including TLC, HPLC, GC/MS and GC/MS/MS, specific experimental details are summarized below. **Table 8** contains the results obtained.

Milk:

Whey/Protein Separation: A subsample of milk was separated into whey and protein. The sample was adjusted to pH 4.2 using 2 N phosphoric acid and centrifuged. Sodium hydroxide (0.1 N) was added to the solids; after mixing, the pH was adjusted to 4.2 using phosphoric acid, and the mixture was centrifuged. The whey supernatants were combined, acetone was added, and the mixture was cooled (4 °C) overnight and filtered. Precipitated lactose was collected by filtration and dissolved in water for radioactivity determination by LSC; the filtrate was concentrated for HPLC analysis. The protein solids remaining following the initial hydrolysis were mixed with MeOH and centrifuged. The supernatant was collected, and MeOH:chloroform (1:3, v:v) was added to the remaining residue and centrifuged following mixing. The supernatants were combined, cooled overnight (4 C), filtered, and an aliquot of the filtrate was concentrated for HPLC analysis.

Microwave Treatment: Another subsample of milk was mixed with acetonitrile (ACN) and concentrated. The concentrate was mixed with acetic acid and subjected to microwave treatment by heating to 170 °C over 5 minutes and then maintaining the temperature at 170 °C for 0.5 hours. The extract was then mixed with water and cleaned up by C18 SPE. Residues were eluted with 100% MeOH, concentrated, and diluted with MeOH:water (8:2, v:v) for HPLC analysis.

Fat:

ACN/iso-Hexane Extraction: A subsample of fat was extracted with ACN:iso-hexane (1:1, v:v; 3x). The ACN and iso-hexane phases were separately combined, and the iso-hexane phase was extracted with ACN (2x). The residue remaining after ACN:iso-hexane extraction was mixed with 2% ascorbic acid and extracted with ACN:iso-hexane (2x). The remaining solids were extracted with water:MeOH (3:7, v:v). The MeOH extract was combined with the ACN extracts and centrifuged for HPLC analysis.

Liver:

Extraction with Organic Solvents and Protease Digestion: A subsample of liver was extracted with MeOH (3x) and water, and the remaining solids were divided into two subsamples. One subsample was sequentially extracted with ethyl acetate, dichloromethane, and toluene and the extracts were isolated by centrifugation. The second subsample was digested with protease (in 0.1 M TRIS buffer, pH 7.3, 37 °C, overnight). The hydrolysate was isolated by centrifugation, and the solids were extracted with MeOH (3x). An additional subsample of liver was directly subjected to protease digestion; however, the resulting mixture could not be separated by centrifugation or cleaned up by SPE.

Trypsin Digestion: A subsample of liver was extracted with MeOH (3x) and water (2x), and the remaining solids were digested with trypsin (0.1 M TRIS buffer, pH 8, 25 °C, overnight); the hydrolysate was isolated by centrifugation, and solids were extracted with MeOH (3x).

Acid Hydrolysis: A subsample of liver was refluxed in 5 M HCl in MeOH (1 h) and the hydrolysate was neutralized with NaOH, mixed with MeOH, and cleaned up by C18 SPE; residues were eluted with MeOH. The remaining solids were refluxed with HCl in MeOH a second time.

Sodium Hydroxide Hydrolysis: A subsample of liver was refluxed in 5 M NaOH (1 h), and the resulting hydrolysate was neutralized for HPLC analysis.

Ammonium Hydroxide Hydrolysis: A subsample of liver was refluxed in 5 M ammonium hydroxide (2 h), and the hydrolysate was split into two aliquots: one was cleaned up by C18 SPE, and the other was neutralized with formic acid, centrifuged, and the resulting solids were redissolved in aqueous ammonium hydroxide.

Microwave Treatment: Subsamples of liver were mixed with ACN and acetic or formic acid, heated to 170 C over a period of 5 minutes, and then maintained at 170 °C for 0.5 h. The resulting solutions were cleaned up by C18 SPE, concentrated, and diluted with MeOH or MeOH:water (8:2, v:v) for HPLC analysis. The petitioner subjected reference compounds to the same microwave treatments.

Extracts of all matrices were analyzed by HPLC using a Hypersil ENV or YMC C30 column, a UV detector, a radioactivity monitor, and a fraction collector. The chromatographic column effluent was split to allow parallel radiodetection and MS analysis. Gradient mobile phases of 10 mM ammonium formate and ACN or MeOH were used. Residues were identified by co-chromatography with the following reference compounds: BAS 510 F, M510F01, M510F04, M510F05, M510F48, and chlorophenylaminobenzene (M510F62). M510F01, M510F04, M510F05, and M510F48 were obtained from the rat metabolism study (MRID 45404918).

M510F02 was also used as a reference standard, although the petitioner did not indicate its source.

LC/MS and LC/MS/MS were used for identification of metabolites isolated from urine and the reaction products of the microwave hydrolysis of reference compounds. Analyses were conducted using electrospray ionization in the positive ion mode.

Table 8. *Summary of Characterization and Identification of Radioactive Residues in Goat Milk and Tissues Following Oral Dosing with [Diphenyl-U-$^{14}$C]BAS 510 F at 32.43 ppm for 5 Consecutive Days.*

| Metabolite or Fraction | Milk, Whey/Protein Separation (TRR = 0.037 ppm) | | Milk, Microwave Treatment (TRR = 0.039 ppm) | | Muscle (TRR = 0.012 ppm) | | Fat (TRR = 0.036 ppm) | | Kidney (TRR = 0.270 ppm) | | Liver, Microwave Treatment (ACN/acetic acid) (TRR = 2.593 ppm) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | %TRR | ppm | %TRR | ppm | %TRR | ppm | %TRR | ppm | %TRR | ppm | %TRR | ppm |
| BAS 510 F | 3.2 | 0.001 | 7.9 | 0.003 | 20.4 | 0.002 | 34.6 | 0.012 | 2.5 | 0.007 | -- | -- |
| M510F02 | 6.4 | 0.002 | -- | -- | 11.9 | 0.001 | -- | -- | 50.3 | 0.136 | -- | -- |
| M510F01 | 14.9 | 0.006 | 19 | 0.007 | 20.6 | 0.003 | 26.3 | 0.009 | 8.6 | 0.023 | 6.4 | 0.166 |
| M510F49[2] | | | 7.7 | 0.003 | -- | -- | -- | -- | -- | -- | 11 | 0.285 |
| M510F51[3] | | | 12.2 | 0.005 | -- | -- | -- | -- | -- | -- | 2.4 | 0.062 |
| M510F53[4] | | | 11.2 | 0.004 | -- | -- | -- | -- | -- | -- | 43.6 | 1.13 |
| Unknowns | 58.7 | 0.025 | 7 | 0.003 | 19.8 | 0.002 | 10.9 | 0.004 | 19.4 | 0.052 | -- | -- |
| Hexane extracts | -- | -- | -- | -- | -- | -- | 4.6 | 0.002 | -- | -- | -- | -- |
| Retained on C18 SPE | -- | -- | 23.5 | 0.009 | 7.1 | 0.001 | -- | -- | -- | -- | 36.7 | 0.952 |
| Precipitates | 4.1 | 0.002 | -- | -- | -- | -- | -- | -- | 0.5 | 0.001 | -- | -- |
| Total Identified (TI) | 24.5 | 0.009 | 58.0 | 0.022 | 52.9 | 0.006 | 60.9 | 0.021 | 61.4 | 0.166 | 63.4 | 1.643 |
| Total Characterized (TC) | 62.8 | 0.027 | 30.5 | 0.012 | 26.9 | 0.003 | 15.5 | 0.006 | 19.9 | 0.053 | 36.7 | 0.952 |
| Total Extractable (TE) | 87.3 | 0.036 | 88.5 | 0.034 | 79.8 | 0.009 | 76.4 | 0.027 | 81.3 | 0.219 | 100.1 | 2.595 |
| Total Bound (TB) | 0 | 0 | Not reported | | 24.1 | 0.003 | 8 | 0.003 | 16.3 | 0.044 | 0 | 0 |
| % Mass Balance | 87.3 | | 88.5 | | 103.9 | | 84.4 | | 97.6 | | 100.1 | |

TC = Sum of all unidentified, extractable residues
TE = Sum of TI and TC
% Mass Balance = TE %TRR +TB % TRR

[1] Identified in the methanol extract (4.97% TRR, 0.129 ppm) with organic solvent and protease digestion, and in the C18 SPE eluent (5.7% TRR, 0.15 ppm) with microwave treatment with ACN and formic acid)

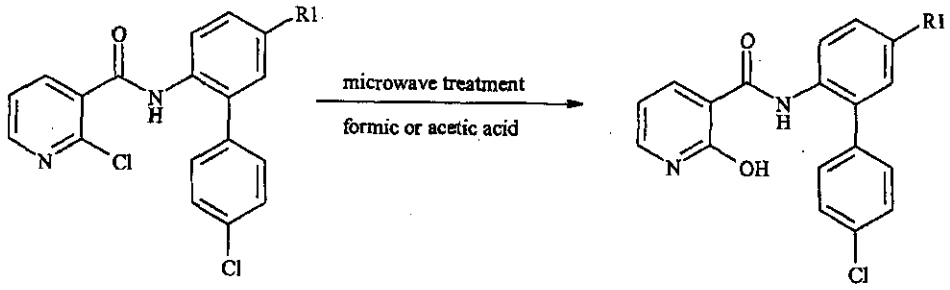
[2] Released during microwave hydrolysis; putatively results from hydrolysis of BAS 510 F.

[3] Released during microwave hydrolysis; putatively results from hydrolysis of M510F01.

[4] Released during microwave hydrolysis; putatively results from hydrolysis of bound/conjugated BAS 510 F.

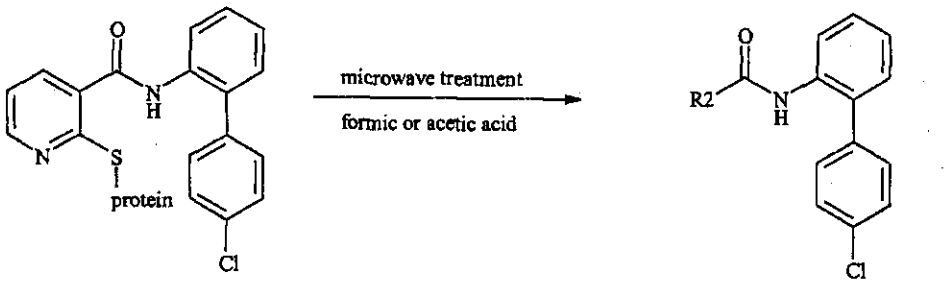
Approximately 76-87% of the TRR were extracted from goat milk, muscle, fat, and kidney using solvents; solvent extraction only released ~14% TRR from liver. The parent, BAS 510 F, was detected in all goat matrices, accounting for 2.5-7.9% TRR (0.003-0.129 ppm) in milk and liver, 20.4% TRR (0.002 ppm) in muscle, and 34.6% TRR (0.012 ppm) in fat. Metabolite M510F01 was detected in all goat matrices, at 6.4-26.35% TRR (0.003-0.166 ppm), and metabolite M510F02 was detected in milk (6.4% TRR, 0.002 ppm), muscle (11.9% TRR, 0.001 ppm), and kidney (50.35% TRR, 0.136 ppm). Material balances were ~84-102% TRR.

Subsamples of milk and liver were also subjected to microwave hydrolysis for further characterization/identification of residues. According the petitioner, this procedure modifies the structure of various metabolites as follows:




R1 = H; BAS 510 F
R1 = OH; M510F01

R1 = H; M510F49
R1 = OH; M510F51




R2 = H when formic acid used; M510F52
R2 = $CH_3$ when acetic acid used; M510F53

Although the petitioner did not provide information which conclusively identifies the metabolites released by microwave extraction, we have no basis on which to disagree with their assumptions about the chemical reactions resulting in the compounds found in the hydrolysate.

