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

MAY 18 1988

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: PP#8G3602, 352-EUP-RUL. **DPX-M6316** (Pinnacle) on **soybeans.** Evaluation of analytical method and residue data. (MRID#'s 404676-01, -02, -03, -04, -10, -11, -12, and -13) [RCB#'s 3400, 3401]

FROM: Richard Loranger, Chemist R. Loranger
Residue Chemistry Branch
Hazard Evaluation Division (TS-769C)

THRU: Andrew Rathman, Section Head
Residue Chemistry Branch
Hazard Evaluation Division (TS-769C)

TO: R. Taylor/V. Walters, PM Team 25
Registration Division (TS-767C)
and
Toxicology Branch
Hazard Evaluation Division (TS-769C)

E.I. du Pont de Nemours and Company has proposed a temporary tolerance of 0.1 ppm for residues of the herbicide **DPX-M6316** (methyl 3-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino] carbonyl]amino]sulfonyl]-2-thiophene carboxylate) on soybeans.

This herbicide is also known by the names **Pinnacle**^{TM} and **Harmony**^{R} (latter formulation for use on wheat and barley). The common name thiometuron-methyl has been rejected by ISO and sulfothiphenuron has been proposed in its place (info. from Charlotte Blalock, 5/2/88).

Residue Chemistry Branch has recently recommended for 0.05 ppm permanent tolerances for DPX-M6316 in wheat and barley grain and 0.1 ppm tolerances in wheat and barley straw (C. Deyrup, 5/3/88, PP# 6F3431).

The temporary tolerance is requested to run concurrently with an experimental use permit (EUP) from 5/1/88 to 5/1/90. Up to 15 total pounds active ingredient will be tested each year on 3850 acres in 17 states. The test sites will compare Pinnacle with crop oil concentrate or surfactant to the same combination plus liquid nitrogen fertilizer.

CONCLUSIONS

1. Radiolabeled studies conducted on wheat and soybeans show that the major metabolic pathways for DPX-M6316 are hydrolysis of the methyl ester and sulfonylurea linkages. With the very low residues observed from the uses on these crops the only residue to be regulated is the parent compound.

2. Based on the goat metabolism study submitted previously, similar metabolic paths appear to be operating in animals. However, with the low dietary burden for livestock ingesting treated soybeans, we reiterate our conclusion (made in connection with the use on wheat) that feeding studies and meat/milk tolerances are not required. This use falls under 40 CFR 180.6(a)(3)-no reasonable expectation of finite residues in meat, milk, poultry or eggs.

3. An adequate analytical method has been submitted to measure residues of DPX-M6316 in soybeans. The procedure involves a basic pre-soak which has been shown to extract aged residues from wheat straw treated with ^{14}C-DPX-M6316.

4. Field trials conducted at 4 and 16 times the proposed rate show residues of DPX-M6316 less than the method's quantitation limit of 0.05 ppm. Therefore, residues from this experimental use are not expected to exceed the proposed 0.1 ppm tolerance.

5. Although soybean processing studies have not been conducted, the results of the field trials (Conclusion 4) show that residues of DPX-M6316 in hulls, meal, oil and soapstock will not exceed the 0.1 ppm tolerance on beans. Food/feed additive tolerances are not necessary.

RECOMMENDATIONS

TOX and EAB considerations permitting, we recommend for the proposed temporary tolerance for DPX-M6316 on soybeans.

For a permanent tolerance on soybeans additional residue trials throughout U.S. soybean production areas will be required. As noted in the Residue Chemistry Guidelines, the procedures described in the FAO Plant Protection Bulletin, 1981, Volume 29, page 12, should be followed in the design and execution of residue field trials. Provided field trials at exaggerated rates (≥4x) continue to show residues below the 0.05 ppm quantitation limit, processing studies will not be required.

Although it is not a data requirement, we request that du Pont obtain Chemical Abstracts Service Registry numbers for metabolites found in the radiolabeled studies. Structures and chemical names for metabolites are given in Attachment 5 to this review.

DETAILED CONSIDERATIONS

Manufacturing Process and Formulation

Information on the manufacture and composition of technical DPX-M6316 is presented in our 2/12/87 and 12/24/87 reviews of PP# 6F3431. Taking into account the impurity levels and very low application rate of Pinnacle, detectable residues of these compounds will not be present in soybeans.

The product to be used on soybeans is Pinnacle™ Herbicide, a 25% dry flowable formulation. The inerts in Pinnacle are approved for use on crops under 40 CFR 180.1001(c) or (d). The petitioner has submitted a reverse phase liquid chromatography method (MRID# 404676-03) with ultraviolet (230 nm) detection for quantitating the active ingredient in DPX-M6316 technical and formulations. We have not reviewed the information on the manufacturing process, discussion of impurity formation, certified limits, and physical/chemical properties of the end-use product (MRID#'s 404676-01, -02, -04).

Proposed Use

Pinnacle Herbicide is a 25% active dispersible granule formulation to be mixed with water and sprayed broadcast for postemergence weed control in soybeans. It can be applied any time after the first trifoliate leaf has fully expanded up to 60 days before harvest. Apply to young actively growing weeds by ground (min. 10 gallons spray per acre) or air (min. 5 gallons) at a maximum rate of 0.25 ounce product per acre (0.0625 oz ai per acre). A surfactant must be included at a concentration of 1 pt/100 gallons (0.125%). Use an EPA approved surfactant with a minimum 80% active ingredient. One gallon of liquid nitrogen fertilizer per acre is needed to control velvetleaf. Do not graze or feed forage, hay or straw from treated areas to livestock.

Nature of the Residue

Plant Metabolism

Metabolism studies utilizing ^{14}C-triazine and ^{14}C-thiophene labeled DPX-M6316 on wheat were reviewed by RCB in PP#6F3431. The herbicide was observed to be metabolized by hydrolysis of the methyl ester and sulfonylurea moieties. Some demethylation of the triazine methoxy group also occurred. Extrapolating from wheat straw to grain we estimated that the various metabolites would be present at levels of 0.07-1.3 ppb each in wheat grain and deferred to Toxicology Branch on the significance of these levels. TB replied there is not a need to include these metabolites in the tolerance expression (M. van Gemert, 3/1/88).

