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OFFICE OF  
HEALTH AND ENVIRONMENTAL  
SCIENCE AND TOXIC SUBSTANCES  
EPA SERIES 361



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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TO: Yan Donovan, Executive Secretary  
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:

<u>Primary (Target) Crops</u>	<u>ppm</u>
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

<u>Rotational Crops</u>	<u>ppm</u>
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 <i>Brassica</i> (crop subgroup 5-A) .....	3.0
Leafy <i>Brassica</i> 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-hydroxybiphenyl-2-yl)nicotinamide [aka M510F01], expressed in BAS 510 F equivalents, in animal commodities, are as follows:

	<u>ppm</u>
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**

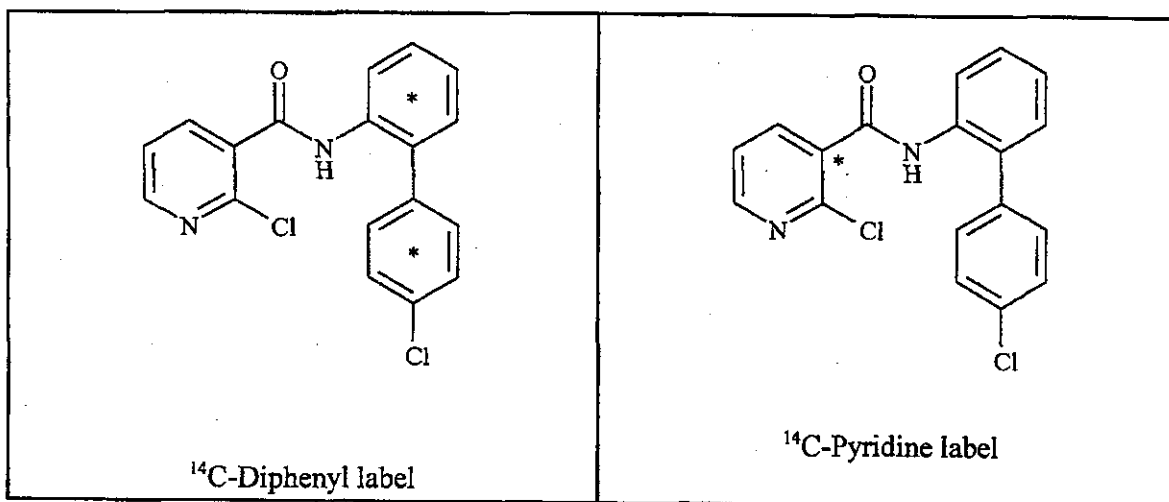
There are three end use products proposed for registration. **BAS 510 02 F Turf Fungicide** is a 70% WG (wetable granule) formulation containing BAS 510 F as sole active ingredient (ai); it is intended for use on golf course turf grass only. **BAS 510 02 F Crop Fungicide** is also a 70% WG formulation 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) to 1.8 (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 [<sup>14</sup>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.



### Grapes

BASF Corporation has submitted a study investigating the metabolism of [<sup>14</sup>C]BAS 510 F in grapes. Grape bunches and leaves were collected 45 days following the last of three foliar applications of [<sup>14</sup>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,  $\approx$  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 <sup>14</sup>C- Labeled BAS 510 F

Label Location	Crop Matrix	Application Rate	PHI, days	TRR, ppm		% Mass Balance <sup>3</sup>
				Combustion <sup>1</sup>	Calculation <sup>2</sup>	
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- <sup>14</sup> 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- <sup>14</sup> 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 [ $^{14}\text{C}$ ]BAS 510 F in lettuce was submitted. Lettuce samples were collected 18 days following the last of three foliar applications of [ $^{14}\text{C}$ ]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 [ $^{14}\text{C}$ ]BAS 510 F and pyridine-label [ $^{14}\text{C}$ ]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 <sup>3</sup>
				Combustion <sup>1</sup>	Calculation <sup>2</sup>	
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%

<sup>1</sup> As determined by direct combustion/L.C.

<sup>2</sup> 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.