Microwave hydrolysis, using formic or acetic acid, released 88.5% of TRR from milk samples and 100% TRR from liver. In addition to BAS 510 F (7.9% TRR in milk) and M510F01 (19%

TRR in milk, 6.4% TRR in liver), microwave hydrolysates of liver and milk were found to contain three components that were not identified in any other extracts: M510F48 (11.0% and 7.7% TRR, respectively), M510F01 (2.4% and 12.2% TRR, respectively), and M510F513 (43.6% and 11.2% TRR, respectively). The petitioner concluded that these components corresponded to BAS 510 F, M510F01, and bound or conjugated BAS 510 F, respectively, in milk and liver because microwave hydrolysis of reference compounds indicated that BAS 510 F was converted to M510F492, M510F01 was converted to M510F01, and reference compounds in which the pyridine chlorine group had already been substituted with sulfur were converted to M510F02 or M510F513. The petitioner stated that total BAS 510 F residues in milk and liver consisted of the sum of BAS 510 F and M510F48 residues, total M510F01 residues consisted of the sum of M510F01 and M510F02 residues, and residues of M510F02 or M510F513 corresponded to bound residues in liver or soluble conjugates of glutathione, cysteine, or mercaptic acid in milk.

Based on the results of the study, the petitioner has proposed that BAS 510 F is metabolized in goats through hydroxylation of the diphenyl portion to form M510F01. M510F01 then undergoes glucuronidation to form M510F02. Further hydroxy and thiol substitutions in the diphenyl portion occur. In addition, the chlorine atom on the pyridine ring is substituted by thiol groups in biomolecules. The proposed metabolic pathway for BAS 510 F in lactating goat is presented in **Figure 2** below. A summary list of all metabolites identified throughout the animal and plant metabolism studies is included in **Attachment 1**.

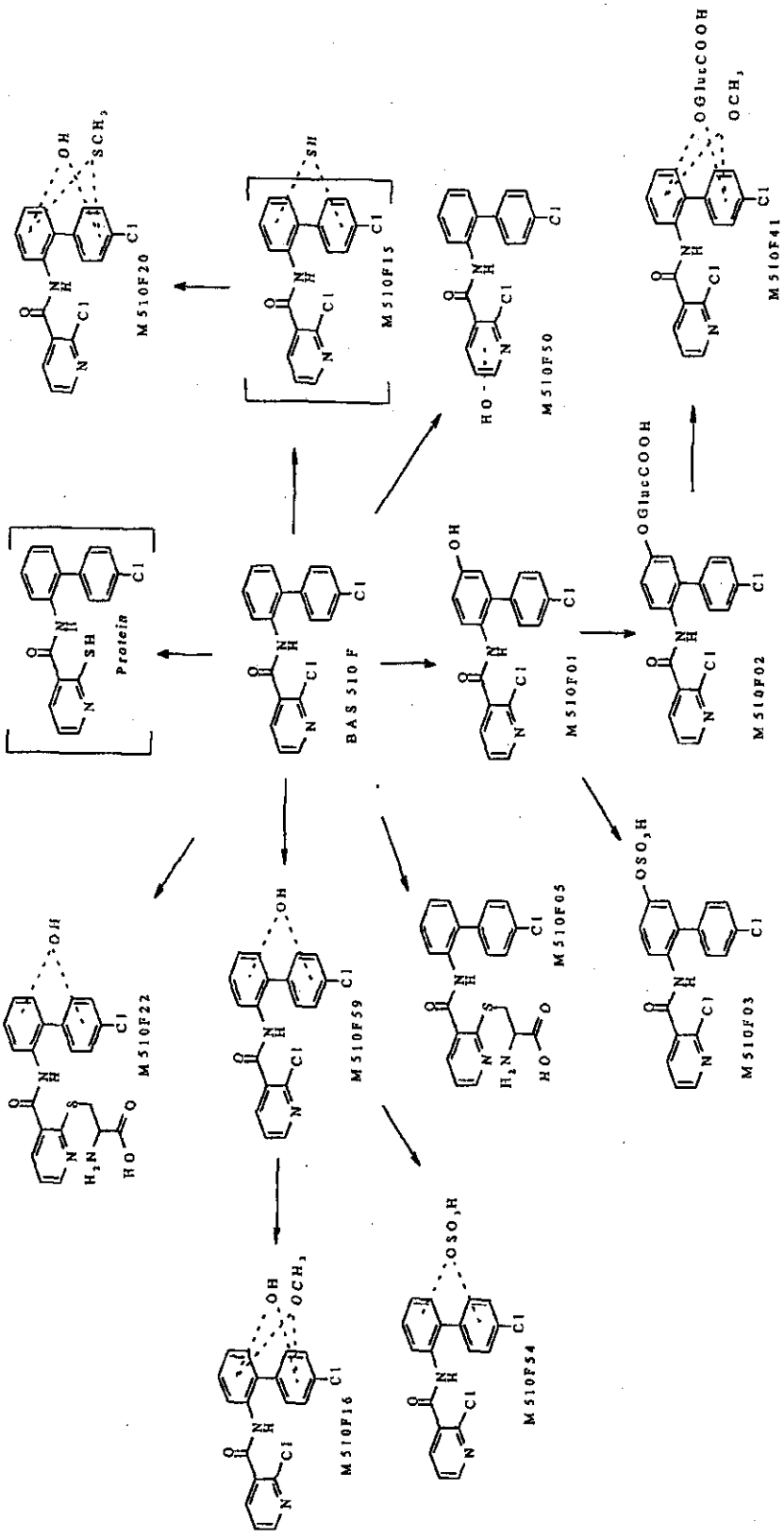



Figure 2. Proposed metabolic pathway for BAS 510 F in the lactating goat.

## Laying Hen

Ten laying hens were orally dosed with [$^{14}$C]BAS 510 F, uniformly labeled in the biphenyl rings, at 12.1 ppm in the diet once daily for 10 consecutive days. Eggs were collected twice daily. The hens were sacrificed 21-23 hours following the last dose, and samples of liver, muscle, and fat were collected. The distribution of the radioactive material is presented below in **Table 9.**

Table 9. Total Radioactive Residues in Laying Hens Following Oral Dosing with [Biphenyl-U-$^{14}$C]BAS 510 F at 12.1 ppm for 10 Consecutive Days.

| Matrix | Treatment Day | TRR (ppm) | Percent of Dose |
|---|---|---|---|
| Eggs | Day 1 | 0 | 0.115 |
| | Day 2 | 0.0239 | |
| | Day 3 | 0.0305 | |
| | Day 4 | 0.0447 | |
| | Day 5 | 0.0521 | |
| | Day 6 | 0.0739 | |
| | Day 7 | 0.0677 | |
| | Day 8 | 0.0752 | |
| | Day 9 | 0.0739 | |
| | Day 10 | 0.0805 | |
| Liver | 23 hours after last dose | 0.1687 | 0.039 |
| Muscle | | 0.0025 | 0.003 |
| Fat | | 0.025 | 0.004 |
| Excreta | Days 1-10 | 4.1675 (10 day average) | 97.68 |
| Cage wash | Day 0 and 23 hours after last dose | 0.3416 | 0.154 |
| Subtotal, excreta | Entire study | – | 97.834 |

The total radioactive residues (TRR) were 0.0239-0.0805 ppm in eggs collected throughout the dosing period, 0.1687 ppm in liver, 0.0250 ppm in fat, and 0.0025 ppm in muscle. Radioactivity in eggs appeared to plateau by the sixth dosing day. Radioactivity in excreta and cage wash collected throughout the dosing period and at sacrifice accounted for 97.68% and 0.154%, respectively, of the applied radioactivity.

Tissue samples were extracted and analyzed as follows:

Eggs: A subsample of whole eggs was subjected to enzyme digestion. The sample was mixed with 25mM potassium dihydrogen phosphate buffer (pH 7.5) and protease, and the mixture was allowed to incubate at 37 C overnight. Acetonitrile (ACN) was added to the protease

hydrolysate, which was placed in the freezer (1 h) to allow precipitation of lipids and denaturation of protein, then centrifuged. This step was repeated, and the ACN extracts were combined and evaporated to near dryness. The solids remaining following centrifugation were washed with methanol (MeOH), and the MeOH and concentrated protease hydrolysate were combined and cleaned up by C18 solid phase extraction (SPE). Residues were sequentially eluted with water, water:MeOH (50:50, v:v), water:MeOH (5:95, v:v), and MeOH. The water:MeOH eluates were combined for HPLC analysis, and the MeOH eluate was subjected to a second C18 SPE cleanup with water:MeOH.

Fat: A subsample of fat was homogenized with hexane, and the mixture was extracted twice with ACN and centrifuged. The ACN fraction was concentrated for HPLC analysis.

A second subsample of fat was mixed with hexane; the mixture was frozen (overnight) and the hexane was decanted. The remaining solids were extracted twice with ACN. The ACN and hexane fractions were mixed together and allowed to separate. The ACN fraction was then washed with hexane. The remaining solids were extracted with hexane, and all hexane fractions were combined and partitioned twice with ACN. The ACN fractions were combined with the previous ACN extracts and applied to three TLC plates (silica gel; solvent system of dichloromethane:MeOH, 95:5, v:v). The bands which corresponded to BAS 510 F were scraped from the plate and extracted with MeOH; the MeOH extracts were combined and reserved for HPLC analysis.

Liver: Liver was subjected to several extraction/hydrolysis procedures in an attempt to release radioactivity. These procedures are described below.

ACN and Water Extraction: A subsample was homogenized in ACN, and the mixture was sonicated to ensure release of any encapsulated metabolites. The ACN extract was isolated by centrifugation.

Protein Denaturing: A first subsample was homogenized in water and then sonicated. The extract was isolated by centrifugation, and ACN was added to denature proteins. The extracts were combined with the ACN extracts from the above extraction, concentrated, and partitioned with ACN. A second subsample was extracted twice with water. Trichloracetic acid was added to the water extracts to precipitate proteins. Following centrifugation, the solids were redissolved in water, and then trichloroacetic acid. The acidic mixture was refrigerated overnight and centrifuged. The third subsample of the water extract was heated at 60 °C for 1 h. The extract was freeze dried and dissolved in water.

Mild Acid and Base Hydrolysis: A subsample was extracted twice with water; the extracts were isolated by centrifugation and combined. The supernatant was heated at 60 C for 1 h and centrifuged. The solids remaining after heating were extracted with 1 N HCl and centrifuged. The remaining solids were then extracted twice with 1 N NaOH; the extracts were isolated by

centrifugation and combined. The basic extract was extracted with ethyl acetate, acidified to pH 1 and extracted with ethyl acetate again. The ethyl acetate fractions were combined.

Protease Digestion and Molecular Weight Cut Off (MWCO): A subsample was extracted with MeOH and centrifuged. The nonretractable residues were digested with protease (in potassium phosphate buffer, pH 7.5, 37 °C, 49 h). ACN was added and the mixture was frozen overnight. Following centrifugation, the solids were extracted with MeOH, and the MeOH extract was combined with the protease hydrolysate. The combined extract was subjected to Molecular Weight Cut Off membrane separation using 30K, 10K, and 3K separators.

Strong Base Hydrolysis: A subsample was refluxed in 10 N NaOH for 2 h. The neutralized hydrolysate was partitioned with hexane. The remaining aqueous phase was separately neutralized to pH 7 and pH 2 and partitioned with ethyl acetate. The ethyl acetate phases were combined.

Accelerated Solvent Extraction (ASE): A subsample was subjected to ASE using ACN at 60 C, ACN in formic acid at 60 C, MeOH in formic acid at 60 C, or MeOH in formic acid at 100 C. The extracts were combined and cleaned up by C18 SPE, using 1% formic acid and MeOH to elute the column. A separate subsample was subjected to ASE using ACN and formic acid at 170 C. The extracts were cleaned up by C18 SPE as described above.

