1-11-94

MEMORANDUM

SUBJECT: Imazalil. Case No. 2325. Registrants' Response to Reregistration. MRID No. 42626901, -02, & -03. CBRS No. 11295. DP Barcodes: D187506.

FROM: Leung Cheng, Chemist
Special Review Section II
Chemistry Branch II - Reregistration Support
Health Effects Division(7509C)

THROUGH: Andrew R. Rathman, Section Head
Chemistry Branch II - Reregistration Support
Health Effects Division(7509C)

TO: Kathleen Depukat, CRM 52
Accelerated Reregistration Branch
Special Review/Reregistration Division(7508W)

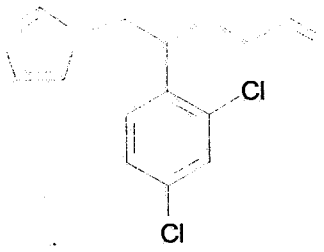
Attached is a review of wheat metabolism, method validation, and frozen storage stability in bananas for imazalil, submitted by the registrant in response to Phase IV review (S. Funk, 10/19/90) and CBRS No. 8846 (A. Aikens, 8/13/92). This information was reviewed by Dynamac Corporation under the supervision of CBRS, HED. The data assessment has undergone secondary review in the branch and has been revised to reflect branch policies.

The submitted wheat metabolism study is adequate. The submitted method validation data and frozen storage stability data for bananas are also adequate.

Attachment: Dynamac review of residue chemistry data

cc(without Attachment):RF
cc(with Attachment):Circ, SF, List B File, Cheng, Dynamac
RDI:ARRathman:1/5/94:MMetzger:1/10/94:EZager:1/10/94
7509C:CBRS:LCheng:CM#2:RM804/810D:1/5/94:03:A\IMAZALIL\WHEATMET

IMAZALIL



Shaughnessy No. 111901; Case 2325

(CBRS No. 11295; DP Barcode D187506)

Task 4

REGISTRANT'S RESPONSE TO RESIDUE CHEMISTRY DATA REQUIREMENTS

BACKGROUND

In response to the Imazalil Phase 4 Reviews (S. Funk, 10/19/90) , Janssen Pharmaceutica submitted data pertaining to the metabolism (1993; MRID 42626901) of [^{14}C]imazalil in spring wheat, and data depicting the storage stability (1993; MRID 42626903) of imazalil residues in/on bananas. In addition, the registrant submitted method validation data (1993; MRID 42626902) for a GC/electron capture detection (ECD) method for determining residues of imazalil [1-[2-(2,4-dichlorophenyl)-2-(2-propenyloxy)ethyl]-1H-imidazole] and its metabolite R14821 [1-(2,4-dichlorophenyl)-2-(1*H*-imidazole-1-yl)-1-ethanol] in/on wheat and barley commodities. These submissions are reviewed here to determine their adequacy in fulfilling residue chemistry data requirements. The Conclusions and Recommendations stated in this review pertain only to the submissions listed above. Other data requirements specified in the Phase 4 Reviews are not addressed herein.

The nature of the residue in plants and animals is not adequately understood. The Phase 4 Reviews required the registrant to prove the stability of the tritium-labeled imazalil used in the banana, orange, and barley metabolism studies. Review of plant metabolism studies submitted for oranges and bananas was postponed pending receipt of data on the stability of tritiated imazalil. The existing goat metabolism study can be upgraded with additional data on residues in liver and supporting storage stability data. A new metabolism study in poultry is required.

Two GC/ECD methods are available for enforcing imazalil tolerances in plants and animals; these methods are listed in PAM, Vol. II as Methods I and II. These methods have undergone successful EPA method tryouts; however, both methods require fortification of control samples for use as external standards in calibrating the GC/ECD response.

Tolerances have been established for the combined residues of imazalil and its metabolite R14821 in/on raw agricultural commodities [40 CFR §180.413(a)]. Food/feed additive tolerances have also been established for the combined residues of imazalil and R14821 in processed plant commodities (40 CFR §185.3650 and §186.3650). Tolerances for animal commodities have been established for the combined residues of imazalil and its metabolites R14821 and R42243 [3-[1-(2,4-dichlorophenyl)-2-(1H-imidazole-1-yl)ethoxyl]-1,2-propanediol] (40 CFR §180.413[b]).

