Read 6-9-95

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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

JUN 9 1995

MEMORANDUM

SUBJECT: **Iprodione.** Confined Rotational Crop Study. Reregistration Case No. 2335
Chemical No. 109801 MRID #43596201 DP Barcode D214277 CBRS #15422

FROM: Steven A. Knizner, Chemist
Special Review Section I
Chemistry Branch II - Reregistration Support
Health Effects Division (7509C)

THRU: Andrew Rathman, Section Head
Special Review Section I
Chemistry Branch II - Reregistration Support
Health Effects Division (7509C)

TO: Bill Wooge, PM Team 52
Special Review and Reregistration Division (7508W)

Rhone-Poulenc has submitted a confined rotational crop study for iprodione (MRID #43596201). This study is reviewed below.

Permanent tolerances have been established for combined residues of iprodione [3-(3,5-dichlorophenyl)-N-(1-methylethyl)-2,4-dioxo-1-imidazolidinecarboxamide], its isomer 3-(1-methylethyl)-N-(3,5-dichlorophenyl)-2,4-dioxo-1-imidazolidinecarboxamide, and its metabolite 3-(3,5-dichlorophenyl)-2,4-dioxo-1-imidazolidine-carboxamide on various raw agricultural commodities at levels ranging from 0.1 to 150.0 ppm (40 CFR 180.399). Food additive tolerances have been established for iprodione, its isomer and its metabolite on dried ginseng (4.0 ppm) and raisins (300 ppm) (40 CFR 185.3750). Feed additive tolerances have been established for iprodione, its isomer, and its metabolite on dried grape pomace (225 ppm), raisin waste (300 ppm), rice bran (30.0 ppm), rice hulls (50.0 ppm), and soapstock (10.0 ppm) (40 CFR 186.3750).

Permanent tolerances for animal commodities have also been established for iprodione, its isomer, its metabolite, and an additional metabolite N-(3,5-dichloro-4-hydroxyphenyl)-ureido-carboxamide at levels ranging from 0.5 to 5.0 ppm (40 CFR 180.399).

The Phase 4 Review of iprodione (C. Olinger, 3/15/91) did not identify a data gap for plant metabolism studies. The review noted that similar metabolism was demonstrated on three different crops (peaches, peanuts, and rice), with iprodione (RP-26019), its isomer (RP-30266) and one metabolite (RP-32490) being identified as the major radioactive residues. CBRS also notes that RP-25040 was also identified in rice and peanuts, but at levels of less than 10% the TRR.

The HED Cancer Peer Review Committee met in 1994 and determined that iprodione should be classified as a Group B2 Carcinogen (probable human carcinogen) (E.Rinde and L.Taylor memo dated 7/27/94). A Q_1^* from a rat study was calculated to be 0.0439 $(mg/kg/day)^{-1}$ (B.Fisher, 7/19/94).

Recommendations

The nature of the residue study in rotational crops is not adequate, but is upgradeable by supplying information requested in Conclusion 11. CBRS concludes that the major residues tentatively identified include iprodione (RP-26019), its isomer (RP-30228), RP-25040, and RP-44247 (see Figure 1, pages 8-9 for structures and chemical names). RP-25040 and RP-44247 are not included in the iprodione tolerance expression for primary crops.

After resolution of the issues raised in Conclusion 11, the results of this study will likely be presented to the HED Metabolism Committee.

Conclusions

1. The test material was adequately described and characterized. ^{14}C-iprodione was uniformly labeled in the phenyl ring. Radiochemical purity was 97.9%, and the specific activity was 7.98 uCi/mg, corresponding to 17,716 dpm/ug. The application rate to soil was 5.6 lb ai/A (1.4X maximal seasonal rate for crops that can be rotated).

2. Except for the 364-DAT wheat, the test system was adequate. Branch policy (Guidance on How to Conduct Studies on Rotational Crops, E.Zager and D.Edwards, 2/23/93) allows a primary crop to be grown on treated soil prior to planting a rotated crop. In this study however, for wheat, prior to the planting of the 364 DAT crops, two crops had been grown on the treated soil (specifically the 31-DAT and 125-DAT radish and mustard crops). Because the third wheat crop was grown in the same soil used to grow two crops of radish and mustard (the 31- and 125-DAT crops), radioactive residues the 364-DAT wheat may be lower than those obtained if the study had been done correctly.

3. The extraction/fractionation procedures used were adequately described. The analytical techniques employed were also adequately described. Sufficient raw data were presented. The reference standards employed were adequately characterized. To determine the stability and partitioning behavior of iprodione throughout the extraction procedure, control wheat

forage and control radish tops were fortified with ^{14}C-iprodione and extracted just as treated tissues. The radiolabeled iprodione was found almost exclusively in the ACN I extract.

4. TRR Determinations - TRR levels for the various crops are presented in Table 3. The TRR levels decreased from the first to second to third harvest interval for mustard and radish. For wheat, TRR levels decreased from the first to second harvest interval. Because the third wheat crop was grown in the same soil used to grow two crops of radish and mustard (the 31- and 125-DAT crops), the results for the 364-DAT wheat may be lower than expected had the study been done correctly. The 125-DAT wheat grain and 364-DAT mustard green samples had TRR levels <0.010 ppm, and therefore did not require characterization of radioactive residues. The 364-DAT radish root and 125-DAT wheat forage and straw samples all had TRR levels greater than 0.010 ppm.

5. Partitioning of Residues - With the exception of radish roots, good extraction of radioactive residues was achieved, with the majority of the radioactivity extracted into ACN. In general, for all samples the extractability of radioactive residues decreased with increasing plant back interval. ^{14}C-residues remaining in radish root PES were higher than those for other samples and increased with increasing plant back interval, ranging from 52.5% TRR (1.36 ppm) at 31-DAT to 81.4% TRR (0.12 ppm) at 364-DAT. Table 4 presents a summary of the partitioning of radioactive residues in the primary extraction solvents.

6. Identification/Characterization of Residues - For residues remaining in the PES, extensive characterization was done using wheat straw because of the high TRR levels and the large amount of material available.

