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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

Memorandum:

SUBJECT: Quizalofop-p ethyl ester. Comparison of the Metabolism of DPX-79376, the R Enantiomer, Optically Active Ingredient, and DPX-Y6202, the Racemic Mixture, in Soybeans. (MRID#24632-01, CB#10606, Barcode #D182751).

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E. I. du Pont de Nemours & Co., Inc., has submitted a metabolism study (MRID#24632-01) for a comparison of the metabolism of DPX-79376, the R enantiomer, the optically active ingredient of the herbicide formulation ASSURE®II, and DPX-Y6202, the racemic mixture used to formulate ASSURE®, in soybeans. The registrant had previously stated that since only the R enantiomer has significant herbicidal activity, the formulation with the optically active ingredient, DPX-79376, would allow the label use rate to be lowered. In response, CBTS informed the registrant that he would need to provide some side by side studies to ensure that there are no differences between the enantiomer and the racemate (See memo of 6/7/84, R. Loranger).

The acceptable ANSI common name for the racemic TGAI mixture is quizalofop ethyl ester. Its acid metabolite is called quizalofop. For the R enantiomer, the respective ANSI names are quizalofop-p ethyl ester and quizalofop-p.

A tolerance is established on cottonseed for the combined residues of the quizalofop-p ethyl ester [ethyl (R)-(2-[4-((6-chloro-

quinoxalin-2-yl)oxy)phenoxy]) propionate], its acid metabolite [(R)-(2-[4-((6-chloroquinoxalin-2-yl)oxy)phenoxy]propanoic acid], and the S enantiomers of the ester and the acid, all expressed as quizalofop-p ethyl ester (40 CFR 180.441). Tolerances are established (40 CFR 180.441, 185.5250, and 186.5250) for the combined residues of the racemic quizalofop ethyl ester and its metabolite quizalofop in/on soybeans (0.05 ppm), soybean flour (0.5 ppm), hulls (0.02 ppm), meal (0.5 ppm), and soapstock (1.0 ppm). Tolerances have been established for the combined residues of quizalofop ethyl ester, quizalofop methyl ester, and quizalofop in eggs (0.02 ppm), milk (0.01 ppm), milk fat (0.05 ppm), and the fat (0.05 ppm), meat (0.02 ppm), and meat byproducts (0.05 pm) of cattle, goats, hogs, horses, poultry, and sheep.

Conclusions/Recommendations:

In summary, the nature of R enantiomer, quizalofop-p ethyl ester, residues appears to follow those determined in the previous soybean metabolic studies using the racemic TGAI, quizalofop ethyl ester with no measurable difference in the rate of metabolism.

This metabolism study is adequate to support the registration of the R enantiomer, quizalofop-p ethyl ester.

Detailed Considerations:

Metabolism of quizalofop ethyl ester:

Previous submissions include metabolism data for the racemic quizalofop ethyl ester (DPX-Y6202) for soybeans, cotton, potato, and sugar beet. These studies have shown the following:

a) The rate of translocation, following foliar application of DPX-Y6202 to soybean, cotton, potato, or sugar beet is rather slow.

b) DPX-Y6202 appears to involve cleavage at three sites, and conjugation with plant sugars:

   1. Hydrolysis of the ethyl ester;

   2. Cleavage of the enol ether linkage between the phenyl and quinoxalinyl rings;

   3. Cleavage of the ether linkage between the phenyl ring and the isopropanoic group.

Previously submitted metabolism studies for soybeans (See PP#5F3252, ACC#073547) using the racemic TGAI have shown the residues of parent quizalofop ethyl ester, the hydrolyzed acid metabolite quizalofop (and conjugates), and Phenols 2 and 4 (and

hydroxyphenoxy)propanoic acid). In addition, two other phenols in the soybean foliage, i.e., Phenol 1: 4-(6-chloroquinoxalin-2-yloxy)phenol [1.4 - 4.2 % of the total C14-activity in 1 week vs. nondetectable (<0.005 ppm) activity in 3 weeks], and Phenol 3: ethyl 2-[(4-hydroxyphenoxy)oxy] propionate [0.9 % of the total C14 activity in 1 week vs. nondetectable activity (<0.005 ppm) in 3 weeks] when treated at an application rate of 4.0 oz a.i./A (1X maximum label rate for ASSURE® formulation). However, the levels of total C14 activity were <0.01 ppm in the mature beans and pods. Another experiment used a rate of 10 oz a.i./A (2.5X maximum label rate for ASSURE® formulation) and measured the 14C-residues in seeds and pods 52 days after treatment. No attempt was made to determine the conjugate (bound) residues.

