UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

Date: July 2, 2003

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RAB2/HED (7509C)

Date: July 16/03
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Petition#: 1F06313

Citation: 45405026 Nietschmann, D.; Lam, W. (2000) Nature of Residues of (carbon 14) BAS 510 F in Laying Hens: Final Report: Lab Project Number: 98084: 2000/5154. Unpublished study prepared by BASF Corporation. 246 p.

Sponsor: BASF Corporation

Background

The information contained herein was compiled by the Dynamac Corporation (20440 Century Boulevard, Suite 100, Germantown MD 20874), contractor, under the supervision of RAB2/ HED. This DER has undergone secondary review by PMRA/Canada, and peer review by RAB2, and reflects current HED and Office of Pesticide Programs (OPP) policies.

Executive Summary

BASF Corporation has submitted a study investigating the metabolism of [^{14}C]BAS 510 F in poultry. The in-life phase of the study was conducted by Southwest Bio-Labs (Las Cruces, NM), and the analytical phase of the study was conducted by BASF Corporation (Research Triangle Park, NC) and BASF Aktiengesellschaft (Limburgerhof, Germany). Ten laying hens were orally dosed with [^{14}C]BAS 510 F, uniformly labeled in the diphenyl 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 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.

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 this 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.

Sufficient data were submitted to demonstrate the stability of residues in hen matrices for the duration of the study.

This metabolism study is **acceptable** to fulfill the data requirement for a metabolism study in poultry. Although it reflects radiolabeling in the diphenyl ring portion of the molecule only, there was no apparent cleavage of the parent molecule in the subject study (or in the goat metabolism study; see DER of MRIDs 45405024/45405025), and only a very small amount of cleavage was observed in the plant metabolism studies.

GLP Compliance

Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. The petitioner noted that not all raw data were collected in compliance with GLP regulations.

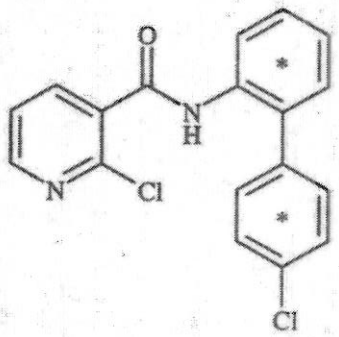
1. Materials and Methods

1.1. Substance

Active Ingredient

Common Name: Nicobifen (ISO, proposed)
IUPAC Name: 2-Chloro-N-(4'-chlorobiphenyl-2-yl)nicotinamide
CAS Name: 3-Pyridinecarboxamide, 2-chloro-N-(4'chloro[1,1'-biphenyl]-2-yl)-
CAS Number: 188425-85-6
Company Name: BAS 510 F
Other Synonyms: BASF Registry No. 300355
Purity of Non-labeled Material: 99.6%

Location of Isotopic Label: Uniformly labeled in both phenyl rings
Radiochemical Purity: 99% (as determined by HPLC)
Specific Activity: 376,000 dpm/μg (labeled compound); 98,564 dpm/μg (dosing material), (μCi/mmol not provided)



[Diphenyl-U-^{14}C]BAS 510 F

1.2. Test Animals and Site

Species/breed: Hen, *Gallus gallus*
Age: Approximately 74 weeks at initiation of dosing
Gender: Female
Number: 10 hens in the treatment group and 10 hens in the control group.
Housing Areas: Individual laying cages housed in a controlled environment.
Diet and Water: Commercial hen feed and fresh tap water were provided *ad libitum*.
Feed consumption was measured daily.
Acclimation period: 51 days prior to treatment
Predosing: None

1.3. Dosing

Type(s) of Dosing: Oral by balling gun
Dosing Vehicle: [^{14}C]BAS 510 F was mixed with non-labeled BAS 510 F and the mixture was dissolved in methanol. The dosing solution was added to capsules containing cornstarch and the solution was allowed to air dry. Control hens were fed dosing capsules containing cornstarch only.
Dosing Rate: Nominal of 12.5 ppm in the diet; actual dose rate, based on average feed consumption, was 12.1 ppm
Number and Duration of Doses: Animals were dosed once daily, after morning egg collection, for 10 consecutive days.