Codex MRLs for residues of imazalil in/on various plant commodities are currently defined in terms of parent imazalil, and as such, are not compatible with the U.S. tolerances. Compatibility issues will be addressed at the issuance of the RED.

CONCLUSIONS AND RECOMMENDATIONS

Qualitative Nature of the Residue in Wheat

1. The submitted plant metabolism study is adequate. Total radioactive residues in wheat grain were ≤0.004 ppm; therefore, residues in grain were not characterized. Extractable radioactive residues accounted for approximately 73% of the TRR in forage and 44% of the TRR in straw grown from wheat seeds treated with [^{14}C]imazalil at 5x the maximum label rate. Imazalil was the major component detected by TLC analysis in both matrices, and accounted for 24.4% of the TRR in forage and 16.9% of the TRR in straw. The metabolites R14821 and R42639 [1-[2-(2,4-dichlorophenyl)-2-(2,3-dihydroxypropoxy)ethyl]-1H-imidazole were also detected in forage (8.3 and 4.6% of the TRR, respectively) and straw (3.8 and 3.7% of the TRR, respectively). Approximately 12-21% of the TRR remained at the origin during TLC analysis of extracts from both matrices. Subsequent HPLC analysis of the forage sample indicated that the radioactivity remaining at the origin consisted of N-[2-(2,4-dichlorophenyl)-2-hydroxyethyl]urea (R44085), 3-[2-(2,4-dichlorophenyl)-2-(2,3-dihydroxypropoxy)ethyl]-2,4-imidazolidinedione (R61000), 1-[2-(2,4-dichlorophenyl)-2-hydroxyethyl]-2,4-imidazolidinedione (R43449), and 2,4-dichloro-β-(2-propenyloxy) benzeneethanamine (R44177), none of which accounted for ≥4.3% of the TRR.

Residue Analytical Methods

2. The submitted method validation data indicate that the GC/ECD method, using either an internal or external standard, is adequate for collecting data on residues of imazalil and R14821 in/on wheat/barley grain, forage, and straw.

Storage Stability Data

3. The submitted storage stability data are adequate and indicate that residues of imazalil and R14821 are stable in bananas stored at ≤-20 C for up to 38 weeks. These data support the available imazalil residue data for bananas (CBRS No. 8846, 8/13/92, A. Aikens); no additional storage stability data on bananas are required.

DETAILED CONSIDERATIONS

Qualitative Nature of the Residue in Wheat

Janssen Pharmaceutica submitted a protocol for determining the nature of the residue of imazalil in wheat grown from imazalil treated seeds. The Agency (CBRS No. 9211, 2/25/92, S. Funk) found the protocol acceptable with minor modifications and stated that validation with the radiolabeled samples would be needed only if new metabolites requiring regulation were found in the study.

Janssen Pharmaceutica subsequently submitted (1993; MRID 42626901) data pertaining to the metabolism of [2-ethyl-^{14}C]imazalil in spring wheat. The [^{14}C]imazalil was applied as an ethanolic spray to spring wheat seeds at a rate of 493 ppm (mg ai/kg seed), 5x the maximum permitted on the label. The radioactive compound had a specific activity of 2.05 GBq/mmol and a radiochemical purity >99%. Control seeds were treated with ethanol:water (9:1, v:v). One day after treatment, treated and control seeds were planted into pots containing a sandy clay loam soil. The pots were placed in an outdoor enclosure covered with a plastic mesh. Forage was harvested 42 days after planting, and grain and straw (plus chaff) were harvested 158 days after planting. The samples were placed in frozen storage (-20 C) until analysis. Analyses were completed within 6-7 months of sampling, therefore no storage stability data are required.

This study was performed by Life Science Research Limited, Suffolk, England.

Total radioactive residues (TRR)

Radioactive residues in liquid fractions were determined directly by liquid scintillation spectrometry (LSS). Radioactivity in seeds, freeze-dried stems and leaves (forage samples), and grain and straw were determined by combustion and liquid scintillation spectrometry (LSS). All samples were radioassayed in duplicate. The detection limit

was 0.08 ppb. Total radioactive residues in forage, straw, and grain were 1.36, 0.15, and ≤0.004 ppm, respectively.

Extraction and hydrolysis of residues

Radioactive residues in grain were not characterized due to the low levels of ^{14}C-residues in grain. Several extraction schemes were attempted on the forage and straw samples. A modified Bligh-Dyer-Gemisch procedure followed by a Soxhlet extraction was found to be the most efficient and is described below.

