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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Date: July 25/03
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DP Barcode: D278386

Petition: 1F06313

Citation: 45405022 Rabe, U.; Schluter, H. (2001) Metabolism of BAS 510 F in Grapevine: Final Report: Lab Project Number: 41837: 2000/1014860. Unpublished study prepared by BASF Aktiengesellschaft. 68 p.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 grapes. The in-life and analytical phases of the study were conducted by BASF Aktiengesellschaft (Limburgerhof, Germany). Grape bunches and leaves were collected 45 days following the last of three foliar applications of [^{14}C]BAS 510 F, uniformly labeled on the phenyl

(1)

rings (diphenyl label) or labeled at the 3-position of the pyridine ring (pyridine label), at 0.713 lb ai/A/application (800 g ai/ha/application for a total of 2.13 kg ai/ha, ≈2X proposed GAP). Grape bunches were separated into grapes and stalks. TRR (calculated by summing extractable and nonextractable residues) were 1.181 and 2.066 ppm in/on grapes, 12.356 and 19.637 ppm in/on grape stalks, and 43.672 and 63.359 ppm in/on grape leaves treated with diphenyl-label [^{14}C]BAS 510 F and pyridine-label [^{14}C]BAS 510 F, respectively. Material balances, based on sample combustion, were 90.6-108.7% for grapes and grape leaves.

The majority of residues (>92% TRR) in grape commodities were extracted with methanol. An additional water extraction step released a small amount of radioactivity (<1% TRR). Extracts from the grape berry were analyzed by HPLC; identification of BAS 510 F was confirmed by LC/MS/MS. 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 (1.049 ppm). Because of the low levels of this unknown, and the fact that grape leaves are not a significant food/feed item, the petitioner chose not to 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.

The petitioner included supporting storage stability data in which the extractions and analyses of samples were repeated at the end of the study and compared to the results obtained at the beginning of the study. The extraction profiles of grapes stored for approximately 16 months were very similar to those of grapes extracted within 2 months of sample collection. In addition, the HPLC profiles of the initial methanol extracts of stored grapes were very similar to those obtained at the beginning of the study. These data are sufficient to support the storage intervals of the RAC samples from this study.

The submitted study is **acceptable** to satisfy data requirements for a plant metabolism study with grapes.

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 guidelines.

1. Materials and Methods

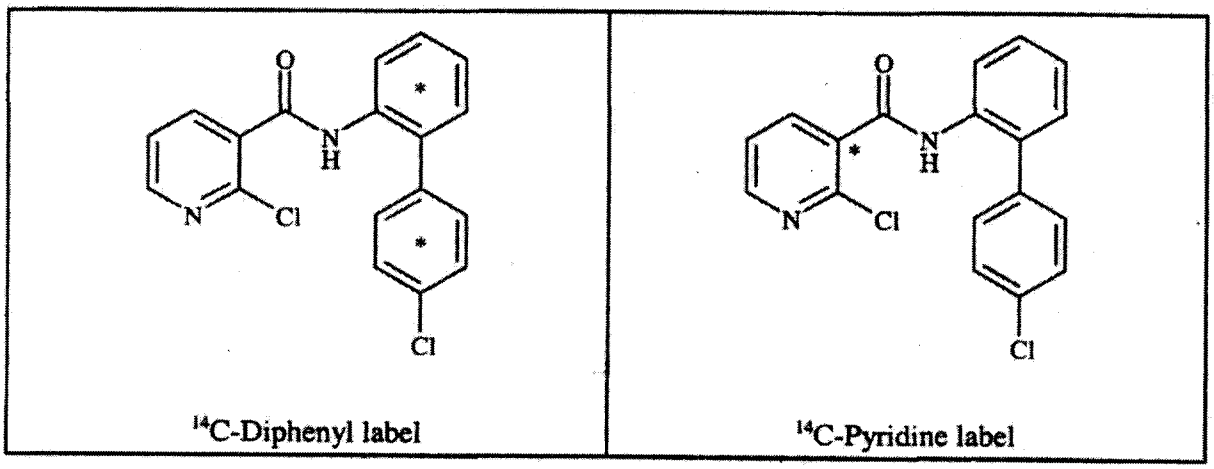
1.1. Substance

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 300355

Location of Isotopic Label (diphenyl label): Uniformly labeled in both phenyl rings
Radiochemical Purity: >99%
Specific Activity: 314,000 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)



^{14}C-Diphenyl label | ^{14}C-Pyridine label

1.2. Crop and Site

Type and Variety of Crop: Grape, *var.* Müller-Thurgau
Growth Environment: Four plots of grapevines, two plots per label, at the BASF agricultural testing facility (Limburgerhof, Germany)
Conditions: Fertilization, herbicide, and fungicides were applied according to standard German agricultural practices. PMRA cannot comment on what these involve.

