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

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

Date: July 2, 2003

Reviewers:

William T. Drew, Chemist — Date: 8/20/03
Reviewer
RAB2/HED (7509C)

Henri P. Bietlot, Chemist — Date: July 16/03
Peer reviewer
FREAS, HED, PMRA

Richard A. Loranger — Date: 8/15/03
Branch Senior Scientist
RAB2/HED (7509C)

Ariff Ally — Date: July 25/03
Section Head
FREAS, HED, PMRA

DP Barcode: D278386

Petition: 1F06313

Citation: 45405028 Jordan, J. (2001) Independent Method Validation of BASF Analytical Method D0008 Entitled "Method for Determining BAS 510 F in Plant and Turf Cloth Matrices Using GC/MS and Dislodgeable Foliar Sample Using HPLC/UV": Lab Project Number: 2001/5000880: 64954: D0008. Unpublished study prepared by BASF Corporation. 61 pages.

45405101 Adbel-Baky, S.; Jones, J. (2001) Validation of BASF Analytical Method D0008, Method for Determining BAS 510 F in Plant and Turf Cloth Matrices Using GC/MS and Dislodgeable Foliar Samples Using HPLC/UV: Final Report: Lab Project Number: 64276: 2001/5000977: D0008. Unpublished study prepared by BASF Agro Research. 97 pages.

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 RAB2, and reflects current HED

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and Office of Pesticide Programs (OPP) policies. This DER has also been peer-reviewed by PMRA/Canada.

Executive Summary

BASF Corporation has proposed a **GC/MS** method, **Method D0008**, for the enforcement of proposed tolerances for residues of BAS 510 F in plant commodities. Briefly, plant matrix samples, except oil, are extracted with methanol:water:2N HCl (70:25:5, v:v:v). For oil matrices, residues are partitioned with hexane and ACN, and the ACN phase is collected. An aliquot of the acidic methanol:water or ACN extract is partitioned with iso-octane and the iso-octane phase is cleaned up by solid phase extraction. The quantitation of BAS 510 F is based on selected ion monitoring (SIM) of major ions. The method lists monitoring ions of *m/z* 342, 142, or 140, and notes that any ion can be used for quantitation based on the cleanness of the chromatograms. Quantitation is performed using an external calibration curve of BAS 510 F standards. The reported limit of quantitation (LOQ) is 0.050 ppm for the residues of BAS 510 F in/on all plant matrices.

Adequate method validation and independent laboratory validation (ILV) studies were conducted for GC/MS method D0008 for the analysis of residues of BAS 510 F in plant matrices. Method validation recoveries ranged from 75 to108% in snap beans, from 81 to 114% in canola seed, from 91 to 108% in canola oil, from 85 to 123% in lettuce, from 78 to 100% in peanut nutmeat, and from 86 to117% in tomato fortified with BAS 510 F at 0.050 and 0.50 ppm. Independent laboratory validation studies were conducted by BASF (Research Triangle Park, NC) on canola seed (representative oil matrix) and tomato (representative non-oily matrix); recoveries ranged from 80 to 96% in canola seed fortified at 0.050 and 3.5 ppm and from 79 to 95% in tomato fortified at 0.050 and 1.0 ppm.

A study to demonstrate the stability of standard solutions of BAS 510 F and various of its metabolites in ACN has been submitted separately (see DER of MRID 45405104). The recoveries of BAS 510 F, M510F01, M510F49, M510F51, and M510F53 following 62 days of storage, either refrigerated in the dark or at room temperature with daylight exposure, indicated no loss of concentration during storage. Based on these data, the petitioner recommended that standard solutions be stored no longer than 60 days. **As a condition of registration,** the petitioner should revise this GC/MS method to recommend a 60-day maximum storage interval for standard solutions of reference standards, and submit a copy of the revised method to the Agency.

Radiovalidation of the GC/MS method was not conducted. However, because the plant metabolism studies (see DERs for MRIDs 45405021-45405023) indicated that residues were readily extractable with methanol, radiovalidation of the proposed enforcement method will not be required.

This GC/MS method for the analysis of residues of BAS 510 F in/on plant matrices has been forwarded to ACB/BEAD for a tolerance method validation (TMV) trial on canola seed, raisin,

and tomato. **Contingent upon** satisfactory results from the BEAD TMV, **and provided** the method is revised to impose a 60-day limitation on the storage of standard solutions of reference standards, the method can be approved for tolerance enforcement purposes in plant matrices.