Freeze-dried forage and straw were extracted with methanol (MeOH):chloroform:0.1 N HCl (2:1:0.8; v:v:v) followed by Soxhlet extraction with MeOH:0.1 N HCl (95:5, v:v). The extracts were combined, concentrated under vacuum, and diluted with distilled water. The resulting forage and straw aqueous fractions were freeze-dried, and the residues were redissolved in MeOH for subsequent TLC and HPLC analysis.

Solids were sequentially hydrolyzed (overnight at room temperature) with 1 N HCl and 1 N NH_4OH followed by refluxing (6 hours) with 6 N HCl and 10 N NaOH.

Characterization of residues

TLC analyses were conducted on silica gel plates using several solvent systems. Reference standards were either spotted alongside the samples or applied on the same spot as the sample. Reference standards were detected by ultraviolet light and radioactive zones were located by autoradiography or by scanning with a linear analyzer. The molecular structures and chemical names of imazalil and suspected metabolites are presented in Figure 1.

HPLC analyses were performed on a system equipped with a radioactivity detector and UV absorbance detector at 230 nm. Reference standards were co-chromatographed with samples. Sample chromatograms and calculations were provided. Characterization of ^{14}C-residues in wheat forage and straw is summarized in Table 1.

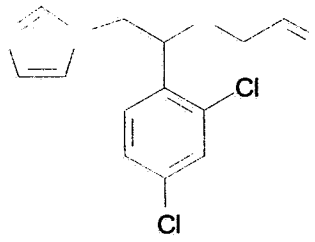
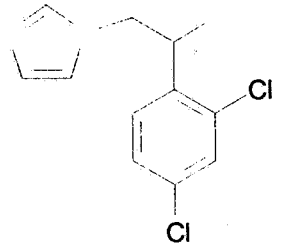
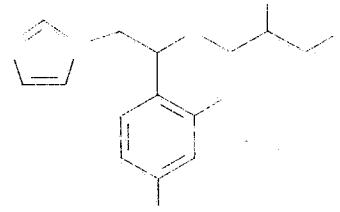
Additional filtration and TLC clean-up steps were required of the straw aqueous fraction prior to analysis. In addition, the initial TLC analysis resolved five peaks that did not correspond to the reference standards. Therefore, the silica gel corresponding to each peak was scraped and the radioactive residues extracted and re-chromatographed using the same solvent system. Several metabolites and unknowns were resolved (Table 1). However, the registrant stated that the viscous nature of the straw sample precluded HPLC analysis.
In summary, approximately 73% and 44% of the TRR was extractable from forage and straw, respectively. Imazalil was the major component detected by TLC analysis in both matrices, accounting for 24.4% of the TRR in forage and 16.9% of the TRR in straw. The metabolites R14821 and R42639 were also detected in forage (8.3% and 4.6% of the TRR, respectively) and straw (3.8% and 3.7% of the TRR, respectively).

Approximately 12-21% of the TRR remained at the origin during TLC analysis of both matrices. Subsequent HPLC analysis of the forage sample indicated that the radioactivity remaining at the origin consisted of R44085, R61000, R43449, and R44177, none of which accounted for ≥4.3% of the TRR. The registrant stated that the viscous nature of the straw sample precluded HPLC analysis. The registrant has proposed that the principal pathway of imazalil metabolism in wheat plants is by hydroxylation of the propenyl side-chain followed by oxidation of the imidazole ring.

Table 1. Distribution of radioactive residues in forage (42 days) and straw (158 days) grown from wheat seeds treated with [^{14}C]imazalil.