Additional radiolabeled studies on soybeans are submitted in the present petition (MRID#'s 404676-10 and -11). Residues were identified in green foliage 0, 7 and 30 days after treatment of young plants. These show the same hydrolytic pathways noted above for wheat (see our attached Data Evaluation Records for more details). Also, these studies demonstrated that the addition of surfactant has a dramatic effect on the penetration of DPX-M6316 into plant tissues. This penetration resulted in a much higher degree of metabolism/hydrolysis of the herbicide.

Considering that total activity in mature soybeans was 0.4-1.6 ppb in these radiolabeled studies conducted at ≈2 and 4x the proposed application rate, we conclude that the residue to be regulated is just the parent compound as was done with wheat. Although the label permits application at a later stage (60 day PHI) than in the ^{14}C studies (100 day PHI), the results of the residue trials lead us to believe that detectable levels of metabolites are unlikely in soybeans at the currently proposed rate (0.0625 oz ai/A).

Animal Metabolism

A goat metabolism study is discussed in our 12/24/87 review of PP#6F3431. ^{14}C-triazine or ^{14}C-thiophene labeled DPX-M6316 was administered to lactating goats for 7 consecutive days at 50 mg/day or 28 ppm in the whole diet. Total radioactivities were as follows: milk 0.08-0.16 ppm, muscle ND-0.03 ppm, fat-ND, kidney 0.10-0.16 ppm, and liver 0.04-0.05 ppm. The parent compound comprised a major portion of the residue in urine (77-92%), milk (69-81%), and kidney (44-50%). Metabolites in milk were the result of hydrolytic cleavage of the methyl ester and sulfonylurea groups. Some O-demethylation was also observed. Thus, based on the milk residues, the same metabolic pathways appear to be operating in plants and animals. Residues in tissues other than kidney were not characterized.

The dietary burdens for cattle and poultry as a result of the use on soybeans will be 0.0125 ppm and 0.025 ppm using the 0.05 ppm quantitation limit of the method as the maximum residue in beans. The goat study represents ingestion of 2240x and 1120x times these levels. A linear extrapolation predicts total residues in milk or tissues of ≈0.1 ppb from consumption of treated soybeans. As in PP#6F3431 (where the dietary burden from wheat straw was 0.01 ppm), we conclude that feeding studies and meat/milk tolerances are not necessary.

Analytical Methods

Method AMR-973-87 has been provided to determine residues of DPX-M6316 in soybeans (MRID# 404676-12). In brief, the procedure entails soaking ground soybeans in sodium bicarbonate solution, acidification, extraction into methylene chloride, cleanup on a silica solid phase column, and quantitation by normal phase liquid chromatography with a photoconductivity detector.

The basic pre-soak has been shown to efficiently extract aged ^{14}C residues from wheat straw in PP#6F3431. The pre-soak, extraction, and determinative steps are the same as methods in that petition. The cleanup procedure differs in that the earlier methods used partitionings instead of the silica column.

Recoveries of 0.05-1.0 ppm DPX-M6316 from soybeans were 56-112% (88% average). See our attached DER for more details. We conclude that the method is satisfactory for measuring DPX-M6316 residues in soybeans down to a quantitation limit of 0.05 ppm.

Storage Stability Data

Data demonstrating the stability of DPX-M6316 residues on wheat straw and grain after 2 and 3 years, respectively, of frozen storage are discussed in our 12/24/87 review of PP#6F3431. Additional data on corn kernels and corn forage are presented in the residue data volume (MRID# 404676-13) of the present petition. Samples were spiked with 0.1 ppm DPX-M6316 and analyzed after 1,3,6,12,18 and 24 months of frozen storage. Recoveries of the initial fortifications were 80-119%. The data in the current petition were obtained on soybeans stored 2-14 months after collection and thus are acceptable.

Residue Data

The petitioner has reported the results of 10 field trials conducted in 1986-87 using 25% or 75% dry flowable formulations of Pinnacle (MRID# 404676-13)(see attached DER for details). The rates examined included 1.0 oz ai/A without surfactant and 0.25 oz ai/A with 0.1-0.25% added surfactant. The latter truly depicts 4x the proposed rate. The former represents 16x the requested rate of active ingredient, but is really not equivalent to such an exaggeration as it does not include the surfactant, which has been shown to greatly increase penetration into plant tissues. Treatments were made 45-68 days prior to harvest, simulating the proposed 60 day PHI. In all 14 samples of beans residues of DPX-M6316 were below the 0.05 ppm quantitation limit of the method. Since both a control and treated soybean chromatogram contained a small peak equivalent to about 0.01-0.02 ppm DPX-M6316, we can not determine whether trace residues are present at levels below the quantitation limit. We can conclude, however, that the 0.1 ppm tolerance will not be exceeded from this experimental use.

Although a processing study was not run to determine whether residues concentrate in soybean hulls, meal, oil or soapstock, the absence of residues in beans from the 4x and 16x field trials allows us to conclude that DPX-M6316 levels in these commodities will not exceed the 0.1 ppm bean tolerance. Food/feed additive tolerances are not required.

Meat, Milk, Poultry and Eggs

As discussed above under the Nature of the Residue section, using the ^{14}C goat study as a reference point we estimate that total residues in milk or tissues from ingestion of treated soybeans would be on the order of ≈0.1 ppb. We consider the proposed use to fall under 180.6(a)(3)-no reasonable expectation of finite residues in meat, milk, poultry and eggs. Tolerances for these commodities are not needed.

