

US EPA ARCHIVE DOCUMENT

BAS 510 F
Bean
PMRA a.i. code (CCH)

Nature of the Residue in Plants
OPPTS 860.1300
DACO 6.3

PC Code: 128008
MRID: 45405023
submission # 2001-1027, 1036, 1043



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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DP Barcode: D278386

Petition#: 1F06313

Citation: 45405023 Veit, P. (2001) Metabolism of (carbon-14)-BAS 510 F in Beans: Final Report: Lab Project Number: 42560: 2000/1014861. Unpublished study prepared by BASF Aktiengesellschaft. 183 p.

Sponsor: BASF Corporation

Background

The information contained herein was compiled by 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 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 [¹⁴C]BAS 510 F in beans. The in-life and analytical phases of the study were conducted by BASF Aktiengesellschaft (Limburgerhof, Germany). Samples were collected following three foliar applications of

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[¹⁴C]BAS 510 F, uniformly labeled on the phenyl rings (diphenyl label) or labeled at the 3-position of the pyridine ring (pyridine label), 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 non-extractable residues) were 49.09 ppm in/on bean plants collected on the day of the last application, 66.24, 1.03, 0.90, and 0.20 ppm in/on bean forage, green beans, bean pods, and bean seeds, respectively, collected 14 days after the last application, and 127.3, 6.12, and 0.21 ppm in/on bean straw, dry pods, and dry seeds, respectively, collected 53 days after the last application for the diphenyl label; for the pyridine label, TRR were 21.25 ppm in/on bean plants collected on the day of the last application, 17.0, 0.09, 0.11, and 0.07 ppm in/on bean forage, green beans, bean pods, and bean seeds, respectively, collected 15 days after the last application, and 93.8, 1.37, and 0.13 ppm in/on bean straw, dry pods, and dry seeds, respectively, collected 51 days after the last application. Material balances, based on sample combustion, were 87.1-136.7% for bean commodities, with the exception of pyridine-label bean straw, which had a material balance of 65.8%.

Based on storage stability data (comparisons of extraction profiles and HPLC profiles of stored samples over time) in this study and also in the grape metabolism study (MRID 45405022), sufficient data are available to support the storage-to-harvest intervals of the bean samples in this metabolism study.

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 post-treatment intervals and more radioactivity released from pyridine-label samples than from diphenyl-label samples. Non-extractable 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-label bean straw by LC/MS/MS.

The unchanged parent, BAS 510 F, was the major component identified in all bean commodities, accounting for 36.9% TRR (0.05 ppm; pyridine label) and 72.0% TRR (0.15 ppm; diphenyl label) in/on bean dry seeds to 98.1% (20.9 ppm; pyridine label) and 99.3% TRR (48.7 ppm; diphenyl label) in/on bean plants (0-day PHI). Additional bound BAS 510 F (<3% TRR) was released by ammonia extraction of non-extractable residues. Chlorophenylaminobenzene and 2-chloronicotinic acid were identified in small amounts, 0.02-0.7% TRR (0.001-0.61 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 derivatives 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). Non-extractable residues accounted for <9% TRR in all bean commodities, except bean dry seed, which had non-extractable residues of 13.5% TRR (0.02 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

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molecule occurred, as demonstrated by the presence of the cleavage products 2-chloronicotinic acid and chlorophenylaminobenzene.

The submitted study is **acceptable** to fulfill data requirements for a plant metabolism study in beans.

GLP Compliance

Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. The petitioner stated that the study was conducted in accordance with the GLP regulations established in Germany (Appendix 1 to §19a Section 1, Chemikaliengesetz of 25-July-1994; Official Bulletin/Federal Republic of Germany I 1994, p. 1703) instead of U.S. EPA GLP regulations or PMRA's GLP requirements.

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

Location of Isotopic Label (diphenyl label): Uniformly labeled in both phenyl rings

Radiochemical Purity: >99%

Specific Activity: 376,000 dpm/μg (μCi/mmol not provided)

Location of Isotopic Label (pyridine label): Labeled at the 3-position in the pyridine ring

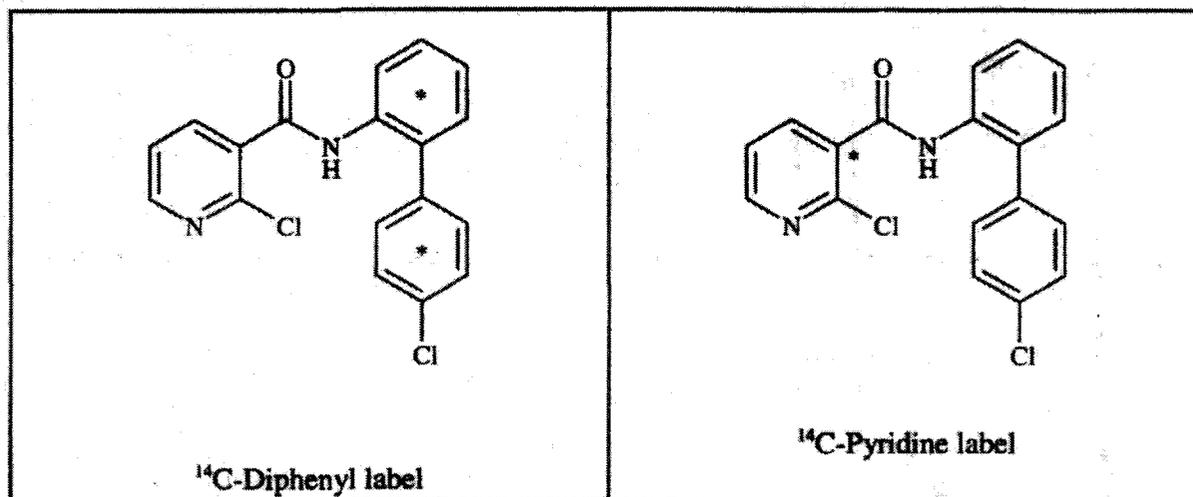
Radiochemical Purity: >99%

Specific Activity: 349,000 dpm/μg (μCi/mmol not provided)

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1.2. Crop and Site

Type and Variety of Crop: Bean, *var.* Hild's Maxi

Growth Environment: 60 individual plastic pots (per label) in a greenhouse or growth chamber (Limburgerhof, Germany)

Conditions: Pots were filled with loamy sand soil. No additional details pertaining to the growing conditions of the bean plants were provided.

