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

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

Date: 03-MAR-2006

Subject: **Chlorothalonil** Technical Fungicide - Review of Residue Chemistry Studies Submitted by Vischim S.R.L.

DP#:	323612	Decision#:	220223
PC Code:	081901	MRID#s:	45710225, 45710226, 45710228
40 CFR	180.275	Chemical Class:	Aromatic Fungicide

From: George F. Kramer, Ph.D., Chemist
Registration Branch 1 (RAB1)
Health Effects Division (HED; 7509C)

Through: P.V. Shah, Ph.D., Branch Senior Scientist
RAB1/HED (7509C)

To: Tony Kish/Rosemary Kearns, RM 22
Herbicide Branch
Registration Division (7505C)

Action Requested: Evaluation of residue chemistry studies for chlorothalonil submitted by Vischim S.R.L.

RAB1's Conclusions: The residue chemistry studies are classified unacceptable. MRID#s 45710225 and 45710228 can be upgraded to acceptable by submission of additional data.

ATTACHMENTS:

I. 45710225.DER.DOC

II. 45710226.DER.DOC

III. 45710228.DER.DOC

RDI: P.V. Shah (3/2/06)
G.F. Kramer:806T:CM#2:(703)305-5079:7509C:RAB1

Primary Evaluator: Date: 03-MAR-2006
George F. Kramer, Ph.D., Chemist
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Approved by: Date: 03-MAR-2006
P.V. Shah, Ph.D. Branch Senior Scientist
RAB1/HED (7509C)

This DER was originally prepared under contract by Dynamac Corporation (2275 Research Boulevard, Suite 300; Rockville, MD 20850; submitted 02/01/2006). The DER has been reviewed by the HED and revised to reflect current Office of Pesticide Programs (OPP) policies.

STUDY REPORT:

45710225 McEwen, A. (1997) Chlorothalonil Metabolism in Peas: Lab Project Number: VCM 68/962010. Unpublished study prepared by Huntingdon Life Sciences Ltd. 124 p.

EXECUTIVE SUMMARY:

Vischim S.r.l has submitted a study investigating the metabolism of [phenyl-U-^{14}C]chlorothalonil (specific activity 14.6 µCi/mg) in pea. The radiolabeled test substance was formulated as a water-dispersible granule (WDG) formulation and applied as a foliar spray at 1.2 lb ai/A (1.4 kg ai/ha) approximately two weeks before green harvest. Applications were made either as a whole-plant treatment or as a leaf-only treatment; for the leaf-only treatment, the pea pods were shielded with polyethylene bags during application. Pea vines and pods were harvested on the day of application (one hour after treatment) and at intervals of 7, 14, 30, and 41 days after treatment. Vines and pods were washed with acetonitrile (ACN) after collection and then the peas were separated from the pods. The in-life phase and analytical phases of the study were conducted by Huntingdon Life Sciences Ltd. (Huntingdon, England).

In vines and pods, total radioactive residues (TRR) were calculated by summing radioactivity in surface washes, extracts, and extracted solids. TRR in pea seeds were determined by combustion/liquid-scintillation counting (LSC). In pea vines from plants receiving whole-plant treatment, TRR were 53.09 ppm in/on samples collected one hour after treatment, 70.87 ppm in/on samples collected 7 days after treatment, 45.93-69.61 ppm in/on samples collected 14 days after treatment, 61.71 ppm in/on samples collected 30 days after treatment, and 62.11-84.28 ppm in/on samples collected 41 days after treatment. TRR in/on pea pods from the same plants were 8.51 ppm in/on samples collected one hour after treatment, 12.79 ppm in/on samples collected 7 days after treatment, 8.61-12.76 ppm in/on samples collected 14 days after treatment, 24.31 ppm in/on samples collected 30 days after treatment, and 16.76-42.66 ppm in/on samples collected 41 days after treatment, and TRR in pea seeds were <0.01 ppm in samples collected one hour after treatment, 0.04 ppm in samples collected 7 days after treatment, 0.03-0.04 ppm in samples collected 14 days after treatment, 0.13 ppm in samples collected 30 days after treatment, and 0.07-0.08 ppm in samples collected 41 days after treatment.

TRR in/on pea vines and seeds from plants receiving leaf-only treatment were similar to those in/on samples from the whole-plant treatment: 44.70 ppm in/on samples collected on the day of treatment, 91.78 ppm in/on samples collected 14 days after treatment, and 84.19 ppm in/on samples collected 41 days after treatment in vines; and <0.01 ppm in samples collected on the day of treatment, 0.03 ppm in samples collected 14 days after treatment, and 0.07 ppm in samples collected 41 days after treatment in seeds. TRR in the pods from these plants, which were shielded during treatment, were much lower than TRR in pod samples from the whole-plant treatment: 1.71 ppm in/on samples collected on the day of treatment, 0.73 ppm in/on samples collected 14 days after treatment, and 2.54 ppm in/on samples collected 41 days after treatment. Radioactivity in the pods on these plants may have resulted from spray droplets inadvertently entering the plastic bags used to protect them during application. The petitioner concluded that the similar levels of radioactivity in seeds from both treatments indicate that residues in peas result from translocation from the rest of the plant.

Only samples from the whole-plant treatment were subjected to residue characterization/identification procedures. In vines and pods, the majority of the TRR were recovered in ACN surface washes, from 95-98% TRR in the 0-day samples to 80% TRR in the 41-day samples. Solvent extraction with ACN and water released a large portion of the remaining radioactivity, from 2-3% TRR in the 0-day samples to 14-15% TRR in the 41-day samples. In pea seeds, solvent extraction with ACN and water released the majority (~57-69%) of the TRR. In seeds from the 41-day sampling interval, an additional 21% TRR were released using base hydrolysis. Nonextractable residues remaining following extraction accounted for <6% TRR in vines and pods, and ≤0.02 ppm in seeds. Because the petitioner normalized the extraction and residue results, accountabilities were ~100%. Residues were identified and quantitated primarily by high-performance liquid chromatography (HPLC) and thin-layer chromatography (TLC). These methods successfully identified the predominant residues in pea matrices.

Approximately 83-93% of the TRR were identified in pea vines and pods. Chlorothalonil was the major residue identified, at 78-91% TRR (47.5-64.6 ppm) in vines and 75-91% TRR (7.5-21.0 ppm) in pods. In general, chlorothalonil accounted for a greater proportion of the TRR in the samples from the shorter sampling intervals. Two other metabolites were identified: 4-hydroxy chlorothalonil (0.3-5.4% TRR) and the diglutathione conjugate of chlorothalonil (2.0-5.5% TRR). The remainder of the radioactivity in vines and pods consisted of unknowns (totaling 1.7-8.5% TRR; up to three unknowns in vines and one unknown in pods) and polar material (totaling 1.1-8.3% TRR). The petitioner noted that a minor unknown metabolite, accounting for up to 5.7% TRR in pods and 3.3% TRR in vines, was identified as a diglutathione mercapturate conjugate of chlorothalonil using liquid chromatography/mass spectroscopy (LC/MS) analyses. However, data pertaining to levels of this metabolite in individual matrices were not provided.