ATTACHMENTS (to all copies)

1. DER-plant metabolism, MRID# 404676-11, 6 pages.
2. DER-plant metabolism, MRID# 404676-10, 6 pages.
3. DER-analytical method, MRID# 404676-12, 3 pages.
4. DER-residue data and storage stability, MRID# 404676-13, 3 pages.
5. Structures and chemical names of DPX-M6316 and metabolites, 2 pages.

cc: Circu, RF, PMSD/ISB, Loranger, PP#8G3602
RDI:Section Head:ARRathman:5/17/88:RDSchmitt:5/17/88
TS-769:RCB:557-7887:RAL:ral:CM#2:Room 810:Date:5/17/88
Disk 01:Files PINNACL1.PP and PINNACL1.DER thru PINNACL4.DER

ATTACHMENT 1 TO RCB# 3400 MAY 1988

Reviewed by: Richard Loranger R. Loranger
Chemist, Residue Chemistry Branch
Secondary Reviewer: Andrew Rathman
Section Head, Residue Chemistry Branch

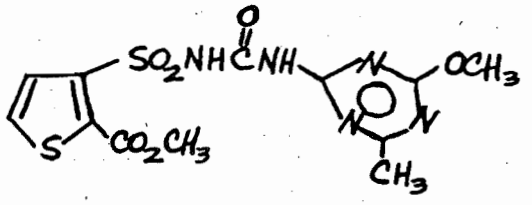
DATA EVALUATION RECORD

STUDY TYPE: Plant (soybean) metabolism

REPORT TITLE: "Metabolism of [Triazine-2-^{14}C]DPX-M6316 in Greenhouse-Grown Soybean Plants"

MRID NUMBER: 404676-11 **RCB#'s** 3400, 3401 **PP#:** 8G3602

TEST MATERIAL: methyl 3-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino]sulfonyl]-2-thiophene carboxylate labeled with ^{14}C in 2-position of triazine ring



other names: DPX-M6316; Pinnacle^{TM}; Harmony^{R} sulfothiphenuron (ISO proposed)
CAS# 79277-27-3

STUDY I.D. #: Laboratory Project ID AMR-547-86

AUTHOR: L. B. Brattsten

TESTING LABORATORY: E.I. du Pont de Nemours & Company, Inc.

DATE OF REPORT: March 23, 1987

STUDY SPONSOR: E.I. du Pont de Nemours & Company, Inc.

CONCLUSIONS:

The residues identified in green soybean foliage show two metabolic pathways operating on DPX-M6316: hydrolysis of the methyl ester group and hydrolysis of the sulfonylurea bridge. Use of a surfactant leads to greater penetration of the herbicide into plant tissues and a higher degree of metabolism. Without surfactant the parent compound is the major residue throughout the sampling period (30 days after treatment). However, the hydrolysis products predominate within 7 days when surfactant is added. For actual levels of each component of the residue refer to the table on page 5 of this DER. A list of the identified components follows on the next page.

List of compounds identified:

methyl 3-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino] carbonyl]amino]sulfonyl]-2-thiophene carboxylate
CAS# 79277-27-3

3-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl] amino]sulfonyl]-2-thiophene carboxylic acid
(CAS# not provided)

4-methoxy-6-methyl-1,3,5-triazine-2-amine
(CAS# not provided)

4-amino-6-methyl-1,3,5-triazin-2-ol (CAS# not provided)

TEST MATERIALS AND METHODS

The radiolabeled DPX-M6316 was synthesized by Du Pont NEN Products, Boston, Mass. The ^{14}C was placed in the 2-position of the triazine ring. The chemical and radiochemical purities were 99% and 100%, respectively. The specific activity was 33.9 μCi/mg=13.14 Ci/mole=74.58 x 10^9 dpm/g.

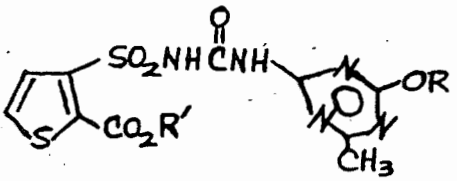
The unlabeled reference standards employed in the study were the following:

L9225; 3-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino] carbonyl]amino]sulfonyl]-2-thiophene carboxylic acid (free acid of DPX-M6316)

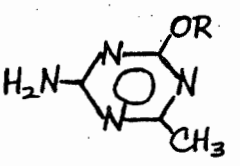
L9226; methyl 3-[[[[(4-hydroxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino]sulfonyl]-2-thiophene carboxylate; "O-demethyl DPX-M6316"

A4098; 4-methoxy-6-methyl-1,3,5-triazine-2-amine; "triazine amine"

B5528; 4-amino-6-methyl-1,3,5-triazin-2-ol; "amino methyltriazinol"



R=CH_3, R'=H L9225
R=H, R'=CH_3 L9226



R=CH_3 A4098
R=H B5528

Soybean seeds (variety Miami) were planted 3/14/86 in unsterilized Sassafras loamy sand in 30 8-inch plastic pots (5 seeds per pot). Watering was done by hand or automatic drip irrigation. 20-20-20 NPK fertilizer was included weekly in the irrigation water. The greenhouse was sprayed or fumigated monthly with assorted insecticides (not applied directly to plants).

The plants were treated at the first trifoliate growth stage on 4/2/86 using a hand-held Preval atomizer. The radiolabeled DPX-M6316 was formulated with certain inert ingredients and dissolved in water to give a concentration of 25.9 ppm (25.9 ug/ml). The high dose treatment entailed spraying 2 ml of this solution per pot. This 51.8 ug DPX-M6316/8-inch diameter pot is equivalent to 16 g/ha or 0.228 oz/A (3.65x the application rate proposed in the present petition). The low dose treatment involved diluting the above solution 1:1 with water and adding 0.25% surfactant (Ortho X-77). Two ml of this solution were sprayed in each pot, giving an application rate equivalent to 8 g/ha or 0.114 oz/A (1.82x the proposed use of 0.0625 oz/A). The spray volume of 2 ml/pot is equivalent to 66 gallons per acre.

Plants were cut at soil level on days 0, 7 and 30 (4/2, 4/9, 5/2) and weighed immediately before rinsing by swirling with 80% ethanol. Rinsed samples were stored at -18°C until analysis. At final harvest (7/10; 100 days) the mature seeds and empty pods were weighed separately. The remaining dry stalks and fallen leaves were apparently not weighed or analyzed in any manner. The rinses of the 0, 7 and 30 day samples were used to quantify any radioactivity on plant surfaces. Aliquots were evaporated in a rotary evaporator, suspended in pH 7.4 phosphate buffer, and analyzed by liquid scintillation counting (LSC) and high performance liquid chromatography (HPLC). The frozen tissues were ground in liquid nitrogen in a mortar and pestle and portions extracted with 80% acetone in a wrist-action shaker. Extracts were stored at -18°C prior to quantifying and identifying the activity inside plants. Extracted tissues were dried and combusted to determine extraction efficiency. Portions of the mature beans and pods were also combusted to determine the total radioactivity.

