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EXPEDITE

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OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: SAN-582H/Dimethenamid. Responses to CBTS Review of 7/29/92.

DP Barcode: D184696. CBTS # 10890.
MRID # 425436-01.

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This review is being expedited at the 11/25/92 request of Lawrence E. Culleen, Acting Director, Registration Division. The due date is 12/21/92.

The present submission is a partial response to our review of 7/29/92. It consists of analytical data previously reported in meetings held 9/17/92 and 10/29/92 and a storage stability study. Responses to deficiencies in the corn metabolism study have been submitted in a separate report, which will be reviewed in another memo.

Conclusions (pertaining to this memo only)

1. Storage stability data will support residue analyses of the sulfonate metabolite for periods up to 16 months. Submitted TLC data from the soybean radiolabeled study suggest that the sulfonate and other metabolites are not stable to 19 months. (See following conclusion.)

2. The analytical method for the sulfonate conjugate of SAN-582H in corn, Method AM-0868-0392-1 produced acceptable recoveries at fortification levels of 0.05-0.1 ppm in grain and 0.13-0.44 ppm in silage. In forage, acceptable recoveries could only be consistently obtained at a fortification level 0.5 ppm.

3. Attempts to develop a method for the sulfoxide of thiolactic acid conjugate of SAN-582H have been unsuccessful. Provided that a satisfactory conclusion can be drawn from the sulfonate residue analyses (not reviewed in this memo), further analysis for the sulfoxide conjugate in corn will be unnecessary.

Recommendation

CBTS continues to recommend against the proposed tolerances for reasons given in conclusions in our 7/29/92 memo. The present memo does not substantively affect these conclusions.

Detailed Considerations

Deficiencies listed in our 7/29/92 memo will be listed together with Sandoz's responses and CBTS' comments.

Deficiency # 4 (Conclusion #4 from our 7/29/92 memo)

> Submitted storage stability data for SAN-582H demonstrate stability under frozen storage for periods up to 21 months. The oxalamide metabolite appears to be stable under frozen conditions for at least one year, but if this metabolite is to appear in the tolerance expression, samples must be reanalyzed using a method giving more reproducible recoveries.
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> Comparative TLC analyses imply that the thioglycolic acid conjugate of SAN-582H, the oxalamide metabolite, the sulfoxide of thioglycolic acid conjugate and the sulfoxide of thiolactic acid conjugate are stable for periods up to 22 months. However, since the precision of the TLC method is not known, results are indicative only and cannot substitute for controlled storage stability studies, should they be necessary.

[In the 9/17/92 conference with Sandoz, we indicated that TLC data from the radiolabeled study would be acceptable if reproducibility could be assessed. We suggested that if suitable validation data were developed for the metabolites in soybeans using the TLC method, we might accept the data already submitted for corn in support of storage stability. (See our Memorandum of Conference, 9/29/92.)]

Sandoz Response

The following report has been submitted:

"Determination of the Stability of Residues of SAN-582H

and Its Metabolites in Stored Soybean Samples;" 11/2/92; K.L. Smith; Sandoz I.D. Number 414108-33; Performing Laboratory -- Sandoz Agro, Des Plaines, IL. (MRID # 425436-01)

Stability was determined in two different approaches. In the first approach, untreated soybeans were fortified with SAN-582H, oxalamide and sulfonate and frozen for up to 16 months. In the second approach, soybean grain and forage samples from the soybean metabolism study were reanalyzed by TLC 11 and 19 months later. Replicates of the soybean forage sample were analyzed 19 months after the original analysis to assess reproducibility.

Analysis of Fortified Control Samples. Samples of soybean grain and forage were fortified at 0.5 ppm with SAN-582H, oxalamide and the sulfonate conjugate and stored at ≤-16°C. Samples were analyzed immediately after fortification and after storage for 1,3, and 16 months. Samples from 0, 1 and 3 months were analyzed by Sandoz's residue analytical method AM-0580-0291-0, "Determination of SAN-582H and the Oxalamide Metabolite in Soybean Forage, Hay, Grain and Straw", and AM-0855-0491-0, "Determination of the Sulfonate Metabolite of SAN-582H in Soybeans". Both these methods were deemed unacceptable in our review of PP#1G3980 (J. Abbotts, memo of 7/19/91). Concerning AM-0580-0291-0, the reviewer stated that..."Fortification samples show unacceptable deviation of recoveries at 0.1 ppm, and no other data were submitted to indicate that the method can detect residues at concentrations lower than 0.1 ppm." Concerning the sulfonate method, he noted that "Data have not been provided to demonstrate that the method submitted can effectively detect the sulfonate at concentrations lower than 0.1 ppm in forage or 0.5 ppm in hay and grain."