<sup>3</sup> 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- <sup>14</sup> C]BAS 510 F		[Pyridin-3- <sup>14</sup> C]BAS 510 F	
	Lettuce (TRR = 17.541 ppm)		Lettuce (TRR = 17.622 ppm)	
	%TRR	ppm	%TRR	ppm
<b>BAS 510 F</b>	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 [<sup>14</sup>C]BAS 510 F in beans. Samples were collected following three foliar applications of [<sup>14</sup>C]BAS 510 F at 0.44 lb ai/A/application (500 g ai/ha, for a total of 1.5 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 <sup>3</sup>
				Combustion <sup>1</sup>	Calculation <sup>2</sup>	
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%

<sup>1</sup> As determined by direct combustion/L.C.<sup>2</sup> Calculated by summing extractable residues and nonretractable residues detected by L.C. The petitioner used the calculated value for all reported results.<sup>3</sup> 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- <sup>14</sup> 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	100.13	100.61	100.61	100.1	100.1	100.1	100.7	100.7	100.11	99.9	99.9	99.9	99.8	99.8
[Pyridin-3- <sup>14</sup> 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	--	--
Chlorogenic 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	

Round 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.

<sup>1</sup> Identified in the hexane phase following liquid/liquid partitioning of the methanol extract.

<sup>2</sup> Identified in the water phase following liquid/liquid partitioning of the methanol extract.

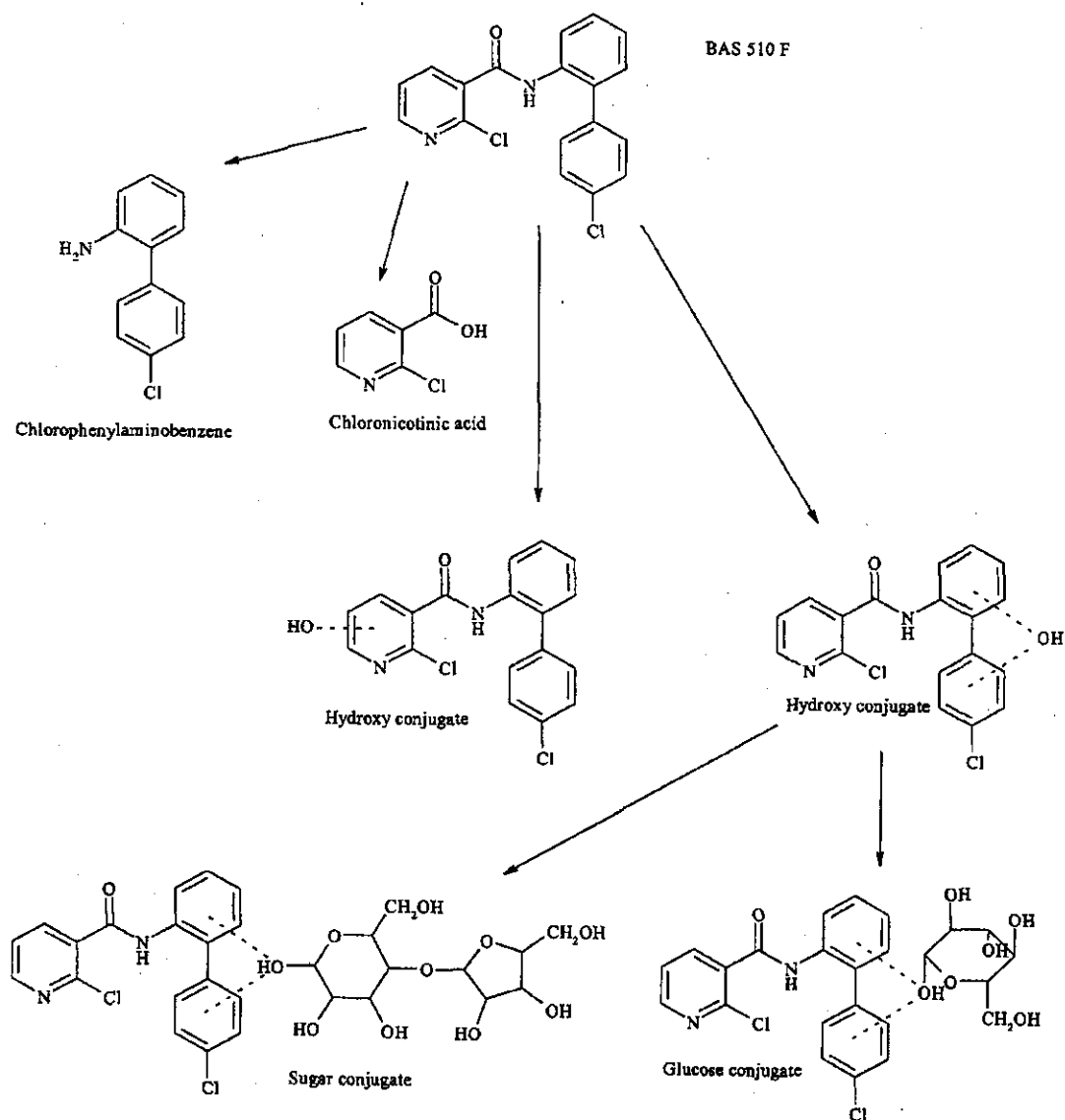
<sup>3</sup> Analysis using a different column indicated the presence of chlorophenylaminobenzene in this fraction at 0.02% TRR.