6.a. Wheat Straw - For each plant back interval, RP-44247 was the major identified residue (ranging from 5.4% TRR, 0.28 ppm to 14.2% TRR, 0.23 ppm at 31- and 125-DAT respectively). RP-26019, RP-30228, and RP-25040 were also identified. A conjugate of RP-25040 or RP-32490 or RP-26019 with mass of 452 amu was also found in the polar extractable and aqueous fractions. For residues in the PES, mild, moderate, and exhaustive acid/base hydrolysis were also carried out. Only under exhaustive hydrolysis conditions (10 N NaOH or 6 N HCl) were ^{14}C-residues released, primarily RP-44247. Cell wall fractionation results indicated that most of the solubilized radioactivity in the PES was found in the lignin fraction, containing 5.7% to 10.1% TRR.

6.b. Wheat Forage - RP-44247 was the major identified residue, with RP-26019, RP-30228, and RP-25040 also identified. Polar metabolites (presumably the same conjugates as those identified in wheat straw) also contributed significantly the TRR.

6.c. Wheat Grain - RP-44247 was the major identified residue, with RP-26019, RP-30228, and RP-25040 also identified. Polar metabolites (presumably the same conjugates as those identified in wheat straw) also contributed significantly the TRR. Radioactive residues in the PES were higher in grain versus straw and forage.

7. Mustard Greens - For all plant back intervals, the major identified residue was RP-25040, ranging from 38.8% TRR (0.31 ppm) at 31-DAT to 9.2% TRR (<0.01 ppm) at 364-DAT. RP-44247, RP-26019, and RP-30228 were also identified (ranging from 1.4% TRR, 0.01 ppm to 11.7% TRR, 0.06 ppm). The amount of radioactivity in the PES increased with increasing plant back intervals.

8. Radishes -
8.a. Tops - As was the case for mustard greens, the major identified residue was RP-25040, ranging from 39.6% TRR (0.37 ppm) at 31-DAT to 3.6% TRR (0.01 ppm) at 364-DAT. RP-44247, RP-26019, and RP-30228 were also identified. Also as was the case with mustard greens, the amount of radioactivity in the PES increased with increasing plant back intervals.

8.b. Roots - The major identified residue was RP-25040, ranging from 8.3% TRR (0.22 ppm) at 31-DAT to 1.9% TRR (<0.01 ppm) at 364-DAT. RP26019, RP-30228, and RP-44247 were also identified. For all plant back intervals, the majority of radioactive residues remained in the PES. The amount of TRR found in the PES increased with increasing plant back interval, from 52.5% TRR (1.36 ppm) at 31-DAT to 81.4% TRR (0.12 ppm) at 364-DAT.

9. The registrant presented a proposed metabolic pathway for iprodione in rotational crops (see Figure 2). The data supplied in this study support this proposed pathway.

10. Storage Stability - CBRS concludes that the registrant has adequately demonstrated the storage stability of radioactive residues over the time periods involved in this study (i.e., up to 2 years of frozen storage).

11. Hydrolysis of Standards - The registrant conducted a series of experiments in which reference standards were subjected to extensive hydrolysis (6 N HCl or **10 N NaOH at 120 C for 3 days**).

11.a. Both acid and base hydrolysis of iprodione resulted in formation of RP-324090 and RP-44247. RP-44247 was also formed from RP-25040, RP-324090, RP-36112, and RP-30228.

11.b. Acid digestion of RP-44247 did not indicates significant degradation, but when RP-44247 was base hydrolyzed and unknown peak with R_t 13 min was observable (HPLC gradient 14-D, Figure 6 of Appendix K; p 684 of report). Although the R_t for RP-25040 in this system is 13.3 min., it would appear to be highly unlikely that RP-25040 could be formed from RP-44247 under base hydrolysis conditions. CBRS notes that no R_t for RP-32596 was given for this HPLC gradient system. **The registrant should provide the R_t for RP-32596 with HPLC gradient 14-D.**

11.c. The registrant concluded that based on the exhaustive hydrolysis results for standards,

it has to be assumed that identification of metabolites following this treatment was not necessarily indicative of the molecular species that was first released from the conjugate or bound material.

11.d. CBRS has concerns over the results of these hydrolysis experiments. In recent residue data submissions for residue field trials (S.Knizner, 1/24/95, CBRS #14497 and 13955, MRID #40244001 and 43262501; and S.Knizner, 1/24/95, CBRS #13956, MRID #43255702) a common moiety analytical method had been employed. In this method, RP-26019, RP-30228, and RP-32490 are hydrolyzed to RP-32596 (3,5-dichloroaniline) following **overnight hydrolysis in 3 N KOH at 100 C**. These hydrolysis conditions appear to be equally harsh to those employed in the confined rotational crop study. **The registrant must explain why RP-32596 was not present following base hydrolysis of standards.**

Detailed Considerations

Rhone-Poulenc was the study sponsor. The field portion of the study was carried out by American Agricultural Services Inc (AASI), Lucama, NC, and the analytical portion of the study was first carried out by IIT Research Institute (IITRI), Newington, VA, (9/27/91 to 12/15/93) and then completed by Analytical Development Corporation (ADC), Colorado Springs, CO. Rhone-Poulenc Ag Co., Research Triangle Park, NC, also provided some analytical support for the study. The study was initiated on 9/27/91 and completed on 2/28/95.

Test Material

^{14}C-iprodione uniformly labeled in the phenyl ring was supplied by Rhone-Poulenc (Lot Nos. 1179-0291 and 1179-0491, radiopurity of both lots >97%, specific activity of both lots 3.3 mCi/mM, total weight of first lot 1175.7 mg and second lot 1375.8 mg). These two lots were combined and purified by IITRI and redesignated Lot No. 94B01. Following purification, radiochemical purity was 97.9%, and the specific activity was 7.98 uCi/mg, corresponding to 17,716 dpm/ug.

Non-radiolabeled technical grade iprodione (Lot No. 8906201, purity 96.2%) was used to dilute the radioactive material (a total of 413 mg cold iprodione was added to the purified radiolabeled material). The final test solution (radiolabeled and cold iprodione in a total of 250 mL of benzene) was analyzed by HPLC and shown to contain 10.61 mg/mL iprodione.

The dose solution was prepared by IITRI and was shipped on blue ice to AASI. The dose solution was shipped on 9/25/91, received on 9/26/91 and stored refrigerated (39 to 55 F) until use on 9/27/91. A portion of the dosing solution was returned to IITRI on 9/30/91.

