

US EPA ARCHIVE DOCUMENT



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

SEP 25 1985

PMSD/TSB
0049

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: PP#5F3252 [RCB#1127]. DPX-Y6202 (Assure®)
Herbicide on Cotton and Soybeans.
Evaluation of Analytical Methodology and
Residue Data (Accession Nos. 073529 and 073547).

FROM: Michael P. Firestone, Ph.D., Chemist
Tolerance Petition Section II
Residue Chemistry Branch
Hazard Evaluation Division (TS-769)

Michael P. Firestone

TO: Robert J. Taylor, Product Manager No. 25
Fungicide-Herbicide Branch
Registration Division (TS-767)

and

Toxicology Branch
Hazard Evaluation Division (TS-769)

THRU: Charles L. Trichilo, Ph.D., Chief
Residue Chemistry Branch
Hazard Evaluation Division (TS-769)

[Signature]

E. I. du Pont de Nemours and Company is proposing that tolerances be established for residues of the herbicide ethyl 2-[4-(6-chloroquinoxalin-2-yl oxy)phenoxy] propanoate (Assure®, DPX-Y6202, NC-302) and its acid metabolite, 2-[4-(6-chloroquinoxalin-2-yl oxy)phenoxy] propanoic acid in or on the raw agricultural commodities soybeans and cotton at 0.05 ppm each.

Temporary tolerances have been established for residues of DPX-Y6202 and its acid metabolite in/on soybeans and cotton at 0.05 ppm each in conjunction with PP#4G2978 and PP#4G2977, respectively.

The subject petition represents the first request for establishing permanent tolerances for the herbicide DPX-Y6202.

Conclusions

- 1a. The petitioner will be required to submit a complete description of the manufacturing process including a discussion of the reaction steps, the reaction conditions, impurities in the starting materials, formation of impurities in the technical product, cleanup (purification) procedures, etc.

- 1b. DuPont has indicated in a June 1, 1984, conference with EPA (see R. Loranger memo of June 7, 1984) that although the data submitted to date were obtained [REDACTED]

Since the present submission does not discuss this topic, information relating to the nature of the isomer(s) in the technical product should be included in a future amendment.

- 1c. All inerts in the formulated product are cleared for use on growing crops except one identified in the Confidential Appendix (see Attachment 3). The petitioner will either need to provide evidence that this inert is cleared under 40 CFR 180.1001(c) or (d), or ask the manufacturer to seek such a clearance.
2. The petitioner will need to revise Section B/proposed label so that the total amount of herbicide applied per season (not to exceed 4 oz ai or 2.5 pints Assure® per acre) is clearly stated. Also, the proposed label should stress that only EPA approved oil concentrates and surfactants should be used.

Finally, the directions for use on soybeans should include the restriction:

Do not apply after pod-set;

and the directions for use on cotton should also include a growth stage restriction in addition to a PHI, considering the long (80-day) PHI proposed.

- 3a. At this time, RCB considers the nature of the residue in soybeans and cotton treated according to the proposed use (i.e., maximum application of 4 oz ai/A/season with an 80-day PHI) to be adequately understood.

With certainty, the residues DPX-Y6202 and its acid metabolite 2-[4-(6-chloroquinoxalin-2-yl oxy)phenoxy] propionic acid (free plus conjugates) should be included in the tolerance expression. The need to include any of the phenol metabolites in the tolerance expression cannot be determined until the petitioner generates residue data for the following compounds (both free plus conjugated):

Phenol 1 = 4-(6-chloroquinoxalin-2-yl oxy) phenol;
 Phenol 2 = 6-chloroquinoxalin-2-ol;
 Phenol 4 = 2-(4-hydroxyphenoxy) propionic acid.

- 3b. Should the use pattern on cotton or soybeans change so as to increase the likely level of residues on the rac's, additional metabolism studies will be required reflecting higher rates of ^{14}C -DPX-Y6202 treatment. Much more of the unidentified residues in/on soybean seeds and cottonseeds will then need to be characterized.
4. No animal metabolism data have been presented in support of the subject petition. The proposed use involves several animal feed items:

soybean hulls, meal, oil and soapstock;
cottonseed hulls, meal, oil and soapstock.

Until issues involving the analytical methodology (see Conclusion 5) and the maximum likely level of residues (parent plus metabolites) in/on various animal feed items have been resolved (see Conclusions 6, 7 and 8), RCB remains unable to reach any final conclusion regarding the need for animal metabolism data in support of the subject petition.

5. The proposed regulatory method (Method No. AMR-153-83 Revision A) is not considered adequate for enforcement purposes because it is not designed to quantitate residues of DPXY6202 Acid conjugates. Depending on the results from the requested residue studies (see Conclusions 7 and 8), methodology for some of the phenol metabolites (free plus conjugates) may need to be submitted and reviewed for regulatory purposes.

The petitioner will need to develop such methodology along with appropriate validation data (fortification/recovery data, control values, representative chromatograms, etc.) for analysis of both cottonseed and soybeans.

Also, the petitioner will need to examine whether any other pesticides registered for use on soybeans and cotton will interfere with the analysis of DPX-Y6202 and its acid and phenol metabolites of concern (free plus conjugates).