1.3. Application

Type of Application: Foliar spray application using a hand sprayer
Application Matrix: The radiolabeled test substances were dissolved in suspension concentrate formulation blank and water.
Application Rate: 0.713 lb/A/application (800 g ai/ha/application)
Number of Applications: Three
Timing of Applications: First application at BBCH growth stage 68-69 (end of flowering); second application 12 days later at growth stage 71; third application 41 days later at growth stage 81 (beginning of ripening)
Pre-harvest Interval(s): Leaves were sampled 15 days after the third application to thin out the foliage. Samples of grape bunches and leaves were collected at grape maturity, 45 days after the final application.

1.4. Harvest/Post-harvest Procedures

The leaf samples collected 15 days after the final application were not subjected to any extractions or analyses. Grape bunches were separated into grapes and stalks. Samples of grapes, stalks, and leaves from mature vines were frozen immediately after sampling and stored frozen (≤-18 C) until analysis.

Table 1.4.1. Summary of Storage Conditions

Matrix	RAC or Extract	Storage Temperature (°C)	Duration (days)
Grape	Grapes (RAC)	≤-18 C	63
	Leaves	≤-18 C	105
	Stalks	≤-18 C	383

The petitioner included supporting storage stability data in which the extractions and analyses of samples were repeated at the end of the study and compared to the results obtained at the beginning of the study. The extraction profiles of grapes stored for approximately 16 months were very similar to those of grapes extracted within 2 months of sample collection. In addition, the HPLC profiles of the methanol extracts of stored grapes were very similar to those collected at the beginning of the study. Although minor amounts of unknown compounds were observed in the extracts of stored grapes, the observed amounts were low (<2% TRR). These data are acceptable and sufficient to support the storage intervals of the RAC samples in this study.

Freezer storage stability information was not provided for the leaves and stalks. Based on the results of the analysis in these commodities and the results from the grape berries, no additional freezer storage stability information will be needed to support this study.

1.5. Analytical Methods

Samples of grape commodities from both labels were homogenized and subjected to combustion/LSC for determination of total radioactive residues (TRR), with the exception of stalks. Because of sample inhomogeneity, TRR in stalks could not be determined by direct combustion/LSC; instead, TRR were determined by summing extractable and nonextractable radioactivity. The reported limits of quantitation (calculated) were 0.006 ppm for diphenyl-label samples and 0.005 ppm for pyridine-label samples.

Subsamples of homogenized grape commodities were extracted with methanol (MeOH; 3x) and water (2x; grapes and grape stalks only). The MeOH extracts were isolated by centrifugation and combined; the water extracts were similarly isolated and combined. The MeOH extracts were mixed with water, concentrated, and partitioned with cyclohexane (3x). The remaining aqueous phase was partitioned with ethyl acetate (EtOAc; 3x).

The MeOH extracts and aqueous grape stalk extracts were analyzed by HPLC. HPLC analyses were conducted on a system equipped with an ODS II or PRP-1 column, 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. The petitioner only used radiolabeled BAS 510 F as a standard in these experiments. No othe known or postulated betaloites of BAS 510 was used.

The identification of BAS 510 F in diphenyl-label grapes was confirmed by electrospray ionization LS/MS/MS. LC/MS/MS analyses were conducted using an ODS II column and a gradient mobile phase similar to that used for HPLC analyses.

2. Results

Table 2.1. Total Radioactive Residues in Grape Commodities Following Three Foliar Applications of Isotopically Labeled BAS 510 F

Label Location	Crop Matrix	Application Rate	PHI, days	TRR, ppm		% Mass Balance[3]
				Combustion[1]	Calculation[2]	
Diphenyl 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%

[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 **Grapes**[2] **(TRR = 1.181 ppm)** Harvested 45 Days Following Three Foliar Applications of **[Diphenyl-U-^{14}C]BAS 510 F** at 0.713 lb ai/A/application.

Fraction ID	% TRR	ppm	Residue ID	% TRR	ppm	Comments
MeOH extract	92.7	1.095	BAS 510 F	92.7	1.095	Identification confirmed by LC/MS/MS. Sequentially partitioned with cyclohexane and EtOAc.
Cyclohexane	95.6	1.129	N/A[1]			
EtOAc	2.3	0.027	N/A			
Water	0.7	0.008	N/A			
Water extract	0.4	0.005	N/A			
Nonextractable	6.8	0.081	N/A			

[1] Not analyzed.
[2] Calculated by summing extractable residues and nonextractable residues. The petitioner used the calculated value for all reported results.