1.3. Application

Type of Application: Foliar spray application using an automated spray track above the pots

Application Matrix: The radiolabeled test substances were dissolved in acetone and mixed with suspension concentrate formulation blank and water.

Application Rate: ~0.44 lb ai/A/application (500 g ai/ha/application)

Number of Applications: Three

Timing of Applications: First application at beginning of flowering, second and third applications at 8- to 10-day retreatment intervals. Only half the plants were treated; the remaining plants served as controls.

Pre-harvest Interval(s): Whole plant samples were collected shortly after the final application; green beans and bean forage samples were collected 14-15 days following the last application; and bean straw, bean dry pods, and dry bean seeds were collected 51-53 days following the last application.

1.4. Harvest/Post-harvest Procedures

A portion of green bean samples (collected 14-15 days following the last application) were separated into pods and seeds. All samples were frozen immediately after sampling and were stored frozen (~-18 C) until analysis.

Matrix	RAC or Extract	Storage Temperature (°C)	Duration (months)
Bean plants	RAC	--18 C	Not specified
Green beans	RAC	--18 C	Not specified
-pods	RAC	--18 C	Not specified
-seeds	RAC	--18 C	Not specified
Bean forage	RAC	--18 C	Not specified
Bean straw	RAC	--18 C	Not specified
Bean dry pods	RAC	--18 C	Not specified
Bean dry seeds	RAC	--18 C	Not specified

The petitioner did not specify the duration of sample storage prior to analysis. However it is known, based on the dates in the GLP statement, that the experimental phase of the study took <31 months. The stability data submitted with this study support the storage of diphenyl-label bean samples for up to 5 months and pyridine-label bean samples for up to 27 months prior to completion of analysis. Samples of green beans (diphenyl and pyridine label) and bean straw (pyridine label) were reextracted and analyzed after storage for 150 days for diphenyl-label green beans and 873 days for pyridine-label green beans and bean straw. The extraction pattern and metabolite profile were similar in the stored samples vs the original analysis. This was also the case when methanol extracts of green bean (both labels) and bean straw (pyridine label) which had been stored for 127 days (diphenyl-label green beans), 830 days (pyridine-label green beans), or 812 days (pyridine-label bean straw) were reanalyzed by HPLC. Analogous findings are reported in the grape metabolism study (MRID 45405022); the extraction and HPLC profiles of grapes stored for 16 months were very similar to those of grapes extracted within 2 months of sample collection. Based on these collective data, sufficient data are available to support the intervals of storage (<31 months) which occurred in this study.

1.5. Analytical Methods

Samples of bean commodities from both labels were homogenized and subjected to combustion/LSC for determination of total radioactive residues (TRR). The reported limits of quantitation were 0.00036-0.00038 ppm for green beans.

Subsamples of homogenized bean commodities were extracted with methanol (MeOH; 3x) and then water. The MeOH extracts were isolated by filtration or centrifugation and combined; the water extracts were similarly isolated and combined. The MeOH extracts were evaporated to aqueous phase, diluted with water, and, for diphenyl-label samples, partitioned with n-hexane (3x) followed by ethyl acetate (EtOAc); MeOH extracts from pyridine-label samples were partitioned with EtOAc only. For samples of diphenyl-label bean plant, forage, straw, and dry pods, the water extracts were similarly partitioned.

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Samples of nonextractable residues of bean plant, forage, straw, dry pods, and dry seeds (both labels) were subjected to extraction with aqueous ammonia (1% for diphenyl-label samples and 0.25 M for pyridine-label samples) at ~40 C for 2 hours (diphenyl label) or overnight (pyridine label). The extracts were isolated by centrifugation. The remaining nonextractable residues from diphenyl-label bean straw were split into subsamples. One subsample was extracted with 10% sodium hydroxide (3 hours at 90-100 C), and the extract was isolated by centrifugation. A second subsample was hydrolyzed with a mixture of cellulases and macerozyme (37 C) for 24 hours. A third subsample was extracted with dimethyl sulfoxide (DMSO):water (1:1, v:v); after centrifugation, the mixture was allowed to separate overnight, and the extraction was repeated. The DMSO extracts were combined, and ethanol was added to precipitate starch (mixture was cooled overnight in refrigerator). The precipitated starch was isolated by centrifugation.

All MeOH extracts, and certain water, hexane, EtOAc, and ammonia extracts were concentrated for analysis by HPLC. For the diphenyl-label samples, the extracts were diluted with acetonitrile:water (1:1, v:v) prior to HPLC analysis; tetrahydrofuran was added to samples which contained chlorophyll. For pyridine-label samples, the extracts were diluted with HPLC mobile phase (mixture of water:acetonitrile:formic acid at 950:50:2 and 50:950:2, v:v:v) for analysis. HPLC analyses were conducted on a system equipped with an ODS II Spherisorb column (all extracts except ammonia extracts from diphenyl-label samples) or a PRP column (ammonia extracts from diphenyl-label samples), a UV detector, a radioactivity monitor, and a fraction collector. A gradient mobile phase of water:acetonitrile:formic acid (950:50:2 and 50:950:2, v:v:v) was used. Metabolites were identified by co-chromatography and/or comparison of retention times with those of the following reference standards: ¹⁴C-BAS 510 F, ¹⁴C-2-chloronicotinic acid (M510F47), and ¹⁴C-chlorophenylaminobenzene (M510F62).

The identification of BAS 510 F in diphenyl-label bean straw was confirmed by electrospray ionization LC/MS/MS. The hexane extract of this sample (after partitioning of the MeOH extract) was purified by solid phase extraction (PRS column) and then HPLC (PRP column); the fraction with BAS 510 F retention time was isolated by fraction collection. The collected fraction was then purified again by HPLC (ODS column), and the major peak was isolated for MS analysis. LC/MS/MS analyses were conducted using an ODS II column and a gradient mobile phase similar to that used for HPLC analyses.