At such time as RCB considers the methodology acceptable, it will be sent to EPA's Analytical Chemistry Laboratory (ACS, COB, BUD) for a method tryout (MTO).

6. Storage stability data will need to be generated for residues of DPX-Y6202 Acid and the phenol metabolites of DPX-Y6202.

7. Considering the lack of residue data reflecting residues of DPX-Y6202 Acid conjugates and the phenol metabolites, and considering limited storage stability of DPX-Y6202 in frozen samples, the petitioner will need to conduct new field trials for soybeans and cottonseed in which the parent compound, and its acid and phenol metabolites (both free and conjugated) are quantitated (i.e., reanalysis of reserve samples is not considered acceptable at this time).

Although the petitioner states that additional cottonseed residue data are currently being generated and will soon be submitted to EPA, unless they reflect quantitation of all residues of concern, RCB will not consider them adequate.

8. Since detectable residues occur in/on soybeans treated even at 1/2x the maximum proposed use rate, the petitioner will need to conduct cottonseed and soybean processing studies in which the treated samples to be processed contain field weathered detectable residues (this may require treatment at greater than 1x and/or PHI's less than 80 days), so that it can be determined quantitatively whether residues concentrate in any processed fractions.
9. At this time, RCB is unable to reach any conclusions concerning the likelihood of secondary residues in animal commodities until issues involving soybean and cottonseed (raw plus processed fractions) residue data, analytical methodology, and possibly animal metabolism of DPX-Y6202 have been resolved.
10. An International Residue Limit Status sheet is included in this review as Attachment 1. Since no Codex, Canadian or Mexican limits/tolerances have been established for DPX-Y6202 on cotton or soybeans, there are no compatibility problems at this time.

Recommendation

At this time, RCB recommends against establishment of the proposed tolerances covering residues of DPX-Y6202 on cottonseed and soybeans for the reasons given under Conclusions 1a, 1b, 1c, 2, 3a, 4, 5, 6, 7, 8, and 9 above.

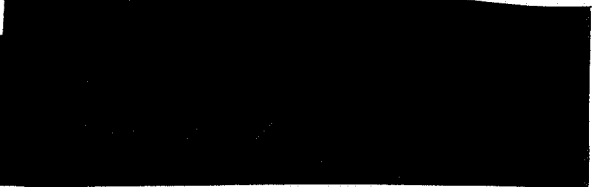
The petitioner must be notified about Conclusion 3b, above.

Detailed Considerations

Manufacture

A schematic diagram of the reaction steps for producing technical DPX-Y6202 (minimum purity = 97%) as well as the results of an analysis of five batches of the technical product have been presented in conjunction with PP#4G2977 (see J. Worthington memo of April 19, 1984). RCB does not expect any residue problems resulting from impurities in the technical product.

The petitioner will be required to submit a complete description of the manufacturing process including a discussion of the reaction steps, the reaction conditions, impurities in the starting materials, formation of impurities in the technical product, cleanup (purification) procedures, etc.

DuPont has indicated in a June 1, 1984, conference with EPA (see R. Loranger memo of June 7, 1984) that although the data submitted to date were obtained using 

Since the present submission does not discuss this topic, information relating to the nature of the isomer(s) in the technical product should be included in a future amendment.

Formulation

The petitioner has submitted a Certification of Ingredient Limits and a Confidential Statement of Formula for DuPont Assure® Herbicide, a 9.5 percent emulsifiable concentrate (EC) equivalent to 0.8 lb ai/gallon.

The composition and level of ingredients in the formulated (9.5% EC) product are detailed in the Confidential Appendix to this review (Attachment 3).

All inerts in the formulated product are cleared for use on growing crops except one identified in the Confidential Appendix. The petitioner will either need to provide evidence that this inert is cleared under 40 CFR 180.1001(c) or (d), or ask the manufacturer to seek such a clearance.

Proposed Use on Cotton and Soybeans

Assure® 9.5 percent Herbicide is to be applied for selective postemergence control of annual and perennial grasses in soybeans and cotton at rates ranging from 0.75 to 2.5 pints (1.2 to 4.0 oz ai) per acre.

In arid regions, two applications of Assure® Herbicide generally provide longer and more effective control than a single application of the same amount.

Apply by ground or aerial equipment in a minimum of 10 gal or 3 gal of water per acre, respectively, and using a nonphytotoxic petroleum oil concentrate or a nonionic surfactant.

Restrictions include:

Do not graze treated fields or harvest for forage or hay;

Do not apply Assure® within 80 days of harvest.

The petitioner will need to revise Section B/proposed label so that the total amount of herbicide applied per season (not to exceed 4 oz ai or 2.5 pints Assure® per acre) is clearly stated. Also, the proposed label should stress that only EPA approved oil concentrates and surfactants should be used.

Finally, the directions for use on soybeans should also include the restriction:

Do not apply after pod-set;

and the directions for use on cotton should include a growth stage restriction in addition to a PHI.