Table 2.2.2. Extraction, Characterization, and Identification of Radioactive Residues in **Grape Stalks** [2] **(TRR = 12.356 ppm)** Harvested 45 Days Following Three Foliar Applications of **[Diphenyl-U-^{14}C]BAS 510 F** at 0.713 lb ai/A/application.

Fraction ID	% TRR	ppm	Residue ID	% TRR	ppm	Comments
MeOH extract	95.6	11.810	BAS 510 F	95.6	11.810	Sequentially partitioned with cyclohexane and EtOAc.
Cyclohexane	61.0	7.542	N/A[1]			
EtOAc	32.6	4.033	N/A			
Water	0.2	0.020	N/A			
Water extract	0.8	0.104	BAS 510 F	0.8	0.104	
Nonextractable	3.6	0.442	N/A			

[1] Not analyzed.
[2] Calculated by summing extractable residues and nonextractable residues. The petitioner used the calculated value for all reported results.

Table 2.2.3. Extraction, Characterization, and Identification of Radioactive Residues in **Grape Leaves** [2] **(TRR = 43.672 ppm)** Harvested 45 Days Following Three Foliar Applications of **[Diphenyl-U-^{14}C]BAS 510 F** at 0.713 lb ai/A/application.

Fraction ID	% TRR	ppm	Residue ID	% TRR	ppm	Comments
MeOH extract	98.0	42.801	BAS 510 F	95.6	41.752	Sequentially partitioned with cyclohexane and EtOAc.
			Unknown	2.4	1.049	
Cyclohexane	99.6	43.488	N/A[1]			
EtOAc	2.2	0.947	N/A			
Water	0.2	0.079	N/A			
Nonextractable	2.0	0.871	N/A			

[1] Not analyzed.
[2] Calculated by summing extractable residues and nonextractable residues. The petitioner used the calculated value for all reported results.

Table 2.2.4. Extraction, Characterization, and Identification of Radioactive Residues in **Grapes** [2] **(TRR = 2.066 ppm)** Harvested 45 Days Following Three Foliar Applications of **[Pyridin-3-^{14}C]BAS 510 F** at 0.713 lb ai/A/application.

Fraction ID	% TRR	ppm	Residue ID	% TRR	ppm	Comments
MeOH extract	92.2	1.905	BAS 510 F	92.2	1.905	Sequentially partitioned with cyclohexane and EtOAc.
Cyclohexane	105.5	2.180	N/A[1]			
EtOAc	1.7	0.035	N/A			
Water	1.1	0.022	N/A			
Water extract	0.5	0.009	N/A			
Nonextractable	7.3	0.150	N/A			

[1] Not analyzed.
[2] Calculated by summing extractable residues and nonextractable residues. The petitioner used the calculated value for all reported results.

Table 2.2.5. Extraction, Characterization, and Identification of Radioactive Residues in **Grape Stalks** [2] **(TRR = 19.637 ppm)** Harvested 45 Days Following Three Foliar Applications of **[Pyridin-3-^{14}C]BAS 510 F** at 0.713 lb ai/A/application.

Fraction ID	% TRR	ppm	Residue ID	% TRR	ppm	Comments
MeOH extract	97.0	19.054	BAS 510 F	97.0	19.054	Sequentially partitioned with cyclohexane and EtOAc.
Cyclohexane	93.7	18.403	N/A[1]			
EtOAc	1.9	0.380	N/A			
Water	0.5	0.102	N/A			
Water extract	0.5	0.108	BAS 510 F	0.5	0.098	
			Unknown	0.1	0.010	
Nonextractable	2.4	0.475	N/A			

[1] Not analyzed.
[2] Calculated by summing extractable residues and nonextractable residues. The petitioner used the calculated value for all reported results.

Table 2.2.6. Extraction, Characterization, and Identification of Radioactive Residues in **Grape Leaves** [1] **(TRR = 63.359 ppm)** Harvested 45 Days Following Three Foliar Applications of **[Pyridin-3-^{14}C]BAS 510 F** at 0.713 lb ai/A/application.