Sample/Fraction	%TRR	PPM	Characterization/Identification
Wheat Forage (1.36 ppm)			
MeOH:$CHCl_3$:HCl	65.8	0.9	Analyzed by TLC; imazalil (0.33 ppm, 24.4% TRR), R14821 (0.11 ppm, 8.3% TRR), and R42639 (0.06 ppm; 4.6% TRR) were identified and confirmed by HPLC. Three unknowns collectively accounted for 5% of the TRR (0.06 ppm). One component, located at the origin, accounted for 21% of the TRR (0.29 ppm), however, subsequent HPLC analysis detected only one unknown that accounted for ≤1.1% of the TRR. The remainder of the residue was accounted for by imazalil (0.26 ppm, 19.3 TRR), R42639 (0.18 ppm, 13.1% TRR), and R14821 (0.1 ppm, 7.5% TRR), confirming the TLC identities, and R44085, R6100, R43449, and R44177 accounting for a total of 11.9% of the TRR.
Solids			Subjected to sequential acid and base hydrolyses.
Hydrolyses 1 N HCl	0.3	>0.01	Not analyzed further.
1 N NH_4OH	4.0	0.05	Not analyzed further.
6 N HCl	5.9	0.08	Not analyzed further.
10 N NaOH	2.4	0.03	Not analyzed further.
Solids	7.7	0.11	Not analyzed further.
Wheat Straw (0.15 ppm)			
MeOH:$CHCl_3$:HCl	43.6	0.05	TLC analysis resolved 5 peaks. Each peak was re-chromatographed separately and imazalil (0.03 ppm, 16.9% TRR), R14821 (<0.01 ppm, 3.8 % TRR), and R42639 (<0.01 ppm, 3.74% TRR) were identified. Two unknowns, each accounting for ≤4.1% TRR, were also detected. ^{14}C-residues remaining at the origin accounted for 11.8% of the TRR (0.02 ppm). It was postulated that this radioactivity was similar in nature to that observed in wheat forage, however, because of the viscous nature of the straw sample, HPLC analysis was not performed.
Solids			Subjected to sequential acid and base hydrolyses.
Hydrolyses 1 N HCl	ND	ND	
1 N NH_4OH	4.7	<0.01	Not analyzed further.
6 N HCl	8.1	0.01	Not analyzed further.
10 N NaOH	3.6	<0.01	Not analyzed further.
Solids	58.5	0.09	Not analyzed further.

Figure 1. Imazalil and its metabolites in plants (MRID 42626901).

Chemical Name	Substrate	Common Name/Chemical Code
1-[2-(2,4-dichlorophenyl)-2-(2-propenyloxy)ethyl]-1H-imidazole	Wheat forage and straw	 **Imazalil**
1-[2-(2,4-dichlorophenyl)-2-hydroxyethyl]-1H-imidazole	Wheat forage and straw	 **R14821**
1-[2-(2,4-dichlorophenyl)-2-(2,3-dihydroxypropoxy)ethyl]-1H-imidazole	Wheat forage and straw	 **R42639**

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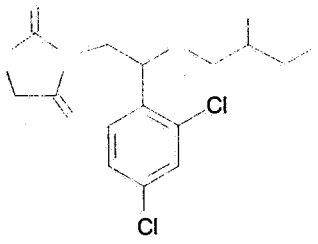
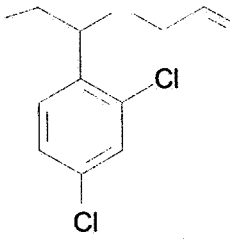
3-[2-(2,4-dichlorophenyl)-2-(2,3-dihydroxypropoxy)ethyl]-2,4-imidazolidinedione
Wheat forage and straw



R61000

(±)-2,4-dichloro-β-(2-propenyloxy)benzeneethanamine
Wheat forage and straw



R44177

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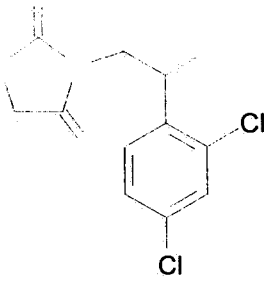
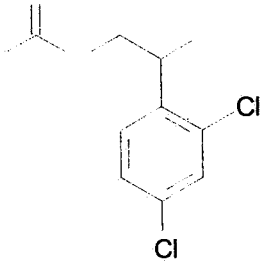
1-[2-(2,4-dichlorophenyl)-2-hydroxyethyl]-2,4-imidazolidinedione
Wheat forage and straw



R43449

(±)-N-[2-(2,4-dichlorophenyl)-2-hydroxyethyl]urea
Wheat forage and straw



R44085

Residue Analytical Methods

Janssen Pharmaceutica submitted validation data (1993, MRID 42626902) for a GC/ECD method for determining residues of imazalil and its metabolite R14821 in/on wheat and barley commodities. This method is a modification of Method I in PAM, Vol. II, and is essentially the same as a GC/ECD method for determining imazalil residues in/on bananas that has been reviewed and deemed adequate by the Agency (S. Funk, CBRS No. 10602, 6/24/93).