Test System

The in-life phase of the study was conducted at AASI, Lucama, NC. Two plots (one treatment, one control) were established in a Lexan-covered, open air structure. Each test plot contained two in-ground galvanized steel tanks, lined with two layers of polyethylene sheets and filled with sandy loam soil. The tanks were 3 ft. wide by 9.5 ft. long and 24 inches deep. A metal barrier was inserted into the tanks 15 inches from each tank end, resulting in a plot area 3 feet wide by 7 feet long within each tank. Plots were further subdivided in half to produce subplots (designated A and B) in each tank approximately 3.5 feet long by 3 feet wide.

The test material was applied on 9/27/91. A CO_2-pressurized total delivery sprayer was used for application of test material to soil. This apparatus delivered all the test material in a 36 inch wide band using a TeeJet 8002E nozzle at 20 psi. A total of 116 mL of test substance were applied to each test plot.

The registrant stated the application rate used was 4.2 lb ai/A, However, based on the concentration of iprodione in the application solution (10.61 mg/mL), the volume applied (116 mL) and the test plot size (21 ft^2), it appears that the application rate was 5.6 lb ai/A.

Prior to each rotational crop planting the soil was tilled 3-4 inches to prepare the seed bed. The test plots were maintained weed free (hand weeding or hoeing). Irrigation was applied to both fallow and crop areas as needed to maintain crop vigor. To prevent cold damage and encourage crop development, plots were covered with clear plastic tent structures as needed during winter months and incandescent and Gro-lites were used to increase light intensity.

Rotational crop plantings were as follows: radish, mustard, and winter wheat at 31 days after soil treatment (DAT), 10/28/91; radish, mustard, and spring wheat at 125 DAT (1/30/95); and radish, mustard and winter wheat at 364 DAT (10/25/92). Each rotational crop at each planting interval occupied one-half of a tank (designated sides A and B), except for the 364 DAT planted wheat which occupied the whole tank. Crops were planted as indicated in Table 1.

Table 1. Planting scheme used in rotational crop study. Tanks 1 and 2 were control (non-treated) crops.

Planting Interval (days)	Planting Date	Tank Number	Crop Growing in Side of Tank	
			Side A	Side B
31-DAT	10/28/91	3 4	Radish Fallow	Mustard Wheat
125-DAT	1/30/92	3 4	Radish Wheat	Mustard Wheat (31-DAT still growing)
364-DAT	9/28/92	3 4	Wheat Radish	Wheat Mustard

Branch policy (Guidance on How to Conduct Studies on Rotational Crops, E.Zager and D.Edwards, 2/23/93) allows a primary crop to be grown on treated soil prior to planting a rotated crop. In this study however, for wheat, prior to the planting of the 364 DAT crops, two crops had been grown on the treated soil (that is, the 31-DAT and 125-DAT radish and mustard crops). Therefore, the results for the 364-DAT wheat crop may be lower than those obtained if the study had been done correctly.

Sampling

Rotational crop samples were collected at maturity except wheat forage. Wheat forage was collected when sufficient quantities were present (~700 g). Radish plants were gently pulled from the soil and shaken to remove adhering soil. The leaves were then cut from the roots. Mustard leaf samples were cut off at least one inch above the soil surface. Wheat forage samples were cut at 6 inches above the soil. For mature wheat samples, grain was obtained by cutting the grain heads off and threshing the grain out by hand. The straw samples were obtained by cutting the plants off at least one inch above the soil. Threshed heads and chaff were included in the straw samples. All samples were placed in a temperature monitored freezer immediately after collection and kept frozen until shipment. Samples were stored at AASI for a maximum of 8 days prior to shipment to IITRI. For shipment, samples were placed in insulated boxes with dry ice.

Reference Standards

The following reference standards were used in the study. RP-26019 (99.7%), RP-25040 (99.9%), RP-30228 (98.5%), RP-32490 (97.1%), RP-32596 (100.0%), RP-36112 (99.6%), RP-35119 (96.7%), RP-36221 (94.3%), RP-37176 (96.3%), RP-37677 (97.1%) and RP-44247 (98.8%). All standards were supplied by Rhone-Poulenc. Structures and full chemical names for the standards are presented in Figure 1 on the following pages.

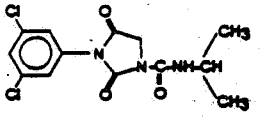
Extraction/Fractionation

In general, approximately 25 g of plant tissue was extracted with ACN:water (10:1) three times and filtered to produce ACN-I and Filter cake. The filter cake was refluxed 4 hours with ACN:water (65:35) to produce another filter cake and ACN extract (ACN-II). ACN-I and ACN-II were combined, concentrated by evaporation and partitioned 3 times with ethyl acetate to yield and Aqueous phase and EtOAc phase. The EtOAc phase was evaporated to dryness, redissolved in ACN, partitioned with hexane to yield fractions ACN-III and Hexane. In order to remove plant pigments, the ACN-III fraction was cleaned up using C-18 solid phase extraction.

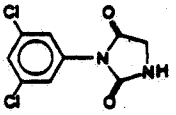
The filter cake was sequentially subjected to Bligh-Dyer extraction, acetone extraction, and acidified water extraction. The remaining nonsoluble residue was subjected to acid/base hydrolysis.

Figure 1. Structures of Reference Standards Used in Study.