Microwave Treatment: Subsamples of liver were mixed with ACN and formic acid, heated to 170 C over a period of 5 min, and then maintained at 170 C for 0.5 h. The resulting solutions were diluted with water and cleaned up by C18 SPE, using water and MeOH to elute the column. The MeOH eluates were combined and concentrated for HPLC analysis (microwave extraction and analysis was conducted by BASF in Germany).

Extracts of all matrices were analyzed by HPLC using a Hypersil ENV or YMC C30 column and a radioactivity monitor; non-labeled standards could not be identified with this method. Gradient mobile phases of 10 mM ammonium formate, ACN, and water were used. Residues were identified by co-chromatography with the following reference compounds: BAS 510 F, M510F01, M510F02, chlorophenylaminobenzene, M510F51, M510F49, and M510F52. Metabolites M510F51 and M510F49 were generated by microwave hydrolysis of M510F01 and BAS 510 F, respectively. Metabolite M510F02 was obtained from the rat metabolism study (MRID 45404918) and metabolite M510F52 was obtained from the goat metabolism study (MRID 45405024). In addition to these reference compounds, one metabolite, M510F54, was isolated from hen excreta, and its identity as a sulfate of BAS 510 F was confirmed by LC/MS. The position of sulfate conjugation could not be determined.

TLC analyses were conducted on silica gel plates using solvent systems of dichloromethane: MeOH or MeOH and 5% ammonium hydroxide or 1% formic acid. Reference standards were visualized under UV light, and radioactivity was visualized using a radioscanner.

LC/MS and LC/MS/MS were used for identification of metabolites isolated from hen excreta (BAS 510 F, M510F01, and M510F54) and confirmation of the parent in the dosing solution following ACN/formic acid extraction. Metabolites identified in microwave treatment of liver by HPLC were co-chromatographed with metabolite reference standards identified by LC/MS. LC/MS analyses were conducted using electrospray ionization in the positive and/or negative ion mode. The chromatographic column effluent was split to allow parallel radiodetection and MS analysis.

The results obtained in these experiments are presented in **Table 10** below.

Table 10 Summary of Characterization and Identification of Radioactive Residues in Hen Eggs and Tissues Following Oral Dosing with [Diphenyl-U-$^{14}$C]BAS 510 F at 12.1 ppm for 10 Consecutive Days.

| Metabolite or Fraction | Eggs, Day 5 (TRR = 0.0521 ppm) | | Fat (TRR = 0.0250 ppm) | | Liver, Microwave Hydrolysis (TRR = 0.1687 ppm) | |
|---|---|---|---|---|---|---|
| | %TRR | ppm | %TRR | ppm | %TRR | ppm |
| BAS 510 F | 33.15 | 0.0173 | 80.77 | 0.0202 | -- | -- |
| M510F02 | 21.94 | 0.0115 | -- | -- | -- | -- |
| M510F01 | 27.41 | 0.0143 | -- | -- | 5.55 | 0.0094 |
| M510F49[1] | | | -- | -- | 12.71 | 0.0214 |
| M510F51[2] | | | -- | -- | 21.69 | 0.0366 |
| M510F52[3] | | | -- | -- | 42.09 | 0.071 |
| Unknowns | 1.68 | 0.0009 | -- | -- | -- | -- |
| Hexane extract | -- | -- | 3.18 | <0.001 | -- | -- |
| Aqueous C18 SPE eluate | -- | -- | -- | -- | 17.96 | 0.0303 |
| Water eluate | 4.92 | 0.0026 | -- | -- | -- | -- |
| Total Identified (TI) | 82.50 | 0.0431 | 80.77 | 0.0202 | 82.04 | 0.1384 |
| Total Characterized (TC) | 6.60 | 0.0035 | 3.18 | <0.001 | 17.96 | 0.0303 |
| Total Extractable (TE) | 89.10 | 0.0466 | 83.95 | 0.0210 | 100.00 | 0.1687 |
| Total Bound (TB) | 3.54 | 0.0018 | 7.03 | 0.002 | -- | -- |
| % Mass Balance | 92.64 | | 90.98 | | 100 | |

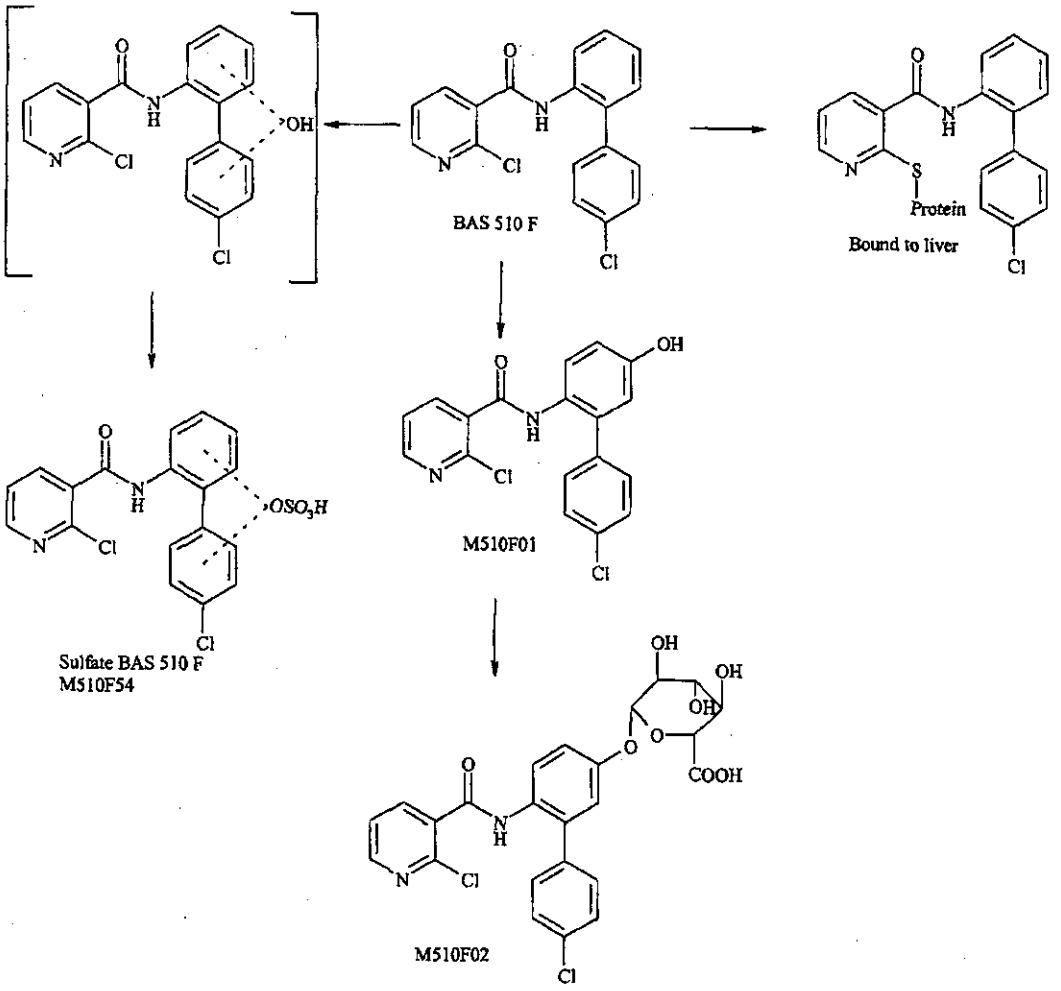
TC = Sum of all unidentified, extractable residues
TE = Sum of TI and TC
% Mass Balance = TE %TRR +TB % TRR
[1] Released during microwave hydrolysis; putatively results from hydrolysis of BAS 510 F.
[2] Released during microwave hydrolysis; putatively results from hydrolysis of M510F01.
[3] Released during microwave hydrolysis; putatively results from hydrolysis of bound/conjugated BAS 510 F.

Because of low radioactivity levels, residues in muscle were not characterized. Approximately 90% of the TRR were extracted from hen fat using acetonitrile. Protease digestion of eggs released 89% TRR, and microwave hydrolysis using formic acid released 100% TRR from liver. The parent, BAS 510 F, was detected in eggs and fat at 33.15% TRR (0.0173 ppm) and 80.77% TRR (0.0202 ppm), respectively. BAS 510 F was the only compound identified in fat. Metabolite M510F01 was detected in eggs and liver at 27.41% TRR (0.0143 ppm) and 5.55% TRR (0.0094 ppm), respectively, and metabolite M510F02 was detected in eggs at 21.94% TRR (0.0115 ppm). Material balances were ~91-100% TRR.

Microwave hydrolysis products M510F49, M510F51, and M510F52 were found in liver hydrolysates at 12.71% TRR (0.0214 ppm), 21.69% TRR (0.0366 ppm), and 42.09% TRR (0.0710 ppm), respectively. In the goat metabolism study (MRID 45405024), the petitioner concluded that M510F49 resulted from microwave hydrolysis of BAS 510 F, M510F51 resulted from hydrolysis of M510F01, and M510F52 resulted from hydrolysis of bound and/or conjugated BAS 510 F.

Based on the results of the study, BAS 510 F is metabolized in hens through hydroxylation of the biphenyl portion to form M510F01. M510F01 then undergoes glucuronidation to form M510F02. A sulfate substitution in the diphenyl portion occurs. In addition, the chlorine atom on the pyridine ring is substituted by thiol groups in biomolecules. The proposed metabolic pathway is presented in **Figure 3**.

Figure 3. Proposed metabolic pathway of BAS 510 in laying hen.



## ROTATIONAL CROPS - CONFINED STUDY

BASF Corporation has submitted a confined rotational crop study (Tier I) with BAS 510 F. Radiolabeled [$^{14}$C]BAS 510 F, labeled at the 3-position of the pyridine ring or uniformly labeled on the phenyl rings, was applied to bare soil at 1.88 lb ai/A (2100 g ai/ha). Rotational crops, lettuce, radish, and wheat, were planted in treated soil 30, 120, 270, and 365 days following treatment of the soil. Immature wheat forage, and mature lettuce, radish root and top, and wheat straw and grain were harvested from each of the plantback intervals (PBIs). TRR were determined by combustion/LSC. The petitioner used the calculated TRR values (extractable radioactivity plus nonextractable residues) for reporting results. Calculated TRR in diphenyl- and pyridine-label samples ranged 0.028-0.084 and 0.022-0.161 ppm in lettuce, 0.150-0.337 and 0.113-0.343 ppm in radish tops, 0.030-0.098 and 0.017-0.066 ppm in radish roots, 0.265-1.575 and 0.230-0.690 ppm in wheat forage, 1.404-9.826 and 1.614-4.008 ppm in wheat straw, and 0.023-0.243 and 0.147-0.285 ppm in wheat grain, respectively. Material balances, based on combustion of the samples, were 77.2-127.3% and 51.7-112.6% for the diphenyl and pyridine labels, respectively; exceptionally low or high material balances were observed for certain matrices: 230% for pyridine-label lettuce (120-day PBI), 178.5% for pyridine-label wheat straw (120-day PBI), 40.0% for pyridine-label wheat forage (270-day PBI) and 564.6% for pyridine-label wheat grain (270-day PBI). The petitioner did not provide any explanations for these values.

For both labels, approximately 62-97% of the TRR were extracted using methanol, and aqueous ammonia in some cases, from all rotational crop matrices except wheat grain; only 18-58% of the TRR were extracted from wheat grain using methanol and aqueous ammonia,. Nonextractable residues of rotational crops were further fractionated using aqueous ammonia hydrolysis, sodium hydroxide hydrolysis, and/or DMSO extraction, to yield fractions that the petitioner attributed to protein, cellulose, lignin, and/or starch. The methanol extracts were analyzed by HPLC, and the results were confirmed by a second HPLC system. Identifications of the parent and the metabolite M510F61 were confirmed by LC/MS/MS in 30-day PBI wheat straw samples.

The results obtained are presented below in **Tables 11-16**.