Metabolites in the ethyl acetate phase of diphenyl-label bean straw (after partitioning of the MeOH extract) were isolated in a similar manner. HPLC analysis (PRP column) was used to separate the extract into four fractions based on retention time. A second HPLC separation (using an ODS column) of each of these fractions yielded a total of 12 fractions; 9 of these fractions were subjected to MS analyses.

Metabolites in the water phase of diphenyl-label bean straw (after partitioning of the MeOH extract) were isolated by HPLC (PRP column), yielding five fractions. Two of the fractions were analyzed by MS, and two of the fractions were subjected to further cleanup (using ODS, Hypercarb, and/or GPC columns) prior to MS analysis.

The identification of 2-chloronicotinic acid in pyridine-label bean dry pods was confirmed by methylation and phase transfer derivatization. The isolated peak fraction from the water phase of the MeOH extract was methylated with diazomethane, and the HPLC retention time was compared to that of methylated standard. In a separate experiment, the isolated peak fraction was mixed with pentafluorobenzyl bromide in dichloromethane and tetrabutylammoniumhydroxide in water. After shaking for an hour, the dichloromethane phase was isolated and analyzed.

2. Results

Label Location	Crop Matrix	Application Rate	PHI, days	TRR, ppm		% Mass Balance ³
				Combustion ¹	Calculation ²	
Diphenyl label	Bean plant	3 x 0.44 lb ai/A	0	49.36	49.09	99.5%
	Bean forage		14	66.45	66.24	99.7%
	Green beans		14	0.891	1.03	115.6%
	Bean pods		14	0.874	0.903	103.3%
	Bean seeds		14	0.212	0.198	93.4%
	Bean straw		53	128.4	127.3	99.1%
	Bean dry pods		53	4.48	6.12	136.6%
	Bean dry seeds		53	0.183	0.205	112.0%
Pyridine label	Bean plant	3 x 0.44 lb ai/A	0	23.28	21.25	91.3%
	Bean forage		15	17.42	17.00	97.6%
	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	142.5	93.8	65.8%
	Bean dry pods		51	1.51	1.37	90.7%
	Bean dry seeds		51	0.13	0.126	96.9%

¹ As determined by direct combustion/LSC.

² Calculated by summing extractable residues and nonextractable residues. The petitioner used the calculated value for all reported results.

³ Based on sample combustion.

Table 2.2.1. Extraction, Characterization, and Identification of Radioactive Residues in Bean Plant (TRR = 49.091 ppm) Harvested 0 Days Following Three Foliar Applications of [Diphenyl-U-¹⁴C]BAS 510 F at 0.44 lb ai/A/application.

Fraction ID	% TRR	ppm	Residue ID	% TRR	ppm	Comments
MeOH extract	99.2	48.714	BAS 510 F	99	48.6	Plus 3 unknowns for a total of 0.3% TRR (0.112 ppm); each 0.1% TRR (≤ 0.049 ppm). Sequentially partitioned with n-hexane and EtOAc.
n-Hexane	100.3	49.254	BAS 510 F	96	47.13	Plus 7 unknowns, each $\leq 1.4\%$ TRR (≤ 0.448 ppm).
EtOAc	1.2	0.565	BAS 510 F	0.5	0.235	Plus 14 unknowns, each $\leq 0.14\%$ TRR (≤ 0.068 ppm).
			Chlorophenyl-aminobenzene	0.02	0	
Water	0.4	0.172	None identified			HPLC analysis resolved 8 peaks, each $\leq 0.19\%$ TRR (≤ 0.082 ppm).
Water extract	0.3	0.137	BAS 510 F	0.28	0.127	Plus 2 unknowns for a total of 0.03% TRR (0.010 ppm); each $\leq 0.02\%$ TRR (≤ 0.008 ppm). Partitioned with n-hexane and EtOAc.
n-Hexane	0.2	0.097	BAS 510 F	0.2	0.1	
EtOAc	<0.1	0.01	N/A ¹			
Water	0.1	0.032	N/A			
Nonextractable	0.5	0.239	N/A			Extracted with ammonia.
Ammonia	0.2	0.077	BAS 510 F	0.05	0.02	Plus 9 unknowns for a total of 0.15% TRR (0.060 ppm); each $\leq 0.09\%$ TRR (≤ 0.035 ppm).
Nonextractable	0.3	0.143	N/A			

¹ Not analyzed.

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Table 2.2.2. Extraction, Characterization, and Identification of Radioactive Residues in Bean Forage (TRR = 66.236 ppm) Harvested 14 Days Following Three Foliar Applications of [Diphenyl-U-¹⁴C]BAS 510 F at 0.44 lb ai/A/application.

Fraction ID	% TRR	ppm	Residue ID	% TRR	ppm	Comments
MeOH extract	98.3	65.117	BAS 510 F	97.8	64.77	Plus 4 unknowns for a total of 0.6% TRR (0.352 ppm); each $\leq 0.2\%$ TRR (≤ 0.124 ppm). Sequentially partitioned with n-hexane and EtOAc.
n-Hexane	95.73	63.415	BAS 510 F	94.7	62.78	Plus 1 unknown at 0.1% TRR (0.063 ppm).
EtOAc	1.3	0.885	BAS 510 F	0.44	0.297	Plus 12 unknowns, each $\leq 0.14\%$ TRR (≤ 0.094 ppm). Analysis using a different HPLC column (PRP) indicated a small amount of chlorophenylamino-benzene (0.02% TRR, 0.010 ppm).
Water	0.6	0.39	None identified			HPLC analysis resolved 7 peaks, each $\leq 0.41\%$ TRR (≤ 0.266 ppm).
Water extract	0.8	0.525	BAS 510 F	0.77	0.504	Plus 4 unknowns for a total of 0.05% TRR (0.021 ppm); each $\leq 0.02\%$ TRR (≤ 0.011 ppm). Partitioned with n-hexane and EtOAc.
n-Hexane	0.6	0.41	BAS 510 F	0.6	0.408	Plus 1 unknown at $<0.01\%$ TRR (0.001 ppm).
EtOAc	0.1	0.036	BAS 510 F	0.09	0.03	Plus 11 unknowns, each $<0.001\%$ TRR (≤ 0.008 ppm)
Water	0.1	0.071	None identified			HPLC analysis resolved 7 peaks, each $\leq 0.05\%$ TRR (≤ 0.035 ppm).
Nonextractable	0.9	0.595	N/A ¹			Extracted with ammonia.
Ammonia	0.3	0.175	BAS 510 F	0.11	0.06	Plus 9 unknowns for a total of 0.20% TRR (0.113 ppm); each $\leq 0.11\%$ TRR (≤ 0.065 ppm).
Nonextractable	0.6	0.37	N/A			

¹ Not analyzed.