1.4. Sample Collection Procedures

Eggs were collected twice daily (a.m. and p.m.). Eggs collected in the afternoon were refrigerated overnight until pooled the following day with the morning egg collection. Eggs were then homogenized for radioactivity analysis; following radioactivity determinations, eggs were stored frozen (≤-15 °C). Excreta were collected once daily, and cage wash samples were collected on the first study day and after sacrifice. Hens were sacrificed 21-23 hours following the last dosing, with control animals sacrificed prior to treated animals. Samples of liver, muscle (composite of breast and thigh), fat (visceral fat and fat adhering to the skin and muscle), and GI tract and contents were collected. Samples were pooled by tissue and stored frozen (≤-15 °C) until analysis.

Table 1.4.1. Summary of Storage Conditions

Hen Matrix	RAC or Extract	Storage Temperature (°C)	Duration (months)
Egg	RAC	≤-15	4.8
Fat	RAC		3.0
Liver	RAC		3.0
	Extract		12.5

For egg and fat samples, extracts were analyzed within one month of sample extraction.

To support the storage conditions and intervals of samples from the study, subsamples of eggs and fat were extracted and analyzed 14 and 6 months, respectively, after the initial extraction and analysis. The chromatograms from the stored samples demonstrated no significant changes in the metabolite profile. Because of the complexity of the liver residues, the petitioner used a spiked sample to demonstrate stability. A subsample of liver was spiked with [^{14}C]BAS 510 F and stored frozen for 7.5 months. The parent was found to be stable in liver over the 7.5-month interval. Though the stability of the parent was not demonstrated in the liver for the full 12.5 months of storage, the results in other plant and animal matrices indicated that the parent was stable during storage. In addition, the extensive binding observed in the liver would also suggest

that the residues are unlikely to break down. These data are sufficient to demonstrate the stability of residues in hen matrices for the duration of the study.

1.5. Analytical Methods

Total radioactive residues (TRR) were determined at the in-life facility (Southwest Bio-Labs, Inc., Las Cruces, NM) and also at the analytical facility (BASF Corporation, RTP, NC). Samples were homogenized and TRR were determined by combustion/LSC (on 3-5 aliquots of each sample). The LSC limits of quantitation (LOQs) were 0.0007-0.0011 ppm for hen matrices. The petitioner used the combustion/LSC data from the in-life facility for muscle and cage wash, and used the combustion/LSC data from the analytical facility for eggs, liver, fat, and excreta.

Subsamples of eggs, fat, and liver were subjected to extraction and characterization of residue at the analytical facility. Because muscle residues were <0.01 ppm, muscle samples were not subjected to extraction procedures.

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 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. The ACN extract was 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 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 mixture was refrigerated overnight and centrifuged. Another 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 nonextractable 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 (80:20, 85:15, 90:10 or 95:5, v:v; normal phase) or MeOH and 5% ammonium hydroxide or 1% formic acid (70:30, v:v; reverse phase). 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.

2. Results

Table 2.1. Total Radioactive Residues in Laying Hens Following Oral Dosing with [Diphenyl-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	0.0000	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		0.0250	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

Table 2.2.1 Extraction, Characterization, and Identification of Radioactive Residues in **Hen Eggs (Day 5; TRR = 0.0521 ppm)** Following Oral Dosing with [Diphenyl-U-^{14}C]BAS 510 F at 12.1 ppm for 10 Consecutive Days.