Nature of the Residue

Included in the subject petition (see Accession No. 073547) are the following plant metabolism studies:

<u>Report Title</u>	<u>DuPont Report No.</u>	<u>Document No.</u>
Metabolism of 14C-DPX-Y6202 in Field Grown Soybean Plants (Revision No. 1 - April 23, 1985).	AMR-149-83	32
Fate of NC-302, Ethyl-[4-(6-chloro-2-quinoxalinyloxy)phenoxy] propionate in Soybean Plants	Nissan 1/85	33

Extraction Characterization of 14C-Residues in Mature Seeds Collected from Soybean Plants Foliage - Treated with 14C-NC 302 at the Early Reproductive Stage	Nissan 2/85	34
Metabolism of 14C-DPX-Y6202 in Field Grown Cotton	AMR-273-85	35
Absorption, Translocation and Metabolism of NC-302 in Potato Plants	Nissan 2/85	36
Fate of NC-302 in Sugarbeet Plants	Nissan 1/85	37
14C-DPX-Y6202 Residue Study in Soybeans	AMR-320-84	38

In the ensuing discussion, various terminal residues will be referred to by the following abbreviations:

DPX-Y6202 = ethyl 2-[4-(6-chloroquinoxalin-2-yl oxy)
phenoxy] propanoate

DPX-Y6202 Acid = 2-[4-(6-chloroquinoxalin-2-yl oxy)
phenoxy] propanoic acid

Phenol 1 = 4-(6-chloroquinoxalin-2-yl oxy) phenol

Phenol 2 = 6-chloroquinoxalin-2-ol

Phenol 3 = ethyl 2-(4-hydroxyphenoxy)oxy] propanoate

Phenol 4 = 2-(4-hydroxyphenoxy) propionic acid

For structures of these residues as well as DuPont's proposed metabolic pathway in plants, see Attachment 2.

A. Soybeans

In DuPont Report No. AMR-149-83, data are presented concerning the decay of 14C-activity with time following treatment of soybeans with [quinoxaline-14C] DPX-Y6202 or [phenyl-14C] DPX-Y6202, and the identification of terminal residues in soybeans is discussed.

Soybean plants were treated at a rate equivalent to 4 oz ai/A, and samples were taken immediately after treatment and at 1, 3, 6, and 13.5 weeks after treatment.

The reported decay data are tabulated below:

Label	DPX-Y6202 Equivalents (ppm) ^a				Beans	Pods
	Day 0 Foliage	Week 3 Foliage	Week 6 Foliage	Week 13.5 Foliage		
phenyl	17.8	4.5	0.48	0.009	< 0.01	< 0.01
quinoxaline	23.2	3.8	0.43	0.009	< 0.01	< 0.01

a) Determined by combustion analysis.

Residue identification was attempted for week 1 and 3 foliage samples. The first step involved an acetone wash. Residues were then extracted sequentially with methylene chloride:acetone (1:1) and acetone:ethanol:water (2:1:1). The aqueous phase was partitioned with n-hexane and methylene chloride. In an attempt to further extract residues, beta-glucosidase hydrolysis and acid (pH 1.5) hydrolysis of aqueous soluble residues were attempted. Residues (¹⁴C-labeled) were identified by co-chromatography using TLC and liquid chromatography. The results are tabulated below:

Compound	Percent of Total ¹⁴ C-Activity in Foliage			
	Phenyl Label		Quinoxaline Label	
	1 Week	3 Weeks	1 Week	3 Weeks
DPX-Y6202	5.6	5.1	3.7	4.5
DPX-Y6202 Acid	26.1	41.5	35.1	35.8
Acid Conjugate	14.1	ND	3.8	ND
Phenol 1	1.4	ND	4.2	ND
Phenol 2 (free)	--	--	1.8	2.0
Phenol 2 (conjugate)	--	--	3.5	ND
Phenol 3	0.9	ND	--	--
Phenol 4	0.7	ND	--	--

Compound	Percent of Total ¹⁴ C-Activity in Foliage			
	Phenyl Label		Quinoxaline Label	
	1 Week	3 Weeks	1 Week	3 Weeks
Phenol 4 (conjugate)	1.9	ND	--	--
Unidentified	49.3	53.4	47.9	57.7

ND = Nondetectable.

In a separate experiment, sixth trifoliolate leaf stage plants were treated with [phenyl-¹⁴C] DPX-Y6202 at a rate equivalent to 10 oz ai/A and foliage was sampled 15 days later. Residue identification was performed as above. The results are tabulated below:

Compound	Percentage of Total ¹⁴ C-Activity in Foliage
DPX-Y6202	8.4
DPX-Y6202 Acid	38.8
Acid Conjugate	11.6
Phenol 1	1.4
Phenol 4	2.5
Phenol 4 conjugate	8.6
Unidentified	28.7

The identification of ¹⁴C-DPX-Y6202-treated soybean beans and pods was not attempted, presumably due to the low level of ¹⁴C-activity resulting from an application at 4 oz ai/A (i.e., < 0.01 ppm DPX-Y6202 equivalents).