TABLE 1. Residue Levels in DPX-Y6202 Equivalents (PPM) in Soybean Seeds and Pods (52 Days Maturity)

Label	seeds	pods
Phenyl	0.21	0.65
Quinoxaline	0.33	0.79

TABLE 2. Percentage 14C-Activity of Extractable Residues in Soybean Seeds and Pods (52 Days Maturity)

Compound	Seeds	Pods
Phenyl-14C		
quizalofop ethyl ester	1.6	4.4
quizalofop	41.9	14.7
Phenol 1	<0.005	2.7
Phenol 4	<0.005	4.5
Unidentified	56.5	73.7
Quinoxaline-14C		
quizalofop ethyl ester	7.4	1.4
quizalofop	26.1	7.1
Phenol 1	0.9	2.0
Phenol 2	<0.005	0.7
Unidentified	65.6	88.8

Additional data for the phenols were submitted for soybeans treated at a rate of 6.4 oz a.i./A (1.6X maximum label rate of the ASSURE® formulation) (See memo of 12/18/87, G. Otakie, MRID#403224-06). Residue samples from all 7 field trial locations were <0.05 ppm for quizalofop ethyl ester, quizalofop, and Phenols 1, 2, and 4 (PHI's: 72 to 80 days). Methodology and validation data for the phenol methods (MRID#403224-11, Phenols 1 and 2; MRID#403224-12, Phenol 4) were adequate. The quantitation limits for Phenols 1 and 2, and for Phenol 4 were 0.05 and 0.02 ppm, respectively. Recoveries of 0.02 - 0.1 ppm spiked residues averaged 93, 82, and 94% for Phenols 1, 2, and 4, respectively.

Metabolism of quizalofop-p ethyl ester:

The registrant has **now** submitted a metabolism study titled "Metabolism of [Quinoxaline(U)-14C]DPX-Y6202, [Phenyl(U)-14C]DPX-Y6202, [Quinoxaline(U)-14C]DPX-79376, and [Phenyl(U)-14C]DPX-79376 in Soybean",(MRID#24632-01) for a comparison of the metabolism of DPX-Y6202, the racemic mixture used to formulate ASSURE®, and DPX-79376, the R enantiomer optically active ingredient of the herbicide formulation ASSURE®II, in soybeans.

Note: In the title of the above study, the petitioner has described compounds DPX-Y6202 and DPX-79376 as uniformly labeled in the phenyl ring of the (hydroxyphenoxy)propanoate moiety and in the quinoxaline ring. With the (hydroxyphenoxy)propanoate moiety, this is true. However, only the benzene ring, which is part of the quinoxaline ring, is uniformly labeled with the other 14C compound; the two remaining carbon atoms in the heterocyclic portion of each compound are not labeled. Within the report, the compounds are named [quinoxaline-phenyl(U)-14C]DPX-Y6202 and [quinoxaline-phenyl(U)-14C]DPX-79376, clearly showing that only the benzene portion of the quinoxlaine ring is uniformly labeled. Therefore, for the purpose of clarity, in this memo, compounds [quinoxaline-phenyl(U)-14C]DPX-Y6202 and [quinoxaline-phenyl(U)-14C]DPX-79376 will be called [quinoxaline-14C]DPX-Y6202 and [quinoxaline-14C]DPX-79376, respectively.

A typical commercial agricultural variety, McCall soybeans, were planted in greenhouse pots containing sandy loam soil and treated postemergently with either [quinoxaline-14C]DPX-Y6202, [phenyl(U)-14C]DPX-Y6202, [quinoxaline-14C]DPX-79376 or [phenyl(U)-14C]DPX-79376. Each test substance was applied as an aqueous solution at the trifoliate stage (20 days after emergence) using a hand-held sprayer as an over-the-top spray at an application rate of 4 oz a.i./A, the maximum label use rate for DPX-Y6202, and two times the proposed maximum label use rate for DPX-79376. Two untreated pots

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served as controls. Two pots each were treated with [quinoxaline-14C]DPX-Y6202, [phenyl(U)-14C]DPX-Y6202, [quinoxaline-14C]DPX-79376, or [phenyl(U)-14C]DPX-79376 at 4 oz a.i./A in a solution containing 0.25% Ortho X-77 surfactant. Whole plant samples of the crop treated with each 14C-labeled solution [radiolabeled test substances were diluted with corresponding unlabeled test substance 1:2 (labeled:unlabeled).] were taken immediately after the spray dried and 7, 14,29, and 63 days (maturity) after treatment.