<sup>4</sup> 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. Chlorophenylaminobenzene 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 [ $^{14}\text{C}$ ]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- $^{14}\text{C}$ ]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-<sup>14</sup>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 <sup>2</sup>			7.7	0.003	--	--	--	--	--	--	11	0.285
M510F51 <sup>3</sup>			12.2	0.005	--	--	--	--	--	--	2.4	0.062
M510F53 <sup>4</sup>			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)

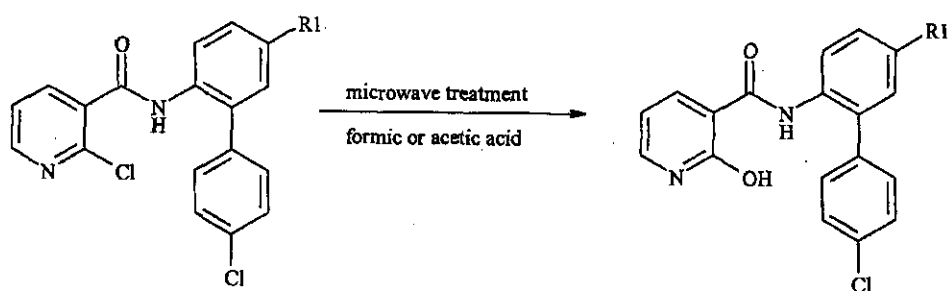
2 Released during microwave hydrolysis; putatively results from hydrolysis of BAS 510 F.

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.

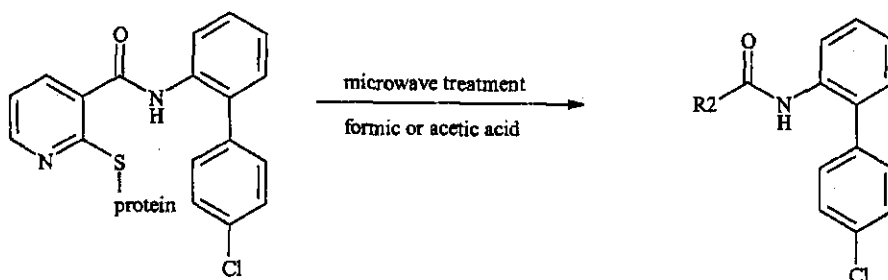
Approximately 76-87% of the TRR were extracted from goat milk, muscle, fat, and kidney using solvents; solvent extraction only released ~14% TRR from liver. The parent, BAS 510 F, was detected in all goat matrices, accounting for 2.5-7.9% TRR (0.003-0.129 ppm) in milk and liver, 20.4% TRR (0.002 ppm) 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 to 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<sub>3</sub> when acetic acid used; M510F53