The 16 month samples were analyzed for SAN-582H by method BS-2304, "A Method for the Determination of SAN-582H in Corn and Soil Samples", and for sulfonate conjugate by method AM-0868-0392-1, "Determination of the Sulfonate Metabolite of SAN-582H in Corn Grain, Forage, Fodder and Silage". The former method produced acceptable recoveries and is undergoing EPA method validation on corn. The latter method reportedly gives more reproducible recoveries. Because this method has not been previously reviewed, it is discussed in this memo.

AM-0868-0392-1. Samples of corn fodder, forage or silage are blended with 98% methanol, then filtered and adjusted to specified volumes, depending on the matrix. Aliquots are extracted with hexane, acidified, then chromatographed on strong cation/anion exchange columns (sulfonic acid cation exchange/SAX anion exchange). The sulfonate is quantitated by HPLC using a UV detector. Two HPLC peaks are observed in the sulfonate standard. NMR has shown that these correspond to rotational isomers "rotamers". Quantitation is based on the combined heights of the

two isomers. Recoveries ("corrected" -- presumably using unfortified controls) from corn fodder samples fortified at 0.1-0.5 ppm averaged 71.7±9.6%; recoveries from forage averaged 72.3±13.2%; recoveries from grain averaged 77.1±10.5%. In the case of forage, there were occasional large discrepancies between "recovery" and "recovery corrected". Recovery from one sample fortified at 0.1 ppm was 163%; corrected recovery was 49%. Recovery from another sample fortified at 0.1 ppm was 123%; corrected recovery was 70%. This implies that occasional samples had major interferences. The few submitted chromatograms with the method showed no interferences; however chromatograms submitted with the residue trials -- not reviewed in this memo -- showed major interferences.

Results. Since the 0, 1 and 3 month analyses were done using unacceptable methods, these results are not reliable. Sulfonate recoveries from soybean grain and forage at 0 Month were barely acceptable (75.8±20.0%); sulfonate recoveries at 3 months from grain varied from 0-106%. Using the improved methods for SAN-582H and sulfonate, 16 month analyses showed recoveries of 91.5±14.0% for SAN-582H and 88.1±12.5% for sulfonate (average of fresh and stored commodities). Recoveries for sulfonate were about 20% higher in stored commodities than in the corresponding fresh commodities. There is no indication that these recoveries were corrected.

Analyses from TLC's. Soybean metabolites were reanalyzed almost 11 months after first analysis by TLC. The distribution of radioactivity on the TLC plate was determined using an Ambis Radioanalytic Imaging System. (In the corn metabolism study the original work was done with a less accurate radioactivity detector.)

Total radioactivity in soybean grain did not differ significantly after 11 months -- 0.1947 ppm (SAN 582 equivalents) initially and 0.1960 ppm after 11 months. Individual metabolites showed declines ranging from 3% to 31%. The sulfonate metabolite, initially present at 0.0089 ppm, was subsequently found at 0.0067 ppm, a decline of 25%.

Decline of total radioactivity in soybean forage was more pronounced. At 11 months total radioactivity was 1.7937 ppm -- a decline of 11% from the initial 2.0224 ppm. At 19 months total residue fell to 1.2514 ppm, a decline of 38%. The sulfonate metabolite concentration declined from 0.494 ppm to 0.360 ppm at 11 months, a 27% decline. At 19 months sulfonate concentration was 0.193 ppm , a decline of 61%. Sandoz has calculated a "normalized" decline based on the decline of total radioactivity. Since 62% of the total radioactivity remained after 19 months, the normalized decline of sulfonate would be [(0.193/0.62)/0.494 - 1] x 100 = - 37%.

Reproducibility of the TLC method was assessed by analyzing four soybean samples from the same batch. The sulfonate conjugate was found at an average concentration of 0.19±0.02 ppm. Four other metabolites were analyzed. Relative standard deviations were lower than for the sulfonate.

CBTS Comment

Sandoz's use of a "normalizing" procedure with TLC samples is based on the assumption that the residue lost over the storage period contains metabolites in the same ratio as in the remaining residue. This is far from certain. As a result we can only accept Sandoz's results to 11 months. The fact that the TLC analytical method gives reproducible results would seem to provide confirmation that the sulfonate metabolite is not stable in soybean forage for 19 months.