The test substances had the following physical properties: [phenyl(U)-14C]DPX-Y6202, a specific activity of 85 μCi/mg, a radiochemical purity of 97.2%; [quinoxaline-14C]DPX-Y6202, a specific activity of 72 μCi/mg, a radiochemical purity of 97.0%; [phenyl(U)-14C]DPX-79376, a specific activity of 62.1 μCi/mg, a radiochemical purity of 98.9%; [quinoxaline-14C]DPX-79376, a specific activity of 102.2 μCi/mg, a radiochemical purity of 99.2%. All radiochemical purities were determined by HPLC.

Mature samples were harvested 63 days after treatment and the beans were separated from the pods, leaves, and straw/foliage. The two mature fractions were weighed and analyzed individually. All replicate samples of day 0 and intermediate sampling days were pooled prior to processing, while the mature sample replicates were processed and analyzed individually. At each sampling date, the harvested plant samples were rinsed briefly with acetone, then air dried. The weights were recorded and the samples were sealed in labeled plastic bags and kept frozen until analyzed. Acetone rinse volumes were measured and aliquots analyzed by LSC. Frozen plant samples were homogenized by pulverizing with dry ice in a blender.

The total radioactivity in the soybeans harvested 0, 7, 14, 29, and 63 days (maturity) after application of the above test substances was measured by combustion analysis. The results are presented in Table 3 as ppm DPX-Y6202 equivalents. Total 14C-residues declined rapidly in soybeans treated with either DPX-Y6202 or DPX-79376. The levels of 14C-residues declined from 14.0 ppm (day 0) to 1.5 ppm at 29 days after [phenyl(U)-14C]DPX-Y6202 application, from 20.3 ppm (day 0) to 26 ppm at 29 days after [quinoxaline-14C]DPX-Y6202, from 9.2 ppm (day 0) to 3.1 ppm at 29 days after [phenyl(U)-14C]DPX-79376 application, and from 5.8 ppm (day 0) to 3.4 ppm at 29 days after [quinoxaline-14C]DPX-79376 application. The 14C-residues in the mature soybeans ranged from 0.06 ppm (both labels of DPX-79376) to 0.14 ppm in the mature soybeans treated with [quinoxaline-14C]DPX-Y6202. Approximately 97-98% of the total 14C-residues in soybean plants harvested immediately after treatment (day 0) were removed by extraction with acetonitrile:water containing 1% acetic acid (3:1). The percentage of unextracted 14C-residues increased

with time to 35-51% in the mature soybeans and 38-60% bound in the mature soybean foliage. The amount of bound radioactive residues, expressed as a percentage of total 14C-residues in each soybean plant sample can be found in Tables 4-7. The concentration of bound residues in the soybean plant samples in ppm (DPX-Y6202 or DPX-79376 equivalents) can also be found in Tables 4-7. These concentrations ranged from 0.02-0.05 ppm in the mature soybeans, and 0.24-0.39 ppm in the mature soybean straw/foliage, and were not significantly different between the DPX-Y6202 and DPX-79376 treatments.

DPX-Y6202 acid, a group of conjugates of DPX-Y6202 acid and Phenol 1, and a group of early eluting unknown polars, were the major metabolites in each of the r.a.c.'s. Representative HPLC chromatograms of day 14 soybean plant extracts were submitted. The conjugates of DPX-Y6202 acid and Phenol 1 were identified by enzymatic hydrolysis of the day 14 soybean plant extracts. After 24 hours of incubation in a buffered solution containing cellulase and p-glucosidase enzymes, the radioactivity in this group of at least five peaks (eluting between 18 and 26 minutes) decreased from approximately 50-60%, to approximately 20-35% of the extractable 14C-residues. Concurrently, the amount of DPX-Y6202 acid in these samples increased from approximately 20-35% of the extractable 14C-residues, to approximately 30-45%, while Phenol 1, which was not present in any of the day 14 extracts before enzyme hydrolysis, increased to approximately 4-12% of extractable residues after enzyme hydrolysis.