Only pea seed samples from the 14-day and 41-day sampling intervals were analyzed (these intervals represent green harvest and dry harvest, respectively). Chlorothalonil was found to account for 14.4% TRR (0.003 ppm) in Day-14 seeds and 3.6% TRR (0.003 ppm) in Day-41 seeds. In Day-41 seeds, TLC analyses of the base hydrolysate of nonextractable residues

indicated that chlorothalonil and 4-hydroxy chlorothalonil may have been present (at a total of 3.0% TRR, 0.002 ppm). The remainder of the radioactivity consisted of unknowns (up to 27% TRR, <0.02 ppm), polar material (up to 29% TRR, 0.02 ppm), and base hydrolysate that was unretained on a cation exchange column (12% TRR, 0.01 ppm). Low radioactivity levels prevented further characterization/identification of radioactivity in pea seeds.

Based on the observed metabolites, chlorothalonil is metabolized in pea to 4-hydroxy chlorothalonil and the di- and triglutathione conjugates of chlorothalonil.

The petitioner did not provide any dates of sample extraction or analysis. Based on the experimental completion date, samples may have been stored for up to 349 days prior to analysis. The petitioner provided data demonstrating that the metabolite profile was stable in the surface washes and extracts of Day-41 vines during up to 5 months of storage.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Pending receipt of additional storage stability, the pea metabolism data are classified as scientifically acceptable. The petitioner should provide the dates of sample extraction and analysis to allow HED to determine whether the submitted storage stability data are adequate to support the storage intervals of samples from the pea metabolism study.

COMPLIANCE:

Signed and dated Good Laboratory Practice (GLP), Quality Assurance and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported which would have an impact on the validity of the study.

A. BACKGROUND INFORMATION

Chlorothalonil is a broad spectrum, non-systemic protectant pesticide mainly used as a fungicide to control fungal foliar diseases of vegetable, field, and ornamental crops. It is also used as a wood protectant, antimold and antimildew agent, bactericide, microbiocide, algaecide, insecticide, and acaricide. The exact mechanism of action is not known.

TABLE A.1. Test Compound Nomenclature.

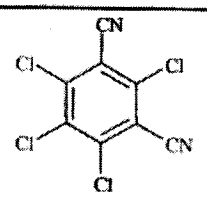
Compound	
Common name	Chlorothalonil
Company experimental name	N/A
IUPAC name	tetrachloroisophthalonitrile
CAS name	2,4,5,6-tetrachloro-1,3-benzenedicarbonitrile

TABLE A.1. Test Compound Nomenclature.	
CAS registry number	1897-45-6

TABLE A.2. Physicochemical Properties of the Technical Grade Test Compound: Chlorothalonil.			
Parameter	Value		Reference
Melting point	250-251 °C		Chlorothalonil Reregistration Eligibility Decision, April 1999
Water solubility	0.6 ppm (25 °C)		Chlorothalonil Reregistration Eligibility Decision, April 1999
Solvent solubility		g/L at 25°C:	The Pesticide Manual, 8th ed.
	acetone	20	
	dimethyl sulfoxide	20	
	cyclohexanone	30	
	dimethylformamide	30	
	kerosene	<10	
	xylene	80	
Octanol/water partition coefficient, Log(K_{OW})	4.37×10^2		TOXNET database

B. EXPERIMENTAL DESIGN

B.1. Test Site and Crop Information

Plants were grown outdoors in a netted tunnel. The seeds used in the study had been treated to reduce seed-borne and seedling diseases (using metalaxyl, thiabendazole, and thiram). Seeds were planted in 7.5-inch pots (treated) or 15-inch pots (control). A total of three pots were treated as a leaf-only foliar application, in which application was only made to the leaves by shielding the pods, and a total of nine pots were treated as a whole-plant foliar application, in which application was made to both pods and leaves.

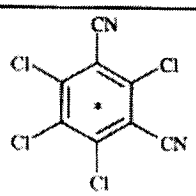
Plants were watered daily using drip irrigation. No unusual weather conditions were reported.

TABLE B.1.1. Test Site Information.					
Type	Method	Soil characteristics [1]			
		Type	%OM	pH	CEC
Leaf-only foliar treatment	Pods were shielded (with polyethylene bags) during application	NA [2]	NA	NA	NA
Whole-plant foliar treatment	The entire plant (pods and leaves) were treated during application	NA	NA	NA	NA

[1] OM = Organic matter, CEC = Cation-exchange capacity. These parameters are optional except in cases where their value affects the use pattern for the chemical.
[2] NA = Not applicable

TABLE B.1.2. Crop Information.				
Crop; crop group	Variety	Growth stage at application	Growth stage at harvest	Harvested Matrix
Pea; Vegetable, legume, group 6, and Vegetable, foliage of legume, group 7	Scout	Estimated to be two weeks before the green harvest.	Matrices were collected 0, 7, 14, 30, and 41 days after application. The petitioner stated that the 14-day harvest interval corresponded to green harvest, and the 41-day harvest interval corresponded to dry harvest.	Pods + seeds; vines

B.2. Test Materials

TABLE B.2.1. Test Material Characteristics.	
Chemical structure	
Radiolabel position	[phenyl -U-^{14}C]chlorothalonil
Lot No.	1021
Purity	97.1-98.9% radiochemical purity
Specific activity	178.6 µCi/mg (test substance) 14.6 µCi/mg (application solution)

The test substance was isotopically diluted with non-labeled chlorothalonil, mixed with formulation blank (WDG), and then mixed with water for application.

B.3. Study Use Pattern

TABLE B.3.1. Use Pattern Information.	
Chemical name	[phenyl -U-^{14}C]chlorothalonil
Application method	Foliar, with pods shielded, or whole-plant
Application rate	1.4 kg ai/ha (1.2 lb ai/A)
Number of applications	1
Timing of applications	approximately 2 weeks prior to green harvest.
Preharvest Interval (PHI)	0, 7, 14, 30, and 41 days; at the 7- and 30-day sampling intervals, samples were only taken from the whole-plant application pots.

B.4. Identification/Characterization of Residues

B.4.1. Sample Handling and Preparation

Plants were separated into pods and vines, and each was surface washed with ACN; the surface washes were radioassayed. The pods were then split open to collect seeds. Samples were stored frozen until preparation for extraction. Prior to extraction, samples were homogenized in the presence of dry ice.

Pod (without seeds) and vine samples were extracted (3x) with ACN/water mixtures (ratios not reported; based on example extraction flowchart included in the submission, some samples were extracted with 9:1 and 1:1 mixtures, as well as with water only), and the extracts were isolated by centrifugation and combined.

Pea seed samples were extracted with ACN:water (1:1. v:v) and water; the combined extracts were reserved for HPLC analysis. The extracts were characterized by partitioning with ethyl acetate at neutral and acidic pH. The remaining aqueous phase was applied to weakly-basic anion-exchange (WBAE) resin, and residues were eluted with water, methanol, and 1 M HCl in methanol. The nonextractable residues in pea seeds (Day 41) were separately treated with cellulase/hemicellulase (in pH 5 acetate buffer), protease (in pH 7 buffer), 0.1 M HCl, 1 M HCl, 0.1 M NaOH, and 1 M NaOH; each treatment was conducted at 37 °C for ~18 hours. The hydrolysate from the base treatment was applied to weakly-acidic cation-exchange (WACE) resin, and residues were eluted with water and methanol.