Fraction ID	% TRR	ppm	Residue ID	% TRR	ppm	Comments
MeOH extract	97.9	62.031	BAS 510 F	96.1	60.859	Sequentially partitioned with cyclohexane and EtOAc.
			Unknown	1.8	1.172	
Cyclohexane	97.4	61.702	N/A[1]			
EtOAc	2.3	1.427	N/A			
Water	0.2	0.141	N/A			
Nonextractable	2.1	1.328	N/A			

[1] Not analyzed.
[2] Calculated by summing extractable residues and nonextractable residues. The petitioner used the calculated value for all reported results.

Table 2.3. 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
[Diphenyl-U-^{14}C]BAS 510 F						
BAS 510 F	92.7	1.095	96.4	11.914	95.6	41.752
Water extract	0.4	0.005	--	--	--	--
Unknown	--	--	--	--	2.4	1.049
Total Identified (TI)	92.7	1.095	96.4	11.914	95.6	41.752
Total Characterized (TC)	0.4	0.005	--	--	2.4	1.049
Total Extractable (TE)	93.1	1.1	96.4	11.914	98.0	42.801
Total Bound (TB)	6.8	0.081	3.6	0.442	2.0	0.871
% Mass Balance	99.9		100.0		100.0	
[Pyridin-3-^{14}C]BAS 510 F						
BAS 510 F	92.2	1.905	97.5	19.152	96.1	60.859
Water extract	0.5	0.009	--	--	--	--
Unknown	--	--	0.1	0.010	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	0.150	2.4	0.475	2.1	1.328
% Mass Balance	100.0		100.0		100.0	

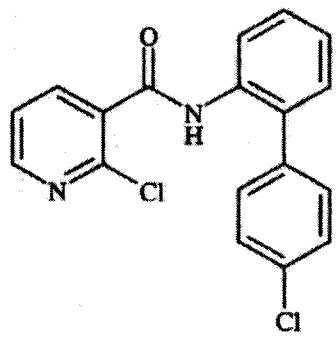
TC = Sum of all unidentified, extractable residues
TE = Sum of TI and TC
% Mass Balance = TE %TRR +TB % TRR. Note that the petitioner calculated TRR by summing extractable and nonextractable residues; therefore, mass balance is at or very close to 100% for all matrices. See Table 2.1 for actual mass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

2.4 Proposed Metabolic Fate of BAS 510 F in Grape.

As borne out by Table 2.3, no significant metabolism of BAS 510 F (>2.5% of the TRR) was observed in grapes.

No metabolites of BAS 510 F were identified in grapes.

Table 2.4. Compounds Identified in Grape.

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 grape commodities, at >92% TRR.

3. Discussion

3.1. Methods

Radiolabeled [^{14}C]BAS 510 F, labeled at the 3-position of the pyridine ring or uniformly labeled on the phenyl rings, was applied three times to grape vines as a foliar spray application at 0.713 lb ai/A/application (800 g ai/ha for a total of 2.4 kg ai/ha/season, ≈2X the proposed label rate), with the first application at the end of flowering, the second application 12 days later, and the third application 41 days after the second, at the beginning of ripening. Grape bunches and leaves were collected at maturity, 45 days following the last application. Grape bunches were separated into grapes and stalks. The TRR were determined by combustion/LSC in grapes and grape leaves. For grape stalks, the petitioner calculated TRR by summing extractable and nonextractable radioactivity because of sample inhomogeneity. The petitioner also used calculated TRR values for grapes and grape leaves for reporting all results. Material balances, based on sample combustion, were 90.6-108.7% for grapes and grape leaves.

The majority of residues (>92% TRR) in grape commodities were extracted with MeOH. An additional water extraction released a small amount of radioactivity (<1% TRR). Extracts of grape commodities were analyzed by HPLC; identification of BAS 510 F was confirmed in grapes by LC/MS/MS. These methods adequately characterized/identified the majority of the residues in grape commodities.

3.2. Results

Following three foliar applications of [^{14}C]BAS 510 F, labeled in the diphenyl portion or in the pyridine ring, at 0.713 lb ai/A/application (800 g ai/ha), TRR were 1.181 and 2.066 ppm, respectively, in/on grapes, 12.356 and 19.637 ppm, respectively, in/on grape stalks, and 43.672 and 63.359 ppm, respectively, in/on grape leaves collected 45 days following the last application.

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 MeOH 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 significant food/feed item, the petitioner did not investigate the identity of this peak. Nonextractable residues accounted for 2.0-7.3% TRR and were not further analyzed.

The submitted study is adequate to satisfy data requirements for a plant metabolism study with grapes.

4. Deficiencies

No deficiencies were identified.

5. References

None.