There was no difference in the retention times of peaks in the group of unknown early-eluting metabolites before and after the enzymatic hydrolysis of the day 14 extracts. This group of polar unknowns consists of at least two or more components, and there was no qualitative difference between the plants treated with 14C-phenyl labeled parent and the ones treated with 14C-quinoxaline labeled parent. This is consistent with results of previous DPX-Y6202 soybean metabolism studies (See PP#5F3252, ACC#073547). Other minor metabolites tentatively identified in plant extracts were Phenol 1 (<1.0% of total 14C-residues), and Phenol 2 (<5.0% of total 14C-residues). The presence of Hydroxy-phenol 2, Phenol 3 and Phenol 4 were not confirmed due to their retention times falling within the range of the larger groups of glucose conjugates and unknown polar metabolites, but their identification in previous DPX-Y6202 soybean metabolism studies above and the presence of these peaks after enzyme hydrolysis suggests that these three metabolites are present in these samples.

TABLE 3. Total 14C-DPX-Y6202 Equivalent Residues (PPM) in Soybean Plant Samples After Treatment with 14C-DPX-Y6202 or 14C-DPX-79376.

	[Phenyl-14C]DPX-Y6202 PPM (avg.)	[Quinoxaline-14C]DPX-Y6202 PPM (avg.)	[Phenyl-14C]DPX-79376 PPM (avg.)	[Quinoxaline-14C]DPX-79376 PPM (avg.)
Day 0	14.0	20.3	9.2	5.8
Day 7	11.9	10.7	7.5	12.5
Day 14	4.1	3.2	1.6	2.4
Day 29	1.5	2.6	3.1	3.4
Mature Soybeans	0.08,0.11 (0.10)	0.18,0.09 (0.14)	0.07,0.04 (0.06)	0.04,0.08 (0.06)
Mature Straw	0.45,0.54 (0.50)	0.84,0.93 (0.89)	0.69,0.57 (0.63)	0.99, 0.93 (0.96)

TABLE 4. Characterization of 14C-Residues in Soybean Plants Treated with [Phenyl(U)-14C]DPX-Y6202.

	Percent of Total Radioactivity (ppm)						
Sample	Bound	Polar Unk.	Unk #1	Unk #2	Phenol 1	DPX-Y6202 Acid	DPX-Y6202
Day 0	2.9 (0.41)	ND (<0.01)	ND (<0.01)	ND (<0.01)	ND (<0.01)	66.2 (9.25)	30.9 (4.32)
Day 7	14.5 (1.72)	4.2 (0.50)	1.1 (0.13)	26.3 (3.12)	ND (<0.01)	30.4 (3.61)	23.4 (2.78)
Day 14	21.3 (0.88)	16.1 (0.67)	ND (<0.01)	43.3 (1.79)	ND (<0.01)	19.4 (0.80)	ND (<0.01)
Day 29	20.5 (0.30)	24.8 (0.36)	1.0 (0.01)	47.6 (0.70)	ND (<0.01)	6.0 (0.09)	ND (<0.01)
Mature Beans	44.3 (0.04)	25.0 (0.02)	ND (<0.01)	14.9 (0.01)	ND (<0.01)	16.0 (0.02)	ND (<0.01)
Mature Straw	48.1 (0.24)	24.4 (0.12)	ND (<0.01)	22.1 (0.11)	ND (<0.01)	5.3 (0.03)	ND (<0.01)

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TABLE 5. Characterization of 14C-Residues in Soybean Plants Treated with [Quinoxaline-14C]DPX-Y6202.