Table 11. Summary of Characterization and Identification of Radioactive Residues in Lettuce Planted Following Application of Isotopically Labeled BAS 510 F at 1.88 lb ai/A to Bare Soil.

| Metabolite or Fraction | 30-day PBI | | 120-day PBI | | 270-day PBI | | 365-day PBI | |
|---|---|---|---|---|---|---|---|---|
| | %TRR | ppm | %TRR | ppm | %TRR | ppm | %TRR | ppm |
| **Diphenyl label** | TRR = 0.050 ppm | | TRR = 0.084 ppm | | TRR = 0.067 ppm | | TRR = 0.028 ppm | |
| BAS 510 F | 93.8 | 0.047 | 85.2 | 0.072 | 94.1 | 0.063 | 55.6 | 0.016 |
| Unknowns | -- | -- | 4 | 0 | -- | -- | 7.2 | 0 |
| Aqueous ($NH_3$) | -- | -- | -- | -- | -- | -- | 15.4 | 0 |
| Aqueous (NaOH) | -- | -- | -- | -- | -- | -- | 16.3 | 0.01 |
| DCM fraction | -- | -- | -- | -- | -- | -- | 2.2 | 0 |
| Precipitate (lignin) | -- | -- | -- | -- | -- | -- | 4.5 | 0 |
| Solids (cellulose) | -- | -- | -- | -- | -- | -- | 5.2 | 0 |
| Total Identified (TI) | 93.8 | 0.047 | 85.2 | 0.072 | 94.1 | 0.063 | 55.6 | 0.016 |
| Total Characterized (TC) | -- | -- | 4.0 | 0 | -- | -- | 50.8 | 0.014 |
| Total Extractable (TE) | 93.8 | 0.047 | 89.2 | 0.075 | 94.1 | 0.063 | 106.4 | 0.030 |
| Total Bound (TB) | 6.2 | 0 | 10.8 | 0.01 | 5.9 | 0 | -- | -- |
| % Mass Balance | 100 | | 100 | | 100 | | 106.4 | |
| **Pyridine label** | TRR = 0.035 ppm | | TRR = 0.161 ppm | | TRR = 0.031 ppm | | TRR = 0.022 ppm | |
| BAS 510 F | 58.5 | 0.02 | 90.8 | 0.146 | 65.1 | 0.02 | 61.6 | 0.014 |
| Unknowns | 22.7 | 0.01 | -- | -- | 9.4 | 0 | 14.5 | 0 |
| Ammonia extract | 1.9 | 0 | -- | -- | -- | -- | -- | -- |
| Total Identified (TI) | 58.5 | 0.020 | 90.8 | 0.146 | 65.1 | 0.020 | 61.6 | 0.014 |
| Total Characterized (TC) | 24.6 | 0.009 | -- | -- | 9.4 | 0.003 | 14.5 | 0.003 |
| Total Extractable (TE) | 83.1 | 0.029 | 90.8 | 0.146 | 74.5 | 0.023 | 76.1 | 0.017 |
| Total Bound (TB) | 11.6 | 0 | 9.2 | 0.015 | 25.5 | 0.01 | 23.9 | 0.01 |
| % Mass Balance | 94.7 | | 100 | | 100 | | 100 | |

TC = Sum of all unidentified, extractable residues
TE = Sum of TI and TC
% Mass Balance = TE %TRR +TB % TRR



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Table 12. Summary of Characterization and Identification of Radioactive Residues in **Radish Tops** Planted Following Application of Isotopically Labeled BAS 510 F at 1.88 lb ai/A to Bare Soil.

| Metabolite or Fraction | 30-day PBI | | 120-day PBI | | 270-day PBI | | 365-day PBI | |
|---|---|---|---|---|---|---|---|---|
| | %TRR | ppm | %TRR | ppm | %TRR | ppm | %TRR | ppm |
| **Biphenyl label** | TRR = 0.337 ppm | | TRR = 0.294 ppm | | TRR = 0.150 ppm | | TRR = 0.207 ppm | |
| BAS 510 F | 90.2 | 0.304 | 71.2 | 0.209 | 73.1 | 0.109 | 69.4 | 0.144 |
| M510F61 | 5.9 | 0.02 | -- | -- | 21.2 | 0.032 | 15.5 | 0.032 |
| Unknowns | -- | -- | 13.2 | 0.039 | -- | -- | 10.2 | 0.022 |
| Aqueous ($NH_3$) | -- | -- | 2 | 0.01 | -- | -- | -- | -- |
| Aqueous (NaOH) | -- | -- | 2.6 | 0.01 | -- | -- | -- | -- |
| DCM fraction | -- | -- | 0.4 | 0 | -- | -- | -- | -- |
| Precipitate (lignin) | -- | -- | 0.4 | 0 | -- | -- | -- | -- |
| Solids (cellulose) | -- | -- | 1.2 | 0 | -- | -- | -- | -- |
| Total Identified (TI) | 96.1 | 0.324 | 71.2 | 0.209 | 94.3 | 0.141 | 84.9 | 0.176 |
| Total Characterized (TC) | 0 | 0 | 19.8 | 0.059 | 0 | 0 | 10.2 | 0.022 |
| Total Extractable (TE) | 96.1 | 0.324 | 91.0 | 0.268 | 94.3 | 0.141 | 95.1 | 0.198 |
| Total Bound (TB) | 3.9 | 0.013 | -- | -- | 5.7 | 0.01 | 4.8 | 0.018 |
| % Mass Balance | 100 | | 91 | | 100 | | 99.9 | |
| **Pyridine label** | TRR = 0.343 ppm | | TRR = 0.211 ppm | | TRR = 0.125 ppm | | TRR = 0.113 ppm | |
| BAS 510 F | 87.6 | 0.301 | 81.8 | 0.172 | 82.5 | 0.104 | 78.2 | 0.088 |
| M510F61 | 4.6 | 0.016 | 7 | 0.015 | 3.6 | 0 | 11.2 | 0.013 |
| Unknowns | -- | -- | -- | -- | -- | -- | 1.7 | 0 |
| Total Identified (TI) | 92.2 | 0.317 | 88.8 | 0.187 | 86.1 | 0.108 | 89.4 | 0.101 |
| Total Characterized (TC) | -- | -- | -- | -- | -- | -- | 1.7 | 0 |
| Total Extractable (TE) | 92.2 | 0.317 | 88.8 | 0.187 | 86.1 | 0.108 | 91.1 | 0.103 |
| Total Bound (TB) | 7.8 | 0.027 | 11.2 | 0.024 | 13.9 | 0.017 | 8.9 | 0.01 |
| % Mass Balance | 100 | | 100 | | 100 | | 100 | |

TC = Sum of all unidentified, extractable residues
TE = Sum of TI and TC
% Mass Balance = TE %TRR +TB % TRR

| Metabolite or Fraction | 30-day PBI | | 120-day PBI | | 270-day PBI | | 365-day PBI | |
|---|---|---|---|---|---|---|---|---|
| | %TRR | ppm | %TRR | ppm | %TRR | ppm | %TRR | ppm |
| **Biphenyl label** | TRR = 0.072 ppm | | TRR = 0.052 ppm | | TRR = 0.098 ppm | | TRR = 0.030 ppm | |
| BAS 510 F | 89.6 | 0.064 | 67.8 | 0.035 | 92.8 | 0.091 | 78.4 | 0.024 |
| M510F61 | -- | -- | 10.9 | 0.01 | -- | -- | 4 | 0 |
| Unknowns | 3.5 | 0 | -- | -- | -- | -- | 7.5 | 0 |
| Aqueous (NaOH) | -- | -- | 4.7 | 0 | -- | -- | -- | -- |
| Aqueous ($NH_3$) | -- | -- | 2.1 | 0 | -- | -- | -- | -- |
| DCM fraction | -- | -- | 0.9 | <0.001 | -- | -- | -- | -- |
| Precipitate (lignin) | -- | -- | 1 | 0 | -- | -- | -- | -- |
| Precipitate (protein) | -- | -- | 0.3 | <0.001 | -- | -- | -- | -- |
| Solids (cellulose) | -- | -- | 2 | 0 | -- | -- | -- | -- |
| Total Identified (TI) | 89.6 | 0.064 | 78.7 | 0.041 | 92.8 | 0.091 | 82.4 | 0.025 |
| Total Characterized (TC) | 3.5 | 0 | 11.0 | 0.01 | -- | -- | 7.5 | 0 |
| Total Extractable (TE) | 93.1 | 0.067 | 89.7 | 0.047 | 92.8 | 0.091 | 89.9 | 0.027 |
| Total Bound (TB) | 6.9 | 0.01 | -- | -- | 7.2 | 0.01 | 10.1 | 0 |
| % Mass Balance | 100 | | 89.7 | | 100 | | 100 | |
| **Pyridine label** | TRR = 0.048 ppm | | TRR = 0.038 ppm | | TRR = 0.017 ppm | | TRR = 0.066 ppm | |
| BAS 510 F | 62.7 | 0.03 | 60.1 | 0.023 | 52.6 | 0.01 | 91 | 0.06 |
| M510F61 | -- | -- | -- | -- | -- | -- | -- | -- |
| Unknowns | 18 | 0.01 | 21.5 | 0.01 | 24.5 | 0 | -- | -- |
| Ammonia extract | 1.2 | 0 | -- | -- | -- | -- | -- | -- |
| Total Identified (TI) | 62.7 | 0.03 | 60.1 | 0.023 | 52.6 | 0.01 | 91.0 | 0.06 |
| Total Characterized (TC) | 19.2 | 0.01 | 21.5 | 0.01 | 24.5 | 0 | -- | -- |
| Total Extractable (TE) | 81.9 | 0.04 | 81.6 | 0.031 | 77.1 | 0.013 | 91.0 | 0.06 |
| Total Bound (TB) | 17.4 | 0.01 | 18.4 | 0.01 | 22.9 | 0 | 9 | 0.01 |
| % Mass Balance | 99.3 | | 100 | | 100 | | 100 | |

TC = Sum of all unidentified, extractable residues
TE = Sum of TI and TC
% Mass Balance = TE %TRR +TB % TRR

Table 13. Summary of Characterization and Identification of Radioactive Residues in **Radish Roots** Planted Following Application of Isotopically Labeled BAS 510 F at 1.88 lb ai/A to Bare Soil.