In Accession No. 073547 - Document No. 33, the ability of residues of DPX-Y6202 to translocate is discussed. Plants were treated during early second trifoliolate stage (¹⁴C-labeled DPX-Y6202 was applied to first trifoliolate leaves), and sampled up to 60 days later. The results are tabulated below:

PHI (days)	Percent of Total Applied 14C-Activity					
	Treated Leaves	Roots	Stems	Other Leaves	Pods	Seeds
1	98.1	< 0.1	0.3	0.1	--	--
3	98.3	0.2	0.8	0.2	--	--
7	88.7	0.4	1.3	1.0	--	--
14	82.0	0.5	1.3	1.8	--	--
28	82.4	0.6	1.4	2.6	0.1	--
42	78.4	0.7	1.8	3.1	0.2	0.1
60	71.2	0.6	1.9	1.7	0.3	0.2

As can be seen in the above table, the rate of translocation through the leaves of soybean plants is very slow.

A study of the effectiveness of various hydrolytic techniques showed that hydrolysis with cellulase was more effective in releasing conjugated residues than treatment with beta-glucosidase, NaOH (0.1-0.2 N) or HCl (0.25-1.0 N).

In Accession No. 073547 - Document No. 34, an attempt at qualitatively determining the terminal residues in soybean seeds, 7 weeks after ¹⁴C-DPX-Y6202 treatment, is discussed. Seeds were extracted with 70 percent aqueous acetone, the acetone was evaporated, the pH was adjusted to 2-3 with concentrated HCl, and residues were partitioned first with ether:hexane (1:1) and then with ethyl acetate. The ethyl acetate fraction underwent either alkali: (0.1 N NaOH) or cellulase hydrolysis. Residue identification was performed by TLC.

Regardless of label (phenyl or quinoxaline), approximately 70 percent of the ¹⁴C-activity in seeds was extractable in aqueous acetone. Residues identified (but not quantitated) included parent, DPX-Y6202 Acid (free plus cellulase hydrolyzable conjugates, Phenols 1 and 2 (free plus conjugates)).

In DuPont Report No. AMR-320-85, residue data are presented reflecting ¹⁴C-activity as DPX-Y6202-equivalents, as well as an attempt to identify terminal residues in mature soybean seeds and pods 52 days after treatment at a rate of 10 oz ai/A.

The ¹⁴C-activity residue data are tabulated below:

PHI (days)	Label	Appl. Rate (oz ai/A)	Residue Levels - (ppm) ¹⁴ C-DPX-Y6202 Equivalents	
			Beans	Pods
92	phenyl	4	< 0.005	< 0.005
92	quinoxaline	4	< 0.005	< 0.005
79	phenyl	4	< 0.005	0.008
66	phenyl	4	0.02	0.04
52	phenyl	4	0.10	0.39
52	phenyl	10	0.21	0.65
52	quinoxaline	10	0.33	0.79

In order to identify the terminal residues, beans and pods were successively extracted with n-hexane, methylene chloride:acetone (1:1) and acetone:ethanol:water (2:1:1). Residues in the n-hexane phase were partitioned into acetonitrile. Organic phase residues were analyzed by TLC while aqueous phase residues were analyzed by reverse-phase liquid chromatography. No attempt was made to determine conjugate residues. The results are presented below:

Compound	Percent ¹⁴ C-Activity:			
	Phenyl Labeled Beans	Quinoxaline Labeled Pods	Phenyl Labeled Beans	Quinoxaline Labeled Pods
DPX-Y6202	1.6	4.4	7.4	1.4
DPX-Y6202 Acid	41.9	14.7	26.1	7.1
Phenol 1	< 0.005	2.7	0.9	2.0
Phenol 2	--	--	< 0.005	0.7
Phenol 4	< 0.005	4.5	--	--
Unidentified	56.5	73.7	65.6	88.8

Presumably, the percentage identified could have been increased had the petitioner used cellulase hydrolysis to release bound residues (see previous discussion on the translocation of DPX-Y6202 residues; Accession No. 073547 - Document No. 33).

B. Cotton

In DuPont Report No. AMR-273-85, the results of a cotton metabolism study are presented. Cotton plants, about 15 inches in height, were foliarly treated with either phenyl-¹⁴C or quinoxaline-¹⁴C DPX-Y6202 at a rate equivalent to 3.7 oz ai/A, and were harvested on day zero, and at 3, 6, and 13.5 weeks later.

The decay of ¹⁴C-activity is summarized in the table below:

Label	DPX-Y6202 Equivalent ^a (ppm)					
	Day 0	Week 3	Week 6	Week 13.5		
	Foliage	Foliage	Foliage	Foliage	Fiber	Seed
Phenyl	10.1	1.50	1.10	0.03	0.095	0.086
Quinoxaline	20.6	1.50	0.009	0.10	0.09	0.08

a) Determined by combustion analysis.

Samples of week 3 and 6 foliage were analyzed in an attempt to determine the nature of the terminal residues in cotton plants. Samples were rinsed with acetone prior to successive extraction with methylene chloride:acetone (1:1) and acetone: ethanol:water (2:1:1). Aqueous extracts were exposed to beta-glucosidase hydrolysis, partitioning with methyl ethyl ketone, acidification to pH 1.5 (6 hrs at 100 °C) and further extraction with ethyl acetate in order to release conjugated residues.