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Table 2.2.3. Extraction, Characterization, and Identification of Radioactive Residues in Green Beans (TRR = 1.027 ppm) Harvested 14 Days Following Three Foliar Applications of [Diphenyl-U-¹⁴C]BAS 510 F at 0.44 lb ai/A/application.

Fraction ID	% TRR	ppm	Residue ID	% TRR	ppm	Comments
MeOH extract	97.6	1.003	BAS 510 F	97.2	0.999	Plus 2 unknowns for a total of 0.4% TRR (0.004 ppm); each ≤0.3% TRR (≤0.003 ppm). Sequentially partitioned with n-hexane and EtOAc.
n-Hexane	99.9	1.027	BAS 510 F	98.9	1.02	Plus 3 unknowns, each ≤0.2% TRR (≤0.006 ppm). Analysis of the extract using the PRP column resolved chlorophenylaminobenzene at 5.3% TRR (0.055 ppm).
			Chlorophenylaminobenzene	0.6	0.01	
EtOAc	1.4	0.014	N/A ¹			
Water	1.1	0.012	N/A			
Water extract	0.7	0.01	N/A			
Nonextractable	1.7	0.017	N/A			

¹ Not analyzed.

Table 2.2.4. Extraction, Characterization, and Identification of Radioactive Residues in Bean Pods (TRR = 0.903 ppm) Harvested 14 Days Following Three Foliar Applications of [Diphenyl-U-¹⁴C]BAS 510 F at 0.44 lb ai/A/application.

Fraction ID	% TRR	ppm	Residue ID	% TRR	ppm	Comments
MeOH extract	97.3	0.878	BAS 510 F	96.7	0.872	Plus 3 unknowns for a total of 0.6% TRR (0.006 ppm), each 0.2% TRR (0.002 ppm). Sequentially partitioned with n-hexane and EtOAc.
n-Hexane	92.2	0.832	BAS 510 F	91.9	0.829	Plus 3 unknowns, each ≤0.2% TRR (0.001 ppm).
EtOAc	2.2	0.02	N/A ¹			
Water	1.6	0.014	N/A			
Water extract	0.9	0.01	N/A			
Nonextractable	1.9	0.017	N/A			

¹ Not analyzed.

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Table 2.2.5. Extraction, Characterization, and Identification of Radioactive Residues in Bean Seeds (TRR = 0.198 ppm) Harvested 14 Days Following Three Foliar Applications of [Diphenyl-U-¹⁴C]BAS 510 F at 0.44 lb ai/A/application.

Fraction ID	% TRR	ppm	Residue ID	% TRR	ppm	Comments
MeOH extract	89	0.176	BAS 510 F	87.5	0.173	Plus 1 unknown at 1.5% TRR (0.003 ppm). Sequentially partitioned with n-hexane and EtOAc.
n-Hexane	96.4	0.191	BAS 510 F	95.7	0.19	Analysis using a different HPLC column (PRP) yielded parent at 91.5% TRR and chlorophenylaminobenzene at 4.9% TRR.
			Chlorophenylaminobenzene	0.7	0	
EtOAc	2.6	0.01	N/A ¹			
Water	2.1	0	N/A			
Water extract	2.4	0.01	N/A			
Nonextractable	8.6	0.017	N/A			

¹ Not analyzed.

Table 2.2.6. Extraction, Characterization, and Identification of Radioactive Residues in Bean Straw (TRR = 127.3 ppm) Harvested 53 Days Following Three Foliar Applications of [Diphenyl-U-¹⁴C]BAS 510 F at 0.44 lb ai/A/application.

Fraction ID	% TRR	ppm	Residue ID	% TRR	ppm	Comments
MeOH extract	95.9	122.0	BAS 510 F	94.3	120.0	Plus 4 unknowns for a total of 1.2% TRR (1.440 ppm); each ≤0.4% TRR (≤0.476 ppm). Sequentially partitioned with n-hexane and EtOAc.
			Chlorophenylaminobenzene	0.5	0.61	
n-Hexane	94.2	119.9	BAS 510 F	94.2	119.9	Identification of BAS 510 F in this fraction confirmed by LC/MS/MS.
EtOAc	1.8	2.30	BAS 510 F	0.11	0.144	Plus 10 unknowns, each ≤0.61% TRR (≤0.783 ppm). LC/MS/MS analyses of isolated fractions of this extract indicated the presence of a glucose conjugate of BAS 510 F and two hydroxy derivatives of BAS 510 F; the position of conjugation could not be defined. No quantitative data were provided.

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Table 2.2.6. Extraction, Characterization, and Identification of Radioactive Residues in Bean Straw (TRR = 127.3 ppm) Harvested 53 Days Following Three Foliar Applications of [Diphenyl-¹⁴C]BAS 510 F at 0.44 lb ai/A/application.

Fraction ID	% TRR	ppm	Residue ID	% TRR	ppm	Comments
Water	0.5	0.646	None identified			HPLC analysis resolved 6 peaks, each $\leq 0.35\%$ TRR (≤ 0.458 ppm). LC/MS/MS analyses of the major peak in this extract (0.35% TRR, 0.458 ppm) indicated that it was a sugar conjugate of BAS 510 F (with two sugar molecules).
Water extract	0.9	1.10	BAS 510 F	0.8	0.98	Plus 8 unknowns for a total of 0.08% TRR (0.120 ppm); each $\leq 0.03\%$ TRR (≤ 0.040 ppm). Partitioned with n-hexane and EtOAc.
n-Hexane	0.5	0.56	BAS 510 F	0.5	0.56	
EtOAc	<0.1	0.055	BAS 510 F	<0.01	0.017	Plus several unknowns, each $\leq 0.01\%$ TRR (≤ 0.011 ppm).
Water	0.2	0.248	None identified			HPLC analysis resolved 4 peaks, each $\leq 0.08\%$ TRR (≤ 0.096 ppm).
Nonextractable	3.3	4.147	N/A ¹			Extracted with ammonia.
Ammonia	0.5	0.641	BAS 510 F	0.13	0.164	Plus 12 unknowns for a total of 0.35% TRR (0.477 ppm); each $\leq 0.16\%$ TRR (≤ 0.203 ppm).
Nonextractable	2.4	3.06	N/A			Split into three subsamples for separate NaOH extraction, macerozyme and cellulase treatment, and DMSO:water extraction.
-NaOH extract	0.7	0.952	N/A			Corresponds to ¹⁴ C-cellulose.
-NaOH residue	0.6	0.715	N/A			
-Macerozyme extract	0.4	0.533	N/A			Corresponds to ¹⁴ C-cellulose.
-Macerozyme residue	1.9	2.42	N/A			
-DMSO extract	Not reported		N/A			Ethanol added to precipitate starch.