The HPLC analyses employed a Zorbax ODS column with acetonitrile-water gradient elution. One ml fractions were collected and analyzed by LSC. Major peaks and reference standards were located by UV light (245 nm).

Portions of the 7 day extracts were also examined by thin layer chromatography (TLC). The cold reference standards listed earlier in this section as well as radiolabeled DPX-M6316 were spotted on the same silica gel plates as the extracts. After development the plates were examined under UV light and by a TLC radioscanner.

REPORTED RESULTS AND REVIEWER'S DISCUSSION

The low dose treatment was observed to injure the soybean plants. This can be seen by the plant weights which increased from 3.43 to only 9.69 grams over days 0-30 (versus 3.34 to 22.22 grams in the high dose plants). The injury was apparently due to the added X-77 surfactant aiding penetration of the DPX-M6316 into plant tissues. This is consistent with the results of the ethanol rinses and acetone extractions discussed below.

As can be seen in the following table, total activity in both the high and low dose plants was in the 0.5-0.7 ppm range on the day of application and 7 days later. By 30 days after treatment, however, ^{14}C residues had declined about 8-fold and 3.5-fold for the 16 g and 8 g plants, respectively. These decreases in total residues parallel the growth of the plants as the average weights increased about 7x and 3x (previous paragraph). Most notably, total radioactivity in the mature soybeans was only 0.0004 ppm (0.4 ppb) and 0.0010 ppm (1 ppb) for the 16 g and 8 g treatments, respectively. The empty pods had somewhat higher residues (0.0018 ppm and 0.0042 ppm, respectively).

In the following table we have summarized the total activities (ppm DPX-M6316 equivalents) in whole plants and the amounts of these residues that went into the ethanol rinses and acetone extractions.

RATE	DAY	^{14}C TOTAL PPM	% INTO ETHANOL RINSE	% INTO ACETONE EXTRACT	% BOUND
	0	0.588	84.6	14.9	0.5
16 g/ha	7	0.525	75.6	20.7	3.6
	30	0.068	58.4	34.9	6.7
8 g/ha +	0	0.687	42.9	54.8	2.3
X-77	7	0.638	29.8	61.2	9.0
	30	0.183	16.8	74.3	8.9

The above ppm and %'s are consistent with the raw data (dpm, sample weights, specific activity) given in the tables in the report. The petitioner provided sample calculations of the ppm radioactivity in several samples.

We concur with du Pont that the rinsing data show "considerable augmentation of the penetration of the radioactivity into the plant tissues by the surfactant". With the addition of 0.25% Ortho X-77, only 43% of the 0 day activity was rinsed off by 80% ethanol (versus 85% rinsed off the 16 g/ha plants). The greater penetration is a likely explanation of the plant injury from the lower application rate. With time the % of penetrated ^{14}C increased in both sets of plants. However, the 80% acetone extraction was able to remove most of this penetrated activity as the bound residues were ≤9% of the total ^{14}C in all cases.

Metabolites were identified by matching HPLC retention times with those of reference standards. Identities were also confirmed by TLC. Copies of chromatograms were not provided in the report. (Liquid chromatograms were shown in the concurrrent report using thiophene-^{14}C DPX-M6316).

The petitioner has reported the ppm of DPX-M6316 and metabolites in both the ethanol rinse and acetone extracts of the soybean foliage. In most cases the degree of metabolism in the acetone extracted residues is much higher than in the rinsed residues. For example, 30 days after the 8 g/ha plus surfactant treatment, 55% of the rinse activity was still DPX-M6316, but only 7% of the acetone extract was unchanged parent.

From the rinse and extract values we have calculated the total ppm of each component on a whole plant basis. These are presented in the following table along with the % of the total residue they represent in each case. The ppm are in terms of DPX-M6316 equivalents.

		M6316		L9225		A4098		B5528	
RATE	DAY	ppm	%	ppm	%	ppm	%	ppm	%
	0	0.528	90	0.029	5	0.019	3	0.005	1
16 g/ha	7	0.357	68	0.040	8	0.026	5	0.035	7
	30	0.030	44	0.005	7	0.006	9	0.006	9
8 g/ha	0	0.437	64	0.037	5	0.053	8	0.012	2
+ 0.25%	7	0.220	35	0.128	20	0.083	13	0.046	7
X-77	30	0.018	10	0.050	28	0.027	15	0.024	13

.047

.119

The total % identified in the above 6 soybean foliage samples are 99, 88, 69, 79, 75, and ~~76~~ 66.

From the above results we can concur with du Pont that the use of the surfactant aids the metabolism of DPX-M6316 by the soybean plants. Without surfactant the parent is by far the predominant residue 30 days after treatment. With addition of X-77 the parent comprises only 10% of the 30 day residue while the identified metabolites constitute 56%. The surfactant can thus be seen to have a dramatic effect on the behavior of Pinnacle as also shown by the plant injury and rinsability of residues discussed earlier.

The identified residues show two metabolic pathways at work in the soybean foliage. The free acid L9225 is formed by cleavage of the methyl ester moiety, while the triazines A4098 and B5528 are the result of hydrolysis of the sulfonylurea. Although L9225 is the major individual metabolite when surfactant is used, the sum of the two triazines is ≥ the % of L9225. Therefore, the two hydrolytic pathways appear to be operating at similar rates in soybean foliage.

We note that the same two hydrolytic pathways were observed in a soybean study conducted with ^{14}C-thiophene ring labeled DPX-M6316 (see concurrent DER). The latter study also showed the same effects of surfactant: greater plant injury, more penetration of the herbicide into plant tissues, and a greater degree of metabolism.

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With respect to the soybeans, the radioactivity was so low (0.0004-0.0010 ppm) that characterization of the residue was not possible according to the petitioner. Considering that these very low total activities were observed following applications at 1.82 and 3.65x the rate proposed in the present petition, we will not require actual identification of residues in the beans. The residues identified in foliage are consistent with hydrolytic pathways that could be anticipated for DPX-M6316.

