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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: PP#5F3252/FAP#6H5479 [RCB Nos. 487 and 488]—
DPX-Y6202 (Assure®) Herbicide on Cotton and
Soybeans. Evaluation of a Goat Metabolism
Study Protocol Dated February 5, 1985 (No
Accession Number)

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

TO: Robert J. Taylor, PM 25
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Registration Division (TS-767C)

and

Toxicology Branch
Hazard Evaluation Division (TS-769C)

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

Background

E.I. du Pont de Nemours and Company has submitted this protocol in response to Deficiency No. 4 of the Residue Chemistry Branch (RCB) September 25, 1985 review of PP#5F3252 (M. Firestone).

RCB has previously recommended against establishment of the proposed DPX-Y6202 tolerances for cottonseed, soybeans, and processed soybean fractions (hulls, meal, soapstock, flour)

because of numerous deficiencies involving product chemistry (nos. 1a, 1b, and 1c), Section B/proposed use (no. 2), definition of the DPX-Y6202 tolerance expression (no. 3a), animal metabolism (no. 4), analytical methodology (no. 5), storage stability (no. 6), residue data (noš. 7a and 7b), processing studies for soybeans and cottonseed (no. 8), and possible secondary residues in animal commodities (no. 9) (see M. Firestone memorandums of September 25, 1985, October 23, 1985, and November 9, 1985 re: Deficiencies 1 through 6, 7a/b, and 8, respectively).

Current Considerations

Deficiency 4 states the following:

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.

In response, the petitioner has decided to conduct a goat metabolism study. The present submission consists of a protocol for such a study as well as a chart of the metabolic pathway of DPX-Y6202 in rats and a short discussion of comparative metabolism in rats versus cotton and soybeans.

In cotton and soybean plants, the metabolism of DPX-Y6202 involves cleavage at three sites and conjugation with plant sugars (see M. Firestone memorandum of September 25, 1985). The three cleavage sites are as follows:

1. Hydrolysis of the ethyl ester;
2. Cleavage of the enol ether linkage between the phenyl and quinoxaliny rings;

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

Hydrolysis of DPX-Y6202 at site 1 leads to formation of the primary metabolite DPX-Y6202 acid, which can be conjugated to plant sugars. The petitioner reports that plant conjugates of DPX-Y6202 acid are rapidly cleaved by cow rumen fluid back to the acid.

Phenol metabolites in plants are formed to a lesser degree than the acid metabolite by cleavage at sites 2 and 3.

In rats, DPX-Y6202 acid and the phenol metabolites are all formed, in addition to hydroxylated metabolites of DPX-Y6202 and DPX-Y6202 acid.

Since plant metabolites are common to rats, and since cow rumen fluid can cleave conjugated forms of the primary metabolite DPX-Y6202 acid back to its free (acid) form, RCB concludes that the material fed to goats in the proposed metabolism study should consist of parent compound only. This conclusion is in accord with recent RCB policy which states that animal metabolism studies should reflect feeding of one compound, usually parent, especially if plant metabolites are also found to be animal metabolites (see C. Trichilo memorandum of April 16, 1985 re: Material Fed in Animal Metabolism Studies).

Since the results of plant and rat metabolism studies have shown that metabolic cleavage can occur between the phenyl and quinoxindinyl rings, each portion of DPX-Y6202 should be labeled in separate experiments.

In the current amendment, the petitioner (DuPont) has included a protocol developed by Hazleton Laboratories America, Inc. (Protocol LA-143), entitled "Metabolism Study of Three ¹⁴C-Labeled Test Materials in Lactating Goats" (dated February 1984).

The study will involve four lactating goats, which includes one control animal. The three test goats will be fed 50 mg DPX-Y6202 per day, administered orally in gelatin capsules, for seven consecutive days.

Milk, urine, and feces samples will be collected twice daily during the study. All test animals will be sacrificed within 24 hours following the last dose.

The protocol provides information concerning animal health examination, housing and environmental conditions, animal diet, acclimation period, dose preparation, milking, urine and feces collection, and sacrifice, blood and tissue (muscle, liver, kidney and fat - renal and omental) collection.

According to the protocol, all samples will be weighed and stored frozen pending analysis. Solid samples (feces and tissues) will be homogenized, minced, or otherwise prepared prior to freezing.

RCB concludes that the protocol is in accord with the Pesticide Assessment Guidelines - Subdivision O - Residue Chemistry (§171-4(a)(3)). However, the petitioner should consider the following:

1. Separate experiments should be performed involving feeding of either ^{14}C -phenyl or ^{14}C -quinoxalinyll labeled DPX-Y6202.
2. The petitioner should attempt to free conjugated residues using acid, base and enzymatic hydrolysis techniques.
3. The petitioner should report results in terms of percent of total administered dose, percent of the total radioactivity residue in each tissue, urine, feces, or milk sample, and actual ppm ^{14}C -activity level for each terminal residue.
4. The goat metabolism report should include photographs of thin-layer chromatographic (TLC) plates, paper chromatograms and/or gas-liquid chromatography (GLC) chromatograms used to identify ^{14}C -containing residues.
5. The study should result in elucidation of the efficiency of extraction of the various components of the residue so that extraction/residue release procedures can be developed as part of the regulatory methods. Also, representative samples from the ^{14}C -metabolism studies

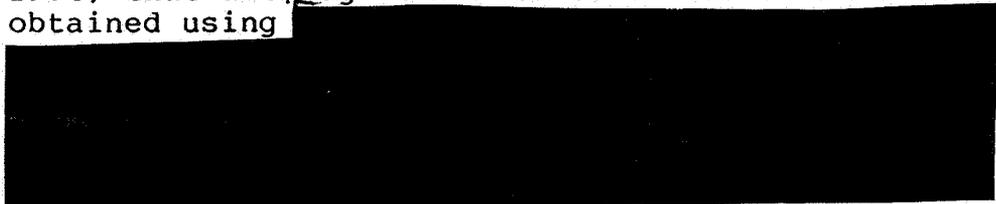
should be analyzed by the proposed enforcement methodology so that a determination of the percent of total radioactive residue determined by the enforcement methodology can be made.