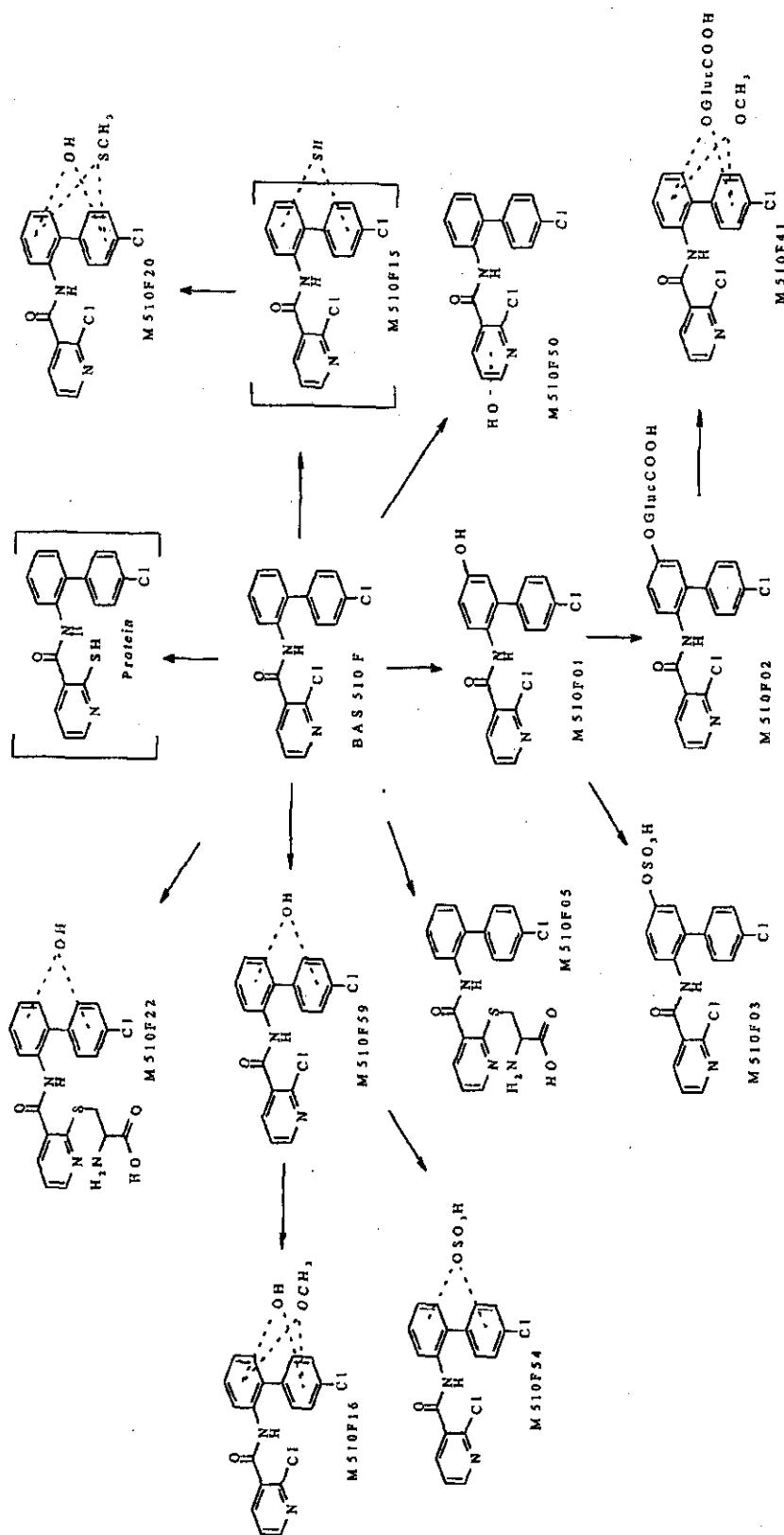
Although the petitioner did not provide information which conclusively identifies the metabolites released by microwave extraction, we have no basis on which to disagree with their assumptions about the chemical reactions resulting in the compounds found in the hydrolysate.

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 [ $^{14}\text{C}$ ]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- $^{14}\text{C}$ ]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. Trichloroacetic 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 radioscaner.

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- <sup>14</sup> 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 <sup>1</sup>			--	--	12.71	0.0214
M510F51 <sup>2</sup>			--	--	21.69	0.0366
M510F52 <sup>3</sup>			--	--	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

<sup>1</sup> Released during microwave hydrolysis; putatively results from hydrolysis of BAS 510 F.

<sup>2</sup> Released during microwave hydrolysis; putatively results from hydrolysis of M510F01.