Following successive soxhlet, enzyme (cellulase, protease) and acid (1% HCl, 1% phosphoric acid) extraction, 22 to 26 percent of the total ¹⁴C-activity could be extracted from the bound residues. Residues were identified by co-chromatography employing both TLC and liquid chromatography.

The results of analyses of week 3 and 6 cotton foliage are tabulated below:

Compound	Percentage of Total Radioactive Residue in Foliage			
	Week 3		Week 6	
	Phenyl Label	Quinoxaline Label	Phenyl Label	
DPX-Y6202	8.3	12.6	1.8	
DPX-Y6202 Acid	17.0	6.8	6.2	
Acid Conjugate	11.3	--	ND	
Phenol 2	--	0.3	--	
Phenol 2 Conjugate	--	5.4	--	

Compound	Percentage of Total Radioactive Residue in Foliage		
	Week 3	Week 3	Week 6
	Phenyl Label	Quinoxaline Label	Phenyl Label
Phenol 4	0.6	--	10.1
Phenol 4 Conjugate	1.3	--	9.7
Unidentified	61.5	74.9	72.2

ND = not detected.

At 6 weeks following DPX-Y6202 treatment, residues of Phenol 4 (free plus conjugates) represent the majority of the identified terminal residue.

At week 3, Phenol 2 represents about 20 percent of the total identified terminal residue. No attempt was made to identify residues in treated cottonseed, presumably due to the low level of ^{14}C -activity (< 0.1 ppm DPX-Y6202 equivalents) resulting from application at a rate of 3.7 oz ai/A.

C. Root Crops

Reports prepared by Nissan Chemical Industries Ltd., have been submitted concerning the metabolism of DPX-Y6202 in potato plants and sugarbeet plants (Accession No. 073547 - Document Nos. 36 and 37, respectively).

In potato plants treated at the "10-15 cm leaf stage" (^{14}C -DPX-Y6202 in ethanol was applied using a microsyringe onto second and third terminal leaflets), more than 90 percent of the applied ^{14}C -activity remained on treated leaflets 14 days after treatment (i.e., translocation is very slow). Identification of ^{14}C -residues in potato plant leaves was attempted, but residue levels were not quantitated. The following terminal residues were found by TLC co-chromatography:

DPX-Y6202; DPX-Y6202 Acid (free plus conjugates);
Phenols 1, 2, 3, and 4 (free plus conjugates).

Conjugates of DPX-Y6202 acid, Phenol 2, Phenol 3 and Phenol 4 were released using cellulase and beta-glucosidase.

Residues in potato tubers were not identified.

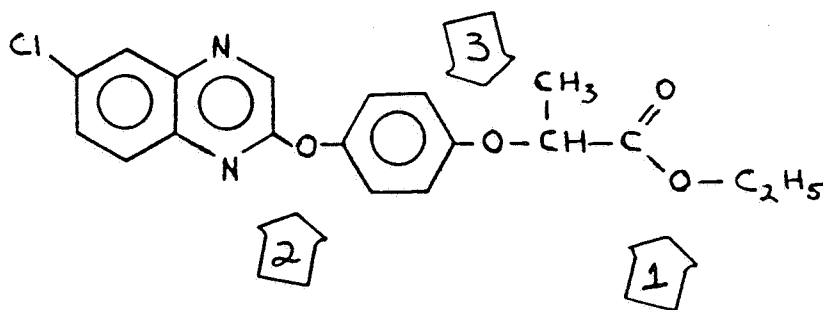
In sugarbeet plants treated at the early 5th leaf stage (^{14}C -DPX-Y6202 was applied to first through fourth true leaves by microsyringe), 71.9 to 89.7 percent of the total applied ^{14}C -activity remained on treated leaves 28 days after treatment. Residues identified in potato leaves were the same as those identified in soybean, cotton and potato leaves. No attempt was made to identify residues in sugarbeet roots.

RCB's Comments/Conclusions Re: Plant Metabolism of DPX-Y6202

The metabolism studies submitted to date indicate that the rate of translocation following foliar application of DPX-Y6202 to soybean, cotton, potato, or sugarbeet plants is rather slow.

The metabolism of DPX-Y6202 appears to involve cleavage at three sites and conjugation with plant sugars. The three cleavage sites are as follows:

1. Hydrolysis of the ethyl ester;
2. Cleavage of the enol ether linkage between the phenyl and quinoxalinyll rings;
3. Cleavage of the ether linkage between the phenyl ring and the isopropanoic group.



(See Attachment 2 for the metabolic pathway of DPX-Y6202 in plants as proposed by DuPont.)