Fraction ID	% TRR	ppm	Residue ID	%TRR	ppm	Comments
Protease hydrolysate	89.47	0.0466	N/A[1]			Combined with MeOH rinse, applied to C18 SPE column.
Nonextractable	Not determined					Washed with MeOH.
MeOH rinse	7.94	0.0041	N/A			Combined with protease hydrolysate; applied to C18 SPE column.
Water eluate	4.92	0.0026	N/A			
Combined water:MeOH eluate	70.64	0.0368	BAS 510 F	27.72	0.0145	
			M510F02	21.94	0.0115	
			M510F01	20.98	0.0110	
MeOH eluate	12.92	0.0067	N/A			Applied to C18 SPE column

Table 2.2.1 Extraction, Characterization, and Identification of Radioactive Residues in **Hen Eggs (Day 5; TRR = 0.0521 ppm)** Following Oral Dosing with [Diphenyl-U-^{14}C]BAS 510 F at 12.1 ppm for 10 Consecutive Days.						
Fraction ID	% TRR	ppm	Residue ID	%TRR	ppm	Comments
Water:MeOH eluate	13.54	0.0071	BAS 510 F	5.43	0.0028	Plus 1 unknown at 1.68% TRR (0.0009 ppm).
			M510F01	6.43	0.0033	
Nonextractable	3.54	0.0018	N/A			

[1] Not analyzed.

The petitioner subjected eggs from days 2 through 10 to extraction and analysis; only the results from day 5 are presented above (the petitioner only provided detailed extraction and analysis data for day 5 eggs). The extraction profile and the levels of the identified metabolites were similar in eggs from the different days of the study; however, parent BAS 510 F accounted for a greater portion of the radioactivity, and metabolite M510F02 a lesser portion, in the earlier days of the study. Metabolite M510F54 was identified in some egg samples (Days 4, 6, and 7-9) at 0.59-6.74% TRR (0.0004-0.0045 ppm).

Table 2.2.2 Extraction, Characterization, and Identification of Radioactive Residues in **Hen Fat (TRR = 0.0250 ppm)** Following Oral Dosing with [Diphenyl-U-^{14}C]BAS 510 F at 12.1 ppm for 10 Consecutive Days.						
Fraction ID	% TRR	ppm	Residue ID	%TRR	ppm	Comments
1st subsample						
ACN extract	93.32	0.0233	BAS 510 F	93.32	0.0230	
Hexane	Not determined		N/A[1]			Radioactivity level was too low for quantification.
Nonextractable	9.32	0.0023	N/A			
2nd subsample						
Combined ACN extracts	88.81	0.0222				Applied to TLC plates; single radioactive band scraped and extracted with MeOH.
MeOH	80.77	0.0202	BAS 510 F	80.77	0.0202	
Hexane	3.18	<0.001				
Nonextractable	7.03	0.002				

[1] Not analyzed.

Table 2.2.3 Extraction, Characterization, and Identification of Radioactive Residues in **Hen Liver (TRR = 0.1687 ppm)** Following Oral Dosing with [Diphenyl-U-^{14}C]BAS 510 F at 12.1 ppm for 10 Consecutive Days.

Fraction ID	% TRR	ppm	Residue ID	%TRR	ppm	Comments
Microwave hydrolysis						
Aqueous C18 SPE eluate	17.96	0.0303	N/A[1]			
MeOH C18 SPE eluate	82.04	0.1384	M510F51	21.69	0.0366	
			M510F01	5.55	0.0094	
			M510F52	42.09	0.0710	
			M510F49	12.71	0.0214	

[1] Not analyzed.

The results of the different extractions of liver that were not presented in Table 2.2.3 are summarized below:

ACN and Water Extraction: Extraction with ACN released 12.04% TRR (0.0203 ppm). A separate extraction with water released 23.99% TRR (0.0405 ppm). HPLC analysis of these combined extracts was inconclusive, resolving two broad peaks.

Protein Denaturing: Addition of trichloroacetic acid to water extracts yielded 20.63% TRR (0.0349 ppm). Heating of a separate sample of water extract yielded 29.20% TRR (0.0493 ppm). HPLC analysis of the heated extract resolved multiple peaks. The petitioner subjected [^{14}C]BAS 510 F to heating under similar conditions and HPLC analysis demonstrated that the parent was stable to heating at 60 C.