ATTACHMENT 2 TO RCB# 3400 MAY 1988

Reviewed by: Richard Loranger R. Loranger
Chemist, Residue Chemistry Branch
Secondary Reviewer: Andrew Rathman
Section Head, Residue Chemistry Branch

DATA EVALUATION RECORD

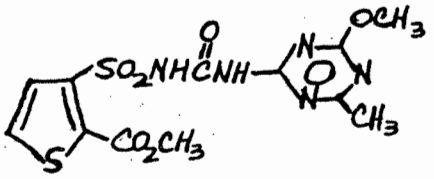
STUDY TYPE: Plant (soybean) metabolism

REPORT TITLE: "Metabolism of [Thiophene-2-^{14}C]DPX-M6316 in Greenhouse-Grown Soybeans"

MRID NUMBER: 404676-10 **RCB#'s** 3400, 3401

PP#: 8G3602

TEST MATERIAL: methyl 3-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino]sulfonyl]-2-thiophene carboxylate labeled with ^{14}C in 2-position of thiophene ring



other names: DPX-M6316; Pinnacle™; Harmony®
sulfothiphenuron (ISO proposed)
CAS# 79277-27-3

STUDY I.D. #: Laboratory Project ID AMR-572-86

AUTHOR: H. M. Brown

TESTING LABORATORY: E.I. du Pont de Nemours & Company, Inc.

DATE OF REPORT: June 8, 1987

STUDY SPONSOR: E.I. du Pont de Nemours & Company, Inc.

CONCLUSIONS:

The results of this thiophene ring label study are consistent with the triazine ^{14}C experiment (see concurrent DER). The metabolic routes for DPX-M6316 in green soybean foliage are hydrolytic cleavage of the methyl ester and sulfonylurea linkages. The ester sulfonamide formed by hydrolysis of the urea can undergo further hydrolyses and internal cyclization. The use of surfactant aids penetration of the herbicide into plant tissue and results in a much higher degree of metabolism.

Actual levels of each component of the residue are shown in the table on page 5 and a list of the full chemical names of identified residues appears below. The corresponding trivial names are given below under Test Materials and Methods.

methyl 3-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino] carbonyl]amino]sulfonyl]-2-thiophene carboxylate
CAS# 79277-27-3

3-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl] amino]sulfonyl]-2-thiophene carboxylic acid ("A")

Methyl 3-(aminosulfonyl)-2-thiophenecarboxylate ("B")

3-(aminosulfonyl)-2-thiophenecarboxylic acid ("C")

Thieno[2,3-d]isothiazol-3(2H)one 1,1-dioxide ("D")

3-sulfo-2-thiophenecarboxylic acid ("E")

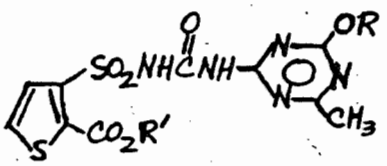
CAS#'s were not provided for any of the metabolites.

TEST MATERIALS AND METHODS

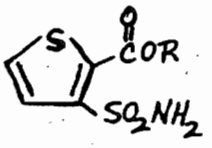
The radiolabeled DPX-M6316 was synthesized by Du Pont NEN Products, Boston, Mass. The ^{14}C was placed in the 2-position of the thiophene ring. The radiochemical purity was >98% with a specific activity of 23.3 μCi/mg.

The reference standards employed in the study included the following (from Table 14-"Metabolite Standards and Their Chromatographic Behavior"):

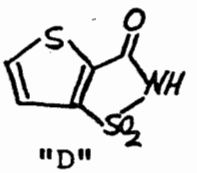
"A"	3-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino] carbonyl]amino]sulfonyl]-2-thiophene carboxylic acid (free acid of DPX-M6316)		
"B"	2-ester-3-sulfonamide	"C"	2-acid-3-sulfonamide
"D"	thiophene sulfonimide	"E"	2-acid-3-sulfonic acid
"F"	O-demethyl-DPX-M6316		
"G"	2-ester-3-sulfonic acid	"H"	2-ester-3-sulfonylurea



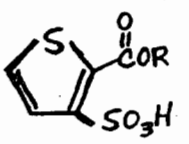
R=CH_3, R'=H "A"
R=H, R'=CH_3 "F"



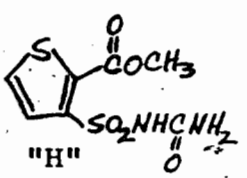
R=CH_3 "B"
R=H "C"



"D"



R=H "E" ; R=CH_3 "G"



"H"

The soybeans (variety Miami) were grown in a greenhouse at the Du Pont Experimental Station (Wilmington, DE). The seeds were planted 3/14/86 in unsterilized Sassafras loamy sand in 30 8-inch plastic pots (5 seeds per pot). Watering was done by hand or automatic drip irrigation. 20-20-20 NPK fertilizer was included weekly in the irrigation water. The greenhouse was sprayed or fumigated monthly with assorted insecticides (not applied directly to plants).

The plants were treated at the first to third trifoliate growth stage on 4/1/86 using a hand-held Preval atomizer. The radiolabeled DPX-M6316 was formulated with certain inert ingredients and dissolved in water to give a concentration of 25.9 ppm (25.9 ug/ml). The high dose treatment entailed spraying 2 ml of this solution per pot. This 51.8 ug DPX-M6316/8-inch diameter pot is equivalent to 16 g/ha or 0.228 oz/A (3.65x the application rate proposed in the present petition). The low dose treatment involved diluting the above solution 1:1 with water and adding 0.25% surfactant (Ortho X-77). Two ml of this solution were sprayed in each pot, giving an application rate equivalent to 8 g/ha or 0.114 oz/A (1.82x the proposed use of 0.0625 oz/A). The spray volume of 2 ml/pot is equivalent to 66 gallons per acre.

Whole plants were harvested on the day of application and 7 and 30 days later. The entire 0 and 7 day plants were rinsed with 80% ethanol and frozen at -20°C until analysis. Only the lower leaves (those likely to have been sprayed) were rinsed in the 30 day plants. Aliquots of the rinses were analyzed by liquid scintillation counting (LSC). Other aliquots were concentrated and suspended in pH 7.5 phosphate buffer by a wrist-action shaker. Aliquots of these solutions were analyzed by LSC and HPLC (Zorbax ODS column, CH_3CN-H_2O gradients). One mL fractions were collected off the latter and counted by LSC.