At the 14- and 41-day sampling intervals, three samples each of pea vines, pods, and seeds were collected, and the samples were subjected to extraction procedures individually. Surface washes and extracts from like samples were then pooled for analysis.

Representative flowcharts of the extraction procedures are presented in the figures below, which were copied without alteration from MRID 45710225.

FIGURE B.4.1. Extraction Procedures for Pea Vines and Pods (without seeds).

(Data shown is taken from a subsample of Day 41 vine)

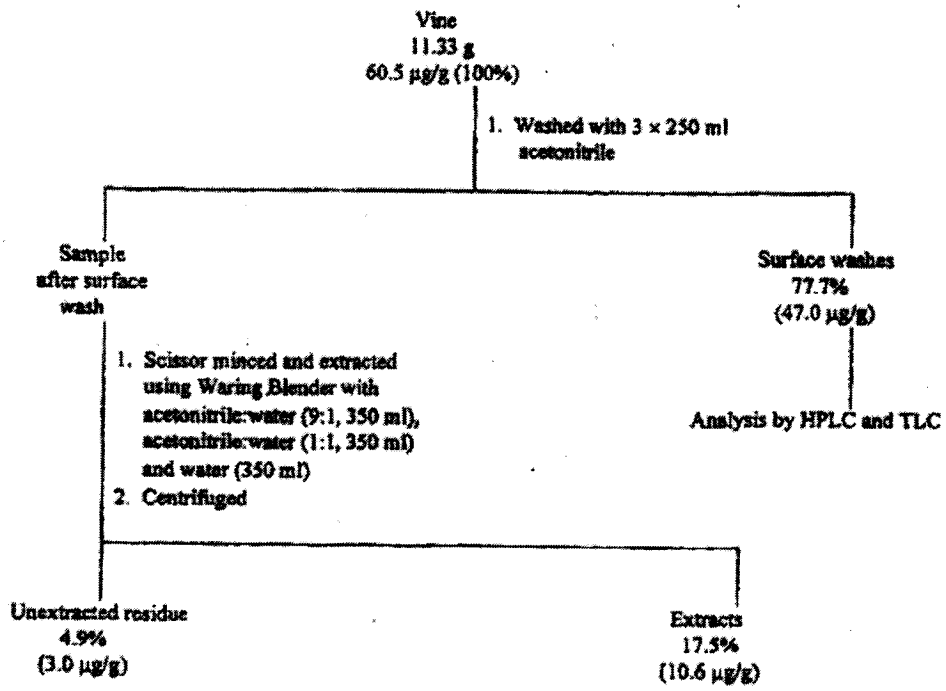
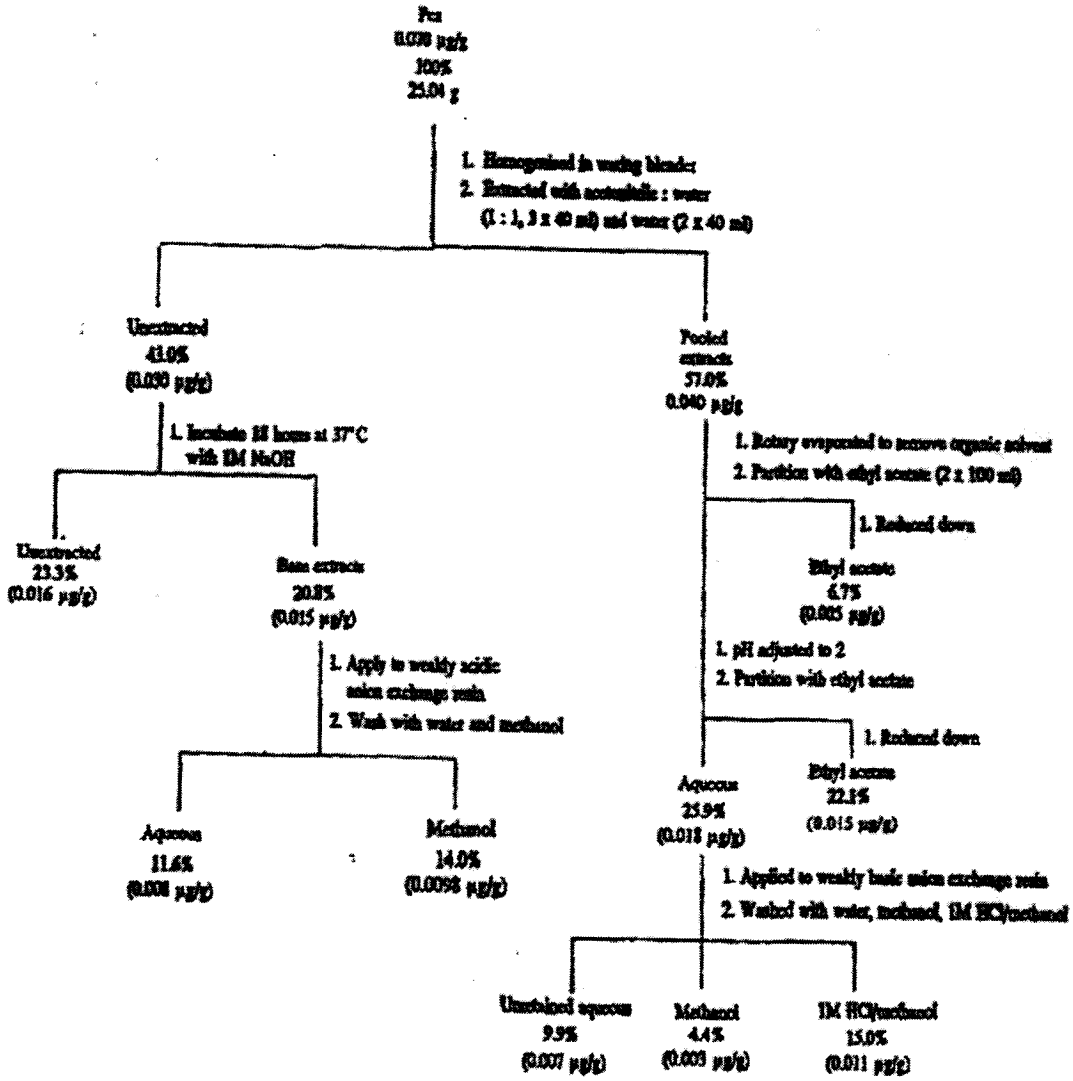


FIGURE B.4.2. Extraction Procedures for Pea Seeds.



B.4.2. Analytical Methodology

TRR were measured in surface washes, extracts, and hydrolysates by LSC. TRR in pea seeds and extracted solids were determined by combustion/LSC. The petitioner determined TRR in pods, vines, and pea seeds by summing radioactivity in surface washes, extracts, and extracted solids; TRR in pea seeds were also determined by combustion/LSC. No limits of detection or quantitation were reported.

