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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: PP #4G2977. Assure™ on cotton. Evaluation of the analytical methods and residue data, including the amendment of 4/4/84. Accession Numbers 072023, 072024, 250071 and 252848.

FROM: John M. Worthington, Chemist
Residue Chemistry Branch
Hazard Evaluation Division (TS-769)

TO: Robert Taylor, PM. No. 25
Registration Division (TS-767)
and
Toxicology Branch
Hazard Evaluation Division (TS-769)

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

E.I. du Pont de Nemours and Company proposes the establishment of temporary tolerances for residues of the herbicide, ethyl 2-[4-(6-chloroquinoxalin-2-yl-oxy)phenoxy] propanoate, (Assure™ or DPX-Y6202) in or on cotton at 0.05 ppm.

No tolerances have been previously established or proposed for Assure™. PP #4G2978 which proposes temporary tolerances for residues of Assure™ in or on soybeans is also currently pending. The proposed two year experimental program involves the application of approximately 750 lbs. a.i. to 3000 acres of cotton in the first year, and 1750 lbs. a.i. to 7000 acres in the second year.

## Conclusions

1a. The fate of Assure™ in plants has been adequately delineated for the purpose of the proposed temporary tolerance. The parent compound and DPX-Y6202 acid are considered the principal residues of concern.

1b. The proposed tolerance expression must be modified to include the acid metabolite.

2. Adequate methodology is available to determine residues of both the parent compound and its acid metabolite.

3a. The available residue data are adequate to demonstrate that the combined residues of the parent compound and its acid metabolite in cottonseed will not exceed the proposed 0.05 ppm temporary tolerance from the proposed use.

3b. No tolerance proposal for residues in forage is needed because a label restriction prohibiting the feeding or grazing of treated fields has been imposed.

3c. For the purpose of the proposed temporary tolerance no cottonseed fraction data will be needed.

4. For the purpose of the proposed temporary tolerance, the proposed use falls under Category 3 of Section 180.6(a).

## Recommendations

1. Contingent upon the revision tolerance expression as indicated in conclusion (1b) above, RCB recommends that the proposed temporary tolerance be granted.

2. For a future permanent tolerance the following will be required:

   a) A complete description of the manufacturing process including a discussion of the reactions, the reaction conditions, impurity of the starting materials and cleanup procedures.

   b) Inclusion of the significant conjugated metabolites in the tolerance expression.

   c) Analytical methodology and appropriate validation data to determine residues of the significant conjugated metabolites.

   d) Residue data determining the levels of the conjugated metabolites in treated cottonseed.

   e) Appropriate cottonseed fraction residue data determining if there is any concentration of residues of DPX-Y6202 or its significant metabolites in cottonseed hulls, meal, oil, or oil soapstock upon processing.

   f) Depending upon the level of residues of conjugated metabolites found in livestock feed items, a ruminant metabolism, conventional animal feeding studies and possibly tolerance proposals for residues in milk, meat, poultry and eggs may be required for a future permanent tolerance.

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g) Information on the interval and storage conditions between harvest analysis, and storage stability data demonstrating that residues are stable under the conditions of storage.

## Detailed Considerations

### Formulation

Assure™ is formulated as a 75% active ingredient dry flowable powder which is made to be mixed with water. Its ingredients are listed as follows:

| | Percent by weight |
|---|---|
| Technical Assure™ | 10.7 |
| [redacted] | |

The inert ingredients of the proposed formulation are cleared under Section 180.1001(c) or (d).

The analysis of five samples of technical Assure™ are are summarized below:

| | Percent by weight |
|---|---|
| Assure™ | 97.7% |
| [redacted] | |

We do not anticipate any specific residue problems with the technical impurities when present at the above levels and applied at the proposed rates.



Impurity Information not included

Only a schematic of the technical Assure™ manufacturing process has been submitted. A discussion of the reactions, the reaction conditions, impurity of the starting materials and cleanup procedures will be required for a future permanent tolerance.

Proposed Use

One or two applications of Assure™ at rates ranging from 1 to 4 oz. active ingredient per acre are proposed for the control of a variety of weeds in cotton

Minimum spray volumes of 15 and 5 gallons for ground and aerial applications, respectively, are required.

A restriction against the grazing or harvesting of treated fields has been imposed and an 80 day preharvest interval is required.

Nature of the Residue

Plant Metabolism: An interim radiotracer soybean metabolism study using both $^{14}$C-phenyl ring and $^{14}$C-quinoxaline ring labeled DPX-F6025 has been submitted. Field grown beans were treated post-emergence with labeled Assure™ at a rate equivalent to 4.0 oz act./acre and periodically analyzed. A short term greenhouse experiment was also conducted at two times the maximum proposed rate.

The field grown plants were treated by spraying with a hand held sprayer before any beans were present. Samples were taken for analysis on day 0, and at 3 and 5 weeks after treatment. The experimental protocol also calls for analysis of the mature soybeans. The greenhouse grown soybeans were treated 19 days after germination with either the $^{14}$C phenyl or quinoxaline ring labeled DPX-F6025 and sampled at 0, 6 and 10 days after treatment.