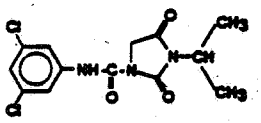
Iprodione, RP 26019
3-(3,5-Dichlorophenyl)-N-(1-methylethyl)-2,4-dioxo-1-imidazolidinecarboxamide
[3-(3,5-Dichlorophenyl)-N-isopropyl-2,4-dioxoimidazolidine-1-carboxamide]
Analytical Lot# EA 2002SD9
CAS # 367-34-19-7
Purity 99.7%



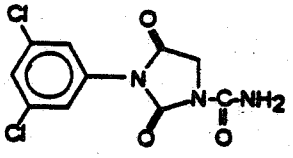
RP 25040
3-(3,5-Dichlorophenyl)-N-2,4-dioxo-1-imidazolidine
[3-(3,5-Dichlorophenyl) hydantoin]
Analytical Lot# EA 2046RF1
CAS # 27387-87-7
Purity 99.9%



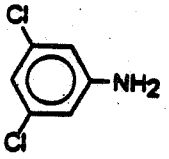
RP 30228
3-(1-Methylethyl)-N-(3,5-dichlorophenyl)-2,4-dioxo-1-imidazolidinecarboxamide
'3,5-dichlorophenyl)carbamoyl-3-isopropyl hydantoin]
lytical Lot# EA 2025RF1
.S # 63637-89-8
Purity 98.5%



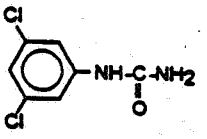
RP 32490
3-(3,5-Dichlorophenyl)-2,4-dioxo-1-imidazolidinecarboxamide
[1-(3,5-Dichlorophenyl)-3-carbamoyl hydantoin]
Analytical Lot# EA 2026RF1
CAS # N/A
Purity 97.1%



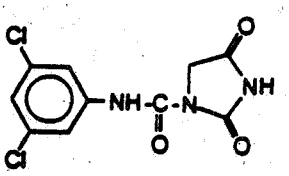
RP 32596
3,5-Dichloroaniline
Analytical Lot# EA 2099RF1
CAS # 626-43-7
urity 100.0%



RP 44247
3,5-Dichlorophenylurea
[1-(3,5-Dichloro phenyl) urea]
Analytical Lot# EA 2092RF
CAS # N/A
Purity 98.8%



RP 36112
N-(3,5-Dichlorophenyl)-2,4-dioxo-1-imidazolidinecarboxamide
[(dichloro-3,5 phenyl)carbamoyl-1 hydantoin]
Analytical Lot# EA 2058RF
CAS # N/A
Purity 99.6%



RP 37677
3-(3,5-Dichloro-4-hydroxyphenyl)-N-(1-methylethyl)-2,4-dioxo-1-imidazolidinecarboxamide
[isopropylcarbamoyl-1(dichloro-3,5 hydroxy-4 phenyl)-3 hydantoin]
Analytical Lot# EA 2084RF
CAS # N/A
Purity 97.1%

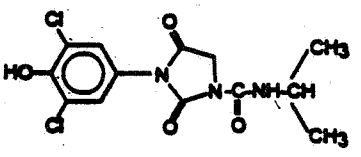
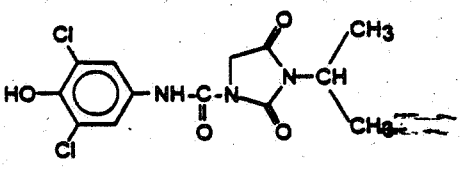
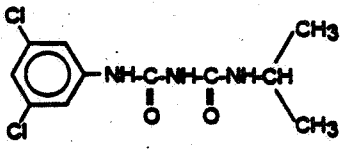


Figure 1 (cont.). Structures of Reference Standards Used in Study.

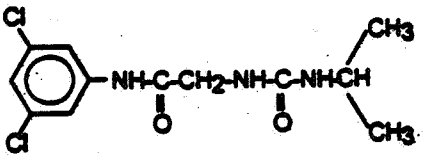
RP 36119
3-(1-Methylethyl)-N-(3,5-dichloro-4-hydroxyphenyl)-2,4-dioxo-1-imidazolidinecarboxamide
[(dichloro-3,5 hydroxy-4 phenyl) carbamoyl-1 isopropyl-3 hydantoin]
Analytical Lot# EA 2080RF
CAS # N/A
Purity 96.7%



RP 36221
3-(1-Methylethyl)-N-(3,5-dichlorophenyl)-1-ureylenecarboxamide
[1-(3,5-dichlorophenyl)-5-isopropyl biuret]
Analytical Lot# EA 2060RF1
CAS # N/A
Purity 94.3%



RP 37176
N-(3,5-Dichlorophenyl)-2-(1-methylethyl)ureylenecacetamide
[1-[N-(3,5-Dichlorophenyl)acetamide]-3-isopropyl urea]
Analytical Lot# EA 2062RF1
CAS # N/A
Purity 96.3%



Cell Wall Fractionation - Characterization of bound ^{14}C-residues of wheat straw was attempted using the following cell wall fractionation scheme. The PES remaining after extraction of wheat straw were treated sequentially with alpha-amylase, pronase-E, pectinase or EGTA, dioxane/2N HCl, hemicellulase or 24% KOH, and lastly cellulase or 72% H_2SO_4. These treatment resulted in the solubilization of the starch, protein, pectin, lignin, hemicellulose, and cellulose fractions of the cell wall respectively.

Hydrolysis of Polar Soluble Metabolites in Aqueous Extract and PES - The polar soluble metabolites in the aqueous extract and the PES of the 31-DAT wheat straw were subjected to hydrolysis with the following enzymes: beta-glucosidase, pronase-E, glusulase, papain, urease, trypsin, and pepsin.

Certain extracts, PES, and standards were also subjected to ~~mild,~~ moderate, and exhaustive acid and base digestion. Exhaustive digestion consisted of hydrolysis with either 10 N NaOH or 6 N HCl at 120 C for 3 days. Mild hydrolysis consisted of digestion with 0.2 N NaOH or 0.1 N HCl at room temperature or 70-80 C. Moderate hydrolysis consisted of digestion with 2 N NaOH or 1 N HCl at room temperature or 70-80 C.

Analytical Techniques

TLC - Both one and two dimensional TLC were used. Representative data for standards and extracts were provided.

HPLC - HPLC using UV and radiochemical (flow through and fraction collection followed by LSC) were used. Several different solvent gradients were used. A table was provided listing the R_fs of standards in the various solvent systems (Table II, page 324). Representative chromatograms were also provided for standards and sample extracts. The solvent system most relied upon was designated solvent system 14D. The reason for this is that RP-25040 and RP-44247 co-eluted in the other solvent systems. System 14D consisted of a formic acid/MeOH gradient. CBRS notes that R_ts for RP-37677, RP-36119, and RP-32596 were **not** provided for this solvent system as they were not included in the standard mixture.