Extracts from the whole-plant treatment were analyzed by HPLC and TLC; the petitioner did not analyze extracts from leaf-only treatment plants. HPLC analyses were conducted on a system equipped with a C8 column, a ultraviolet (UV) detector (254 nm), and a radioactivity detector, using a gradient mobile phase of ACN and 2 mM ammonium formate adjusted to pH 3-4 using

formic acid. Metabolites were identified by co-chromatography with non-labeled reference standards. Radioactivity was quantified using fraction collection/LSC. The chemical names and structures of the reference standards used in this study are presented in Appendix I.

TLC analyses were conducted in the normal phase, using Kieselgel 60 F_{254} plates, or in the reversed phase, using C-18 (LKC18F) plates. The following solvent systems were used: (1) hexane:ethyl acetate:acetic acid (90:10:1, v:v:v; normal phase); (2) hexane:dichloromethane (1:1, v:v; normal phase); (3) methanol:water (9:1, v:v; reverse phase); (4) toluene:acetone (4:1, v:v; normal phase); (5) ACN:water (7:3, v:v; normal phase); (6) butan-1-ol:acetic acid:water (6:2:2,v:v:v; normal phase); and (7) chloroform:ethyl acetate:formic acid (5:4:1, v:v:v; normal phase). Radioactivity was detected and quantified using a bioimaging analyzer. Non-labeled standards were visualized by quenching the UV-fluorescent indicator on the plate. Metabolites were identified by co-chromatography with reference standards.

Electrospray ionization LC/MS and LC/MS/MS analyses were conducted using LC conditions similar to those used for the HPLC analyses. The metabolites were first isolated from the extract of Day-14 vines using TLC, and the components of interest were removed from the TLC plates by scraping.

C. RESULTS AND DISCUSSION

Sample storage information is presented in Table C.1. Samples were stored frozen prior to analysis. The petitioner did not provide any dates of sample extraction or analysis. Based on the experimental completion date, samples may have been stored for up to 349 days prior to analysis. The petitioner provided data demonstrating that the metabolite profile was stable in the surface washes and extracts of Day-41 vines during up to 5 months of storage. The petitioner should provide the dates of sample extraction and analysis to allow HED to determine whether the submitted storage stability data are adequate to support the storage intervals of samples from the pea metabolism study.

TRR in pea matrices are reported in Table C.2.1. In vines and pods (without seeds), TRR were calculated by summing radioactivity in surface washes, extracts, and extracted solids. TRR in pea seeds were determined by combustion/LSC. In pea vines from plants receiving whole-plant treatment as a foliar application at 1.2 lb ai/A, TRR were 53.09 ppm in/on samples collected one hour after treatment, 70.87 ppm in/on samples collected 7 days after treatment, 45.93-69.61 ppm in/on samples collected 14 days after treatment, 61.71 ppm in/on samples collected 30 days after treatment, and 62.11-84.28 ppm in/on samples collected 41 days after treatment. TRR in/on pea pods from the same plants were 8.51 ppm in/on samples collected one hour after treatment, 12.79 ppm in/on samples collected 7 days after treatment, 8.61-12.76 ppm in/on samples collected 14 days after treatment, 24.31 ppm in/on samples collected 30 days after treatment, and 16.76-42.66 ppm in/on samples collected 41 days after treatment, and TRR in pea seeds were <0.01 ppm in samples collected one hour after treatment, 0.04 ppm in samples collected 7 days after treatment, 0.03-0.04 ppm in samples collected 14 days after treatment, 0.13 ppm in samples collected 30 days after treatment, and 0.07-0.08 ppm in samples collected 41 days after treatment. The

petitioner observed that the slight increase in radioactivity levels with increased sampling intervals was probably due to desiccation of the crop.

TRR in pea vines and seeds from plants receiving leaf-only treatment, as a foliar application at 1.2 lb ai/A, were similar to those from the whole-plant treatment: 44.70 ppm in/on samples collected on the day of treatment, 91.78 ppm in/on samples collected 14 days after treatment, and 84.19 ppm in/on samples collected 41 days after treatment in vines; and <0.01 ppm in samples collected on the day of treatment, 0.03 ppm in samples collected 14 days after treatment, and 0.07 ppm in samples collected 41 days after treatment in seeds. TRR in the pods from these plants, which were shielded during treatment, were much lower than TRR in pod samples from the whole-plant treatment: 1.71 ppm in/on samples collected on the day of treatment, 0.73 ppm in/on samples collected 14 days after treatment, and 2.54 ppm in/on samples collected 41 days after treatment. Radioactivity in the pods on these plants may have resulted from spray droplets inadvertently entering the plastic bags used to protect them during application. The petitioner concluded that the similar levels of radioactivity in seeds from both treatments indicate that residues in peas result from translocation from the rest of the plant.

The distribution of the radioactivity in pea matrices is presented in Tables C.2.2.1 (leaf-only treatment), C.2.2.2 (vines and pods, whole-plant treatment) and C.2.2.3 (seeds, whole-plant treatment). Only samples from the whole-plant treatment were subjected to residue characterization/identification procedures. In vines and pods from the whole-plant treatment, the majority of the TRR was recovered in ACN surface washes, from 95-98% TRR in the 0-day samples to 80% TRR in the 41-day samples. Solvent extraction with ACN and water released a large portion of the remaining radioactivity, from 2-3% TRR in the 0-day samples to 14-15% TRR in the 41-day samples. In pea seeds, solvent extraction with ACN and water released the majority (~57-69%) of the TRR. In seeds from the 41-day sampling interval, an additional 21% TRR was released using base hydrolysis. Nonextractable residues remaining following extraction accounted for <6% TRR in vines and pods, and ≤0.02 ppm in seeds. Because the petitioner normalized the extraction and residue results, accountabilities were ~100%. Residues were identified and quantitated primarily by HPLC and TLC co-chromatography. These methods successfully identified the predominant residues in pea matrices.

The characterization and identification of residues in pea matrices following whole-plant treatment are summarized in Tables C.2.3.1 (vines), C.2.3.2 (pods), and C.2.3.3 (seeds). Approximately 83-93% of the TRR was identified in pea vines and pods. Chlorothalonil was the major residue identified, at 78-91% TRR (47.5-64.6 ppm) in vines and 75-91% TRR (7.5-21.0 ppm) in pods. In general, chlorothalonil accounted for a greater proportion of the TRR in the samples from the shorter sampling intervals. Two other metabolites were identified: 4-hydroxy chlorothalonil (0.3-5.4% TRR) and the diglutathione conjugate of chlorothalonil (2.0-5.5% TRR). The remainder of the radioactivity in vines and pods consisted of unknowns (totaling 1.7-8.5% TRR; up to three unknowns in vines and one unknown in pods) and polar material (totaling 1.1-8.3% TRR).