Mild Acid and Base Hydrolysis: The water extract comprised 91.13% TRR (0.1537 ppm); heating of this extract yielded 28% TRR (0.0472 ppm). Mild acid hydrolysis of the remaining solids released 3.12% TRR (0.0053 ppm) and mild base hydrolysis released 51.14% TRR (0.0863 ppm). The ethyl acetate extracts of the base hydrolysate contained 25.35% TRR (0.0428 ppm); however, HPLC analysis of these extracts resolved broad peaks and was inconclusive.

Protease Digestion and Molecular Weight Cut off (MWCO): Protease digestion released 84.80% TRR (0.1431 ppm). HPLC analysis resolved multiple peaks. MWCO separation demonstrated that 18.96% TRR was filtered into the 30K fraction, 17.58% TRR was filtered into the 10K fraction, and 8.36% was filtered into the 3K fraction.

Strong Base Hydrolysis: The hexane extract of the base hydrolysis was found to contain 57.52% TRR (0.097 ppm). Normal and reverse phase TLC of the extract indicated the presence of the hydrolysis product chlorophenylaminobenzene, at 21-29% TRR; its presence was confirmed by HPLC. Ethyl acetate partitioning of the aqueous extract at neutral and acidic pH yielded 32.68% TRR (0.0551 ppm) in the combined extracts; HPLC analysis indicated the presence of BAS 510 F at 26.62% TRR (0.0449 ppm).

Accelerated Solvent Extraction (ASE): The combined ASE extracts contained 31.58% TRR; however, after concentration and SPE cleanup, the extracts contained 14.1% TRR (0.0238 ppm). HPLC analysis indicated the presence of BAS 510 F (1.18-1.63% TRR, 0.002-0.0027 ppm) and M510F01 (3.86-6.03% TRR, 0.0065-0.0102 ppm). ASE of [^{14}C]BAS 510 F for 0.5, 1.0, 2.0, and 4.0 h indicated that BAS 510 F was rapidly converted to M510F49; 40%

conversion was observed after 0.5 h and conversion was complete after 2 h. LC/MS analysis was used to confirm the identification of M510F49.

Microwave Treatment: In the goat metabolism study (MRID 45405024), the petitioner had demonstrated that microwave hydrolysis caused the substitution of the chlorine atom on the pyridine ring with a hydroxy group, thus converting BAS 510 F to M510F49 and converting M510F01 to M510F51. For reference compounds in which the pyridine chloro group had already been substituted with sulfur, microwave hydrolysis caused the cleavage of the amide bond and acylation of this bond, yielding M510F52 when formic acid was used. The petitioner noted that cleavage of the amide bond was not observed when BAS 510 F or M510F01 were subjected to microwave treatment.

Table 2.3 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	0.0710
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.

Figure 1. Proposed Metabolic Fate of BAS 510 F in Hens.