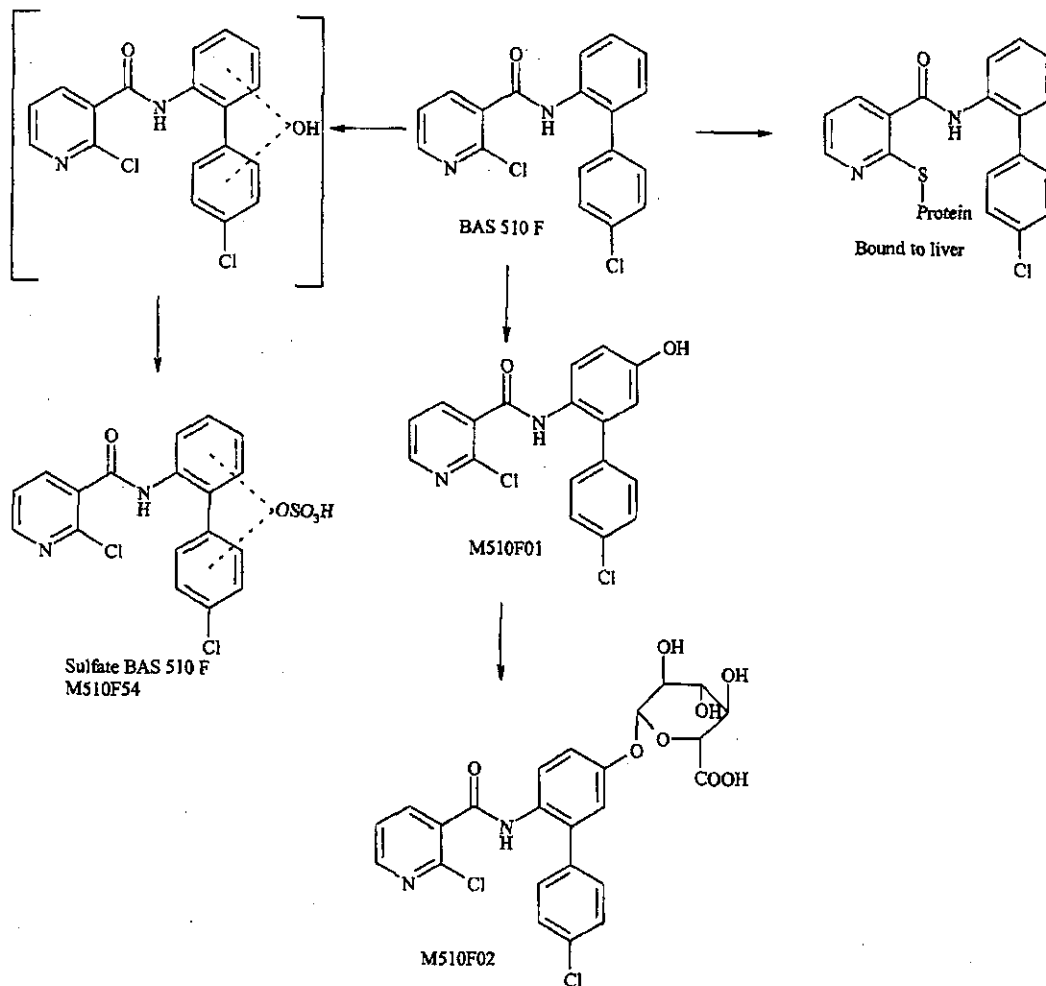
<sup>3</sup> Released during microwave hydrolysis; putatively results from hydrolysis of bound/conjugated BAS 510 F.

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 [ $^{14}\text{C}$ ]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
<b>Diphenyl label</b>	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 <sub>3</sub> )	--	--	--	--	--	--	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	
<b>Pyridine label</b>	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

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
<b>Biphenyl label</b>	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 <sub>3</sub> )	--	--	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	
<b>Pyridine label</b>	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
<b>Biphenyl label</b>	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 <sub>3</sub> )	--	--	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	
<b>Pyridine label</b>	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
<b>Diphenyl label</b>	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 <sub>3</sub> )	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	
<b>Pyridine label</b>	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 <sub>3</sub> )	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
<b>Biphenyl label</b>	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 <sub>3</sub> )	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	
<b>Pyridine label</b>	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 <sub>3</sub> )	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
<b>Diphenyl label</b>	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 <sub>3</sub> )	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	
<b>Pyridine label</b>	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 <sub>3</sub> )	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