Investigations into the nature of the activity involved removing the surface residues with a methylene chloride/acetone wash and extraction of the plant material with 1:1 methylene chloride/acetone solution. The extract was filtered and reduced in volume by evaporation until only the water remained. The residues in the aqueous extract were then partitioned into methylene chloride. The extracted plant material was reextracted with an acetone/ethanol/water solution to remove the polar residues. The residual extract was then filtered.

The level of unextractable activity was determined by combustion. The total activity in initial washes and the polar and organic fractions was determined by liquid scintillation counting. The various fractions were cleaned up further on a Bond-Elut® column and analyzed by thin layer chromatography.

About 80% of the the activity found in the greenhouse samples analyzed at days 6 and 10 was extracted using the above extraction procedures. Approximately 90% of the recovered activity was extractable on day 0. The average amounts of DPX-Y6202, per se, at 0, 6 and 10 days were 48, 8.5 and 4.3% of the total activity, respectively.

The major metabolite, 2-[4-(6-chloroquinoxalin-2-yl-oxy)phenoxy] propionic acid (DPX-Y6202 acid), accounted for about 41.8, 49.2 and 27.6% at 0, 6 and 10 days, respectively. After 6 days about 20% of the activity was not extractable using the above procedures. At day 6 and 10 approximately 23 and 43%, respectively, of the activity remained at the origin of the of the chromatographic plate.

This study made only preliminary investigations into the nature of the residues in the field treated soybeans. The available data indicate that about 0.3 ppm phenyl ring labeled activity and about 0.4 ppm quinoxaline ring labeled activity was found in in the three week old beans and pods. At five weeks the pods could be separated from the beans. The level of activity in the pods treated with phenyl ring labeled Assure™ was 0.10 ppm while the level in the beans was 0.05 ppm. The corresponding levels for the quinoxaline ring labeled activity were 0.06 and 0.03 ppm respectively. Generally, about 30% of the recovered activity was found to be bound or unextractable.

A second metabolism study investigating the fate of Assure in corn, and soybeans has also been submitted. The corn and soybean plants were grown from seed under greenhouse conditions. The leaves of the plants were treated individually with 2.3 ug. (in 2 ul drops). The plants were harvested 2, 4, 8 and 14 days after treatment and divided into treated leaves, untreated leaves, germ, stem, cotyledons and root fractions.

Surface residues were removed from the treated leaves with a chloroform wash. The various fractions were homogenized with a 70% aqueous methanol solution and the homogenate centrifuged. The total activity in the residual precipitate was determined by combustion.

The leaf washings were concentrated and analyzed by TLC. The extracts were reduced to a small volume, diluted with distilled water and partitioned against chloroform. Both the aqueous and organic fractions were also analyzed by TLC. The aqueous soluble residues were also subjected to beta-glucosidase enzymatic hydrolsis. After hydrolysis, the mixture was reextracted with ether and the residues analyzed by TLC.

The radioautographs showed that DPX-Y6202 is absorbed and translocated more rapidly by soybeans than corn plants. After 14 days about 11% of the recovered activity in soybean plants had moved from the treated leaves. The corresponding figure for corn was only 4.7%.

In soybeans after 14 days, the parent compound accounted for 11.4% of the total recovered activity. The two principal metabolites DPX-Y6202 acid and 4-(6-chloroquinoxalin-2-yl-oxy)phenol (DPX-Y6202 phenol) accounted for 13% and 1.8% of the activity. About 20% of the recovered activity was found in a band containing glucosidic conjugates of the phenol and acid metabolites. Therefore, about 34% of the activity present in the soybean plants at 14 days has been characterized. The majority of the activity was unextractable and remains uncharacterized. A similar pattern of metabolites was also found in the corn plants.

In a third study the fate of DPX-Y6202 was investigated in sugar beets. The sugar beets were treated at the rate of either 40 or 60 ug per plant in 40 and 60 ul of solution, respectively. The plants were harvested 1, 3, 7, 14 and 28 days after treatment and divided into treated leaves, untreated leaves and root fractions.

The treated leaves were washed with aqueous methanol and then extracted with aqueous methanol. The activity present in the insoluble residue was determined by combustion. The total activity present in the extracts and the washings were determined LSC. The washings and the extracts (separately) were then reduced in volume by evaporation, acidified and partitioned against chloroform. The activity present in the organic layer was concentrated and analyzed by TLC.

Translocation within the sugar beet plant was very limited. After 28 days only about 1% of the recovered activity had moved from the treated leaves. Approximately 10-27% of the applied activity was lost due to volatilization.

After 28 days, the parent compound still accounted for 70-90%. DPX-Y6202 acid and DPX-Y6202 phenol only amounted to 0.9 and 0.1%, respectively. of the total recovered activity. None of the remaining bands, including those containing the conjugated metabolites accounted to more than 3% of the recovered activity.