Percent of Total Radioactivity (ppm)							
Sample	Bound	Polar Unk.	Phenol 2	Unk #2	Phenol 1	DPX-Y6202 Acid	DPX-Y6202
Day 0	1.9 (0.39)	ND (<0.01)	ND (<0.01)	ND (<0.01)	ND (<0.01)	54.4 (11.06)	43.7 (8.89)
Day 7	9.4 (1.00)	4.9 (0.52)	1.9 (0.20)	48.7 (5.20)	ND (<0.01)	25.0 (2.67)	10.1 (1.08)
Day 14	16.2 (0.52)	13.2 (0.42)	3.2 (0.10)	43.9 (1.41)	ND (<0.01)	23.5 (0.75)	ND (<0.01)
Day 29	19.9 (0.53)	17.8 (0.47)	2.8 (0.07)	51.7 (1.36)	ND (<0.01)	7.8 (0.21)	ND (<0.01)
Mature Beans	34.5 (0.05)	20.2 (0.03)	ND (<0.01)	24.2 (0.03)	ND (<0.01)	21.1 (0.03)	ND (<0.01)
Mature Straw	37.9 (0.34)	23.1 (0.21)	4.0 (0.04)	25.5 (0.23)	ND (<0.01)	9.4 (0.08)	ND (<0.01)

TABLE 6. Characterization of 14C-Residues in Soybean Plants Treated with [Phenyl(U)-14C]DPX-79376.

Percent of Total Radioactivity (ppm)							
Sample	Bound	Polar Unk.	Unk #1	Unk #2	Phenol 1	DPX-79376 Acid	DPX-79376
Day 0	1.8 (0.17)	ND (<0.01)	ND (<0.01)	ND (<0.01)	ND (<0.01)	92.3 (8.50)	5.9 (0.54)
Day 7	16.7 (1.25)	5.2 (0.39)	ND (<0.01)	63.2 (4.72)	ND (<0.01)	12.9 (0.96)	2.1 (0.16)
Day 14	28.7 (0.46)	13.8 (0.22)	ND (<0.01)	45.1 (0.72)	ND (<0.01)	12.4 (0.20)	ND (<0.01)
Day 29	29.0 (0.89)	12.9 (0.39)	ND (<0.01)	51.8 (1.59)	ND (<0.01)	6.4 (0.20)	ND (<0.01)
Mature Beans	51.3 (0.03)	21.3 (0.01)	ND (<0.01)	21.6 (0.01)	ND (<0.01)	5.8 (<0.01)	ND (<0.01)
Mature Straw	60.3 (0.38)	12.8 (0.08)	ND (<0.01)	21.0 (0.13)	0.7 (<0.01)	5.2 (0.03)	ND (<0.01)