Based on the metabolism data previously described, the maximum percentage of total identified ^{14}C -activity can be calculated. These results are tabulated below:

Sample	Max. % of Total Identified ¹⁴ C-Activity				
	Parent	DPX-Y6202 Acid	Phenol 1	Phenol 2	Phenol 4
Soybean foliage (3 weeks)	10.9	89.1	0.0	4.7	0.0
Soybean foliage (1 week)	11.0	79.3	8.1	10.7	5.1
Soybean foliage (2 weeks)	11.8	70.7	2.0	NA	15.6
Soybean seeds (7 1/2 weeks)	21.5	96.3	2.6	0.0	0.0
Soybean pods (7 1/2 weeks)	16.7	63.4	17.9	6.3	17.1
Cotton foliage (3 weeks)	50.2	73.5	0.0	22.7	4.9
Cotton foliage (6 weeks)	6.5	22.3	0.0	NA	71.2

NA - Not analyzed.

It should be noted that identification of residues in/on cottonseeds was not attempted.

At this time, RCB considers the nature of the residue in soybeans and cotton treated according to the proposed use (i.e., maximum application of 4 oz ai/A/season with an 80-day PHI) to be adequately understood.

With certainty, the residues of DPX-Y6202 and its acid metabolite 2-[4-(6-chloroquinoxalin-2-yl oxy)phenoxy] propionic acid (free plus conjugates) should be included in the tolerance expression. The need to include any of the phenol metabolites in the tolerance expression cannot be determined until the petitioner generates residue data for Phenols 1, 2, and 4 (free plus conjugates).

Should the use pattern on cotton or soybeans change so as to increase the likely level of residues on the rac's, additional metabolism studies will be required reflecting higher rates of ¹⁴C-DPX-Y6202 treatment. Much more of the unidentified residues in/on soybean seeds and cottonseeds will then need to be characterized.

D. Animal Metabolism

No animal metabolism data have been presented in support of the subject petition. The proposed use involves several animal feed items:

soyben hulls, meal, oil and soapstock;
cottonseed hulls, meal, oil and soapstock.

Until issues involving the analytical methodology and the maximum likely level of residues (parent plus metabolites) in/on various animal feed items has been resolved (see Residue Data section of this review), RCB is unable to reach any final conclusion regarding the need for animal metabolism data in support of the subject petition.

Analytical Methodology

In conjunction with PP#'s 4G2977 and 4G2978, the petitioner has previously submitted analytical methods for independently determining residues of DPX-Y6202 and its acid metabolite in/on cottonseed and soybeans, respectively (see J. Worthington memos of April 19, 1984, re: PP#4G2977 and PP#4G2978). Both methods employ quantitation by HPLC using a UV detector.

In the subject petition, DuPont has submitted a new method entitled "Determination of Residues of DPX-Y6202 and DPX-Y6202 Acid in Soybeans" (Report No. AMR-153-83 Revision A) which determines both the parent compound and its acid metabolite.

Soybean samples are extracted with acetone:water:glacial acetic acid (78:20:2). Residues are then partitioned into chloroform from the aqueous solution, and the chloroform is evaporated. The residues are dissolved in acetonitrile, and hexane partitioning is used to remove any oil. The acetonitrile is evaporated and residues are taken up in a solution of hexane:acetone:glacial acetic acid (58:40:2). Residues of DPX-Y6202 and its acid metabolite are cleaned up and fractioned by medium pressure liquid chromatography (LiChroprep Si60 - 40-63 μ m - packing), and quantitated by high performance liquid chromatography (Zorbax[®] CN and SIL columns) using a UV detector at 335 nm.

The following fortification/recovery data are reported:

Compound	Fortification (ppm)	Recovery Range	Percentage (Average)
DPX-Y6202	0.02-0.10	85-118	94
DPX-Y6202 acid	0.02-0.10	65-114	88

All control values were reported as < 0.020 ppm for both parent compound and its acid metabolite.

RCB's Comments/Conclusions Re: Analytical Methodology

Method No. AMR-153-83 Revision A is not considered adequate for enforcement purposes because it is not designed to quantitate residues of DPX-Y6202 Acid conjugates. Depending on the results from the requested residue studies (see the Residue Data section of this review), methodology for some of the metabolites (free plus conjugates) may need to be submitted and reviewed for regulatory purposes.

The petitioner will need to develop such methodology along with appropriate validation data (fortification/recovery data, control values, representative chromatograms, etc.) for analysis of both cottonseed and soybeans.

Also, the petitioner will need to examine whether any other pesticides registered for use on soybeans and cotton will interfere with the analysis of DPX-Y6202 and its acid and phenol metabolites of concern (free plus conjugates).

At such time as RCB considers the methodology acceptable, it will be sent to EPA's Analytical Chemistry Laboratory (ACS, COB, BUD) for a method tryout (MTO).

Residue Data

a. Previous Considerations

Cottonseed residue data submitted with PP#4G2977 (see J. Worthington memo of April 19, 1984) reflected 13 field trials conducted in 10 different States. Cottonseed samples were analyzed for residues of DPX-Y6202 and its acid metabolite after treatments of up to 8 oz ai/A (i.e., 2x proposed maximum application rate) and PHI's of 24 to 150 days. None of these samples reportedly contained detectable (> 0.02 ppm) residues of either the parent compound or DPX-Y6202 Acid (free).