The storage stability data from the fortified field trial samples suffer because the analytical methods employed during the initial analyses were not really acceptable -- recoveries were extremely variable. The sulfonate data do indicate, however, that there is stability under frozen conditions for up to 16 months.

Relevance to Storage Stability in Corn Matrices

As noted in our 7/29/92 memo, Sandoz performed similar TLC analyses on corn samples treated with radiolabeled SAN-582H. Samples were reanalyzed 24 months after the first analysis for the following metabolites: thiolactic acid conjugate, sulfoxide of thiolactic acid conjugate, oxalamide, sulfoxide of thioglycolic acid conjugate. Concentration of all but oxalamide actually increased over this period -- an observation that could be due to the more accurate detector used in the later analysis. Analysis for the sulfonate was not reported. These results combined with the soybean analyses -- which did show reproducibility -- suggest stability of these metabolites over the period. We note however, that analysis for sulfonate was not carried out; and, more practically, the residue analyses for sulfonate have a much higher limit of quantitation than the levels expected to be present from the radiolabeled study. Further comment is deferred until the actual residue analyses are reviewed.

Deficiency #5b (Conclusion #5b from our 7/29/92 memo)

> Residue data for the sulfonate conjugate of SAN-582H and the sulfoxide of thiolactic acid conjugate of SAN-582H in corn grain, forage and fodder should be generated from field trials held in six states. Analyses should be supported by appropriate storage stability data. This requirement is made because of the absence of a suitable common moiety analytical method and is provisional, pending review by the HED Metabolism Committee.
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> If these metabolites (or common moiety) are non-detected in residue samples, the appropriate tolerance will be for parent only, pending concurrence by the HED Metabolism

Committee.

Should it be necessary to regulate these metabolites, the analytical methods must undergo independent laboratory validation and EPA method validation.

Sandoz Response

With cover letter dated 10/28/92, Sandoz has submitted some residue analyses on the sulfonate metabolite and has described its analytical problems with the sulfoxide of thiolactic acid conjugate. As noted above, this information was previously presented at meetings with EPA on 9/17/92 and 10/29/92. The complete report of residue analyses for sulfonate and the residue analytical method have been submitted separately (MRID # 425160-03). CBTS will review these data in a separate submission.

Attachment II of the 10/28/92 submission describes the company's efforts to develop an analytical method for the sulfoxide of thiolactic acid conjugate. Two types of procedures were used.

In the first, the matrix was extracted with 98% methanol followed by hexane and methylene chloride washes. The methanol solution was passed through cation and anion exchange solid phase extraction (SPE) columns. The eluate was analyzed by HPLC. Various column modifications were employed, but recoveries were low and variable at the 0.1 and 0.05 fortification levels. Recoveries at 0.05 ppm were 12-21%.

In the second type, derivatization techniques were attempted. Derivatizing the conjugate with p-bromophenacyl-8 reagent to make the corresponding ester for HPLC analysis produced multiple products, as shown by HPLC. Methylation of the conjugate to make the methyl ester was attempted with diazomethane. Detectable products were not observed in the GC with either a NPD detector or GCR radiochromatography detector when ^{14}C was used. Fischer esterification (heating with alcohol in the presence of mineral acid) produced a single compound as analyzed by HPLC at 240 nm. "The HPLC assay is currently being optimized for lower level reactions to overcome interference problems." Derivatization to make the pentafluorobenzyl ester was not successful.

Development of a residue analysis method for the sulfoxide of thiolactic acid conjugate is still being pursued.

CBTS Comment

Results from the radiolabeled study on corn suggest that the sulfoxide of thiolactic acid conjugate could be present in forage or fodder at levels not exceeding 0.05 ppm. That study also indicated that the three principal metabolites were the sulfonate conjugate, the sulfoxide of thiolactic acid conjugate and the

oxalamide metabolite. The oxalamide metabolite has not been found at a level of 0.01 ppm; the sulfonate conjugate is apparently not present at a level of 0.05 ppm -- the residue data have not as yet been formally reviewed. We conclude that further analysis for the sulfoxide conjugate in corn is unnecessary, provided that a satisfactory conclusion can be drawn from the sulfonate residue analyses.

This deficiency is partially -- and provisionally -- resolved.

cc: SF (SAN-582H), RF, Circu., PP#0F3918, PP##1G3980, Mike Flood, E. Haeberer.
H7509C:CBTS:Reviewer(MTF):CM#2:Rm800A:305-6362:typist(mtf):12/16/92.
RDI:SectionHead:ETHaeberer:12/16/92:BranchSeniorScientist:RALoranger: 12/16/92.