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Table 2.2.6. Extraction, Characterization, and Identification of Radioactive Residues in Bean Straw (TRR = 127.3 ppm) Harvested 53 Days Following Three Foliar Applications of [Diphenyl-U-¹⁴C]BAS 510 F at 0.44 lb ai/A/application.

Fraction ID	% TRR	ppm	Residue ID	% TRR	ppm	Comments
EtOH precipitate	<0.1	0.031	N/A			The low amount of radioactivity indicates very little incorporation of radioactivity into starch.
EtOH supernatant	1.4	1.84	N/A			
-DMSO residue	0.8	1.04	N/A			

¹ Not analyzed.

Table 2.2.7. Extraction, Characterization, and Identification of Radioactive Residues in Bean Dry Pods (TRR = 6.12 ppm) Harvested 53 Days Following Three Foliar Applications of [Diphenyl-U-¹⁴C]BAS 510 F at 0.44 lb ai/A/application.

Fraction ID	% TRR	ppm	Residue ID	% TRR	ppm	Comments
MeOH extract	94.3	5.77	BAS 510 F	94.1	5.76	Plus 1 unknown at 0.2% TRR (0.010 ppm). Sequentially partitioned with n-hexane and EtOAc.
n-Hexane	90.9	5.56	BAS 510 F	90.9	5.56	
EtOAc	2.7	0.162	BAS 510 F	1.96	0.117	Plus 10 unknowns, each $\leq 0.17\%$ TRR (≤ 0.010 ppm).
Water	1.1	0.07	BAS 510 F	0.09	0.01	Plus 8 unknowns, each $\leq 0.47\%$ TRR (≤ 0.030 ppm).
Water extract	1.1	0.068	BAS 510 F	0.42	0.026	Plus 5 unknowns for a total of 0.68% TRR (0.042 ppm); each $\leq 0.24\%$ TRR (≤ 0.015 ppm). Partitioned with n-hexane and EtOAc.
n-Hexane	0.3	0.019	N/A ¹			
EtOAc	0.1	0	N/A			
Water	0.7	0.042	N/A			
Nonextractable	4.6	0.279	N/A			Extracted with ammonia.
Ammonia	1.2	0.075	BAS 510 F	0.25	0.016	Plus 10 unknowns for a total of 0.95% TRR (0.058 ppm); each $\leq 0.55\%$ TRR (≤ 0.034 ppm).
Nonextractable	3.3	0.201	N/A			

¹ Not analyzed.

BAS 510 F
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Table 2.2.8. Extraction, Characterization, and Identification of Radioactive Residues in Bean Dry Seeds (TRR = 0.205 ppm) Harvested 53 Days Following Three Foliar Applications of [Diphenyl-¹⁴C]BAS 510 F at 0.44 lb ai/A/application.

Fraction ID	% TRR	ppm	Residue ID	% TRR	ppm	Comments
MeOH extract	75	0.154	BAS 510 F	72	0.148	Plus 6 unknowns for a total of 3.0% TRR (0.004 ppm); each $\leq 2.01\%$ TRR (≤ 0.004 ppm). Sequentially partitioned with n-hexane and EtOAc.
n-Hexane	70	0.143	BAS 510 F	68.5	0.14	Plus 1 unknown at 1.5% TRR (0.003 ppm).
EtOAc	5.7	0.012	N/A ¹			
Water	2.2	0	N/A			
Water extract	5.5	0.011	N/A			
Nonextractable	19.5	0.04	N/A			Extracted with ammonia.
Ammonia	10.4	0.021	N/A			
Nonextractable	8.9	0.018	N/A			

¹ Not analyzed.

Table 2.2.9. Extraction, Characterization, and Identification of Radioactive Residues in Bean Plant (TRR = 21.25 ppm) Harvested 0 Days Following Three Foliar Applications of [Pyridin-3-¹⁴C]BAS 510 F at 0.44 lb ai/A/application.

Fraction ID	% TRR	ppm	Residue ID	% TRR	ppm	Comments
MeOH extract	97.4	20.70	BAS 510 F	97.4	20.70	Partitioned with EtOAc.
EtOAc	104.7	22.26	BAS 510 F	104.7	22.26	
Water	0.7	0.146	BAS 510 F	0.7	0.146	
Water extract	0.7	0.158	BAS 510 F	0.69	0.156	Plus 1 unknown at 0.01% TRR (0.002 ppm).
Nonextractable	1.8	0.387	N/A ¹			Extracted with ammonia.
Ammonia	0.5	0.109	BAS 510 F	0.48	0.104	Plus 2 unknowns for a total of 0.02% TRR (0.005 ppm); each 0.01% TRR (≤ 0.003 ppm).
Nonextractable	0.7	0.145	N/A			

¹ Not analyzed.

Table 2.2.10. Extraction, Characterization, and Identification of Radioactive Residues in **Bean Forage** (TRR = 16.97 ppm) Harvested 15 Days Following Three Foliar Applications of [Pyridin-3-¹⁴C]BAS 510 F at 0.44 lb ai/A/application.