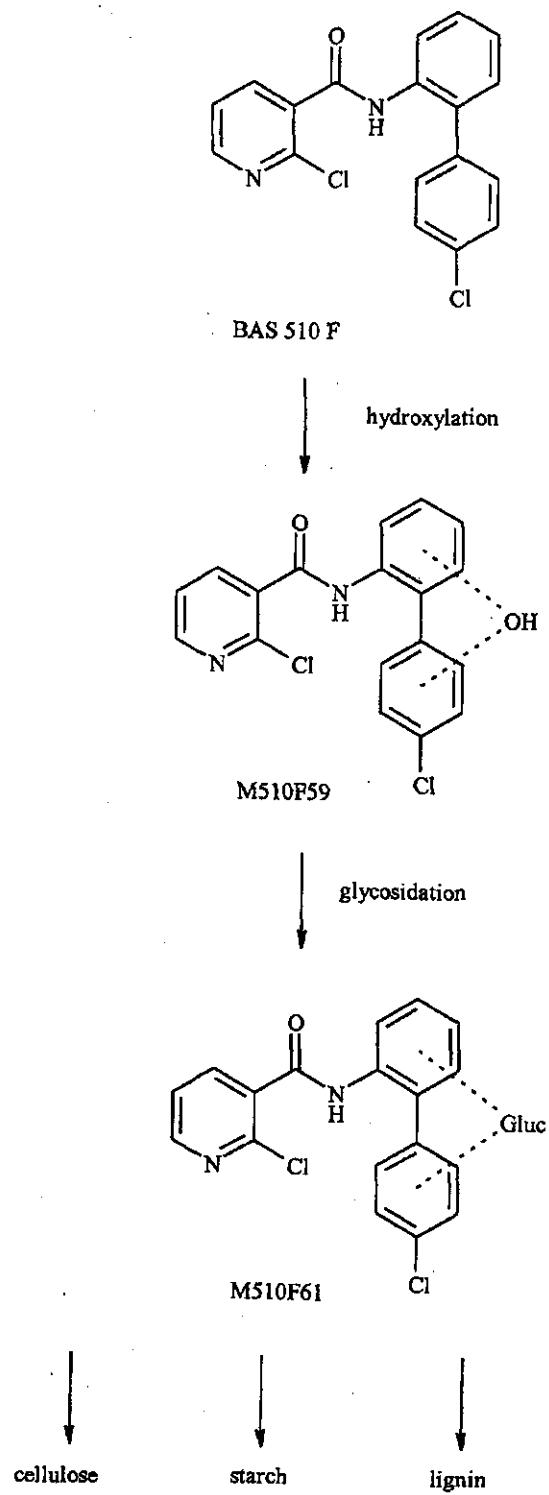
% Mass Balance = TE %TRR + TB % TRR.

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.



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  $^{14}\text{C}$ -starch or  $^{14}\text{C}$ -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  $97 \pm 11\%$  ( $n=60$ ). 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  $79 \pm 8\%$  for canola seed and  $84 \pm 10\%$  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 ( $< -10^\circ\text{C}$ ) for  $\leq 1.5$  to  $\leq 8$  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 ( $< -10^\circ\text{C}$ ) 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 ( $< -10^\circ\text{C}$ ) for  $\leq 1$  to  $\leq 7$  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**

<b>Table 17 - Summary of Residue Data from Cattle Feeding Study with BAS 510 F</b>							
Matrix	Feeding Level (ppm)	Pre-Slaughter Interval (days)	Analyte	Residue Levels (ppm)			
				Maximum	Highest Average	Mean <sup>1</sup>	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 <sup>1</sup>	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 <sup>2</sup>	<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 <sup>2</sup>	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

<sup>1</sup> Mean calculated using LOQ for values below the LOQ.<sup>2</sup> 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.

**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**

<b>Table 18 - Summary of Residue Data from Poultry Feeding Study with BAS 510 F</b>							
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 <sup>1</sup>	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 <sup>1</sup>	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

<sup>1</sup> 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.

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  $\pm$  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 1<sup>st</sup> at 0.72 lb ai/A and the 2<sup>nd</sup> and 3<sup>rd</sup> 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  $\geq 1000$  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  $<1-2 \mu\text{g eq./g}$  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- <sup>14</sup> C]-BAS 510F <sup>a</sup> .				
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 M510F01)	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

<sup>a</sup> 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- <sup>14</sup> C]-BAS 510F <sup>a</sup>				
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 M510F01)	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

<sup>a</sup> 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- <sup>14</sup> C]-BAS 510F or [pyridine- <sup>14</sup> C]-BAS 510F <sup>a</sup> .				
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

<sup>a</sup> 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- <sup>14</sup> C]-BAS 510F <sup>a</sup> .		
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

<sup>a</sup> 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-<sup>14</sup>C]-BAS 510F or 50 mg/kg [diphenyl-<sup>14</sup>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**