The ethanol rinsed foliage was homogenized by grinding with liquid nitrogen. Aliquots were combusted to $^{14}CO_2$ to determine the total activity still remaining in the plant tissues. Other portions were extracted 3 times with 80% acetone in a wrist-action shaker. The extracts were isolated by centrifugation and examined by LSC, HPLC and thin layer chromatography (TLC) (silica gel plates, UV and autoradiography detection). The activity remaining in the extracted foliage was determined by combustion.

The mature soybeans and pods were collected 100 days after treatment with DPX-M6316 and homogenized in a Waring blender prior to combustion to determine total radioactivity.

Metabolites were identified by their HPLC retention time and confirmed by R_f values obtained by the silica gel TLC.

REPORTED RESULTS AND REVIEWER'S DISCUSSION

"Some injury was observed" in both sets of plants as the rates used are stated to be the highest tolerated by soybeans under greenhouse conditions. However, "the soybeans grew out of this initial injury." We note that the injury was more severe with the lower application rate (8 g/ha), which included the surfactant X-77. Plant weights increased about 7x (3.5 to 24 g) over the 30 days following the 16 g/ha application, but only about 3.5x (3.35 to 11.6 g) after use of the surfactant with 8 g DPX-M6316 per hectare. These results are very similar to those obtained with the triazine labeled metabolism study (discussed in a concurrent DER).

The following table includes the total activities (ppm DPX-M6316 equivalents) in whole plants and the amounts of these residues that went into the ethanol rinses and acetone extractions.

RATE	DAY	^{14}C TOTAL PPM	% INTO ETHANOL RINSE	% INTO ACETONE EXTRACT	% BOUND
	0	0.872	85.6	14.1	0.2
16 g/ha	7	0.478	78.2	18.1	3.3
	30	0.092	56.5	34.8	8.7
8 g/ha + X-77	0	0.574	38.7	60.6	0.7
	7	0.550	25.1	67.5	7.4
	30	0.130	18.5	67.7	13.8

The total ppm shown above were obtained by summing the ppm in the ethanol rinses, the acetone extracts, and those remaining in the extracted tissue (i.e., bound). These ppm agree well (89-101%) with those obtained by summing the activities found in the ethanol rinses plus those remaining in the rinsed plants (not extracted with acetone). The above ppm are also consistent with the raw data (dpm, sample weights, etc.) presented in the tables in the report.

Total activity can be seen to have declined more in the 16 g plants (≈10x) than in the lower rate samples (≈4.5x). A similar effect was seen in the triazine label study where the residue declines paralleled the differences in growth rates (see above discussion on injury and plant weights).

Total activities in the mature beans were very low with only 0.0016 ppm (1.6 ppb) and 0.0015 ppm (1.5 ppb) DPX-M6316 equivalents found in the 16 g/ha and 8 g/ha samples, respectively. The empty pods contained similar total ^{14}C residues (0.0089 and 0.010 ppm). Characterization of these residues was not attempted. Du Pont believes these residues are higher than those expected in field-grown soybeans since the

careful watering prevented wash-off of surface residues. However, we point out that the proposed Pinnacle label permits applications at a much later date (60 day PHI) than those made in this radiolabeled study.

The dramatic effect of the surfactant on penetration of the herbicide can be seen in the above table. While 86% of the 0 day activity could be rinsed off the 16 g/ha plants, only 39% was removed by the ethanol rinse when X-77 was used in the spray. This is a probable explanation of the greater injury to the soybean plants receiving the lower dose of DPX-M6316. The % of rinsable residues decreased with time in both sets of plants, while the %'s of penetrated (acetone extractable) and bound residues increased.

The petitioner has reported the ppm of DPX-M6316 and identified metabolites in both the ethanol rinses and acetone extracts of soybean foliage. The degree of metabolism is much higher in the extracts than in the rinses. The highest level of any one metabolite in the rinses is 0.027 ppm (whole plant basis), whereas up to 0.164 ppm metabolite A (free acid of DPX-M6316) was found in an extract. Looking at this from a percent viewpoint, only 8% of the extract from the 30 day-8 g/ha foliage was parent compound, while 80% of the rinse of this sample consisted of the intact herbicide.

From the reported ppm in rinses and extracts we have calculated the <u>total ppm</u> (DPX-M6316 equivs) of each metabolite and parent compound on a <u>whole plant</u> basis. These are shown below along with the % of the total residue represented in each case. The total residues used in these calculations were those obtained by summing the rinses, extracts, and bound activities (see earlier table), except in the case of the 0 day-8 g/ha sample. In the latter we used the total ppm (0.648 ppm) from the ethanol rinse plus combustion of the rinsed plant to avoid the total % identified being >100. The letters A-E refer to the standards given above under <u>Test Materials and Methods</u>.

		M6316		"A"		"B"		OTHERS	
RATE	DAY	ppm	%	ppm	%	ppm	%	ppm	%
	0	0.749	86	0.031	4	0.027	3	-	-
16 g/ha	7	0.394	82	0.020	4	0.028	6	0.002	<1 C
	30	0.061	66	0.005	5	0.004	4	0.004	4 E
8 g/ha +	0	0.541	83	0.025	4	0.031	5	-	-
0.25%	7	0.224	41	0.164	30	0.029	5	0.011	2 C
X-77	30	0.026	20	0.045	35	0.009	7	0.014	10*

* = sum of 0.007 ppm D plus 0.007 ppm E

The total % identified in the above six soybean foliage samples are 93, 92, 80, 92, 78, and 72.

Reconstructed radiochromatograms shown in the report for acetone extracts are consistent with the above results. The degree of metabolism is much higher with the surfactant. This is especially shown by the 30 day results where the surfactant treated plants had 62% of the residue identified as metabolites with only 20% DPX-M6316. In contrast, without surfactant the parent is by far the major component (66%) of the residue. We agree with the petitioner that the surfactant aids uptake of DPX-M6316 into plant cells where it is available for enzymatic metabolism.

The results of this thiophene ring label study are consistent with the triazine ^{14}C experiment (see concurrent DER). The two metabolic routes are cleavage of the ester and urea linkages. Hydrolysis of the methyl ester produces metabolite A, the free acid of DPX-M6316. Metabolite B (2-ester-3-sulfonamide) results from hydrolysis of the urea bridge. Metabolite C is the result of both cleavages having occurred. Internal cyclization of C produces metabolite D (thiophene sulfonimide), while additional hydrolysis of C (cleavage of sulfonamide group) leads to the 2-acid-3-sulfonic acid (metabolite E).