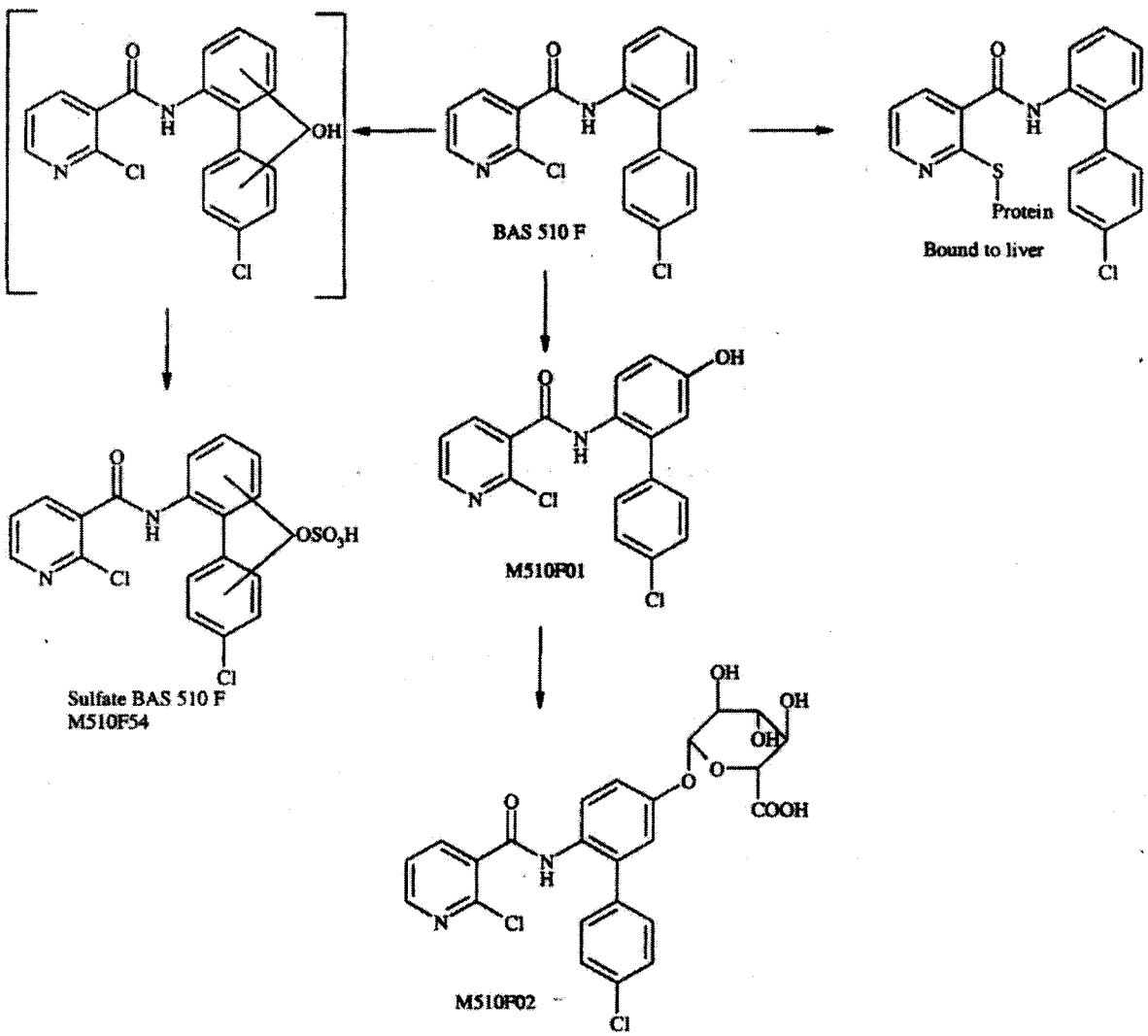


Table 2.4. Metabolites of BAS 510 F in Laying Hens.