It is noted that the data submissions (MRIDs 45405028 and 45405101) for Method D0008 include method descriptions, validation, and ILV for turf cloth matrices using GC/MS and for dislodgeable foliar samples using HPLC/UV. These data are not reviewed herein as they are not applicable to OPPTS 860.1340 guideline requirements.

GLP Compliance

Signed and dated GLP, quality assurance, and data confidentiality statements were provided.

1. Materials and Methods

1.1. Test Substances

Table 1.1.1. List of Analytes Tested with the GC/MS Method.	
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

Table 1.1.2. Matrices Tested with the GC/MS Method.	
Matrix	Matrix Form
Canola oil	Untreated samples obtained from a canola processing study (BASF Study 95017). Samples were not further processed, but frozen until extraction and analysis.
Canola seed	Untreated samples obtained from field trials (BASF Studies 97104, 64114, 97042, 94039, and 98024). Samples were homogenized with dry ice and frozen until extraction and analysis.
Lettuce	
Peanut, nutmeat	
Snap bean	
Tomato	

Table 1.1.3. Fortification Levels Tested with the GC/MS Method.	
Matrices	BAS 510 F (ppm)
Canola seed, canola oil, lettuce, peanut nutmeat, snap bean, and tomato	0.050 (LOQ)
	0.50

1.2. Methods

A description of GC/MS method D0008 follows. For method validation, canola oil and homogenized samples of canola seed, lettuce, peanut nutmeat, snap bean, and tomato were fortified with a solution containing BAS 510 F in acetonitrile (ACN). Samples were fortified prior to method extraction.

Briefly, samples of plant matrices, except oil, are extracted with methanol:water:2N HCl (70:25:5, v:v:v) and centrifuged. For oil samples, residues are partitioned with hexane and ACN. The ACN phase is collected and partitioned again with a new aliquot of hexane. The ACN phase is collected and diluted with additional ACN. An aliquot of the acidic methanol:water or ACN extract is concentrated, 0.05 N HCl is added, and residues partitioned with iso-octane. An aliquot of the iso-octane phase is subjected to silica gel solid phase extraction (SPE) under vacuum for residue cleanup. Residues are eluted with 4% ethyl acetate in dichloromethane. The eluate is evaporated to dryness. Residues are redissolved with 0.01% polyethylene glycol (M_n ca. 400) in toluene and analyzed using GC/MS with selected ion monitoring (SIM). The method lists monitoring ions of *m/z* 342, 142, or 140, and notes that any ion can be used for quantitation based on the cleanness of the chromatograms. Quantitation is performed using an external calibration curve of BAS 510 F standards.

2. Results

2.1. Stability of Reference Materials

The reference substance was stored frozen (<-5° C) until used in the validation study. Stock solutions of the BAS 510 F standard in ACN were stored refrigerated, in amber bottles, except when in use. The method recommends that stock solutions of the standard be made fresh every three months and that dilutions of the stock solutions be stored refrigerated for no longer than one month. Fortification standards were prepared in ACN from the stock solution and the final calibration standards were prepared in 0.01% polyethylene glycol (M_n ca. 400) in toluene from the stock solution.

A study to demonstrate the stability of standard solutions of BAS 510 F and various of its metabolites in ACN has been submitted separately (see DER of MRID 45405104). The recoveries of BAS 510 F, M510F01, M510F49, M510F51, and M510F53 following 62 days of storage, either refrigerated in the dark or at room temperature with daylight exposure, indicated

no loss of concentration during storage. Based on these data, the petitioner recommended that standard solutions be stored no longer than 60 days. **As a condition of registration,** the petitioner should revise this GC/MS method to recommend a 60-day maximum storage interval for standard solutions of reference standards, and submit a copy of the revised method to the Agency.

2.2. Method Characteristics

2.2.1. Chromatography

The representative GC/MS total ion chromatograms for plant matrices indicate that the peak shape was generally good for BAS 510 F. No interferences were observed in the control samples. The quantitation of BAS 510 F is based on selected ion monitoring (SIM) of major ions. The method lists monitoring ions of *m/z* 342, 142, or 140, and notes that any ion can be used for quantitation based on the cleanness of the chromatograms. It was noted that high recoveries may result because of standard loss on the GC/MS instrumentation, therefore, polyethylene glycol is added to the toluene (in the final analysis solution) to help decrease this phenomenon. The method stated that it may be necessary to condition the column with several injections of the matrix sample at the beginning of each run, and recommended that no more than 5 samples be injected between injections of standards.

2.2.2. Linearity

The method linearity was good. The coefficient of determination (r^2) from a representative calibration standard curve was 0.993 for BAS 510 F standard concentrations ranging 2.0-20.0 ng/mL.