LC/MS - A Perkin-Elmer Sciex API LC/MS.MS system was used for analysis of certain wheat straw extracts. A table providing the LC/MS retention times of standards was provided. Representative total ion chromatograms for standards were presented. Mass spectra for each standard were also presented. A table listing characteristic ions as well as masses of resulting daughter ions for each standard was presented

Results

Validation of Extraction Scheme

To determine the stability and partitioning behavior of iprodione throughout the extraction

procedure, control wheat forage and control radish tops were fortified with ^{14}C-iprodione and extracted just as treated tissues. Results are presented in Table 2. The radiolabeled iprodione was found in the ACN I extract.

Table 2. Recovery Data for Control Samples Fortified with ^{14}C-Iprodione.

Sample	^{14}C-Iprodione Spike (dpm)	ACN-I (dpm and %TRR)	ACN-II (dpm and %TRR)
Wheat Forage	367,940	370,987 (100.8%)	1,529 (0.4%)
Radish Tops	367,940	363,489 (98.8%)	None Detected

TRR Determinations

TRR levels for the various crops are presented in Table 3. The TRR levels decreased from the first to second to third harvest interval for mustard and radish. For wheat, TRR levels decreased from the first to second harvest interval. Because the third wheat crop was grown in the same soil used to grow two crops of radish and mustard (the 31- and 125-DAT crops), results for the 364-DAT wheat may be lower than those obtained if the study had been done correctly (see discussion above).

The 125-DAT wheat grain and 364-DAT mustard green samples had TRR levels <0.010 ppm, and therefore did not require characterization of radioactive residues.

Table 3. TRR levels (ppm iprodione equivalents) in various rotational crops. Soil was treated with iprodione at 5.6 lb ai/A and crops were planted at indicated plant back intervals.

Planting Interval	Mustard Greens	Radish Tops	Radish Roots	Wheat Forage	Wheat Straw	Wheat Grain
31-DAT	0.81	0.94	2.59	1.02	5.17	0.14
125-DAT	0.49	0.34	0.64	0.37	1.62	0.09
364-DAT	0.02	0.19	0.14	0.06[a]	0.65[a]	0.02[a]

[a] Because the third wheat crop was grown in the same soil used to grow two crops of radish and mustard (the 31- and 125-DAT crops), results for the 364-DAT wheat may be lower than normally expected (see discussion above).

Partitioning of Radioactive Residues

Table 4 presents a summary of the partitioning of radioactive residues in the primary extraction solvents. With the exception of radish roots, good extraction of radioactive

residues was achieved with the majority of the radioactivity extracted into the ACN/H_2O fraction. In general, for all samples the extractability of radioactive residues decreased with increasing plant back interval.

^{14}C-residues remaining in radish root PES were higher than those for other samples and increased with increasing plant back interval, ranging from 52.5% TRR (1.36 ppm) at 31-DAT to 81.4% TRR (0.12 ppm) at 364-DAT.

Table 4. Summary of the partitioning of radioactive residues in the primary extraction solvents. Values presented are percent TRR with ppm in parentheses.

DAT	Sample	TRR (ppm)	Soluble Residues				PES	Total
			ACN/H_2O	DCM/MeOH/H_2O	Acetone	H_2O/HCl		
31-DAT	W.Forage	1.016	83.8% (0.85)	0.9% (0.01)	0.2% (<0.01)	0.4 (<0.01)	15 (0.15)	100.3 (1.01)
	M.Greens	0.807	72.4% (0.58)	0.7% (0.01)	0.3% (<0.01)	0.7% (0.01)	14.4% (0.12)	88.5% (0.72)
	R.Tops	0.937	84.1% (0.79)	0.4% (<0.01)	0.2% (<0.01)	0.6% (0.01)	16.8% (0.16)	102.1% (0.96)
	R.Root	2.586	26.3% (0.68)	0.2% (0.01)	0.1% (<0.01)	0.2% (0.01)	52.5% (1.36)	79.3% (2.06)
	W.Grain	0.138	66.6% (0.09)	5.3% (0.01)	1.3% (<0.01)	2.1% (<0.01)	28.5% (0.04)	103.8% (0.14)
	W.Straw	5.171	69.1 (3.57)	4.2% (0.22)	0.5% (0.03)	1.4% (0.07)	14.4% (0.74)	89.6% (4.63)
125-DAT	W.Forage	0.368	65.6% (0.24)	0.6% (<0.01)	0.1% (<0.01)	0.5% (<0.01)	12.4% (0.05)	79.2% (0.29)
	M.Greens	0.490	72.6% (0.36)	0.5% (<0.01)	0.2% (<0.01)	0.3% (<0.01)	10.7% (0.05)	84.3% (0.41)
	R.Tops	0.342	85.4% (0.29)	0.3% (<0.01)	0.1% (<0.01)	<0.1% (<0.01)	11.7% (0.04)	97.5% (0.33)
	R.Root	0.635	22.0% (0.14)	0.2% (<0.01)	0.2% (<0.01)	<0.1% (<0.01)	69.4% (0.44)	91.8% (0.58)
	W.Grain	0.092	40.9% (0.04)	3.2% (<0.01)	1.5% (<0.01)	2.6% (<0.01)	41.9% (0.04)	90.1% (0.08)
	W.Straw	1.623	60.3% (0.98)	1.5% (0.02)	0.1% (<0.01)	0.8% (0.01)	19.8% (0.32)	82.6% (1.34)

DAT	Sample	TRR (ppm)	Soluble Residues				PES	Total
			ACN/H_2O	DCM/MeOH/H_2O	Acetone	H_2O/HCl		
364-DAT	W.Forage	0.057	81.7% (0.05)	2.0% (<0.01)	0.7% (<0.01)	2.4% (<0.01)	13.1% (0.01)	99.9% (0.06)
	M.Greens	0.023	83.3% (0.02)	1.0% (<0.01)	2.0% (<0.01)	0.7% (<0.01)	16.5% (<0.01)	103.5% (0.02)
	R.Tops	0.191	27.7% (0.05)	0.3% (<0.01)	0.2% (<0.01)	0.4% (<0.01)	46.2% (0.09)	74.8% (0.14)
	R.Root	0.137	22.7% (0.03)	0.2% (<0.01)	0.3% (<0.01)	<0.1% (<0.01)	81.4% (0.12)	104.7% (0.15)
	W.Grain	0.021	51.0% (0.01)	13.4% (<0.01)	4.5% (<0.01)	8.2% (<0.01)	47.5% (0.01)	124.6% (0.02)
	W.Straw	0.649	55.2% (0.36)	3.3% (0.02)	0.2% (<0.01)	1.8% (0.01)	21.1% (0.14)	81.5% (0.53)

Identification/Characterization of Radioactive Residues

Iprodione (RP-26019), its isomer (RP-30228), RP-25040, and RP-44247 were the major non-polar identifiable ^{14}C-residues for all crops. Other metabolites tentatively identified include RP-37176, RP-32490, RP-36112, and RP-36221 (these metabolites were observed on TLC analysis were below the limit of detection for HPLC).