Only pea seed samples from the 14-day and 41-day sampling intervals were analyzed (these intervals represent green harvest and dry harvest, respectively). Chlorothalonil was found to

account for 14.4% TRR (0.003 ppm) in Day-14 seeds and 3.6% TRR (0.003 ppm) in Day-41 seeds. In Day-41 seeds, TLC analyses of the base hydrolysate of nonextractable residues indicated that chlorothalonil and 4-hydroxy chlorothalonil may have been present (at a total of 3.0% TRR, 0.002 ppm). The remainder of the radioactivity consisted of unknowns (up to 27% TRR, <0.02 ppm), polar material (up to 29% TRR, 0.02 ppm), and base hydrolysate that was unretained on a cation exchange column (12% TRR, 0.01 ppm). Low radioactivity levels prevented further characterization/identification of radioactivity in pea seeds.

The diglutathione conjugate of chlorothalonil was identified by isolating the component from the extracts of Day-14 vines. The isolated metabolite was then analyzed by LC/MS which confirmed the identification. The petitioner also noted that a minor unknown metabolite, accounting for up to 5.7% TRR in pods and 3.3% TRR in vines, was confirmed to be diglutathione mercapturate conjugate of chlorothalonil using LC/MS analyses. However, data pertaining to levels of this metabolite in individual matrices were not provided.

C.1. Storage Stability

Samples were stored frozen prior to extraction and analysis. The petitioner did not report any dates of extraction or analysis. Based on the dates of sample collection and the reported date of experimental completion, samples may have been stored for up to 349 days prior to completion of analysis.

To support sample storage intervals, the petitioner reanalyzed the extracts of Day-41 pea vines. Pea vine samples from the 41-day harvest interval were extracted and analyzed within 3 months of sample collection. The surface washes and extracts were reanalyzed approximately 5 months later. The petitioner provided the HPLC radiochromatograms from the initial and final analyses of the surface washes and extract; the chromatograms indicated that the profile was stable in surface washes and extracts for at least 5 months of frozen storage.

The petitioner should provide the dates of sample extraction and analysis to allow HED to determine whether the submitted storage stability data are adequate to support the storage intervals of samples from the pea metabolism study.

TABLE C.1. Summary of Storage Conditions.

Matrix (RAC or Extract)	Storage Temperature (°C)	Actual Storage Duration	Interval of Demonstrated Storage Stability
Pea vines, pods, and seeds	Frozen (temperature unspecified)	Not reported; based on experimental completion date, samples may have been stored up to 349 days prior to completion of analyses.	The metabolite profile in the surface washes and extracts of day-41 vines was shown to be stable for approximately 5 months.

C.2. Identification, Characterization, and Distribution of Residues

TABLE C.2.1. TRR in Pea Matrices.

Type of Application	PHI (days)	ppm, ^{14}C-chlorothalonil equivalents [1]		
		Vines	Pods	Seeds
Whole-plant foliar treatment	0	53.09	8.51	<0.01
	7	70.87	12.79	0.04
	14 [2]	45.93 53.44 69.61	8.61 9.90 12.76	0.03 0.04 0.04
	30	61.71	24.31	0.13
	41 [2]	65.43 62.11 84.28	16.76 24.72 42.66	0.07 0.08 0.07
Leaf-only foliar treatment	0	44.70	1.71	<0.01
	14	91.78	0.73	0.03
	41	84.19	2.54	0.07

[1] TRR in vines and pods were determined by summing radioactivity in surface washes, extracts, and extracted solids; TRR in seeds were determined by combustion/LSC.
[2] Three samples were collected at this interval.

TABLE C.2.2.1. Distribution of the Radioactivity in Pea Matrices Following Foliar Application of Radiolabeled Chlorothalonil to Leaves Only (Pods Shielded) at 1.2 lb ai/A.

Metabolite Fraction	Vines		Pods		Seeds	
	%TRR	ppm	%TRR	ppm	%TRR	ppm
Day-0 Harvest	**TRR = 44.70 ppm**		**TRR = 1.71 ppm**		**TRR = <0.01 ppm**	
Surface wash	94.0	42.02	97.4	1.67	Not applicable	
ACN/water extracts	3.5	1.56	1.9	0.03	Not analyzed	
Unextractable	2.5	1.12	0.7	0.01	Not analyzed	
Day-14 Harvest	**TRR = 91.78 ppm**		**TRR = 0.73 ppm**		**TRR = 0.03 ppm**	
Surface wash	89.1	81.78	72.9	0.53	Not applicable	
ACN/water extracts	9.8	8.99	21.5	0.16	59.6	0.02
Unextractable	1.1	1.01	5.6	0.04	40.5	0.01
Day-41 Harvest	**TRR = 84.19 ppm**		**TRR = 2.54 ppm**		**TRR = 0.07 ppm**	
Surface wash	83.7	70.46	63.5	1.61	Not applicable	
ACN/water extracts	9.6	8.08	26.7	0.68	66.6	0.05
Unextractable	6.7	5.64	9.9	0.25	33.4	0.02

TABLE C.2.2.2. Distribution of the Radioactivity in Pea Vines and Pods Following Foliar Application of Radiolabeled Chlorothalonil to the Whole Plant at 1.2 lb ai/A.

Metabolite Fraction	Vines		Pods	
	%TRR	ppm	%TRR	ppm
Day-0 Harvest	**TRR = 53.09 ppm**		**TRR = 8.51 ppm**	
Surface wash	94.6	50.22	97.9	8.33
Chlorothalonil	90.6	48.10	88.4	7.52
4-Hydroxy chlorothalonil	1.5	0.80	3.8	0.32
Unknowns	2.4	1.27	5.3	0.45

TABLE C.2.2.2. Distribution of the Radioactivity in Pea Vines and Pods Following Foliar Application of Radiolabeled Chlorothalonil to the Whole Plant at 1.2 lb ai/A.

Metabolite Fraction	Vines		Pods	
	%TRR	ppm	%TRR	ppm
Polar material	0.1	0.05	0.3	0.03
ACN/water extracts	2.7	1.43	1.7	0.14
Chlorothalonil	0.3	0.16	0.1	0.01
4-Hydroxy chlorothalonil	0.3	0.16	--	--
Unknowns	1.2	0.64	0.5	0.04
Polar material	1.0	0.53	1.2	0.10
Unextractable	2.6	1.38	0.4	0.03
Day-7 Harvest	**TRR = 70.87 ppm**		**TRR = 12.79 ppm**	
Surface wash	93.2	66.05	94.9	12.14
Chlorothalonil	90.5	64.14	90.3	11.55
4-Hydroxy chlorothalonil	1.3	0.92	1.5	0.19
Unknowns	1.3	0.92	2.8	0.36
Polar material	0.2	0.14	0.2	0.03
ACN/water extracts	5.0	3.54	4.5	0.58
Chlorothalonil	0.6	0.43	0.2	0.03
4-Hydroxy chlorothalonil	--	--	--	--
Unknowns	2.8	0.99	1.9	0.24
Polar material	1.6	1.14	2.3	0.29
Unextractable	1.8	1.24	0.6	0.08
Day-14 Harvest	**TRR = 56.33 ppm**		**TRR = 10.42 ppm**	
Surface wash	87.0	49.0	84.2	8.77
Chlorothalonil	81.7	46.02	77.0	8.02
4-Hydroxy chlorothalonil	4.1	2.31	1.7	0.18
Diglutathione conjugate of chlorothalonil	<0.1	0.06	--	--
Unknowns	0.4	0.22	5.0	0.52
Polar material	0.3	0.17	0.5	0.05
ACN/water extracts	9.7	5.46	13.2	1.38
Chlorothalonil	2.6	1.46	1.6	0.17
4-Hydroxy chlorothalonil	0.4	0.23	1.0	0.10
Diglutathione conjugate of chlorothalonil	2.0	1.13	5.5	0.57
Unknowns	1.6	0.90	1.2	0.13
Polar material	3.3	1.86	4.0	0.42
Unextractable	3.2	1.80	2.7	0.28
Day-30 Harvest	**TRR = 61.71 ppm**		**TRR = 24.31 ppm**	
Surface wash	84.0	51.84	78.8	19.16
Chlorothalonil	79.4	49.0	78.5	19.1
4-Hydroxy chlorothalonil	2.4	1.48	0.3	0.07
Diglutathione conjugate of chlorothalonil	--	--	--	--
Unknowns	1.9	1.18	--	--
Polar material	0.3	0.19	--	--
ACN/water extracts	13.4	8.27	15.4	3.74
Chlorothalonil	2.1	1.3	--	--