Table 14. Summary of Characterization and Identification of Radioactive Residues in **Wheat Forage** Planted Following Application of Isotopically Labeled BAS 510 F at 1.88 lb ai/A to Bare Soil.

| Metabolite or Fraction | 30-day PBI | | 120-day PBI | | 270-day PBI | | 365-day PBI | |
|---|---|---|---|---|---|---|---|---|
| | %TRR | ppm | %TRR | ppm | %TRR | ppm | %TRR | ppm |
| **Diphenyl label** | TRR = 1.575 ppm | | TRR = 0.980 ppm | | TRR = 0.562 ppm | | TRR = 0.265 ppm | |
| BAS 510 F | 93.5 | 1.472 | 86.4 | 0.846 | 62.8 | 0.352 | 75 | 0.199 |
| M510F61 | 2 | 0.032 | 2.1 | 0.021 | 18.1 | 0.102 | 9.8 | 0.026 |
| Unknowns | -- | -- | -- | -- | 7.5 | 0.042 | 8.3 | 0.022 |
| Aqueous (NaOH) | 0.9 | 0.015 | 2.4 | 0.023 | 1.3 | 0.01 | 2.9 | 0.01 |
| Aqueous ($NH_3$) | 0.9 | 0.014 | 1.6 | 0.015 | 1.6 | 0.01 | 1.2 | 0 |
| DCM fraction | 0.6 | 0.01 | 2.3 | 0.022 | 0.6 | 0 | 0.8 | 0 |
| Precipitate (lignin) | 0.6 | 0.01 | 1.5 | 0.015 | 1.4 | 0.01 | 1.6 | 0 |
| Precipitate (protein) | 1.3 | 0.021 | 0.3 | 0 | 0.5 | 0 | 0.3 | 0 |
| Solids (cellulose) | 0.9 | 0.014 | 1.8 | 0.018 | 0.3 | 0 | 0.7 | 0 |
| Total Identified (TI) | 95.5 | 1.504 | 88.5 | 0.867 | 80.9 | 0.454 | 84.8 | 0.225 |
| Total Characterized (TC) | 5.2 | 0.083 | 9.9 | 0.096 | 13.2 | 0.075 | 15.8 | 0.042 |
| Total Extractable (TE) | 100.7 | 1.587 | 98.4 | 0.963 | 94.1 | 0.529 | 100.6 | 0.267 |
| Total Bound (TB) | -- | -- | -- | -- | -- | -- | -- | -- |
| % Mass Balance | 100.7 | | 98.4 | | 94.1 | | 100.6 | |
| **Pyridine label** | TRR = 0.690 ppm | | TRR = 0.433 ppm | | TRR = 0.230 ppm | | TRR = 0.255 ppm | |
| BAS 510 F | 89.8 | 0.619 | 87.5 | 0.379 | 92.8 | 0.214 | 74.7 | 0.191 |
| M510F61 | 3.4 | 0.024 | -- | -- | 2.3 | 0.01 | 2.9 | 0.01 |
| Unknowns | -- | -- | -- | -- | 2.2 | 0.01 | 5.8 | 0.015 |
| Aqueous (NaOH) | 2.7 | 0.018 | 5.8 | 0.025 | -- | -- | -- | -- |
| Aqueous ($NH_3$) | 1.1 | 0.01 | 1.5 | 0.01 | -- | -- | -- | -- |
| Precipitate (lignin) | 1 | 0.01 | 1.9 | 0.01 | -- | -- | -- | -- |
| Precipitate (protein) | <0.1 | <0.001 | 0.1 | <0.001 | -- | -- | -- | -- |
| Solids (cellulose) | 1.2 | 0.01 | 1.5 | 0.01 | -- | -- | -- | -- |
| Total Identified (TI) | 93.2 | 0.643 | 87.5 | 0.379 | 95.1 | 0.219 | 77.6 | 0.199 |
| Total Characterized (TC) | 6.1 | 0.042 | 10.8 | 0.047 | 2.2 | 0.01 | 5.8 | 0.015 |
| Total Extractable (TE) | 99.3 | 0.685 | 98.3 | 0.426 | 97.3 | 0.224 | 83.4 | 0.214 |
| Total Bound (TB) | -- | -- | -- | -- | 2.7 | 0.01 | 16.5 | 0.042 |
| % Mass Balance | 99.3 | | 98.3 | | 100 | | 99.9 | |

TC = Sum of all unidentified, extractable residues
TE = Sum of TI and TC
% Mass Balance = TE %TRR +TB % TRR

Table 15. Summary of Characterization and Identification of Radioactive Residues in **Wheat Straw** Planted Following Application of Isotopically Labeled BAS 510 F at 1.88 lb ai/A to Bare Soil.

| Metabolite or Fraction | 30-day PBI | | 120-day PBI | | 270-day PBI | | 365-day PBI | |
|---|---|---|---|---|---|---|---|---|
| | %TRR | ppm | %TRR | ppm | %TRR | ppm | %TRR | ppm |
| **Biphenyl label** | TRR = 9.826 ppm | | TRR = 3.912 ppm | | TRR = 3.226 ppm | | TRR = 1.404 ppm | |
| BAS 510 F | 81.3 | 7.991 | 84.6 | 3.311 | 70.8 | 2.283 | 77.6 | 1.088 |
| M510F61 | 4.3 | 0.423 | 4.8 | 0.187 | 0.9 | 0.03 | 1.8 | 0.025 |
| Unknowns | -- | -- | -- | -- | 5.4 | 0.174 | 10 | 0.14 |
| Aqueous (NaOH) | 1.8 | 0.177 | 1.4 | 0.054 | 2 | 0.066 | 1.4 | 0.019 |
| Aqueous ($NH_3$) | 5.5 | 0.538 | 3.8 | 0.149 | 4.7 | 0.153 | 3.1 | 0.044 |
| DCM fraction | 1.2 | 0.112 | 0.9 | 0.033 | 3.6 | 0.117 | 1 | 0.014 |
| Precipitate (lignin) | 2.2 | 0.216 | 1.3 | 0.052 | 4.6 | 0.15 | 2.5 | 0.036 |
| Precipitate (protein) | 0.2 | 0.190 | 0.4 | 0.014 | 1.4 | 0.044 | 0.5 | 0.01 |
| Solids (cellulose) | 1.2 | 0.121 | 1 | 0.04 | 3.5 | 0.114 | 1 | 0.014 |
| Total Identified (TI) | 85.6 | 8.414 | 89.4 | 3.498 | 71.7 | 2.313 | 79.4 | 1.113 |
| Total Characterized (TC) | 12.1 | 1.354 | 8.8 | 0.342 | 25.2 | 0.818 | 19.5 | 0.274 |
| Total Extractable (TE) | 97.7 | 9.768 | 98.2 | 3.84 | 96.9 | 3.131 | 98.9 | 1.387 |
| Total Bound (TB) | -- | -- | -- | -- | -- | -- | -- | -- |
| % Mass Balance | 97.7 | | 98.2 | | 96.9 | | 98.9 | |
| **Pyridine label** | TRR = 3.609 ppm | | TRR = 4.008 ppm | | TRR = 1.614 ppm | | TRR = 1.925 ppm | |
| BAS 510 F | 87.5 | 3.156 | 64.8 | 2.598 | 50 | 0.808 | 77.3 | 1.488 |
| M510F61 | 2.8 | 0.102 | 2.9 | 0.117 | 4.4 | 0.071 | -- | -- |
| Unknowns | -- | -- | -- | -- | 2 | 0.032 | -- | -- |
| Aqueous (NaOH) | 3.4 | 0.124 | 10.1 | 0.405 | 10.1 | 0.164 | 9.2 | 0.178 |
| Aqueous ($NH_3$) | 2.4 | 0.088 | 4.1 | 0.162 | 5.2 | 0.084 | 4.1 | 0.092 |
| Precipitate (lignin) | 4.9 | 0.176 | 2.7 | 0.11 | 2.5 | 0.041 | 3 | 0.057 |
| Precipitate (protein) | 0.1 | 0 | 0.4 | 0.012 | 0.4 | 0.01 | 0.4 | 0.01 |
| Solids (cellulose) | 1.5 | 0.054 | 1.9 | 0.076 | 2.6 | 0.041 | 1.6 | 0.03 |
| Total Identified (TI) | 90.3 | 3.258 | 67.7 | 2.715 | 54.4 | 0.879 | 77.3 | 1.488 |
| Total Characterized (TC) | 12.3 | 0.446 | 19.2 | 0.765 | 22.8 | 0.369 | 18.3 | 0.365 |
| Total Extractable (TE) | 102.6 | 3.704 | 86.9 | 3.480 | 77.2 | 1.248 | 95.6 | 1.853 |
| Total Bound (TB) | -- | -- | -- | -- | -- | -- | -- | -- |
| % Mass Balance | 102.6 | | 86.9 | | 77.2 | | 95.6 | |

TC = Sum of all unidentified, extractable residues
TE = Sum of TI and TC
% Mass Balance = TE %TRR +TB % TRR

Table 16. Summary of Characterization and Identification of Radioactive Residues in **Wheat Grain** Planted Following Application of Isotopically Labeled BAS 510 F at 1.88 lb ai/A to Bare Soil.

| Metabolite or Fraction | 30-day PBI | | 120-day PBI | | 270-day PBI | | 365-day PBI | |
|---|---|---|---|---|---|---|---|---|
| | %TRR | ppm | %TRR | ppm | %TRR | ppm | %TRR | ppm |
| **Diphenyl label** | TRR = 0.166 ppm | | TRR = 0.243 ppm | | TRR = 0.023 ppm | | TRR = 0.048 ppm | |
| BAS 510 F | 16.8 | 0.028 | 9.6 | 0.023 | 35.4 | 0.01 | 23.6 | 0.011 |
| Unknowns | 1.6 | 0 | 2.7 | 0.01 | -- | -- | 1.5 | 0 |
| Aqueous ($NH_3$) | 8 | 0.013 | 6.9 | 0.017 | 8.9 | 0 | 8.1 | 0 |
| DCM fraction | 2.1 | 0 | 0.3 | 0 | 2.3 | 0 | 1 | <0.001 |
| Ethanol fraction | 5.7 | 0.01 | 4.6 | 0.011 | 11.3 | 0 | 6.4 | 0 |
| Precipitate (protein) | 6.5 | 0.011 | 4.9 | 0.012 | 9.6 | 0 | 6.2 | 0 |
| Precipitate (starch) | 0.6 | 0 | 1.6 | 0 | 4.3 | 0 | 2.1 | 0 |
| Total Identified (TI) | 16.8 | 0.028 | 9.6 | 0.023 | 35.4 | 0.01 | 23.6 | 0.011 |
| Total Characterized (TC) | 24.5 | 0.040 | 21.0 | 0.052 | 36.4 | 0.01 | 25.3 | 0.013 |
| Total Extractable (TE) | 41.3 | 0.068 | 30.6 | 0.075 | 71.8 | 0.017 | 48.9 | 0.024 |
| Total Bound (TB) | 65.1 | 0.108 | 71.6 | 0.174 | 30.3 | 0.01 | 54.2 | 0.026 |
| % Mass Balance | 106.4 | | 102.2 | | 102.1 | | 103.1 | |
| **Pyridine label** | TRR = 0.147 ppm | | TRR = 0.285 ppm | | TRR = 0.271 ppm | | TRR = 0.148 ppm | |
| BAS 510 F | 6.1 | 0.01 | 5.3 | 0.015 | 1.9 | 0.01 | 4.2 | 0.01 |
| Unknowns | 5.6 | 0.01 | 3.6 | 0.01 | 2.1 | 0.01 | 2.6 | 0 |
| Aqueous ($NH_3$) | 11.2 | 0.016 | 9.1 | 0.027 | 8.8 | 0.025 | 8.5 | 0.013 |
| Ethanol fraction | 6.7 | 0.01 | 5.5 | 0.016 | 6 | 0.016 | 6.5 | 0.01 |
| Precipitate (protein) | 3.1 | 0.01 | 9.6 | 0.027 | 5.9 | 0.016 | 5.7 | 0.01 |
| Precipitate (starch) | 48.4 | 0.071 | 41.4 | 0.118 | 36.2 | 0.098 | 41 | 0.061 |
| Total Identified (TI) | 6.1 | 0.01 | 5.3 | 0.015 | 1.9 | 0.01 | 4.2 | 0.006 |
| Total Characterized (TC) | 75.0 | 0.111 | 69.2 | 0.198 | 59.0 | 0.161 | 64.3 | 0.096 |
| Total Extractable (TE) | 81.1 | 0.120 | 74.5 | 0.213 | 60.9 | 0.166 | 68.5 | 0.102 |
| Total Bound (TB) | 17.4 | 0.026 | 22.3 | 0.064 | 26.4 | 0.072 | 23.3 | 0.035 |
| % Mass Balance | 98.5 | | 96.8 | | 87.3 | | 91.8 | |