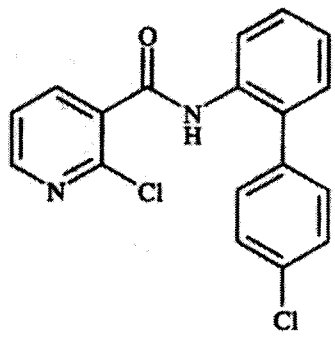
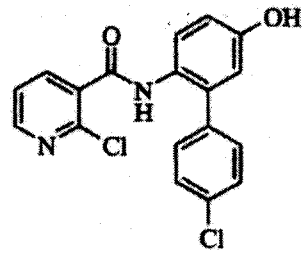
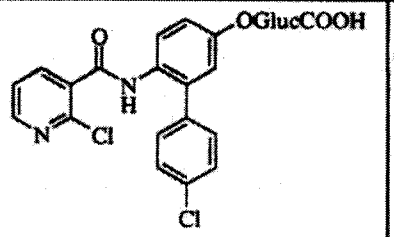
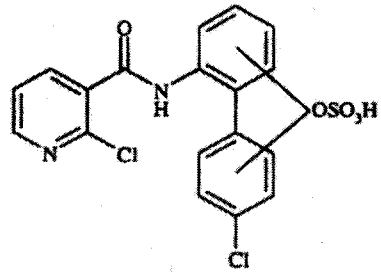
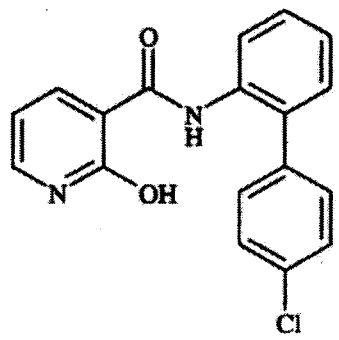
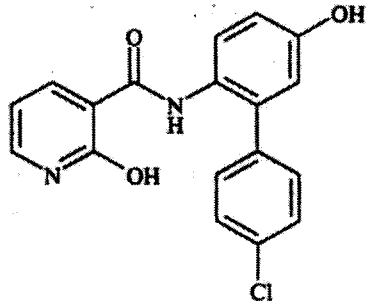
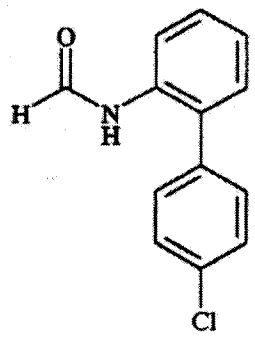
Metabolite Identifier	Chemical Name	Structure	Comments
BAS 510 F (Parent Compound)	3-Pyridinecarboxamide, 2-chloro-N-(4'chloro[1,1'-biphenyl]-2-yl)-		Identified in hen egg and fat
M510F01 (Hydroxy metabolite)	2-chloro-N-(4'-chloro-5-hydroxy-biphenyl-2-yl) nicotinamide		Identified in hen egg and liver
M510F02 (Bound hydroxy metabolite)	glucuronic acid conjugate of M510F01		Identified in hen egg
M510F54	sulfate of BAS 510 F		Identified in hen egg [1]

Table 2.4. Metabolites of BAS 510 F in Laying Hens.

Metabolite Identifier	Chemical Name	Structure	Comments
M510F49	N-(4'-chloro-biphenyl-2-yl)-2-hydroxy-nicotinamide		Identified in hen liver; putatively results from microwave hydrolysis of BAS 510 F
M510F51	N-(4'-chloro-5-hydroxy-biphenyl-2-yl)-2-hydroxy-nicotinamide		Identified in hen liver; putatively results from microwave hydrolysis of M510F01
M510F52	N-(4'-chlorobiphenyl)-formamide		Identified in hen liver; putatively results from microwave hydrolysis of bound/conjugated BAS 510 F

[1] Identified in Day 4, 6, and 7-9 eggs.

3. Discussion

3.1. Methods

Ten laying hens were orally dosed with [^{14}C]BAS 510 F, uniformly labeled in the diphenyl 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. Total radioactivity was determined using combustion/LSC.

Residues in fat were adequately extracted with ACN and residues in eggs were extracted using protease digestion and methanol extraction. Liver residues were extracted using hydrolysis with formic acid with microwave heating. Residues in muscle were <0.01 ppm and were not extracted. The extractable residues were analyzed by HPLC. Material balance was ~91-100% TRR. The methods used to extract and characterize/identify residues adequately elucidated the nature of the residue in the eggs and tissues of hens.

3.2. Results

The 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.

Because of low radioactivity levels, residues in muscle were not characterized. Approximately 90% of the TRR was 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).

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.

4. Deficiencies

No deficiencies were identified.

5. References

None.