Soybean residue data submitted with PP#4G2978 (see J. Worthington memo of April 19, 1984) reflected 18 field trials conducted in 11 different States. Soybean samples were analyzed for residues of DPX-Y6202 and its acid metabolite (free) after treatments of up to 8 oz ai/A (i.e., 2x proposed maximum use rate) and PHI's of 88 to 145 days. Residue levels of both the parent compound and DPX-Y6202 Acid (free) were reportedly < 0.02 ppm in all samples analyzed.

b. Present Considerations

Storage stability data have been submitted reflecting treated soybeans fortified at 100 ppb with DPX-Y6202 and stored under frozen conditions for up to 12 months in one study and 22 months in a second study. The results are tabulated below:

Months	1	3	6	9	13	18	22
% Recovery	97	92	79	69	72	59	68

Months	2	4	9	12
% Recovery	94	96	62	92

Beyond 4 months, the stability of DPX-Y6202 in frozen soybean samples declines.

The above studies need to be expanded to include the storage stability of the acid and phenol metabolites.

Information submitted along with the additional residue data included in the subject petition indicates that some samples were stored at "ambient" temperatures for as long as 23 days. The value of the residue data generated on such samples is considered questionable.

It is not clear exactly how long samples were stored frozen at the laboratory prior to residue analysis, however, based on the earliest sampling date of October 9, 1984, and a Residue Report date of April 26, 1984, the storage period could not have exceeded 6 months.

In conjunction with the subject petition, residue data were generated on treated soybeans grown in the states of TX, GA, MS, AL, DE, AK, and SD. These data do not reflect conjugated residues of DPX-Y6202 Acid or any of the phenol metabolites of concern.

Soybeans were treated at rates ranging from 1.6 to 16 oz ai/A (0.4 to 4x) and harvested from 52 to 89 days after treatment (proposed PHI = 80 days).

Residue levels of parent compound in all samples were undetectable (< 0.02 ppm). Maximum residue levels of unconjugated DPX-Y6202 acid were 0.024, 0.070, and 0.062 ppm at application rates of 2, 4, and 8 oz ai/A, respectively (note: treated soybeans from three trials reflecting soybean storage at ambient temperatures for periods of time from 7 to 23 days were found to contain no detectable DPX-Y6202 Acid residues, while samples from 3 of the other 4 trials contained detectable residue of DPX-Y6202 Acid).

Considering the lack of residue data reflecting DPX-Y6202 Acid conjugates and the phenol metabolites of concern, and considering the limited storage stability of DPX-Y6202 in frozen samples, the petitioner will need to conduct new field trials for soybeans and cottonseed in which the parent compound, its acid, and phenol metabolites both free and conjugated are quantitated (i.e., reanalysis of reserve samples is not considered acceptable at this time).

Although the petitioner states that additional cottonseed residue data are currently being analyzed and will soon be submitted to EPA, unless they reflect quantitation of all residues of concern, RCB will not consider them adequate.

Finally, since detectable residues occur in soybeans treated at even 1/2x the maximum proposed rate (see also the Nature of the Residue Section of this review), the petitioner will need to conduct cottonseed and soybean processing studies in which the treated samples to be processed contain field weathered detectable residues (this may require treatment at greater than 1x and/or PHI's less than 80 days), so that a quantitative determination can be made as to whether residues concentrate in any processed fractions.

Residues in Meat, Fat, Milk, Poultry and Eggs

At this time, RCB is unable to reach any conclusions concerning the likelihood of secondary residues in animal commodities until issues involving soybean and cottonseed (rac plus processed fractions) residue data, analytical methodology, and possibly animal metabolism of DPX-Y6202 have been resolved.

No animal feeding studies have been submitted to date for DPX-Y6202.

Potential animal feed items include cottonseed and soybean meal, oil, hulls, and soapstock.

Other Considerations

An International Residue Limit Status sheet is included in this review as Attachment 1. Since no Codex, Canadian or Mexican limits/tolerances have been established for DPX-Y6202 on cotton or soybeans, there are no compatibility problems at this time.

Attachment 1: International Residue Limit Status Sheet
Attachment 2: Proposed Metabolic Pathway of DPX-Y6202 in Plants
Attachment 3: Confidential Appendix (copies only to TOX, PM#25,
R.F., MPFirestone, PMSD/ISB, PP#5F3252)

cc: Circu, R.F., MPFirestone, EAB, EEB, FDA, PP#5F3252,
PMSD/ISB

RDI:J.H.Onley:9/1/85:R.D.Schmitt:9/5/85
TS-769:RCB;Reviewer:M.P.Firestone:CM#2:RM:800:X557-7484:
Typed by Kendrick:9/13/85:edited by:wh:9/21/85
HED/RCB:JOB-94532:M.Firestone:C.Disk:Kendrick:898-1270:
9/13/85:Kim

INTERNATIONAL RESIDUE LIMIT STATUS

CHEMICAL Assure® (DPX-Y6202)

PETITION NO. PP#5F3252

CCPR NO. _____

Reviewer: M. P. Firestone (7/10/85)

Codex Status

Proposed U.S. Tolerances

☒ No Codex Proposal
Step 6 or above

ethyl 2-[4-(6-chloroquinoxaline-
2-yl oxy) phenoxy] propanoate

Residue (if Step 9): _____

Residue: _____

Crop(s) Limit (mg/kg)