TABLE C.2.2.2. Distribution of the Radioactivity in Pea Vines and Pods Following Foliar Application of Radiolabeled Chlorothalonil to the Whole Plant at 1.2 lb ai/A.

Metabolite Fraction	Vines		Pods	
	%TRR	ppm	%TRR	ppm
4-Hydroxy chlorothalonil	0.9	0.6	--	--
Diglutathione conjugate of chlorothalonil	2.5	1.54	3.7	0.90
Unknowns	--	--	8.5	2.07
Polar material	8.0	4.9	3.2	0.78
Unextractable	2.6	1.60	5.8	1.41
Day-41 Harvest	**TRR = 70.61 ppm**		**TRR = 28.05 ppm**	
Surface wash	80.1	56.6	79.6	22.32
Chlorothalonil	76.3	53.88	74.6	20.93
4-Hydroxy chlorothalonil	3.5	2.47	5.0	1.40
Diglutathione conjugate of chlorothalonil	--	--	--	--
Unknowns	0.1	0.07	--	--
Polar material	0.2	0.14	--	--
ACN/water extracts	14.1	9.96	14.5	4.07
Chlorothalonil	1.5	1.06	0.2	0.06
4-Hydroxy chlorothalonil	0.9	0.64	0.4	0.11
Diglutathione conjugate of chlorothalonil	2.9	2.05	3.1	0.87
Unknowns	2.8	1.97	6.4	1.79
Polar material	6.0	4.2	4.4	1.23
Unextractable	5.8	4.10	5.9	1.65

TABLE C.2.2.3. Distribution of the Radioactivity in Pea Seeds Following Foliar Application of Radiolabeled Chlorothalonil to the Whole Plant at 1.2 lb ai/A.

Metabolite Fraction	Seeds	
	%TRR	ppm
Day-7 Harvest	**TRR = 0.04 ppm**	
ACN/water extracts	64.2	0.03
Unextractable	35.9	0.01
Day-14 Harvest	**TRR = 0.03 ppm**	
ACN/water extracts	61.1	0.02
Organosoluble at pH 7	42.6	0.010
Chlorothalonil	14.4	0.003
Unknowns	14.4	0.003
Polar material	0.6	<0.001
Aqueous soluble	31.6	0.007
Unextractable	38.9	0.01
Day-30 Harvest	**TRR = 0.07 ppm**	
ACN/water extracts	68.8	0.05
Unextractable	31.2	0.02
Day-41 Harvest	**TRR = 0.07 ppm**	
ACN/water extracts	57.0	0.04
Chlorothalonil	3.6	<0.01

TABLE C.2.2.3. Distribution of the Radioactivity in Pea Seeds Following Foliar Application of Radiolabeled Chlorothalonil to the Whole Plant at 1.2 lb ai/A.

Metabolite Fraction	Seeds	
	%TRR	ppm
Polar material	21.2	0.015
Unknown	23.8	0.017
Organosoluble at pH 7	6.7	0.005
Chlorothalonil/4-hydroxy chlorothalonil [1]	3.3	0.002
Unknowns	2.8	0.001
Polar material	--	--
Organosoluble at pH 2	22.1	0.015
Chlorothalonil/4-hydroxy chlorothalonil [1]	5.5	0.004
Unknowns	13.8	0.010
Polar material	--	--
Aqueous soluble	25.9	0.018
WBAE unretained	9.9	0.007
WBAE methanol eluate	4.4	0.003
WBAE 1M HCl/methanol eluate	15.0	0.011
Unextractable	43.0	0.03
1 M NaOH hydrolysate	20.8	0.015
WACE unretained	11.6	0.008
WACE methanol eluate	14.0	0.010
Chlorothalonil/4-hydroxy chlorothalonil [1]	3.0	0.002
Unknowns	2.7	0.002
Polar material	7.5	0.005
Solids	23.3	0.016

[1] Chlorothalonil and 4-hydroxy chlorothalonil co-chromatographed in TLC analyses.

TABLE C.2.3.1. Summary of Characterization and Identification of Radioactive Residues in Pea Vines Following Foliar Application of Radiolabeled Chlorothalonil to the Whole Plant at 1.2 lb ai/A.

Compound	Day 0		Day 7		Day 14		Day 30		Day 41	
	TRR= 3.09 ppm		TRR=70.87 ppm		TRR=56.33 ppm		TRR=61.71 ppm		TRR=70.61 ppm	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
Chlorothalonil	90.9	48.3	91.1	64.6	84.3	47.5	81.5	50.3	77.8	54.9
4-Hydroxy chlorothalonil	1.8	1.0	1.3	0.9	4.5	2.5	3.3	2.0	4.4	3.1
Diglutathione conjugate	--	--	--	--	2.0	1.1	2.5	1.5	2.9	2.0
Unknowns	3.6	1.9	4.1	2.9	2.5	1.4	1.7	1.1	2.9	2.0
Polar material	1.1	0.6	1.8	1.3	3.5	2.0	8.3	5.1	6.2	4.4
Total identified	92.7	49.3	92.4	65.5	90.8	51.1	87.3	53.8	85.1	60.0
Total characterized	4.7	2.5	5.9	4.2	6.0	3.4	10.0	6.2	9.1	6.4
Total extractable	97.3	51.7	98.2	69.6	96.7	54.5	97.4	60.1	94.2	66.6
Unextractable [1]	2.6	1.4	1.8	1.3	3.2	1.8	2.6	1.6	5.8	4.1
Accountability	100.0		100.0		99.9		100.0		100.1	

[1] Residues remaining after exhaustive extractions.

TABLE C.2.3.2. Summary of Characterization and Identification of Radioactive Residues in Pea Pods Following Foliar Application of Radiolabeled Chlorothalonil to the Whole Plant at 1.2 lb ai/A.