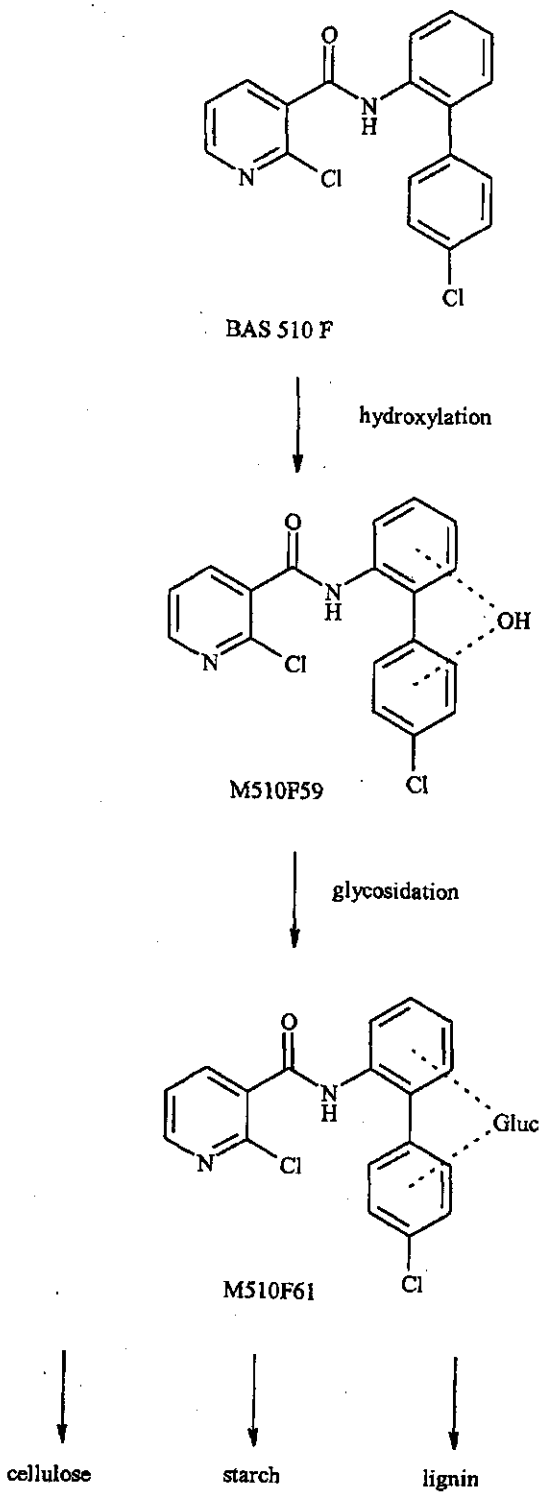
TC = Sum of all unidentified, extractable residues
TE = Sum of TI and TC
% Mass Balance = TE %TRR +TB % TRR.

A total of 52.6-96.1% of the TRR was identified in **lettuce, radish roots and tops, and wheat forage and straw** from the 30-, 120-, 270-, and 365-day PBIs. **The parent, BAS 510 F was the major residue identified** in these commodities, accounting for 50.0-96.1% TRR (0.009-3.156 ppm). The glucoside metabolite M510F61 was identified in radish tops (all PBIs), radish roots (120- and 365-day PBIs only), wheat forage (all PBIs), and wheat straw (all PBIs); M510F61 accounted for 0.9-21.2% TRR (0.001-0.423 ppm). Unknown metabolites accounted for up to 22.7% TRR; however, individual peaks that were present at >10% TRR were each <0.04 ppm. Bound residues in certain samples of lettuce, radish tops and roots, and wheat forage and straw were tentatively demonstrated to be due to the incorporation of radioactivity into protein, lignin, and cellulose at levels totaling up to 9.7% TRR. The remaining nonextractable residues were <0.05 ppm and were not further characterized.

A total of 1.9-35.4% of the TRR was identified in **wheat grain** from the 30-, 120-, 270-, and 365-day PBIs. The parent, BAS 510 F, was the only compound identified in wheat grain at all PBIs, accounting for 9.6-35.4% TRR (0.008-0.028 ppm) in diphenyl-label grain and 1.9-6.1% TRR (0.005-0.015 ppm) in pyridine-label grain. Unknown metabolites accounted for ≤5.6% TRR (≤0.010 ppm). Bound radioactivity in wheat grain was tentatively demonstrated to be due to incorporation of radioactivity into starch, at 0.6-4.3% TRR (0.001-0.004 ppm) for the diphenyl label samples and 36.2-48.4% TRR (0.061-0.118 ppm) for the pyridine label samples, and protein, at 4.9-9.6% TRR (0.002-0.012 ppm) for the diphenyl label samples and 3.1-9.6% TRR (0.005-0.027 ppm) for the pyridine label samples. Nonextractable residues were 30.3-71.6% TRR (0.007-0.174 ppm) in diphenyl-label grain samples and 17.4-26.4% TRR (0.026-0.072 ppm) in pyridine-label grain samples; these residues were not further characterized.

The TRR in soil decreased over the duration of the study, with only the parent identified. Therefore, the petitioner proposed that BAS 510 F is not metabolized in the soil, and that the metabolite M510F61 identified in several crop matrices was a result of metabolism of BAS 510 F in the plants. The proposed metabolic pathway in rotated crops is presented below in **Figure 4**.

Figure 4.Proposed metabolic pathway of BAS 510 F in rotated crops.



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Comments. Based on our evaluation of the confined rotational crop study, the following additional information is requested from the petitioner:

1. Additional analytical work should be conducted to provide direct evidence of incorporation of radioactivity into biomolecules (e.g., isolation and identification of $^{14}$C-starch or $^{14}$C-glucose derived from starch). In addition, significant radioactivity remained nonextractable in wheat grain samples (diphenyl label) following the petitioner's extraction and hydrolysis procedures. If the petitioner cannot provide direct evidence of incorporation of radioactivity into the biomolecules of wheat grain, then additional analytical work to characterize these nonextractable residues may be required.

2. The petitioner should provide additional details regarding the study, including more detailed descriptions of the extraction, hydrolysis, and liquid/liquid partitioning procedures, identification of the source of the M510F59 and M510F61 reference standards used in the study, identification of the detector used in the HPLC analyses, and discussion of the identification of M510F59 in wheat straw samples.

3. The petitioner should provide an explanation for all cases in which there was a large difference between the TRR determined by combustion/LSC and TRR determined by calculation (120-day PBI diphenyl-label wheat grain; 120-day PBI pyridine-label lettuce; 120-day PBI pyridine-label wheat straw; and 270-day PBI pyridine-label wheat forage and grain). The petitioner should also address the large difference in radioactivity attributed to starch between the two labels (~40-50% TRR for the pyridine label, and <5% TRR for the diphenyl label).

## ANALYTICAL METHODS

**Proposed Enforcement Method - Plants (D0008)**

Method D0008 quantitates residues of BAS 510 F in plant matrices. The parent residue is extracted using an aqueous organic solvent mixture followed by liquid:liquid partitioning and a column clean-up. Quantitation is by GC/MS. The validated limit of quantitation (LOQ) of BAS 510 F in plant matrices is 0.05 ppm. Recoveries of BAS 510 F in plant matrices over the fortification range of 0.05-0.50 ppm averaged 97 ± 11% (n=60). Method D0008 was validated successfully at these fortification levels by an independent laboratory using canola seed and tomatoes as representative oily and non-oily matrices. Recoveries were 79±8% for canola seed and 84±10% for tomato. The method has been submitted to ACB/BEAD for a method validation trial.

**Proposed Enforcement Method - Animal Matrices (DFG S19)**

Method DFG S19 quantitates residues of BAS 510 F and its metabolite M510F01 in animal matrices. Residues are extracted with methanol. The extract is treated with enzymes in order to release the conjugated glucuronic acid metabolite. The residues are then isolated by liquid:liquid partitioning followed by column chromatography. The hydroxylated metabolite is acetylated followed by a column clean-up. The parent and acetylated metabolite are quantitated by GC/ECD. M510F01 is reported in terms of BAS 510 F equivalents. The validated LOQ for the residues of BAS 510 F and M510F01 is 0.01 ppm for each analyte in milk and 0.025 ppm for each analyte in eggs and other animal matrices. Recoveries of BAS 510 F and M510F01 in animal matrices over the fortification range of 0.01-0.10 ppm for milk and 0.025-0.25 ppm for eggs and other animal matrices by this method were at acceptable levels (means ranged from 79-98%; n=10 for each analyte for each matrix). Method DFG S19 was validated successfully at these fortification levels by an independent laboratory using milk and bovine liver as the test matrices. Mean recoveries were 95-115% (n=20) for milk and 74-97% (n=40) for liver. The method has been submitted to ACB/BEAD for a method validation trial.

## STORAGE STABILITY DATA

**Plant Matrices - Target Crops**

The samples from the various field trial studies were stored frozen (<-10C) for ≤1.5 to ≤8 months prior to residue analysis. A freezer storage stability study has shown BAS 510 F residues to be stable (84-102%) for at least one year (interim report from an ongoing two year study) in a variety of crop matrices, including wheat (forage, grain, straw), canola seed, sugar beet roots, cabbage, peach, and peas.

**Plant Matrices - Rotational Crops**

Cabbage, radish, and winter wheat samples from the Tier II limited field trials in rotational crops study were maintained in frozen storage (< -10C) for 3.8-12.5 months prior to residue analysis. Samples from the various Tier III large scale field trials in rotational crops study were kept in frozen storage (<-10C) for ≤ 1 to ≤ 7 months prior to residue analysis. As discussed above, BAS 510 F residues have been shown to be stable in plant matrices for at least a year (thus far).

**Animal Matrices**

Samples from the ruminant feeding study were stored frozen (-18 C) for 25-104 days (0.8-3.4 months) prior to analysis for residues of BAS 510 F and M510F01. A freezer storage stability study has shown that residues of BAS 510 F and its metabolite M510F01 are relatively stable (72-93%) for up to 5.5 months (duration of study) in cow milk, liver, and muscle.

Samples from the hen feeding study were stored frozen (< -10C) for 10-80 days (0.3-2.6 months) for eggs and 49-69 days (1.6-2.3 months) for other matrices prior to analysis for residues of BAS 510 F and M510F01. A limited storage stability study (44 days) was conducted concurrently with the hen feeding study for residues of BAS 510 F and its metabolite M510F01 in eggs. Recoveries of 70-102% were reported.

## MAGNITUDE OF RESIDUE STUDIES - PLANTS

Crop field and residue decline trials were carried out in 1999-2001 on the target crops/crop groups (and some rotational crops) in order to determine the magnitude of BAS 510 F residues. The number and geographic distribution of these trials was (for most crops) in accordance with EPA and/or PMRA Guidelines. The trials were carried out using the maximum seasonal use pattern; i.e., maximum single application rate, maximum number of applications, shortest interval between applications, and the minimum preharvest interval for each crop or crop group. A spray adjuvant was also included for all uses. The maximum residues of BAS 510 F reported in subject crops are very close to the tolerance levels being proposed.

## PROCESSING STUDIES

Processing studies were conducted on canola seed, grapes, peanuts, plums, tomatoes, sunflower, mint, wheat, rice, and soybeans to determine concentration factors during normal processing of the raw agricultural commodity into the processed commodities. Concentration of residues was only reported in raisins (2.4x), and (perhaps) in peanut meal or oil (flawed study) for target crops; and, soybean hulls (1.7x) and rice hulls (2.6x) for the rotational crops.

## MAGNITUDE OF THE RESIDUE STUDIES - ANIMALS

**Ruminant**

A feeding study with BAS 510 F was conducted in dairy cows. The complete results are shown in Table 17. The dosing levels, which were based on the petitioner's estimate of the maximum theoretical dietary burden to livestock, were 1.8 (1x), 5.9 (3.3x), and 20.2 (11.2x) mg/kg (ppm) in feed (dry matter; based on actual daily consumption). Combined residues of BAS 510 F (BAS 510 F + M510F01, including its conjugates) were detected in fat, liver, and kidney at the 3.3x and 11.2x dose levels.; in milk, at the 11.2x dose level; and, in fat, not at any of these dose levels.

Tolerances are being proposed for milk, meat, fat, and meat byproducts of cattle. No tolerances are currently being proposed for goats, hogs, horses, or sheep.