Fraction ID	% TRR	ppm	Residue ID	% TRR	ppm	Comments
MeOH extract	97.7	16.58	BAS 510 F	97.62	16.57	Plus 1 unknown at 0.08% TRR (0.013 ppm). Partitioned with EtOAc.
EtOAc	108	18.32	BAS 510 F	108	18.32	
Water	0.8	0.14	BAS 510 F	0.8	0.14	
Water extract	0.8	0.133	BAS 510 F	0.77	0.129	Plus 4 unknowns for a total of 0.02% TRR (0.003 ppm); each ≤0.02% TRR (≤0.003 ppm).
Nonextractable	1.5	0.255	N/A ¹			Extracted with ammonia.
Ammonia	0.5	0.079	N/A			
Nonextractable	0.6	0.108	N/A			

¹ Not analyzed.

Table 2.2.11. Extraction, Characterization, and Identification of Radioactive Residues in **Green Beans** (TRR = 0.090 ppm) Harvested 15 Days Following Three Foliar Applications of [Pyridin-3-¹⁴C]BAS 510 F at 0.44 lb ai/A/application.

Fraction ID	% TRR	ppm	Residue ID	% TRR	ppm	Comments
MeOH extract	97.4	0.088	BAS 510 F	78.09	0.071	Plus 3 unknowns for a total of 16.52% TRR (0.015 ppm); each ≤15.33% TRR (≤0.014 ppm). Partitioned with EtOAc.
			Chloronicotinic acid	2.8	0	
EtOAc	86.4	0.078	BAS 510 F	86.4	0.078	
Water	17.1	0.015	BAS 510 F	5.84	0.01	Plus 3 unknowns, each ≤2.57% TRR (≤0.002 ppm).
			Chloronicotinic acid	7.61	0.01	
Water extract	0.5	<0.001	N/A ¹			
Nonextractable	2.1	0	N/A			

¹ Not analyzed.

BAS 510 F
 Bean
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Table 2.2.12. Extraction, Characterization, and Identification of Radioactive Residues in Bean Pods (TRR = 0.108 ppm) Harvested 15 Days Following Three Foliar Applications of [Pyridin-3-¹⁴C]BAS 510 F at 0.44 lb ai/A/application.

Fraction ID	% TRR	ppm	Residue ID	% TRR	ppm	Comments
MeOH extract	97.6	0.106	BAS 510 F	87.02	0.095	Plus 4 unknowns for a total of 8.44% TRR (0.008 ppm); each ≤ 7.74% TRR (≤ 0.008 ppm). Partitioned with EtOAc.
			Chloronicotinic acid	2.15	0	
EtOAc	86	0.093	BAS 510 F	86	0.093	
Water	15.7	0.017	BAS 510 F	5.49	0.01	Plus 3 unknowns, each ≤ 1.07% TRR (0.001 ppm).
			Chloronicotinic acid	7.45	0.01	
Water extract	0.4	<0.001	N/A ¹			
Nonextractable	2	0	N/A			

¹ Not analyzed.

Table 2.2.13. Extraction, Characterization, and Identification of Radioactive Residues in Bean Seeds (TRR = 0.067 ppm) Harvested 15 Days Following Three Foliar Applications of [Pyridin-3-¹⁴C]BAS 510 F at 0.44 lb ai/A/application.

Fraction ID	% TRR	ppm	Residue ID	% TRR	ppm	Comments
MeOH extract	94.7	0.063	BAS 510 F	64.88	0.043	Plus 3 unknowns for a total of 19.85% TRR (0.014 ppm); each ≤ 13.13% TRR (≤ 0.009 ppm). Partitioned with EtOAc.
			Chloronicotinic acid	9.97	0.01	
EtOAc	27.7	0.019	BAS 510 F	27.7	0.019	
Water	51.4	0.034	BAS 510 F	19.07	0.013	Plus 4 unknowns, each ≤ 13.65% TRR (≤ 0.009 ppm).
			Chloronicotinic acid	11.16	0.01	
Water extract	1.2	0	N/A ¹			
Nonextractable	4.1	0	N/A			

¹ Not analyzed.

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Table 2.2.14. Extraction, Characterization, and Identification of Radioactive Residues in Bean Straw (TRR = 93.78 ppm) Harvested 51 Days Following Three Foliar Applications of [Pyridin-3-¹⁴C]BAS 510 F at 0.44 lb ai/A/application.

Fraction ID	% TRR	ppm	Residue ID	% TRR	ppm	Comments
MeOH extract	85.1	79.80	BAS 510 F	84.79	79.51	Plus 6 unknowns for a total of 0.32% TRR (0.296 ppm); each ≤0.13% TRR (≤0.120 ppm). Partitioned with EtOAc.
EtOAc	89.9	84.28	BAS 510 F	66.01	61.89	Plus 1 unknown at 23.89% TRR (22.394 ppm).
Water	0.6	0.553	BAS 510 F	0.41	0.38	Plus 2 unknowns, each ≤0.10% TRR (≤0.090 ppm).
Water extract	9.3	8.71	BAS 510 F	8.83	8.27	Plus 6 unknowns for a total of 0.47% TRR (0.441 ppm); each ≤0.25% TRR (≤0.235 ppm). The petitioner noted that this water extract released a significant portion of the TRR compared with other bean commodities.
Nonextractable	5.6	5.27	N/A ¹			Extracted with ammonia.
Ammonia	0.7	0.622	BAS 510 F	0.68	0.601	Plus 6 unknowns for a total of 0.02% TRR (0.020 ppm); each ≤0.01% TRR (≤0.006 ppm).
Nonextractable	4	3.79	N/A			

¹ Not analyzed.

Table 2.2.15. Extraction, Characterization, and Identification of Radioactive Residues in Bean Dry Pods (TRR = 1.37 ppm) Harvested 51 Days Following Three Foliar Applications of [Pyridin-3-¹⁴C]BAS 510 F at 0.44 lb ai/A/application.

Fraction ID	% TRR	ppm	Residue ID	% TRR	ppm	Comments
MeOH extract	75.8	1.037	BAS 510 F	72.04	0.986	Plus 3 unknowns for a total of 2.64% TRR (0.036 ppm); each ≤2.56% TRR (≤0.035 ppm). Partitioned with EtOAc.
			Chloronicotinic acid	1.11	0.015	
EtOAc	67.6	0.925	BAS 510 F	67.6	0.925	
Water	11	0.151	BAS 510 F	4.09	0.056	
			Chloronicotinic acid	6.91	0.095	
Water extract	14.6	0.199	BAS 510 F	7.64	0.104	Plus 2 unknowns, at 6.71% TRR (0.091 ppm) and 0.26% TRR (0.0003 ppm).