Crop(s) Tol. (ppm)

Soybeans 0.05

Cotton 0.05

CANADIAN LIMIT

MEXICAN TOLERANCIA

Residue: _____

Residue: _____

Crop Limit (ppm)

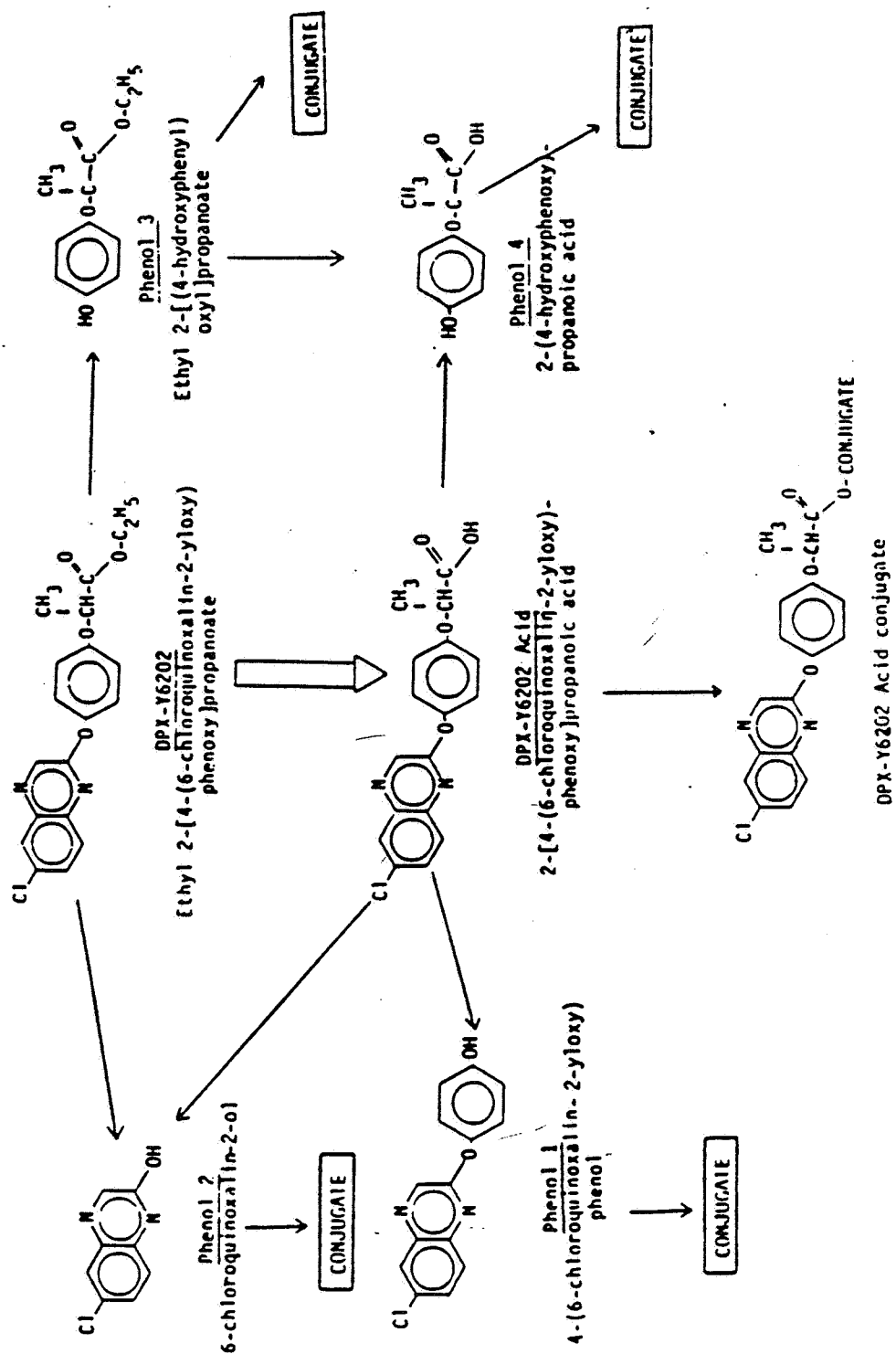
None

Crop Tolerancia (ppm)

None

NOTES:

PROPOSED METABOLIC PATHWAY OF DPX-Y6202 IN PLANTS



Assure residue chemistry review

Page 24 is not included in this copy.

Pages _____ through _____ are not included in this copy.

The material not included contains the following type of information:

- ☐ Identity of product inert ingredients
 - ☐ Identity of product impurities
 - ☐ Description of the product manufacturing process
 - ☐ Description of product quality control procedures
 - ☐ Identity of the source of product ingredients
 - ☐ Sales or other commercial/financial information
 - ☐ A draft product label
 - ☒ The product confidential statement of formula
 - ☐ Information about a pending registration action
 - ☐ FIFRA registration data
 - ☐ The document is a duplicate of page(s) _____
 - ☐ The document is not responsive to the request
-

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.