The major polar metabolite identified (using LC/MS) was a conjugate of iprodione or one of its metabolites (possibly RP-32490 or RP-25040).

Wheat - A summary of the identification/characterization of radioactive residues found in wheat is presented in Table 5.

Straw - For each plant back interval, RP-44247 appeared to be the major identified residue. LC/MS and LC/MS/MS analyses were conducted on the ACN III/C-18 ethyl acetate phase and aqueous phase extracts. The EtOAC extract was shown to contain RP-36112 (R_t 24.0 min), RP-36221 (R_t 26.1 min), and RP-30228 (R_t 27.5 min). The major peak in the EtOAC extract was an unknown with R_t of 19.6 min. The full scan MS of this unknown indicates that it has a molecular weight of 452 amu (based on Cl-35).

LC/MS analysis of the aqueous extract also indicated the presence of unknown with molecular weight of 452 amu. RP-36221 and RP-30228 were also identified in this extract.

The MS/MS daughter spectrum of the 451 amu peak yielded strong peaks at amu 271, 243,

and 160. The MS/MS daughter spectrum of the 453 amu peak yielded strong peaks at amu 273, 245, and 162. The peaks with amu 243/245 are indicative of the possible presence of RP-25040 or RP-32490 or RP-26019. The registrant postulated that the peak with 452 amu was a conjugate of RP-25040 or RP-32490 or RP-26019.

Attempts to characterize residues in the polar soluble fractions by mild and exhaustive acid/base hydrolysis and by enzymatic treatments produced inconclusive results. Small amounts of RP-31162, RP-44247, RP-25040 and possibly RP-32490 appeared to be released. The results from enzyme treatments suggested that glycosides, protein conjugates, glucuronide conjugates, sulfate conjugates and conjugates that could be hydrolyzed with urease or papain were not present in any significant amounts, if at all.

For residues in the PES, mild, moderate, and exhaustive acid/base hydrolysis were also carried out. Only under exhaustive hydrolysis conditions (10 N NaOH or 6 N HCl) were ^{14}C-residues released, primarily RP-44247. Cell wall fractionation results indicated that most of the solubilized radioactivity in the PES was found in the lignin fraction, containing 5.7% to 10.1% TRR.

Forage - Iprodione, RP-30228, RP-25040 and RP-44247 were the major non-polar identifiable ^{14}C-residues. Polar metabolites (presumably the same as those identified in wheat straw) also contributed significantly the TRR.

Grain - Iprodione, RP-30228, RP-25040 and RP-44247 were the major non-polar identifiable ^{14}C-residues.

Mustard Greens - Table 6 presents a summary of identification/characterization of radioactive residues in mustard greens. For all plant back intervals, the major identified residue was RP-25040, ranging from 38.8% TRR (0.31 ppm) at 31-DAT to 9.2% TRR (<0.01 ppm) at 364-DAT.

Radishes - Table 7 summarizes results for identification/characterization of radioactive residues found in radish tops and roots.

Tops - As was the case for mustard greens, the major identified residue was RP-25040, ranging from 39.6% TRR (0.37 ppm) at 31-DAT to 3.6% TRR (0.01 ppm) at 364-DAT. Also as was the case with mustard greens, the amount of radioactivity in the PES increased with increasing plant back intervals.

Roots - The major identified residue was RP-25040, ranging from 8.3% TRR (0.22 ppm) at 31-DAT to 1.9% TRR (<0.01 ppm) at 364-DAT. Iprodione, RP-30228, and RP-44247 were also identified. For all plant back intervals, the majority of radioactive residues remained in the PES. The amount of TRR found in the PES increased with increasing plant back interval, from 52.5% TRR (1.36 ppm) at 31-DAT to 81.4% TRR (0.12 ppm) at 364-DAT.

Proposed Metabolic Pathway

The registrant presented a proposed metabolic pathway for iprodione in rotational crops (see Figure 2). The data supplied in this study support this proposed pathway.

Hydrolysis of Standards

In order to determine if RP-44247 can be formed from other metabolites under exhaustive hydrolysis conditions and to determine if other metabolites are stable under these conditions, the nonradioactive standards of iprodione, RP-25040, RP-32490, RP-36112, RP-26019, RP-30228, and RP-44247 were treated with 6 N HCl and 10 N NaOH at 120 C for 3 days. The neutralized reaction mixtures were extracted with EtOAc and subjected to HPLC analysis.

Both acid and base hydrolysis of iprodione resulted in formation of RP-32490 and RP-44247. RP-44247 was also formed from RP-25040, RP-324090, RP-36112, and RP-30228.

CBRS notes that acid digestion of RP-44247 did not indicates significant degradation, but when RP-44247 was base hydrolyzed and unknown peak with R_t 13 min was observable (HPLC gradient 14-D, Figure 6 of Appendix K, p 684 of report). Although the R_t for RP-25040 in this system is 13.3 min., it would appear to be highly unlikely that RP-25040 could be formed from RP-44247 under base hydrolysis conditions. CBRS notes that no R_t for RP-32596 was given for this HPLC gradient.

The registrant concluded that based on the exhaustive hydrolysis results for standards, it has to be assumed that identification of metabolites following this treatment was not necessarily indicative of the molecular species that was first released from the conjugate or bound material.

Storage Stability - Storage stability data for wheat forage, mustard greens, and radish tops were presented. Initial extractions were performed approximately 6 months after the first samples were collected. Additional extractions were performed after approximately 2 years of frozen storage. Both extraction and partitioning results were similar. TLC analyses of the initial and 2-year extracts also yielded comparable results.

CBRS concludes that the registrant has adequately demonstrated the storage stability of radioactive residues over the time periods involved in this study (i.e., up to 2 years of frozen storage).