In brief, residues of imazalil and R14821 are extracted overnight from wheat/barley grain and straw with MeOH and from forage with 0.1 N HCl. The extracts of grain and straw are evaporated to dryness and reconstituted in 0.1 N HCl. The acidified residues from all matrices are then made basic (pH ≥9) using concentrated ammonia and are extracted into heptane:isoamyl alcohol (95:5, v:v). For determination of R14821, the organosoluble extracts are evaporated to dryness and then derivatized with N,O-bis (trimethylsilyl)acetamide (BSA) in toluene. Residues of imazalil and derivatized R14821 are quantified by GC/ECD. The limit of quantitation (LOQ) for imazalil in grain, forage, and straw is 0.05, 0.06, and 0.08 ppm, respectively; and the LOQ for R14821 in grain, forage, and straw is 0.03, 0.02, and 0.04 ppm, respectively. The reported limits of detection for imazalil and R14821 are 0.02 and 0.01 ppm, respectively.

Validation data were presented for this method using imazalil and R14821 as external standards or using

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R35162 (1-[2-(2,4-dichlorophenyl)-cyclopentyl-methyl]-1H-imidazole) as an internal standard. The current enforcement method uses an external standard, whereas, both external and internal standards were used for data collection in the magnitude of the residue studies for wheat and barley.

For method validation, samples of wheat grain, forage, and straw were each fortified with imazalil at approximately 0.05, 0.1, 0.5, 1, and 2 ppm and with R14821 at 0.07, 0.14, 0.21, 0.28, and 0.42 ppm. Four replicates of each commodity at each fortification level were analyzed using the GC/ECD method with both external and internal standards. The recoveries of imazalil and R14821 are reported in Table 3. The data indicate that the GC/ECD method, using either an internal or external standard, is adequate for collecting data on residues of imazalil and R14821 in/on wheat/barley grain, forage, and straw.

Table 3. GC/ECD method recoveries from wheat commodities fortified with imazalil at 0.05-2 ppm and R14821 at 0.07-0.42 ppm using external and internal standard calibrations.

Analyte	Calibration Method [a]	Wheat Commodity	Fortification Levels (ppm)	No. of Samples [b]	Percent Recovery
Imazalil	ES	Grain	0.05-2.0	20	66-126
		Forage	0.05-2.0	16	74-122
		Straw	0.05-2.0	14	76-126
Imazalil	IS	Grain	0.05-2.0	18	76-157 [c]
		Forage	0.05-2.0	16	62-134
		Straw	0.05-2.0	16	69-125
R14821	ES	Grain	0.07-0.42	20	78-111
		Forage	0.07-0.42	20	95-127
		Straw	0.07-0.42	18	80-136
R14821	IS	Grain	0.07-0.42	18	84-124
		Forage	0.07-0.42	18	84-109
		Straw	0.07-0.42	20	76-123

[a] ES=external standard calibration; IS=internal standard calibration.

[b] Sample numbers of less than 20 occurred when the registrant excluded data due to injection and/or fortification errors.

[c] Six of the 18 samples had recoveries ≥130%.

Storage Stability Data

The Phase 4 Reviews (S. Funk, 10/19/90) required storage stability data for all crops and their processed commodities and for representative animal commodities. In a subsequent review of residue data for bananas (CBRS No. 8846, 8/13/92, A. Aikens), CBRS requested storage stability data for residues of imazalil and R14821 in/on banana matrices. Previously reviewed storage stability data for citrus commodities (S. Funk, 3/2/93; CBRS No. 11335) indicate that imazalil and its metabolite R14821 are stable in whole oranges and processed orange commodities stored at -20 °C for up to 34 weeks.

Janssen Pharmaceutica (1993; MRID 42626903) has submitted data depicting the frozen storage stability of imazalil and its metabolite R14821 in whole ripe bananas. Untreated, whole ripe (yellow) bananas were homogenized and fortified with imazalil at 0.05, 0.2, and 3.0 ppm and with R14821 at 0.05 and 0.2 ppm.