Compound	Day 0		Day 7		Day 14		Day 30		Day 41	
	TRR = 8.51 ppm		TRR = 12.79 ppm		TRR = 10.42 ppm		TRR = 24.31 ppm		TRR = 28.05 ppm	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
Chlorothalonil	88.5	7.53	90.5	11.57	78.6	8.19	78.6	19.1	74.8	21.0
4-Hydroxy chlorothalonil	3.8	0.32	1.5	0.19	2.7	0.28	0.3	0.07	5.4	1.5
Diglutathione conjugate	--	--	--	--	5.5	0.57	3.7	0.90	3.1	0.87
Unknowns	5.8	0.49	4.7	0.60	6.0	0.63	8.5	2.1	6.4	1.8
Polar material	1.5	0.13	2.5	0.32	4.5	0.47	3.2	0.78	4.4	1.2
Total identified	92.3	7.9	92.0	11.8	86.8	9.0	82.6	20.1	83.3	23.4
Total characterized	7.3	0.6	7.2	0.9	10.5	1.1	11.7	2.9	10.8	3.0
Total extractable	99.6	8.5	99.4	12.7	97.4	10.2	94.2	22.9	94.1	26.4
Unextractable [1]	0.4	0.03	0.6	0.1	2.7	0.28	5.7	1.4	5.9	1.7
Accountability	100.0		100.0		100.1		100.0		100.1	

[1] Residues remaining after exhaustive extractions.

TABLE C.2.3.3. Summary of Characterization and Identification of Radioactive Residues in Pea Seeds Following Foliar Application of Radiolabeled Chlorothalonil to the Whole Plant at 1.2 lb ai/A.

Compound	Day 14		Day 41	
	TRR = 0.03 ppm		TRR = 0.07 ppm	
	% TRR	ppm	% TRR	ppm
Chlorothalonil	14.4	0.003	3.6	0.003
Chlorothalonil/4-hydroxy chlorothalonil	--	--	3.0	0.002
Unknowns	14.4	0.003	26.5	0.019
Polar material	0.6	<0.001	28.7	0.020
Aqueous soluble	31.6	0.007	--	--
Base hydrolysate, unretained on WACE	--	--	11.6	0.008
Total identified	14.4	0.003	6.6	0.005
Total characterized	46.6	0.010	66.8	0.047
Total extractable	61.1	0.02	77.8	0.055
Unextractable [1]	38.9	0.01	23.3	0.016
Accountability	100.0		101.4	

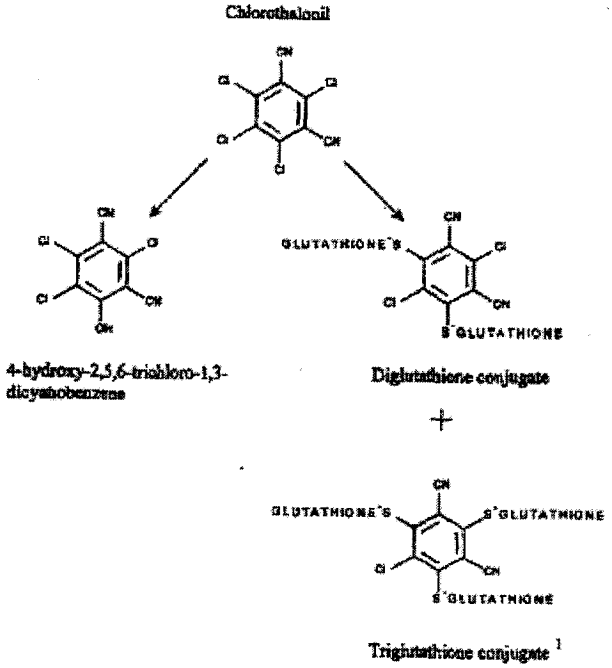
[1] Residues remaining after exhaustive extractions.

C.3. Proposed Metabolic Profile

Based on the observed metabolites, chlorothalonil is metabolized in pea to 4-hydroxy chlorothalonil and the di- and triglutathione conjugates of chlorothalonil.

The figure below was copied without alteration from MRID 45710225.

FIGURE C.3.1. Proposed Metabolic Profile of Chlorothalonil in Pea.



1 may be a minor metabolite

TABLE C.3.1. Identification of Compounds from Metabolism Study.

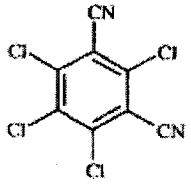
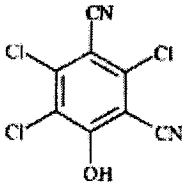
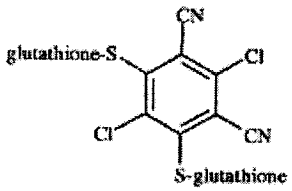
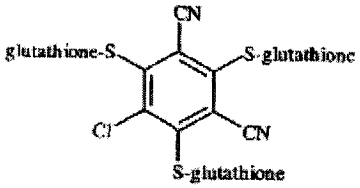
Common name/code Figure C.3.1 ID No.	Chemical name	Chemical structure
chlorothalonil	2,4,5,6-tetrachloro-1,3-benzenedicarbonitrile	
4-hydroxy chlorothalonil	4-hydroxy-2,5,6-trichloro-1,3-dicyanobenzene	

TABLE C.3.1. Identification of Compounds from Metabolism Study.		
Common name/code Figure C.3.1 ID No.	Chemical name	Chemical structure
diglutathione conjugate of chlorothalonil		
triglutathione conjugate of chlorothalonil		

D. CONCLUSION

In pea vines harvested from plants receiving whole-plant treatment with [^{14}C]chlorothalonil as a foliar application at 1.2 lb ai/A, TRR were 53.09 ppm in samples collected one hour after treatment, 70.87 ppm 7 days after treatment, 45.93-69.61 ppm 14 days after treatment, 61.71 ppm 30 days after treatment, and 62.11-84.28 ppm 41 days after treatment. TRR in/on pea pods from the same plants were 8.51 ppm in samples collected one hour after treatment, 12.79 ppm 7 days after treatment, 8.61-12.76 ppm 14 days after treatment, 24.31 ppm 30 days after treatment, and 16.76-42.66 ppm 41 days after treatment, and TRR in pea seeds were <0.01 ppm in samples collected one hour after treatment, 0.04 ppm 7 days after treatment, 0.03-0.04 ppm 14 days after treatment, 0.13 ppm 30 days after treatment, and 0.07-0.08 ppm 41 days after treatment.

TRR in pea vines and seeds from plants receiving leaf-only treatment with [^{14}C]chlorothalonil, as a foliar application at 1.2 lb ai/A, were similar to those from the whole-plant treatment: 44.70 ppm on the day of treatment, 91.78 ppm 14 days after treatment, and 84.19 ppm 41 days after treatment in vines; and <0.01 ppm on the day of treatment, 0.03 ppm 14 days after treatment, and 0.07 ppm 41 days after treatment in seeds. TRR in pods from these plants, which were shielded during treatment, were much lower: 1.71 ppm on the day of treatment, 0.73 ppm 14 days after treatment, and 2.54 ppm 41 days after treatment. The petitioner concluded that the similar levels of radioactivity in seeds from both treatments indicate that residues in peas result from translocation from the rest of the plant.