Figure 2. Proposed metabolic pathway for iprodione in rotational crops (taken directly from Figure 1. of study, page 370).

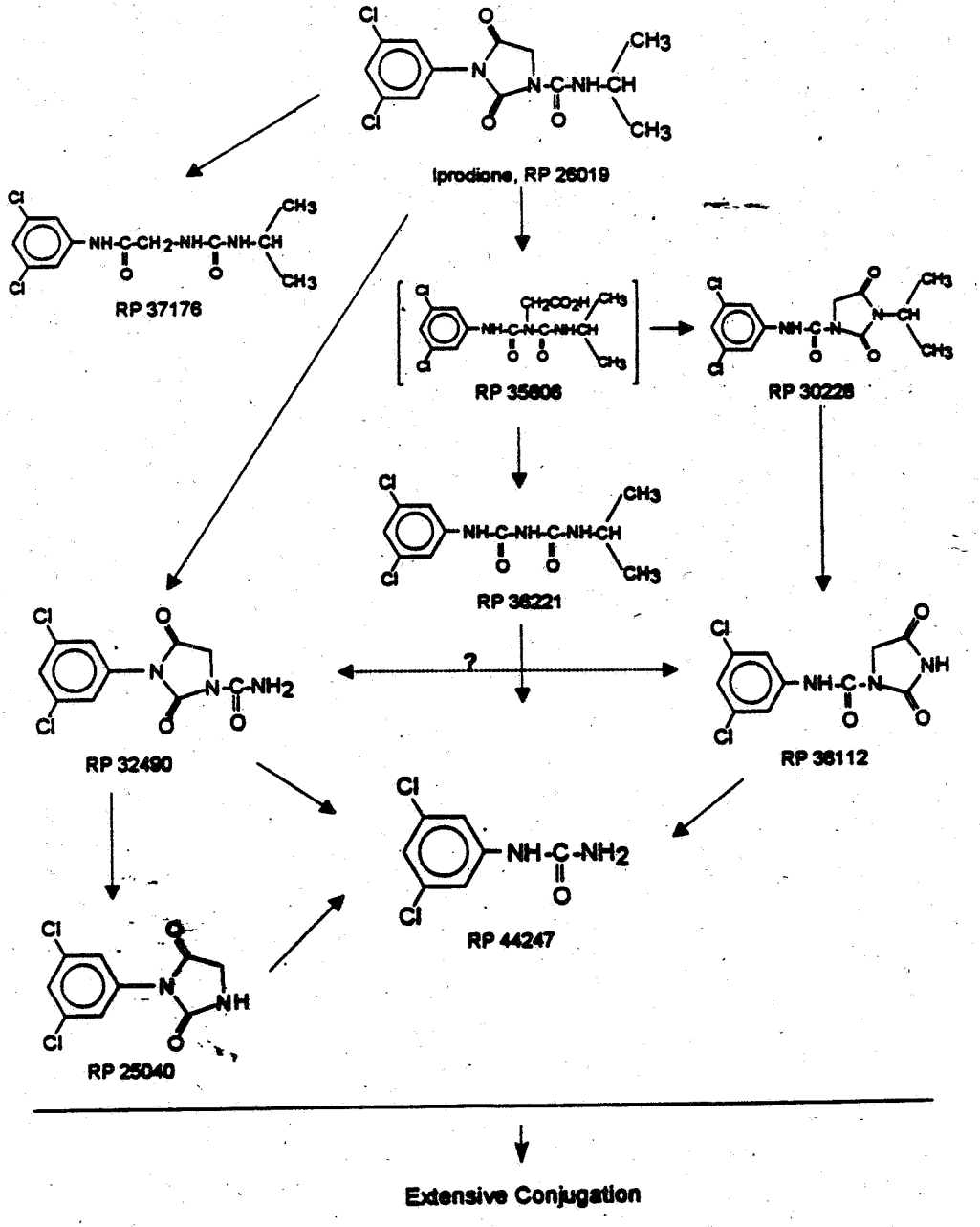


Table 5. Summary of identified/characterized residues found in wheat forage, straw and grain.

Sample	DAT	TRR (ppm)	ACN:Water Extract							Aq.[a]	Hexane	Add'tl Extracts		PES	Total Recovery
			ACN-III/C-18 Fraction									Organ. Extr.[b]	Aq. Extr		
			Identified Nonpolar Residues					Un-Id'd Non-Polar	Polar Metab.[a]						
			HPLC			TLC									
			RP 26019	RP 30228	RP 25040	RP 44247	Other Id'd[c]								
Forage	31	1.016	3.3% 0.03	1.5% 0.02	3.5% 0.04	3.5% 0.04	1.0% 0.01	3.7% 0.04	16.6% 0.17	40.8% 0.41	2.1% 0.02	1.1% 0.01	0.4% <0.01	15.0% 0.15	92.5% 0.93
	125	0.368	5.9% 0.02	1.8% 0.01	3.3% 0.01	7.7% 0.03	3.4% 0.02	4.9% 0.02	13.4% 0.05	25.1% 0.09	1.3% <0.01	0.7% <0.01	0.5% <0.01	12.4% 0.05	80.4% 0.30
	364	0.057	3.2% <0.01	3.3% <0.01	2.0% <0.01	4.8% <0.01	1.0% <0.01	4.0% <0.01	8.1% <0.01	55.5% 0.03	2.8% <0.01	2.7% <0.01	2.4% <0.01	13.1% 0.01	102.9 0.04
Straw	31	5.171	1.6% 0.08	1.5% 0.08	1.4% 0.07	5.4% 0.28	1.4% 0.07		20.3% 1.05	26.7% 1.38	0.5% 0.03	4.7% 0.25	1.4% 0.07	14.4% 0.74	79.3% 4.09
	125	1.623	3.0% 0.05	2.0% 0.03		14.2% 0.23	5.6% 0.09		9.6% 0.16	23.1% 0.37	0.6% 0.01	1/6% 0.02	0.8% 0.01	19.8% 0.32	80.2% 1.29
	364	0.649	2.3% 0.02	2.1% 0.02		6.5% 0.04	1.8% 0.02	2.1% 0.02	11.0% 0.07	25.0% 0.16	<0.1% <0.01	3.5% 0.02	1.8% 0.01	21.1% 0.14	77.2% 0.50
Grain	31	0.138	1.1% <0.01	0.5% <0.01	0.2% <0.01	0.8% <0.01	2.8% <0.01	0.5% <0.01	24.1% 0.04	28.1% 0.04	1.1% <0.01	6.6% <0.01	2.1% <0.01	28.5% 0.04	96.4% 0.14
	125	0.092	0.9% <0.01	0.4% <0.01		4.0% <0.01	2.6% <0.01	0.9% <0.01	5.6% 0.01	24.2% 0.02	1.1% <0.01	4.7% <0.01	2.6% <0.01	41.9% 0.04	88.9% 0.07
	364	0.021	2.0% <0.01	1.6% <0.01		2.6% <0.01		1.7% <0.01	4.9% <0.01	33.3% 0.01	1.3% <0.01	17.9% <0.01	8.2% <0.01	47.5% 0.01	121.0% 0.02