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Samples were analyzed by Janssen Research Foundation (Beerse, Belgium) using a GC/ECD method that uses R30617 (1-[2-(2,4-dichlorophenyl)heptyl]-1H-imidazole mononitrate) as an internal standard. This method was previously reviewed and deemed adequate (CBRS No. 10602, 6/24/93, S. Funk). The LOQ for imazalil is 0.05 ppm and for R14821 is 0.08 ppm. At each fortification level, four replicate samples were extracted and analyzed within 24 hours (zero-time) of fortification and after 12, 24, and 38 weeks of storage at ≤ -20 C. Untreated control samples and concurrent method recovery samples were not analyzed in conjunction with the stored samples. The recoveries of imazalil and R143821 from bananas following frozen storage are presented in Table 4. Representative chromatograms and sample calculations were provided.

Table 4. Storage stability of imazalil and its metabolite R14821 in fortified, whole, ripe (yellow) bananas.

Analyte	Fortification Level (ppm)	Storage Interval (Weeks)	Percent Recovery [a]
Imazalil	0.05	0	98-119
		12	74-92
		24	90-96
		38	124-138
Imazalil	0.2	0	81-95
		12	75-79
		24	80-89
		38	87-97
Imazalil	3.0	0	109-124
		12	91-100
		24	73-90
		38	75-87
R14821	0.05	0	60-86
		12	118-120
		24	102-110
		38	166-172
R14821	0.2	0	74-86
		12	88-94
		24	79-91
		38	105-129

[a] Data represent the analysis of four fortified samples at each sampling interval.

The submitted storage stability data are adequate and indicate that residues of imazalil and R14821 are stable in whole yellow bananas stored at -20 C for up to 38 weeks. The registrant stated that treated field samples were stored for a maximum of 93 days under conditions identical to the storage stability samples. The storage stability data support the available residue data for bananas (CBRS No. 8846, 8/13/92, A. Aikens).

In conducting storage stability studies, CBRS recommends that freshly fortified samples be analyzed concurrently with stored samples in order to correct recoveries of stored samples for variations in method recoveries. For further information on conducting storage stability studies, the registrant is referred to the

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Agency guidance titled "Pesticide Reregistration Rejection Rate Analysis Residue Chemistry: Follow-up Guidance for Generating Storage Stability Data" (D. Edwards and E. Zager, 2/93).

AGENCY MEMORANDA CITED IN THIS REVIEW

CBRS No.: 8846
Subject: Imazalil Reregistration. Magnitude of the Residue - Post Harvest Treatment of Bananas (Foreign).
From: A. Aikens
To: B. Briscoe and K. Davis
Date: 8/13/92
MRID(S): 42058701.

CBRS No.: 9211
Subject: Reregistration of Imazalil. Protocol for Nature of the Residue in Wheat.
From: S. Funk
To: K. Davis
Date: 2/25/92
MRID(S): None.

CBRS No.: 10602
Subject: Analytical Method for the Determination of Imazalil and R14821 in/on Bananas, Citrus, and Citrus Processed Commodities. Research and Enforcement Method Comparison.
From: S. Funk
To: K. Davis and K. Depukat
Date: 6/24/93
MRID(S): 42454803, 42454804, and 42454805.

CBRS No.: 11335
Subject: Imazalil: Citrus and Citrus Processed Commodities Storage Stability.
From: S. Funk
To: K. Depukat
Date: 3/2/93
MRID(s): 42643301.

CBRS No.: 11586
Subject: Imazalil: (1) Wheat Processing Waiver Request; (2) Poultry Metabolism Protocol Change.
From: S. Funk
To: K. Davis and K. Depukat
Date: 4/6/93
MRID(s): None.

MASTER RECORD IDENTIFICATION NUMBERS

The citations for MRID documents used in this review are presented below.

42626901 O'Connor, J. (1992) Imazalil: Distribution and Metabolism in Spring Wheat: Final Report: Lab Project Number: 92/1046: JST/003/IMAZALIL: 92/JST003/1046. Unpublished study prepared by Life

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Science Research Limited. 166 p.

42626902 Ligtvoet, T. (1992) A Validated Gas Chromatographic Method for the Determination of Imazalil-related Residues in Wheat and Barley (Grain, Forage, and Straw): Final Report: Lab Project Number: 58. Unpublished study prepared by Janssen Research Foundation. 78 p.

42626903 Garnier, A. (1992) Storage Stability of Imazalil-derived Residues in Bananas: Lab Project Number: AGR4. Unpublished study prepared by Janssen Research Foundation. 317 p.

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