In vines and pods from the whole-plant treatment, the majority of the TRR were recovered in ACN surface washes, from 95-98% TRR in the 0-day samples to 80% TRR in the 41-day samples. Solvent extraction with ACN and water released a large portion of the remaining radioactivity, from 2-3% TRR in the 0-day samples to 14-15% TRR in the 41-day samples. In pea seeds from the whole-plant treatment, solvent extraction with ACN and water released the majority (~57-69%) of the TRR. In seeds from the 41-day sampling interval, an additional 21%

TRR were released using base hydrolysis. Nonextractable residues remaining following extraction accounted for <6% TRR in vines and pods, and ≤0.02 ppm in seeds.

Approximately 83-93% of the TRR were identified in pea vines and pods. Chlorothalonil was the major residue identified, at 78-91% TRR in vines and 75-91% TRR in pods. In general, chlorothalonil accounted for a greater proportion of the TRR in the samples from the shorter sampling intervals. Two other metabolites were identified: 4-hydroxy chlorothalonil (0.3-5.4% TRR) and the diglutathione conjugate of chlorothalonil (2.0-5.5% TRR). The remainder of the radioactivity in vines and pods consisted of unknowns (totaling <9% TRR) and polar material (totaling up to ~8% TRR). The petitioner noted that a minor unknown metabolite, accounting for up to 5.7% TRR in pods and 3.3% TRR in vines, was identified as a diglutathione mercapturate conjugate of chlorothalonil using LC/MS analyses. However, data pertaining to levels of this metabolite in individual matrices were not provided.

Only pea seed samples from the 14-day and 41-day sampling intervals were analyzed (these intervals represent green harvest and dry harvest, respectively). Chlorothalonil was found to account for 14.4% TRR in Day-14 seeds and 3.6% TRR in Day-41 seeds. In Day-41 seeds, TLC analyses of the base hydrolysate of nonextractable residues indicated that chlorothalonil and 4-hydroxy chlorothalonil may have been present (at a total of 3.0% TRR). The remainder of the radioactivity consisted of unknowns (up to 27% TRR, <0.02 ppm), polar material (up to 29% TRR, 0.02 ppm), and base hydrolysate that was unretained on a cation exchange column (12% TRR, 0.01 ppm). Based on the observed metabolites, chlorothalonil is metabolized in pea to 4-hydroxy chlorothalonil and the di- and triglutathione conjugates of chlorothalonil.

E. REFERENCES

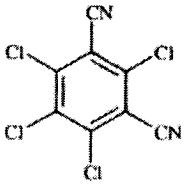
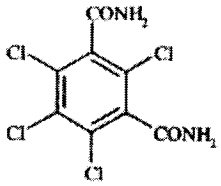
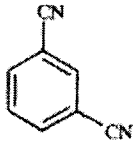
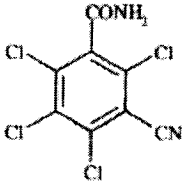
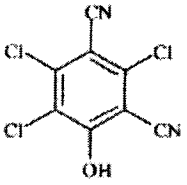
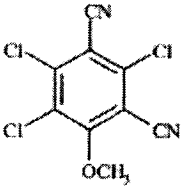
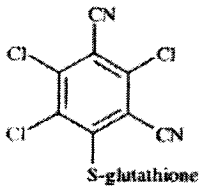
None.

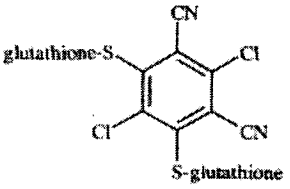
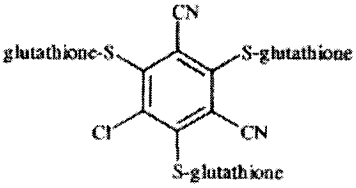
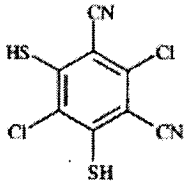
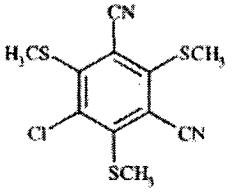
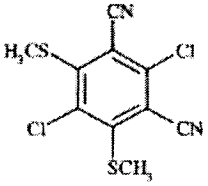
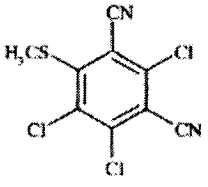
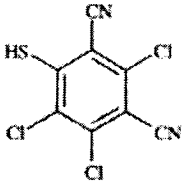
F. DOCUMENT TRACKING

RDI: RAB1 Chemists (3/2/06)
DP#: 323612
PC Code: 081901

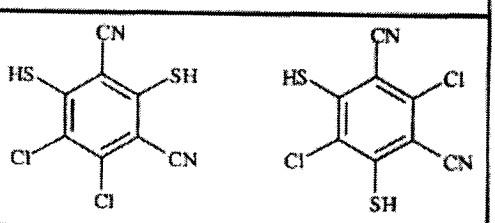
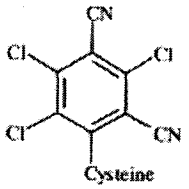
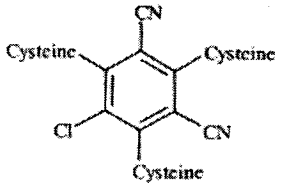
Template Version June 2005

APPENDIX I. Chemical Names and Structures of Reference Standards Used in Chlorothalonil Pea Metabolism Study.

Common name; Company code	Chemical name	Chemical structure
Chlorothalonil	2,4,5,6-tetrachloro-1,3-benzenedicarbonitrile	
Reference 1	1,3-diamino-2,4,5,6-tetrachlorobenzene	
Reference 2	1,3-dicyanobenzene	
Reference 3	1-amino-2,4,5,6-tetrachloro-3-cyanobenzene	
Reference 4	4-hydroxy-2,5,6-trichloro-1,3-dicyanobenzene	
Reference 5	4-methyoxy-2,5,6-trichloroisophthalonitrile	
Reference 6	monoglutathione conjugate	 S-glutathione

APPENDIX I. Chemical Names and Structures of Reference Standards Used in Chlorothalonil Pea Metabolism Study.		
Common name; Company code	Chemical name	Chemical structure
Reference 7	diglutathione conjugate	
Reference 8	triglutathione conjugate	
Reference 9	2,5-dichloro-4,6-dithioisophthalonitrile	
Reference 10	5-chloro-2,4,6-tri(methylthio)isophthalonitrile	
Reference 11	2,5-dichloro-4,6-di(methylthio)isophthalonitrile	
Reference 12	2,4,5-trichloro-6-(methylthio)isophthalonitrile	
Reference 13	2,4,5-trichloro-6-thioisophthalonitrile	

APPENDIX I. Chemical Names and Structures of Reference Standards Used in Chlorothalonil Pea Metabolism Study.

Common name; Company code	Chemical name	Chemical structure
Reference 15 (mixture)	4,5-dichloro-2,6-dimercaptoisophthalonitrile; 2,5-dichloro-4,6-dimercaptoisophthalonitrile	
Reference 16	monocysteine conjugate (supplied as methyl ester)	
Reference 17	tricysteine conjugate (supplied as methyl ester)	
Reference 18	dicysteine conjugate (supplied as methyl ester)