For the purpose of the proposed temporary tolerance, we can consider the fate of Assure™ in plants adequately delineated. The parent compound and DPX-Y6202 acid are considered the principal residues of concern.

However, for a future permanent tolerance, DPX-Y6202 phenol and the conjugates of the two metabolites will have to be included in the tolerance expression.

Animal Metabolism: No animal metabolism data are presented in support of the subject petition. The proposed use involves several animal feed items: cottonseed hulls, meal, oil, and oil soapstock. However, residues are expected to be extremely low in the treated cotton seed after the 80 day preharvest interval.

Therefore, considering the low level of total residues expected in cottonseed, the fact that the feeding of treated forage has restricted and the fact that soybeans and corn rapidly metabolize DPX-Y6202, RCB can conclude that for the purpose of the proposed temporary tolerance that there is no reasonable expectation of secondary residues of DPX-Y6202, per se, or DPX-Y6202 acid in meat, milk, poultry or eggs.

Until additional information is available on the levels of the conjugated metabolites in cottonseed and its fractions, RCB cannot determine whether a ruminant metabolism study will be needed for a future permanent tolerance.

Analytical Methods

The proposed analytical method for residues of Assure™ in crops involves homogenizing a ten gram sample with either ethyl acetate or acetonitrile. The sample is then centrifuged, filtered, reduced to a small volume by evaporation and filtered through a Millex-SR disposable filter unit. After separation on a size exclusion column, the sample is chromatographed on a Florisil column and the level of DPX-Y6202 determined by HPLC using either a UV or fluorescence detector. The proposed procedure is reportedly sensitive to 0.02 ppm DPX-F6025 in cotton. Recoveries of Assure™ from cotton fortified at levels ranging from 20 to 100 ppb ranged between 80 and 110% and averaged 100%. All control values are reported as <0.02 ppm.

The proposed procedure is considered adequate to determine residues of DPX-Y6202, per se, in plant tissue.

A second method has been developed for the determination of the primary metabolite, DPX-Y6202 acid. A 10 g. sample is homogenized with a 50:50:0.2 mixture of acetone:methylene chloride:glacial acetic acid and centrifuged. After the extract is reduced in volume, it is diluted with acetonitrile and washed with hexane. The acetonitrile is cleaned up with a Bond Elut™ cartridge and chromatographed on a medium pressure silica column. The level of DPX-Y6202 acid is finally determined with an HPLC equipped with an UV detector.

The proposed procedure is reportedly sensitive to 0.02 ppm DPX-F6205 in cotton. Recoveries of DPX-Y6202 acid from cotton fortified at levels ranging from 20 to 100 ppb ranged between 62 and 108% and averaged 89%. All control values are reported as <0.02 ppm.

The proposed procedure is considered adequate to determine residues of DPX-Y6202 acid in plant tissue.

Analytical methodology and appropriate validation data to determine residues of the conjugated metabolites will be required for a future permanent tolerance.

Residue Data

A summary of the results from 13 experiments conducted in 10 different states have been submitted. Cottonseed samples were analyzed for residues of Assure™ and the its acid metabolite after treatments at up to two times the maximum proposed rate. The reported preharvest intervals ranged from 24 to 150 days. None of these samples showed any detectable (<0.02 ppm) residues of either the parent compound or DPX-Y6202 acid.

In lieu of tolerance proposals for residues in forage, a label restriction against the harvesting or grazing of treated forage has been imposed.

No residue data have been submitted for cottonseed fractions. However, considering the 80 day preharvest interval, the fact Assure™ is metabolized fairly rapidly and that no detectable residues have been found in cottonseed harvested as early as 28 days after treatment at exaggerated rates, no residue data for cottonseed fractions will be needed for the proposed temporary tolerance.

Residue data that determine the levels of the significant conjugated metabolites in cottonseed will be needed for future permanent tolerance.

Appropriate cottonseed fraction residue data determining if there is any concentration of residues of DPX-Y6202 or its significant metabolites in cottonseed hulls, meal, oil, and oil soapstock upon processing will be required for a future permanent tolerance.

No information on the interval and storage conditions between harvest and analysis has been submitted. This data and data demonstrating that residues are stable under the conditions of storage will be required for a future permanent tolerance.

Meat, Milk, Poultry, and Eggs

Considering the very low levels of residues expected in treated cotton, we can conclude that for the purpose of the proposed temporary tolerance the proposed use falls under Category 3 of Section 180.6(a).

Depending upon the level of residues of conjugated metabolites found found in livestock feed items, appropriate animal feeding studies and possibly tolerance proposals for residues in milk, meat, poultry and eggs will be required for a future permanent tolerance.

cc: Reading file, Circu, Reviewer, PP# No. 4G2977, FDA
EEB, EAB, TOX, R. Thompson

TS-769:Reviewer:JMWORTHINGTON:Date:4/10/84
RDI:Section Head:ARR:Date:4/17/84:RDS:Date:4/18/84

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