Other Considerations

An International Residue Limit Status sheet is included in RCB's previous (November 29, 1985) review of PP#5F3252/FAP#6H5479. Since no Codex, Canadian, or Mexican limits/tolerances have been established for residues of DPX-Y6202 in/on cotton or soybeans, there are no compatibility problems at this time.

Recommendation

At this time, RCB continues to recommend against establishment of the proposed DPX-Y6202 tolerances for cotton, soybeans, and their processed fractions because of the following deficiencies:

- 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 memorandum 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.

- 1c. All inerts in the formulated product are cleared for use on growing crops except one identified in the

Confidential Appendix of RCB's September 25, 1985 review (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 postharvest interval (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 raw agricultural commodities (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 (ruminant, poultry and/or swine) 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 DPX-Y6202 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.
- 7a. 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).
- 7b. RCB can reach no conclusion regarding the acceptability of the supplemental cottonseed residue data submitted in a September 10, 1985 amendment until a detailed description of Method No. AMR-154-83A, as well as representative chromatograms, are submitted. If Method AMR-154-83A does not contain an acceptable hydrolysis step capable of releasing conjugated DPX-Y6202 acid residues, the supplemental cottonseed residue data will probably be considered inadequate (note: plant metabolism studies indicate that a hydrolysis step is needed to release conjugated DPX-Y6202 acid residues).

In any case, the petitioner will still be required to submit residue data for the following three phenol metabolites (free plus conjugates) cited under Deficiency 3a generated on treated cottonseed and soybeans:

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

- 8a. With regard to the soybean processing study:
- i. The data are considered inadequate since they do not reflect residues of DPX-Y6202 acid conjugates and the three possible phenol metabolites of concern.
 - ii. Depending on resolution of the issue of storage stability (see Deficiency 6), either reanalysis of reserve samples should be performed (in which case information concerning the length of storage between harvest, processing, and analysis should be submitted), or a new soybean processing study will be needed in which analysis includes DPX-Y6202, its acid metabolite (free plus conjugates) and Phenols 1, 2, and 4 (free plus conjugates).
 - iii. The petitioner should submit a copy of Method AMR-153-83, Revision A, Appendix A, which was not included in the original petition.
- 8b. The petitioner will still need to conduct a cottonseed processing study in which the treated samples contain field weathered detectable residues (this may require treatment at exaggerated rates and PHI's of less than 80 days), and the residues to be analyzed for include DPX-Y6202, its acid metabolite (free plus conjugates), and its three possible phenol metabolites of concern (free plus conjugates).

With regard to the proposed goat metabolism protocol submitted in response to Deficiency 4 above, RCB recommends the following:

1. Since plant metabolites are common to rats, and since cow rumen fluid can cleave conjugated forms of the primary metabolite DPX-Y6202 acid back to its free (acid) form, RCB concludes that the material fed to goats in the proposed metabolism study should consist of parent compound only. This conclusion is in accord with recent RCB policy which states that animal metabolism studies should reflect feeding of one compound, usually parent, especially if plant metabolites are

also found to be animal metabolites (see C. Trichilo memorandum of April 16, 1985 re: Material Fed in Animal Metabolism Studies).

2. Since the results of plant (cotton and soybean) metabolism and rat metabolism studies have shown that metabolic cleavage can occur at the enol ether linkage between the phenyl and quinoxalinyll rings, separate experiments should be conducted in which either the phenyl or the quinoxalinyll rings are labeled.
3. RCB concludes that the proposed protocol is in accord with the Pesticide Assessment Guidelines - Subdivision - O Residue Chemistry (§171-4(a)(3)). However, the petitioner should consider the following:
 - a. Separate experiments should be performed involving feeding of either ^{14}C -phenyl or ^{14}C -quinoxalinyll labeled DPX-Y6202.
 - b. The petitioner should attempt to free conjugated residues using acid, base and enzymatic hydrolysis techniques.
 - c. The petitioner should report results in terms of percent of total administered dose, percent of the total radioactivity residue in each tissue, urine, feces or milk sample, and actual ppm ^{14}C -activity level for each terminal residue.
 - d. The goat metabolism report should include photographs of TLC plates, paper chromatograms and/or GLC chromatograms used to identify ^{14}C -containing residues.
 - e. The study should result in elucidation of the efficiency of extraction of the various components of the residue so that extraction/residue release procedures can be developed as part of the regulatory methods. Also, representative samples from the ^{14}C -metabolism studies should be analyzed by the proposed enforcement methodology so that a determination of the percent of total radioactive residue determined by the enforcement methodology can be made.

cc: R.F., Circu, MPFirestone, EAB, EEB, PMSD/ISB, FDA,
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