2.2.3. Specificity

An interference study is not required as the method employs an MS detector (m/z 342, 142 and 140) and lists three ions to be monitored. No interferences were observed in the chromatograms of control samples.

2.2.4. Method Limits

The limit of detection (LOD) was 2.0 pg/µL (concentration on the GC column), which is the lowest standard injected onto the GC/MS system. The validated limit of quantitation (LOQ) was 0.050 ppm for the residues of BAS 510 F in/on all plant matrices.

2.2.5. Analyte Recoveries

Table 2.2.5.1. Recovery of BAS 510 F from Plant Matrices Using the GC/MS Method.

Matrix	Fortification Level (ppm)	Recoveries (%)	Mean ± SD (%)
Beans, snap	0.050	75, 95, 104, 106, 108	95 ± 10
	0.50	89, 91, 91, 93, 93	
Canola, seed	0.050	104, 105, 110, 113, 114	100 ± 11
	0.50	81, 89, 89, 94, 97	
Canola, oil	0.050	100, 103, 104, 105, 108	100 ± 5
	0.50	91, 94, 95, 100, 103	
Lettuce	0.050	102, 109, 111, 114, 123	101 ± 13
	0.50	85, 87, 87, 90, 97	
Peanut, nutmeat	0.050	78, 80, 82, 83, 94	88 ± 7
	0.50	86, 87, 93, 96, 100	
Tomato	0.050	109, 110, 114, 115, 117	102 ± 12
	0.50	86, 90, 90, 90, 95	

2.2.6. Independent Laboratory Validation

Independent laboratory validation (ILV) of GC/MS method D0008 was conducted on canola seed and tomato by chemists at BASF Corporation, Agro Research (Research Triangle Park, NC), who were unfamiliar with the method or its development. It was noted that the method was previously developed and validated at the same BASF facility. Canola seed and tomato were chosen because these matrices represent oily and non-oily matrices which are difficult to analyze. Untreated homogenized samples of canola seed and tomato, obtained from field trial studies, were fortified with BAS 510 F at 0.050 ppm (method LOQ) and at the proposed tolerances for canola seed, 3.5 ppm, and tomato, 1.0 ppm.

Method validation on canola seed and tomato was successful with the first attempt. The laboratory noted that some control canola seed and tomato samples had peaks below the method LOD at the retention time of BAS 510 F; recoveries were not corrected for these residues. Ion *m/z* 342 was initially integrated, but due to peak interferences from polyethylene glycol, the monitored ion was changed to *m/z* 140. The laboratory reported that the method (number of samples not specified) took one analyst 5 hours, with an additional hour for data analysis.

Table 2.2.6. Recovery of BAS 510 F from Canola Seed and Tomato Using the GC/MS Method During Independent Laboratory Validation.

Matrix	Fortification Level (ppm)	Recoveries (%)	Mean ± SD
Canola seed	0.050	80, 96	90 ± 8
	3.5	88, 95	
Tomato	0.050	90, 95	88 ± 7
	1.0	79, 86	

3. Discussion

3.1. Recovery and Repeatability

Adequate method validation and independent laboratory validation studies were conducted for the GC/MS method for analysis of residues of BAS 510 F in representative plant matrices. Method validation recoveries ranged 75-108% in snap beans, 81-114% in canola seed, 91-108% in canola oil, 85-123% in lettuce, 78-100% in peanut nutmeat, and 86-117% in tomato fortified with BAS 510 F at 0.050 and 0.50 ppm.

The recoveries obtained from the ILV studies are comparable to the recoveries obtained in method validation. ILV recoveries ranged 80-96% in canola seed fortified with BAS 510 F at 0.050 and 3.5 ppm and 79-95% in tomato fortified with BAS 510 F at 0.050 and 1.0 ppm.

3.2. Method Efficiency

Radiovalidation of the GC/MS method was not conducted. However, because the plant metabolism studies (see DERs for MRIDs 45405021-45405023) indicated that residues were readily extractable with methanol, radiovalidation of the proposed enforcement method will not be required.

4. Deficiencies

As a condition of registration, the petitioner should revise this GC/MS method to recommend a 60-day maximum storage interval for standard solutions of reference standards, and submit a copy of the revised method to the Agency.

Contingent upon satisfactory results from the BEAD TMV, **and provided** the method is revised to impose a 60-day limitation on the storage of standard solutions of reference standards, the method can be approved for tolerance enforcement purposes in plant matrices.

5. References

None.