With respect to the soybeans, the radioactivity was so low (0.0015-0.0016 ppm) that characterization of the residue was not possible according to the petitioner. Considering that these very low total activities were observed following applications at 1.82 and 3.65x the rate proposed in the present petition, we will not require actual identification of residues in the beans. The residues identified in foliage are consistent with hydrolytic pathways that could be anticipated for DPX-M6316.

ATTACHMENT 3 TO RCB# 3400 MAY 1988

Reviewed by: Richard Loranger R. Loranger
Chemist, Residue Chemistry Branch
Secondary Reviewer: Andrew Rathman
Section Head, Residue Chemistry Branch

DATA EVALUATION RECORD

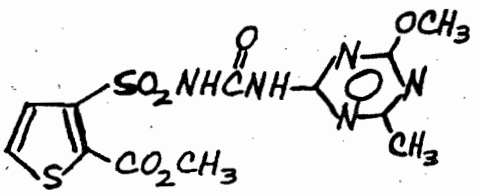
STUDY TYPE: Analytical method

REPORT TITLE: "A Method for Analysis of the Herbicide DPX-M6316 in Soybeans by Liquid Chromatography"

MRID NUMBER: 404676-12 **RCB#'s** 3400, 3401

PP#: 8G3602

TEST MATERIAL: methyl 3-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino]sulfonyl]-2-thiophene carboxylate



other names: DPX-M6316; Pinnacle™; Harmony®; sulfothiphenuron (ISO proposed)
CAS# 79277-27-3

STUDY I.D. #: Laboratory Project ID AMR-973-87

AUTHOR: J. E. Hall, L. M. Reardon

TESTING LABORATORY: E.I. du Pont de Nemours & Company, Inc.

DATE OF REPORT: October 19, 1987

STUDY SPONSOR: E.I. du Pont de Nemours & Company, Inc.

CONCLUSIONS:

This analytical method is satisfactory for measuring residues of DPX-M6316 in soybeans down to a quantitation limit of 0.05 ppm. The method has been validated over the range of 0.05-1.0 ppm. By comparison with the analytical method for wheat (AMR-948-87) in PP#6F3431, the sodium bicarbonate pre-soak should be efficient at extracting aged residues.

SUMMARY OF METHOD

After soaking in sodium bicarbonate solution, ground soybeans are acidified and extracted with methylene chloride. The latter is cleaned up on a silica solid phase column prior to normal phase liquid chromatography with a photoconductivity detector.

PROCEDURE

To 5 grams of ground soybeans in a plastic centrifuge bottle are added 40 mL 0.05M sodium bicarbonate. The beans are soaked for at least two hours and up to overnight if convenient. The pH is adjusted to 3.5 by addition of ≈1 mL 10% HCl before adding 150 mL methylene chloride (DCM) and homogenizing the sample for 5 minutes in a Tekmar Tissumizer. The mixture is centrifuged and the liquid layer passed through glass wool into a separatory funnel. The extraction is repeated with 150 mL DCM and the liquid layer added to the same funnel. After allowing the layers to separate the DCM layer is passed through an Adsorbosil^R silica solid phase column (5g in a 75 mL plastic syringe body). The column is rinsed with additional DCM and the sample eluted from the column with 40 mL acetonitrile (1st 20 mL saved, remainder discarded). The solvent is removed at room temperature (N-EVAP^R evaporator) and the sample resuspended in 5.0 mL 3:1 hexane/isopropanol, filtered, and analyzed by HPLC (μPorasil silica column) with a photoconductivity detector. The eluting solvent is 750:125:125:0.9:0.1 n-hexane:methanol:isopropanol:acetic acid: water with the column temperature maintained at 35°C.

The levels of DPX-M6316 are quantitated by comparing the peak height to that of an external standard.

With regard to the column cleanup step, we note that although 40 mL acetonitrile are added to the column, only the first 20 are collected and the remainder discarded. We contacted Robert Freerksen (302-992-6297) on 5/9/88 for clarification as to why extra solvent is used if the analyte has already eluted. He explained that the column has been sucked dry in the previous step and the additional solvent serves basically to wet the column. Little solvent is actually discarded as most of the excess beyond the 20 mL cut remains on the column. The DPX-M6316 actually elutes in the first 10 mL, but 20 are collected as a precaution.

CONTROL/RECOVERY DATA AND REVIEWER'S COMMENTS

The recovery study described in the report included 5 spiking levels with 5 samples per level. Based on discussion within the report we assume that the "ppm" in Table 2 of the report really should be "ppb". The results are summarized in the table on the following page. "Overall average recovery" is reported as 88%.

PPB SPIKED	% RECOVERY RANGE	AVG ± STD DEV
50	72-100	83 ± 12.5
100	76-110	85 ± 14.5
250	56-112	86 ± 20.5
500	84-106	94 ± 9.3
1000	82-107	93 ± 11.0

Additional recovery data have been submitted in the residue data volume (MRID# 404676-13) of the present petition. For eight samples fortified with 0.05 ppm DPX-M6316 recoveries were 84-124% (95% avg). For four samples fortified with higher levels (0.1-1.0 ppm) recoveries were 82-140%.

Chromatograms are included in the report for a 50 ppb standard, control soybeans, and beans spiked with 50, 100, 500, and 1000 ppb. Inspecting the chromatograms of spiked samples we concur with the petitioner's choice of 0.05 ppm as the method's limit of quantitation. The analyte peak appears on a rather steep baseline and could not be reliably quantitated below this level.

It is noted that "Controls were run and no DPX-M6316 was detected." The chromatogram for a control sample in the present report does not have a measurable peak at the retention time of the analyte. However, one control chromatogram in the residue data volume does have a small peak equivalent to about 0.025 ppm DPX-M6316. In any case, the chromatograms for untreated samples have peaks less than the lower quantitation limit of 0.05 ppm.