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FIGURE 1
PROPOSED METABOLIC PATHWAY IN SOYBEANS

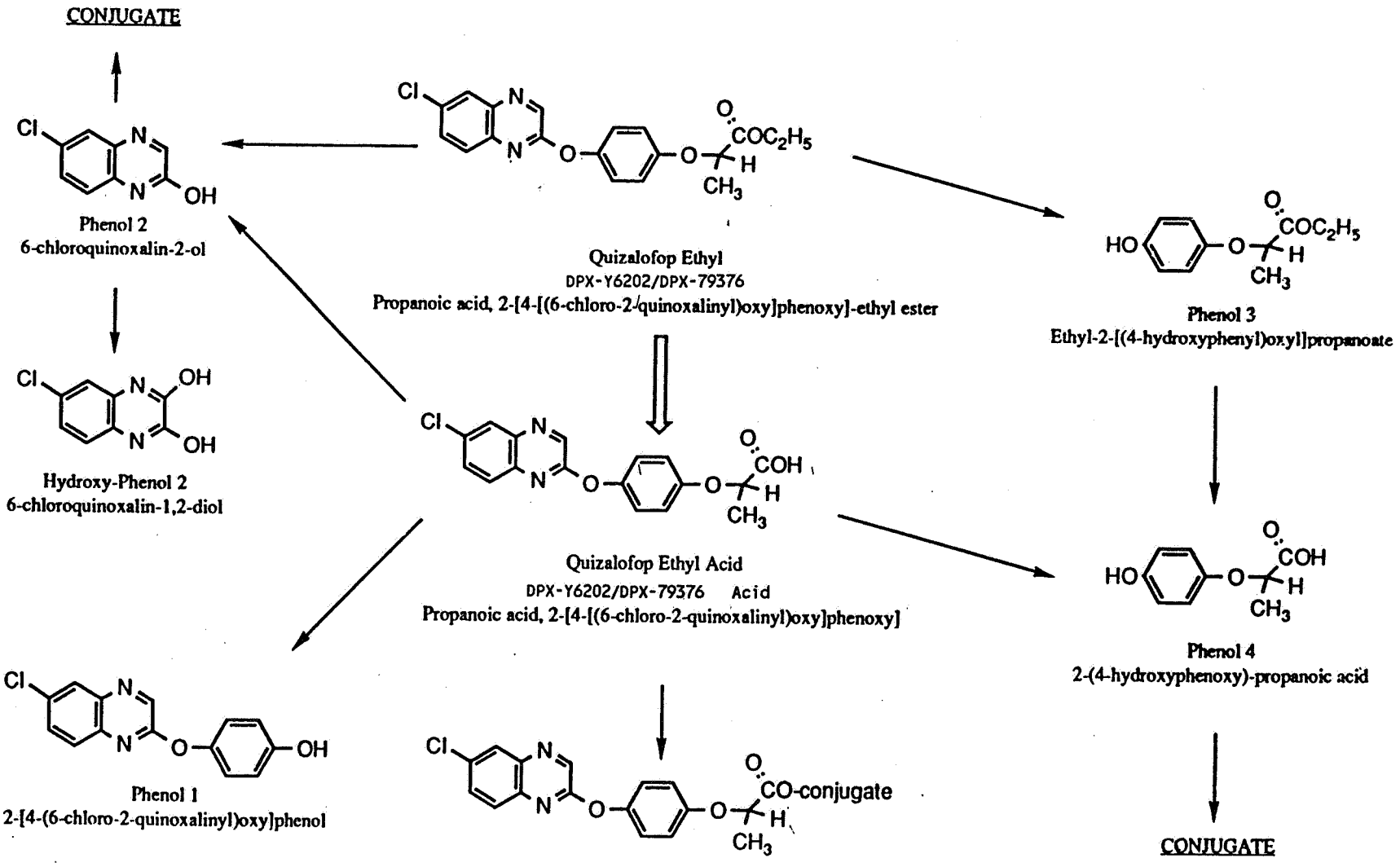

TABLE 7. Characterization of 14C-Residues in Soybean Plants Treated with [Quinoxaline-14C]DPX-79376.

Percent of Total Radioactivity (ppm)							
Sample	Bound	Polar Unk.	Phenol 2	Unk #2	Phenol 1	DPX-79376 Acid	DPX-79376
Day 0	1.7 (0.10)	ND (<0.01)	ND (<0.01)	ND (<0.01)	ND (<0.01)	72.8 (4.19)	25.5 (1.47)
Day 7	11.7 (1.46)	3.6 (0.45)	3.2 (0.40)	60.2 (7.51)	ND (<0.01)	18.4 (2.30)	3.0 (0.37)
Day 14	18.2 (0.44)	10.5 (0.26)	3.2 (0.08)	50.4 (1.41)	ND (<0.01)	17.8 (0.43)	ND (<0.01)
Day 29	23.4 (0.79)	15.0 (0.50)	2.4 (0.08)	52.1 (1.75)	ND (<0.01)	7.1 (0.24)	ND (<0.01)
Mature Beans	35.3 (0.02)	25.0 (0.01)	ND (<0.01)	32.6 (0.02)	ND (<0.01)	7.1 (<0.01)	ND (<0.01)
Mature Straw	41.1 (0.39)	18.6 (0.18)	4.5 (0.04)	25.9 (0.25)	0.6 (0.01)	9.4 (0.09)	ND (<0.01)

In summary, the nature of R enantiomer, quizalofop-p ethyl ester, residues appears to follow those determined in the previous soybean metabolic studies for using the racemic TGAI, quizalofop ethyl ester with no measurable difference in the rate of metabolism. The metabolic pathways for DPX-Y6202 and DPX-79376 are summarized in Figure 1. DPX-Y6202 and DPX-79376 were rapidly hydrolyzed within the soybean plant to DPX-Y6202 acid and DPX-79376 acid respectively, followed by metabolism at the ether linkage producing Phenol 1, Phenol 2, and possibly Hydroxy-phenol 2, Phenol 3, and Phenol 4. After 14 days, the concentration of parent compound in each sample was less than 0.01 ppm. In addition, the hydrolysis data confirm that several of these metabolites undergo glucose conjugation.

Thus, this metabolism study is adequate to support the registration of the R enantiomer, quizalofop-p ethyl ester.

cc: J. Stokes (CBTS); Quizalofop-p ethyl ester S.F.; R.F.; Circu.
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H7509C:CBTS:JStokes:js:Rm 803A:CM#2:305-7561:7/14/93

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