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Table 2.2.15. Extraction, Characterization, and Identification of Radioactive Residues in Bean Dry Pods (TRR = 1.37 ppm) Harvested 51 Days Following Three Foliar Applications of [Pyridin-3-¹⁴C]BAS 510 F at 0.44 lb ai/A/application.

Fraction ID	% TRR	ppm	Residue ID	% TRR	ppm	Comments
Nonextractable	9.7	0.132	N/A ¹			Extracted with ammonia.
Ammonia	3.4	0.046	BAS 510 F	2.57	0.035	Plus 2 unknowns for a total of 0.83% TRR (0.011 ppm); each ≤0.69% TRR (≤0.009 ppm).
Nonextractable	5.9	0.081	N/A			

¹ Not analyzed.

Table 2.2.16. Extraction, Characterization, and Identification of Radioactive Residues in Bean Dry Seeds (TRR = 0.126 ppm) Harvested 51 Days Following Three Foliar Applications of [Pyridin-3-¹⁴C]BAS 510 F at 0.44 lb ai/A/application.

Fraction ID	% TRR	ppm	Residue ID	% TRR	ppm	Comments
MeOH extract	47.6	0.06	BAS 510 F	36.94	0.047	Plus 3 unknowns for a total of 8.93% TRR (0.012 ppm); each ≤6.29% TRR (≤0.008 ppm). Partitioned with EtOAc.
			Chloronicotinic acid	1.72	0	
EtOAc	25.8	0.033	BAS 510 F	25.8	0.033	
Water	17.4	0.022	BAS 510 F	9.92	0.013	Plus 1 unknown at 4.25% TRR (0.005 ppm).
			Chloronicotinic acid	3.23	0	
Water extract	21.3	0.027	N/A ¹			
Nonextractable	31.1	0.039	N/A			Extracted with ammonia.
Ammonia	8.5	0.011	N/A			
Nonextractable	13.5	0.017	N/A			

¹ Not analyzed.

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Table 2.3. Summary of Characterization and Identification of Radioactive Residues in Bean Commodities Following Three Foliar Applications of Isotopically Labeled BAS 510 F at 0.44 lb ai/A/application.

Metabolite or Fraction	Bean plant		Bean forage		Green beans		Bean pods		Bean seeds		Bean straw		Bean dry pods		Bean dry seeds	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
[Diphenyl-U- ¹⁴ C]BAS 510 F																
BAS 510 F	99.28	48.72	98.57	65.27	97.2	0.999	96.7	0.872	87.5	0.173	95.1	120.97	94.52	5.79	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	48.750	98.68	65.330	97.81	1.005	96.7	0.872	88.2	0.174	96.08	122.200	94.8	5.81	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	48.93	99.53	65.816	98.91	1.016	98.2	0.886	92.1	0.182	97.7	124.24	96.63	5.92	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	100.1	100.1	100.1	100.6	100.1	100.7	100.1	99.93	99.8							

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Table 2.3. Summary of Characterization and Identification of Radioactive Residues in Bean Commodities Following Three Foliar Applications of Isotopically Labeled 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
[Pyridin-3- ¹⁴ C]BAS 510 F																
BAS 510 F	98.09	20.86	98.39	16.70	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)	98.14	20.96	98.88	16.78	80.89	0.074	89.17	0.097	74.85	0.05	94.3	88.38	83.36	1.14	38.66	0.049
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)	98.17	20.97	98.99	16.80	97.91	0.09	98.01	0.106	95.9	0.065	95.11	89.14	93.8	1.28	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	98.87		99.59		100.0		100.0		100		99.11		99.7		90.89	

Bound BAS 510 F = BAS 510 F found in ammonia extract

TC = Sum of all unidentified, extractable residues

TE = Sum of TI and TC

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

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

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

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

4 The petitioner used LC/MS/MS analyses to identify three of the unknowns as a glucose conjugate of BAS 510 F and two hydroxy derivatives of BAS 510 F; however, no quantitative data were provided for these metabolites.