With respect to radiovalidation of this analytical method the report states that soybeans with "systemic ^{14}C labeled DPX-M6316 were unavailable since attempts to force quantitatable residues were unsuccessful." However, the extraction efficiency of aged residues by a sodium bicarbonate pre-soak was shown with wheat straw in PP#6F3431. These data have been reviewed by Residue Chemistry Branch and we concluded that weathered residues in wheat straw are adequately accounted for when the extraction procedure uses $NaHCO_3$ (C. Deyrup, 12/24/87). As further stated in our 5/3/88 review of PP#6F3431, the basic presoak serves to both hydrate wheat straw and extract DPX-M6316, which is soluble in base. We do not believe additional radiovalidation data are required for the soybean method.

ATTACHMENT 4 TO RCB# 3400 MAY 1988

Reviewed by: Richard Loranger R. Loranger
Chemist, Residue Chemistry Branch
Secondary Reviewer: Andrew Rathman
Section Head, Residue Chemistry Branch

DATA EVALUATION RECORD

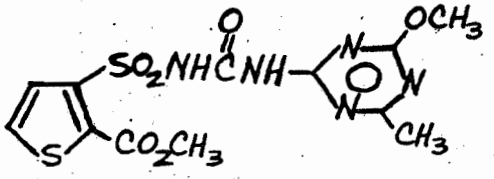
STUDY TYPE: Residue data and storage stability

REPORT TITLE: "Magnitude of the Residues of DPX-M6316 in Field Samples of Soybeans after Treatment with Pinnacle™ Herbicide

MRID NUMBER: 404676-13 **RCB#'s** 3400, 3401

PP#: 8G3602

TEST MATERIAL: methyl 3-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino]sulfonyl]-2-thiophene carboxylate



other names: DPX-M6316; Pinnacle™; Harmony®; sulfothiphenuron (ISO proposed)
CAS# 79277-27-3

STUDY I.D. #: Laboratory Project ID AMR-1022-87

AUTHORS: J. E. Hall, L. M. Reardon

TESTING LABORATORY: E.I. du Pont de Nemours & Company, Inc.

DATE OF REPORT: December 12, 1987

STUDY SPONSOR: E.I. du Pont de Nemours & Company, Inc.

CONCLUSIONS:

In 10 field trials the post-emergence use of 25% or 75% dry flowable formulations of Pinnacle at rates of up to 1.0 oz ai/A without surfactant or 0.25 oz ai/A with surfactant resulted in residues <0.05 ppm, the method's limit of quantitation, in soybeans harvested 45-68 days after treatment.

TEST MATERIALS AND METHODS

DPX-M6316 was applied to soybeans in 10 states (GA, IL, IN, MD, MS, NE, NC, OH, SD, TN) in 1986-87 using ground equipment (one sample received aerial application). The formulations employed were 75% or 25% dry flowables. A single post-emergence application was used in all 10 trials with the following distribution of application rates for the 14 samples: 1.0 oz ai/A with no surfactant (3 samples), 0.25 oz ai/A with 0.1-0.25% added surfactant (8 samples), 0.25 oz ai/A with liquid nitrogen fertilizer added (1 sample), 0.25 oz ai/A with neither additive (1 sample), and 0.5 oz ai/A with no surfactant (1 sample). Spray volumes were 11-39 gallons per acre for the ground applications and 5 GPA for the aerial use. The growth stages for the soybeans at the time of treatment were described by terms such as beginning bloom, full bloom, early pod development, and pod set. Beans were harvested 45-68 days after application.

Two samples were stored at ambient temperature for 3-4 days after harvest and the remainder frozen the day of harvest. Samples were then stored in a freezer for 2 or 13-14 months prior to analysis for residues of DPX-M6316 using analytical method AMR-973-87. The report includes freezer storage stability studies on wheat grain and straw (submitted previously in PP# 6F3431) and on corn kernels and green corn forage.

During the analyses for DPX-M6316 residues "side by side recovery studies" were run on fortifications of 0.05-1.0 ppm. Typical chromatograms are shown for control, spiked, and treated samples.

REPORTED RESULTS AND REVIEWER'S DISCUSSION

The stability of DPX-M6316 residues in wheat grain and straw has been reviewed previously by Residue Chemistry Branch (C. Deyrup, PP#6F3431, 12/24/87). After 18 and 36 months of frozen storage, recoveries of residues from wheat grain were 80-84% and 74-83%, respectively. In the case of wheat straw, 78-89% of 0.1 ppm spikes were recovered after 24 months of storage.

In the present report untreated corn kernels and green forage were fortified with 0.1 ppm DPX-M6316 and placed into frozen storage. Samples were removed after 1, 3, 6, 12, 18, and 24 months for analysis by Method AMR-431-85 (Revision 1). Recoveries of fresh fortifications were run with each set of stored samples. Measured residues on the stored samples were corrected for these fresh recoveries (78-103% for corn kernels, 82-96% for corn forage). Overall, residues were very stable during storage as 80-113% and 85-119% of the original spikes in corn kernels and forage, respectively, were recovered.

The present residue data on soybeans are supported by the existing storage stability data. Beans were kept frozen 2-14 months prior to analysis versus the demonstrated 2-3 year stability of DPX-M6316 residues.

The side by side recovery studies run during the soybean analyses showed the following results:

PPM	N	RANGE	AVG ± STD DEV
0.05	8	84-124	94.8 ± 14.9
0.10	1	82	----------
0.25	2	132-140	136 ± 5.6
1.00	1	98	----------

The overall average of the above recoveries is 101%. We again conclude that Method AMR-973-87 is valid for determining residues of DPX-M6316 in soybeans. See our concurrent Data Evaluation Record for more details on this procedure.

All treated and control soybeans from the 10 field trials are reported as having less than 0.05 ppm DPX-M6316, the limit of quantitation. The submitted chromatograms support this claim. A small peak equal to about 0.01 ppm DPX-M6316 is present in one of the treated sample chromatograms. A control sample has a peak we calculate to be equivalent to ≈0.025 ppm.

We can conclude that in the 10 trials the use of DPX-M6316 at rates of up to 1.0 oz ai/A without surfactant or 0.25 oz ai/A with surfactant resulted in residues <0.05 ppm, the method's limit of quantitation, in soybeans. The submitted chromatograms indicate trace residues on the order of 0.01-0.02 ppm may be present in treated soybeans, but the peak in the control sample prevents a definite conclusion on this point.

RIN 2064-98

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Pages 25 through 26 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

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