[a] Metabolites tentatively identified in these fractions include conjugates of iprodione or one of its metabolites (possibly RP-32490 or RP-25040) and RP-44247.

[b] DCM/MeOH/H_2O and Acetone extracts (see Table 4).

[c] Other identified residues include RP-37176, RP-32490, RP-36112 and RP-36221. These metabolites were observable by 2-D TLC, but were present below the HPLC detection limit.

Table 6. Identification/Characterization of Radioactive Residues in Mustard Greens.

Sample	DAT	TRR (ppm)	ACN:Water Extract							Aq.[a]	Hexane	Add'tl Extracts		PES	Total Recovery
			ACN-III/C-18 Fraction												
			HPLC				TLC	Un-Id'd Non-Polar	Polar Metab.[a]			Organ. Extr.[b]	Aq. Extr		
			RP 26019	RP 30228	RP 25040	RP 44247	Other Id'd[c]								
Greens	31	0.807	1.4% 0.01		38.8% 0.31	8.4% 0.07	1.2% 0.01		4.1% 0.03	13.9% 0.11	1.9% 0.02	1.0% 0.01	0.7% 0.01	14.4% 0.12	85.8% 0.70
	125	0.490	3.3% 0.02		31.6% 0.15	11.7% 0.06	1.6% <0.01		5.1% 0.03	15.6% 0.08	1.9% 0.01	0.7% <0.01	0.3% <0.01	10.7% <0.05	82.5% 0.40
	364	0.023	3.8% <0.01	2.5% <0.01	9.2% <0.01	3.4% <0.01		7.1% <0.01	4.7% <0.01	33.8% 0.01	9.4% <0.01	3.0% <0.01	0.7% <0.01	16.5% <0.01	94.1% 0.02

[a] Metabolites tentatively identified in these fractions include conjugates of iprodione or one of its metabolites (possibly RP-32490 or RP-25040) and RP-44247.
[b] DCM/MeOH/H_2O and Acetone extracts (see Table 4).
[c] Other identified residues include RP-37176, RP-32490, RP-36112 and RP-36221. These metabolites were observable by 2-D TLC, but were present below the HPLC detection limit.

Table 7. Results for identification/characterization of radioactive residues in radish tops and roots.

Sample	DAT	TRR (ppm)	ACN:Water Extract							Aq.[a]	Hexane	Add'tl Extracts		PES	Total Recovery
			ACN-III/C-18 Fraction												
			HPLC				TLC	Un-Id'd Non-Polar	Polar Metab.[a]			Organ. Extr.[b]	Aq. Extr		
			RP 26019	RP 30228	RP 25040	RP 44247	Other Id'd[c]								
Tops	31	0.937	8.2% 0.08	1.4% 0.01	39.6% 0.37	10.8% 0.10	1.6% 0.02		8.9% 0.08	9.8% 0.09	2.0% 0.02	0.6% <0.01	0.6% <0.01	16.8% 0.16	100.3% 0.94
	125	0.342	5.5% 0.02	1.1% <0.01	38.4% 0.13	11.3% 0.04	1.7% 0.02		9.6% 0.03	14.5% 0.05	1.3% <0.01	0.4% <0.01	<0.1% <0.01	11.7% 0.04	95.5% 0.33
	364	0.191	4.5% 0.01	2.5% <0.01	3.6% 0.01	1.0% <0.01	1.1% <0.01	5.3% <0.01	1.4% <0.01	7.2% 0.01	1.6% <0.01	0.5% <0.01	0.4% <0.01	46.2% 0.09	75.3% 0.13

Sample	DAT	TRR (ppm)	ACN:Water Extract							Aq.[a]	Hexane	Add'tl Extracts		PES	Total Recovery
			ACN-III/C-18 Fraction									Organ. Extr.[b]	Aq. Extr		
			HPLC				TLC	Un-Id'd Non-Polar	Polar Metab.[a]						
			RP 26019	RP 30228	RP 25040	RP 44247	Other Id'd[c]								
Roots	31	2.586	3.2% 0.08	1.0% 0.03	8.3% 0.22	0.9% 0.02	0.3% <0.01		7.0% 0.18	3.4% 0.09	0.5% 0.01	0.3% 0.01	0.2% 0.01	52.5% 1.36	77.6% 2.01
	125	0.635	1.7% 0.01	0.6% <0.01	6.7% 0.04				5.0% 0.03	5.3% 0.03	0.3% <0.01	0.4% <0.01	<0.1% <0.01	69.4% 0.44	89.4% 0.55
	364	0.137	2.6% <0.01	2.0% <0.01	1.9% <0.01			3.6% 0.01	1.1% <0.01	8.1% 0.01	0.4% <0.01	0.5% <0.01	<0.01% <0.01	81.4% 0.12	101.6% 0.14

[a] Metabolites tentatively identified in these fractions include conjugates of iprodione or one of its metabolites (possibly RP-32490 or RP-25040) and RP-44247.
[b] DCM/MeOH/H_2O and Acetone extracts (see Table 4).
[c] Other identified residues include RP-37176, RP-32490, RP-36112 and RP-36221. These metabolites were observable by 2-D TLC, but were present below the HPLC detection limit.

cc: S.F., circ., R.F., List B File, S.Knizner
RDI: A.Rathman, 6/8/95 E.Zager, 6/8/95
7509C:CBRS:CM#2:305-6903:SAK:sak:Iprodione:Ipro:5/10/95