**Cattle Feeding Study MRID 45405110**

**Table 17 - Summary of Residue Data from Cattle Feeding Study with BAS 510 F**

| Matrix | Feeding Level (ppm) | Pre-Slaughter Interval (days) | Analyte | Residue Levels (ppm) | | | |
|---|---|---|---|---|---|---|---|
| | | | | Maximum | Highest Average | Mean[1] | Std. Dev. |
| Milk | 1.8 | Not applicable (N/A) | BAS 510 F | <0.01 | N/A | <0.01 | 0 |
| | | | M510F01 | <0.01 | N/A | <0.01 | 0 |
| | | | M510F53 | <0.01 | N/A | <0.01 | 0 |
| Skim milk | 1.8 | N/A | BAS 510 F | <0.01 | N/A | <0.01 | 0 |
| | | | M510F01 | <0.01 | N/A | <0.01 | 0 |
| Cream | 1.8 | N/A | BAS 510 F | 0.0452 | N/A | 0.0310 | 0.012 |
| | | | M510F01 | <0.01 | N/A | <0.01 | 0 |
| Milk | 5.9 | N/A | BAS 510 F | 0.0129 | 0.0110 (Day 18) | 0.0102 | 0.0006 |
| | | | M510F01 | <0.01 | N/A | <0.01 | 0 |
| | | | M510F53 | <0.01 | N/A | <0.01 | 0 |
| Skim milk | 5.9 | N/A | BAS 510 F | <0.01 | N/A | <0.01 | 0 |
| | | | M510F01 | <0.01 | N/A | <0.01 | 0 |
| Cream | 5.9 | N/A | BAS 510 F | 0.1153 | N/A | 0.1094 | 0.0086 |
| | | | M510F01 | <0.01 | N/A | <0.01 | 0 |
| Milk | 20.2 | N/A | BAS 510 F | 0.0862 | 0.0426 (Day 18) | 0.0247 | 0.0137 |
| | | | M510F01 | <0.01 | N/A | <0.01 | 0 |
| | | | M510F53 | <0.01 | N/A | <0.01 | 0 |
| Skim milk | 20.2 | N/A | BAS 510 F | 0.0108 | N/A | 0.0102 | 0.0004 |
| | | | M510F01 | <0.01 | N/A | <0.01 | 0 |
| Cream | 20.2 | N/A | BAS 510 F | 0.3709 | N/A | 0.3300 | 0.0595 |
| | | | M510F01 | <0.01 | N/A | <0.01 | 0 |
| Muscle | 1.8 | 0 | BAS 510 F | <0.025 | N/A | <0.025 | 0 |
| | | | M510F01 | <0.025 | N/A | <0.025 | 0 |
| Muscle | 5.9 | 0 | BAS 510 F | <0.025 | N/A | <0.025 | 0 |
| | | | M510F01 | <0.025 | N/A | <0.025 | 0 |
| Muscle | 20.2 | 0 | BAS 510 F | 0.0326 | N/A | 0.0275 | 0.0044 |
| | | | M510F01 | <0.025 | N/A | <0.025 | 0 |
| Muscle | 20.2 | 7 | BAS 510 F | <0.025 | N/A | <0.025 | 0 |
| | | | M510F01 | <0.025 | N/A | <0.025 | 0 |

**Table 17 - Summary of Residue Data from Cattle Feeding Study with BAS 510 F**

| Matrix | Feeding Level (ppm) | Pre-Slaughter Interval (days) | Analyte | Residue Levels (ppm) | | | |
|---|---|---|---|---|---|---|---|
| | | | | Maximum | Highest Average | Mean[1] | Std. Dev. |
| Fat | 1.8 | 0 | BAS 510 F | 0.0526 | N/A | 0.0342 | 0.0159 |
| | | | M510F01 | <0.025 | N/A | <0.025 | 0 |
| Fat | 5.9 | 0 | BAS 510 F | 0.0991 | N/A | 0.0798 | 0.0214 |
| | | | M510F01 | <0.025 | N/A | <0.025 | 0 |
| Fat | 20.2 | 0 | BAS 510 F | 0.2672 | N/A | 0.2434 | 0.0296 |
| | | | M510F01 | <0.025 | N/A | <0.025 | 0 |
| Fat | 20.2 | 7 | BAS 510 F | <0.025 | N/A | <0.025 | 0 |
| | | | M510F01 | <0.025 | N/A | <0.025 | 0 |
| Kidney | 1.8 | 0 | BAS 510 F | <0.025 | N/A | <0.025 | 0 |
| | | | M510F01 | <0.025 | N/A | <0.025 | 0 |
| Kidney | 5.9 | 0 | BAS 510 F | <0.025 | N/A | <0.025 | 0 |
| | | | M510F01 | 0.0626 | N/A | 0.0491 | 0.0124 |
| Kidney | 20.2 | 0 | BAS 510 F | 0.0443 | N/A | 0.0314 | 0.0111 |
| | | | M510F01 | 0.2930 | N/A | 0.2041 | 0.0787 |
| | | | Total[2] | <0.3180 | N/A | 0.2355 | 0.0759 |
| Kidney | 20.2 | 7 | BAS 510 F | <0.025 | N/A | <0.025 | 0 |
| | | | M510F01 | <0.025 | N/A | <0.025 | 0 |
| Liver | 1.8 | 0 | BAS 510 F | <0.025 | N/A | <0.025 | 0 |
| | | | M510F01 | <0.025 | N/A | <0.025 | 0 |
| | | | M510F53 | <0.05 | N/A | <0.05 | 0 |
| Liver | 5.9 | 0 | BAS 510 F | <0.025 | N/A | <0.025 | 0 |
| | | | M510F01 | 0.0389 | N/A | 0.0318 | 0.0064 |
| | | | M510F53 | <0.05 | N/A | <0.05 | 0 |
| Liver | 20.2 | 0 | BAS 510 F | 0.0795 | N/A | 0.0698 | 0.0099 |
| | | | M510F01 | 0.1221 | N/A | 0.1074 | 0.0158 |
| | | | Total[2] | 0.1818 | N/A | 0.1772 | 0.0061 |
| | | | M510F53 | 0.09 | N/A | 0.09 | 0.006 |
| Liver | 20.2 | 7 | BAS 510 F | <0.025 | N/A | <0.025 | 0 |
| | | | M510F01 | <0.025 | N/A | <0.025 | 0 |
| | | | M510F53 | <0.05 | N/A | <0.05 | 0 |

[1] Mean calculated using LOQ for values below the LOQ.

[2] Total BAS 510 F + M510F01; only included in this table when quantifiable residues were observed for both analytes.

Apparent residues of BAS 510 F and M510F01 were below the LOQ (0.01 ppm for milk, skim milk, and cream, and 0.025 ppm for muscle, fat, liver, and kidney) in 18 samples of milk, and 3 samples each of skim milk, cream, muscle, fat, liver, and kidney from undosed cattle.



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**Poultry**

A feeding study with BAS 510 F was conducted in laying hens. The results are shown in Table 18. The (actual) dosing levels, which were based on the petitioner's estimate of the maximum theoretical dietary burden to poultry, were 1.0 (1.0x), 5.3 (5.3x), and 19.6 (19.6x) ppm (administered daily by balling gun). Combined residues of BAS 510 F (BAS 510 F + M510F01, including its conjugates) were detected in liver at all dose levels; in eggs and fat, at the 5.3x and 19.6x dose levels; and, in muscle, not at any of these dose levels. Tolerances are being proposed for eggs, meat, fat, and meat byproducts of poultry at the combined LOQ of BAS 510 F + M510F01 in each matrix.

**Poultry Feeding Study MRID: 45643801**

**Table 18 - Summary of Residue Data from Poultry Feeding Study with BAS 510 F**

| Matrix | Feeding Level (ppm) | Pre-Slaughter Interval (days) | Analyte | Residue Levels (ppm) | | | |
|---|---|---|---|---|---|---|---|
| | | | | Maximum | Highest Average | Mean | Std. Dev. |
| Egg | 1.02 | Not applicable (N/A) | BAS 510 F | <0.01 | N/A | <0.01 | 0 |
| | | | M510F01 | <0.01 | N/A | <0.01 | 0 |
| Egg | 5.31 | N/A | BAS 510 F | <0.01 | N/A | <0.01 | 0 |
| | | | M510F01 | 0.01 | N/A | <0.01 | 0 |
| Egg | 19.63 | N/A | BAS 510 F | 0.037 | 0.025 (Days 7 & 21) | 0.017 | 0.008 |
| | | | M510F01 | 0.039 | 0.032 (Day 28) | 0.021 | 0.008 |
| | | | Total [1] | 0.065 | 0.054 (Days 21& 28) | 0.038 | 0.015 |
| Muscle | 1.02 | 0 | BAS 510 F | <0.025 | N/A | <0.025 | 0 |
| | | | M510F01 | <0.025 | N/A | <0.025 | 0 |
| Muscle | 5.31 | 0 | BAS 510 F | <0.025 | N/A | <0.025 | 0 |
| | | | M510F01 | <0.025 | N/A | <0.025 | 0 |
| Muscle | 19.63 | 0 | BAS 510 F | <0.025 | N/A | <0.025 | 0 |
| | | | M510F01 | <0.025 | N/A | <0.025 | 0 |
| Muscle | 19.63 | 3 | BAS 510 F | <0.025 | N/A | <0.025 | 0 |
| | | | M510F01 | <0.025 | N/A | <0.025 | 0 |
| Muscle | 19.63 | 10 | BAS 510 F | <0.025 | N/A | <0.025 | 0 |
| | | | M510F01 | <0.025 | N/A | <0.025 | 0 |
| Fat | 1.02 | 0 | BAS 510 F | <0.025 | N/A | <0.025 | 0 |
| | | | M510F01 | <0.025 | N/A | <0.025 | 0 |
| Fat | 5.31 | 0 | BAS 510 F | 0.099 | 0.056 | 0.056 | 0.038 |
| | | | M510F01 | <0.025 | N/A | <0.025 | 0 |

| Table 18 - Summary of Residue Data from Poultry Feeding Study with BAS 510 F | | | | | | | |
|---|---|---|---|---|---|---|---|
| Matrix | Feeding Level (ppm) | Pre-Slaughter Interval (days) | Analyte | Residue Levels (ppm) | | | |
| | | | | Maximum | Highest Average | Mean | Std. Dev. |
| Fat | 19.63 | 0 | BAS 510 F | 0.171 | 0.138 | 0.138 | 0.031 |
| | | | M510F01 | 0.053 | 0.034 | 0.034 | 0.016 |
| | | | Total [1] | 0.196 | 0.172 | 0.172 | 0.033 |
| Fat | 19.63 | 3 | BAS 510 F | <0.025 | N/A | <0.025 | 0 |
| | | | M510F01 | <0.025 | N/A | <0.025 | 0 |
| Fat | 19.63 | 10 | BAS 510 F | <0.025 | N/A | <0.025 | 0 |
| | | | M510F01 | <0.025 | N/A | <0.025 | 0 |
| Liver | 1.02 | 0 | BAS 510 F | <0.025 | N/A | <0.025 | 0 |
| | | | M510F01 | 0.026 | <0.025 | <0.025 | 0.001 |
| Liver | 5.31 | 0 | BAS 510 F | <0.025 | N/A | <0.025 | 0 |
| | | | M510F01 | 0.150 | 0.111 | 0.111 | 0.036 |
| Liver | 19.63 | 0 | BAS 510 F | <0.025 | N/A | <0.025 | 0 |
| | | | M510F01 | 0.441 | 0.379 | 0.379 | 0.078 |
| Liver | 19.63 | 3 | BAS 510 F | <0.025 | N/A | <0.025 | 0 |
| | | | M510F01 | <0.025 | N/A | <0.025 | 0 |
| Liver | 19.63 | 10 | BAS 510 F | <0.025 | N/A | <0.025 | 0 |
| | | | M510F01 | <0.025 | N/A | <0.025 | 0 |

[1] Total BAS 510 F and M510F01; only included in this table when quantifiable residues were observed for both analytes.

Apparent residues of BAS 510 F and M510F01 were each below the LOQ (<0.01 ppm for eggs and <0.025 ppm for muscle, fat, and liver) in 16 samples of eggs, and 2 samples each of muscle, fat, and liver from undosed hens.