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

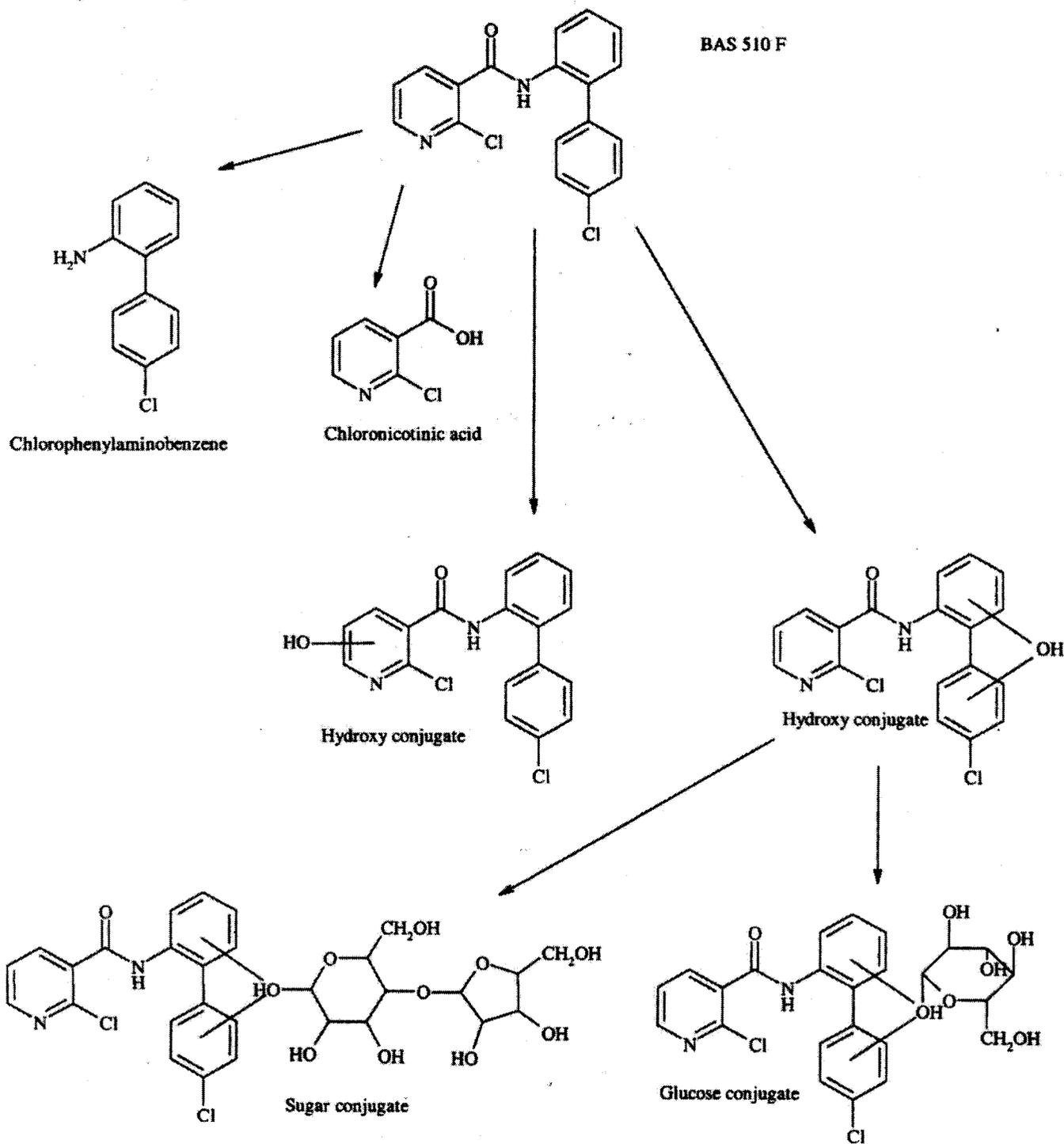
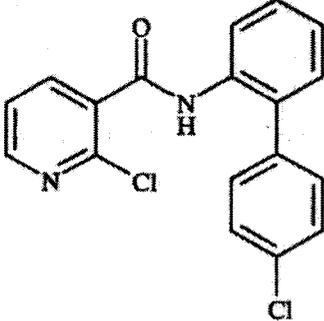
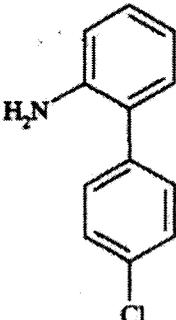
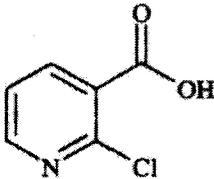


Table 2.4. Metabolites of BAS 510 F in Bean			
Metabolite Identifier	Chemical Name	Structure	Comments
BAS 510 F (Parent Compound)	3-Pyridinecarboxamide, 2-chloro-N-(4'chloro[1,1'- biphenyl]-2-yl)-		Identified in all bean commodities
Chlorophenylamino- benzene	4-Chlorobiphenyl-2- amine		Identified in bean plant, green beans, bean seeds, and bean straw
2-Chloronicotinic acid	2-Chloronicotinic acid		Identified in green beans, bean pods, bean seeds, bean dry pods, and bean dry seeds

3. Discussion

3.1. Methods

Radiolabeled [¹⁴C]BAS 510 F, labeled at the 3-position of the pyridine ring or uniformly labeled on the phenyl rings, was applied three times to bean plants as a foliar spray application at 0.44 lb ai/A/application (500 g ai/ha), with the first application at beginning of flowering, the second application 8-10 days later, and the third application 8-10 days after the second. Whole plant samples were collected shortly after the final application; green beans and bean forage samples were collected 14-15 days following the last application; and bean straw, bean dry pods, and dry bean seeds were collected 51-53 days following the last application. A portion of green bean

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samples (collected 14-15 days following the last application) were separated into pods and seeds. The TRR were determined by combustion/LSC; all analytical results were based on the TRR values calculated by summing extractable and nonextractable residues. Material balances, based on samples combustion, were 87.1-136.7% for bean commodities with the exception of pyridine-label bean straw, which had a material balance of 65.8%.

The majority of residues (47.6-99.2% TRR) in bean commodities were extracted with MeOH. An additional water extraction released a small amount of radioactivity in samples, with increasing amounts released in samples with longer posttreatment intervals, and more radioactivity released from pyridine-label samples than from diphenyl-label samples. Nonextractable residues were subjected to an additional extraction with aqueous ammonia, which released some additional radioactivity. Extracts of all harvested bean commodities were analyzed by HPLC. Identification of BAS 510 F was confirmed in diphenyl-label bean straw by LC/MS/MS. These methods adequately characterized/identified the majority of the residues in bean commodities.

3.2. Results

For the diphenyl label, TRR were 49.091 ppm in/on bean plants collected on the day of the last application, 66.236, 1.027, 0.903, and 0.198 ppm in/on bean forage, green beans, bean pods, and bean seeds, respectively, collected 14 days after the last application, and 127.285, 6.118, and 0.205 ppm in/on bean straw, dry pods, and dry seeds, respectively, collected 53 days after the last application; for the pyridine label, TRR were 21.249 ppm in/on bean plants collected on the day of the last application, 16.967, 0.090, 0.108, and 0.067 ppm in/on bean forage, green beans, bean pods, and bean seeds, respectively, collected 15 days after the last application, and 93.775, 1.369, and 0.126 ppm in/on bean straw, dry pods, and dry seeds, respectively, collected 51 days after the last application.

The petitioner noted that there were differences in the levels of radioactive residues found in bean commodities from the two labels, with higher residues observed in diphenyl-label samples (up to 9x those found in the pyridine-label samples) for all commodities except bean straw (Table 2.1). The petitioner stated that the studies with the two labels were conducted a year apart, which may explain some of the differences. The petitioner also noted that extractable radioactivity from the pyridine-label samples appeared to partition more into water than that from the diphenyl-label samples. Though these differences are noted, they do not adversely affect the validity of these studies. It was concluded that the parent compound was cleaved in bean plants and that the two resulting parts of the molecule followed different degradation pathways.

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 2-chloronicotinic acid were identified in small amounts, 0.02-0.7% TRR (0.001-0.610 ppm) and

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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 derivatives 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).

4. Deficiencies

None identified in this study.

5. References

None.