## FIELD ACCUMULATION IN ROTATIONAL CROPS

**Tier II - Limited Field Trials - Rotational Crops (Winter Wheat, Cabbage, Radish)**

Two field trials were conducted (GA, CA). Five foliar applications of the 70% WG formulation were made to the primary crop (strawberries), with 6- to 8-day retreatment intervals, at 0.37 lb ai/A/application for a total application rate of 1.85 lbs ai/A. Strawberries were harvested at normal maturity, and representative rotational crops, cabbage, radish, and winter wheat, were planted 14, 30, and 45 days following the last application to the primary crop. Samples of rotational crop commodities, cabbage heads with and without wrapper leaves, radish roots and tops, and wheat forage, hay, straw, and grain, were harvested at normal maturity and analyzed for residues of BAS 510 F.



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In rotational crops planted 14 days following the last application, residues of BAS 510 F were non-quantifiable (<0.05 ppm) in/on cabbage heads ± wrapper leaves and in/on wheat grain; analyses were therefore not conducted on these matrices at the longer plantback intervals. Quantifiable residues of BAS 510 F were observed in radish (tops, roots) and wheat (forage, hay, and straw) at all the plantback intervals studied (14, 30, and 45 days). Thus, extensive rotational crop field trials are required to establish tolerances for rotational crop commodities.

**Tier III - Extensive (Large Scale) Field Trials - Rotational Crops**

Field accumulation studies were conducted on legume foliage, cereal grains, forage and fodder of cereal grains, grasses, non-grass animal feeds, and cotton to support indirect/inadvertent residue (rotational crop) tolerances on these food/feed crops. BAS 510 70 WG formulation was applied to bare ground in 3 applications: the 1st at 0.72 lb ai/A and the 2nd and 3rd at 0.55 lb ai/A, for a maximum seasonal rate of 1.82 lbs ai/A. Fourteen days later, the rotational crop was planted into the soil and grown to maturity. Samples were collected at normal harvest; a processing study was conducted, if appropriate; and all samples were analyzed for residues of BAS 510 F. Indirect/ inadvertent residue tolerances are proposed on these commodities.

For those crops for which target crop uses may be sought in future (*brassicas*, cucurbits, edible peas, root crops, sunflower, and mint), the 70 WG was applied foliarly to each crop (as if it were a target crop) according to its maximum proposed seasonal use pattern; i.e., maximum single application rate, maximum number of applications, shortest interval between applications, and shortest PHI. Maximum seasonal rates ranged from 0.80 (*brassicas*, sunflowers) to 1.6 (mint) lbs ai/A. Samples were collected at normal harvest; a processing study was conducted, if appropriate; and all samples were analyzed for residues of BAS 510 F. For purposes of this present petition, indirect/inadvertent residue tolerances are proposed on these commodities.

## INTERNATIONAL HARMONIZATION

There are currently no established Codex, Canadian, or Mexican maximum residue limits (MRLs) for residues of BAS 510 F in/on plant or animal commodities.

## NATURE AND MAGNITUDE OF THE RESIDUE IN DRINKING WATER

See **Attachment 2**: Questionnaire prepared by C. Sutton for presentation to the MARC.

## TOXICOLOGY CONSIDERATIONS

See **Attachment 3**: DRAFT memo prepared by A. Levy for presentation to the HIARC, 9/5/02, for more details. In summary, the proposed chronic RfD is based on increased alkaline phosphatase activity and hepatic weights occurring at the LOAELs of 78.1/81.7 mg/kg/day. The NOAEL is 7.6 mg/kg/day. No appropriate endpoint is being proposed for an acute reference dose.

There was no evidence of neurotoxicity in any of the following studies: acute neurotoxicity in rats, subchronic neurotoxicity in rats, developmental neurotoxicity in rats, subchronic or chronic toxicity in rats or mice, subchronic or chronic toxicity in dogs, developmental toxicity in rats or rabbits or a 2-generation reproduction in rats.

In the rat developmental study, the maternal and developmental toxicity NOAELs were ≥1000 mg/kg/day and LOAELs could not be established. In rabbits the maternal and developmental NOAELs were 300 mg/kg/day based on abortions and early delivery occurring at the LOAELs of 1000 mg/kg/day. Based on these studies and the two generation reproduction study, it is proposed that the hazard-based FQPA safety factor be removed.

BAS 510F is not genotoxic in a series of *in vitro* and *in vivo* assays. Although there was not a treatment related increase in the incidence of any tumor type or in the total number of tumors in the **mouse** carcinogenicity study, there was a small but not statistically significant increase in thyroid follicular cell adenomas in 2500 ppm male and female **rats** when compared to controls. This fungicide will be evaluated by the Cancer Assessment Review Committee on 9/25/02.

**Rat Metabolism Study MRID 45404918, 45404919.**

Two studies were conducted to examine the metabolism and disposition of BAS 510F in Wistar (Chbb-THOM, SPF) rats following a single 50 or 500 mg/kg oral dose, or a 14-day repeated dose (500 mg/kg/day). Overall recovery of administered radioactivity in the mass balance experiments was 93.87-102.75% for the high dose groups (for both diphenyl and phenyl label as well as the repeat dose group) and 96.06-101.54% for the low dose groups (both labels).

Excretion via the feces accounted for most (~80-98%) of the administered radioactivity in all treatment groups. Elimination via the urine accounted for ~16% of the administered low dose and 3-5% of the high dose. Neither BAS 510F nor its metabolites appear to undergo any significant sequestration in most tissues. Tissue concentrations were <1-2 µg eq./g regardless of the dosing regimen. In total, liver metabolites accounted for 0.6% of the pyridine label dose and 1% of the diphenyl label dose.

Metabolites were detected in the urine, feces, bile, and liver. The most prominent metabolites in the urine, bile, and liver were **M510F01** (an hydroxylation product) and **M510F02** (a glucuronide conjugate). Minor metabolites (sulfate and glutathione conjugates, and secondary derivatives of conjugation products) were also found. In the feces, **parent** compound (representing unabsorbed material) was the most prevalent component.

The study authors concluded that BAS 510F is metabolized primarily by oxidation or conjugation with glutathione followed by secondary conjugation, and excreted primarily in the urine or unchanged in the feces. Cleavage of the amide bond in the parent compound, although verified, was considered negligible as a biotransformation pathway.

| Table 19 - Metabolite profile (% of administered radioactivity) in urine of rats (Group DX) dosed with [diphenyl-$^{14}$C]-BAS 510F [a]. | | | | |
|---|---|---|---|---|
| Compound | 500 mg/kg | | | |
| | 0-24 hrs | | 0-48 hrs | |
| | Males | Females | Males | Females |
| M510F01 (4-hydroxyl BAS 510F) | 0.48 | 2.07 | 0.57 | 2.23 |
| M510F02 (glucuronide conjugate of M51F01) | 1.09 | 0.65 | 1.73 | 1.6 |
| M510F03 (sulfate derivative of M510F01) | – | 0.08 | – | 0.08 |
| M510F04 | – | 0.13 | – | 0.13 |
| M510F05 | 0.33 | 0.34 | 0.34 | 0.34 |
| M510F20 | 0.01 | 0.02 | 0.02 | 0.02 |
| M510F42 | 0.03 | – | 0.1 | 0.03 |
| M510F48 | 0.31 | 0.3 | 0.37 | 0.39 |
| Total | 2.25 | 3.59 | 3.13 | 4.82 |

[a] Data obtained from Table 7, p. 48 MRID 45404918

For rats given the pyridine label (Group DX), up to nine components were detected (Table 20). Parent compound was not detected in the urine. The urinary metabolic profiles for the diphenyl labeled test article (Table 19) exhibited up to eight metabolites. Metabolite **M510F02** was the most prominent component from both labels..

| Table 20 - Metabolite profile (% of administered radioactivity) in urine of rats Group DX) receiving a single high dose of [pyridine-$^{14}$C]-BAS 510F[a] | | | | |
|---|---|---|---|---|
| Compound | 500 mg/kg | | | |
| | 0-24 hrs | | 0-48 hrs | |
| | Males | Females | Males | Females |
| M510F01 (4-hydroxyl BAS 510F) | 0.45 | 0.85 | 0.51 | 1.02 |
| M510F02 (glucuronide conjugate of M51F01) | 2.28 | 1.59 | 2.74 | 2.14 |
| M510F03 (sulfate derivative of M510F01) | – | 0.12 | – | 0.13 |
| M510F04 | – | 0.1 | – | 0.1 |
| M510F05 | 0.4 | 0.36 | 0.4 | 0.37 |
| M510F20 | – | 0.01 | – | 0.01 |
| M510F42 | 0.07 | 0.02 | 0.13 | 0.04 |
| M510F47 (2-chloronicotinic acid) | 0.07 | 0.06 | 0.07 | 0.06 |
| M510F48 | 0.4 | 0.27 | 0.44 | 0.33 |
| Total | 3.67 | 3.38 | 4.29 | 4.2 |

[a] Data obtained from Table 8, p. 49 MRID 45404918

| Table 21 - Metabolite profile (% of administered radioactivity over 48 hrs) in feces of rats (Group DX) receiving a high dose of [diphenyl-$^{14}$C]-BAS 510F or [pyridine-$^{14}$C]-BAS 510F[a]. | | | | |
|---|---|---|---|---|
| Compound | 500 mg/kg | | | |
| | Diphenyl label | | Pyridine label | |
| | Males | Females | Males | Females |
| M510F01 (4-hydroxyl BAS 510F) | 4.32 | 9.06 | 8.18 | 10.54 |
| M510F05 | 0.64 | 0.7 | 4.87 | 1.52 |
| M510F06 | 6.1 | 10.29 | 1.04 | 12.24 |
| M510F20 | 0.8 | 0.57 | 1.05 | 0.73 |
| M510F48 | 1.26 | 1.05 | 6.5 | 0.38 |
| BAS 510F | 80.46 | 64.96 | 75.63 | 66.96 |
| Total | 93.58 | 86.63 | 97.27 | 82.57 |

[a] Data obtained from Tables 7 and 8, pp. 48-49 MRID 45404918

| Table 22 - Metabolite profile (percent of administered radioactivity over 48 hrs) *in feces of rats receiving a single low dose of [diphenyl-$^{14}$C]-BAS 510F*[a]. | | |
|---|---|---|
| Compound | 50 mg/kg | |
| | Males | Females |
| M510F01 (4-hydroxyl BAS 510F) | 21.81 | 18.99 |
| M510F05 | – | 1.85 |
| M510F06 | 4.88 | 7.57 |
| M510F11 | 2.31 | 0.53 |
| M510F20 | 6.21 | 3.79 |
| M510F48 | – | 2.84 |
| M510F63 | 0.6 | 4.01 |
| BAS 510F | 41 | 30.45 |
| Total | 76.81 | 70.03 |

[a] Data obtained from Table 9 and , p. 50 MRID 45404918

Tissues: Metabolites were characterized in extracts of liver and kidney at 8 hours following a single 500 mg/kg dose of [pyridine-$^{14}$]-BAS 510F or 50 mg/kg [diphenyl-$^{14}$C]-BAS 510F. In **liver** extracts, three to four components were detected in the water extract and eight to nine components (including parent compound) were detected in the methanol extract. No individual metabolite accounted for more than 0.35% of the administered dose and there did not appear to be appreciable differences in the profiles attributable to gender or label position. In total, liver metabolites accounted for 0.6% of the pyridine label dose and 1% of the diphenyl label dose. In the **kidney**, six to eight components (including unchanged BAS 510F) were detected in the methanol extracts (no data for water extracts) but individual fractions represented only 0.01 to 0.06% of the administered radioactivity. Metabolites detected in kidney tissue, in total, accounted for only 0.02 to 0.14% of the administered radioactivity. No appreciable differences could be attributed to gender or label position.

## ATTACHMENTS

1. Appendix of Chemical Names and Structures
2. EFED Questionnaire
3. DRAFT document for presentation to the HIARC, 9/5/02.

**cc: RAB2 Reading File, M. Nelson**