#4171

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

SEP 29 1993

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: PP#3F04177. Dimethenamid (129051 SAN 582H) in Soybeans. Evaluation of Analytical Methods and Residue Data.
DP Barcode:D187839, D191922, D187839.
CBTS No. 11329, 11978, 12216
Case:284369: Submission:S434923, S442012, S444474.

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Health Effects Division (H7509C)

TO: Cynthia Giles-Parker/James Stone, PM 22
Fungicide-Herbicide Branch
Registration Division (H7505C)

THRU: Debra Edwards, Chief Debra Edwards
Chemistry Branch 1 - Tolerance Support
Health Effects Division (H7509C)

Attached is a review of PP#3F04177, Dimethenamid on Soybeans prepared by the Dynamac Corp. under supervision of Chemistry Branch I (CBTS). This review has undergone secondary review and revision in CBTS and reflects current Branch policies.

Sandoz Crop Protection Corporation is proposing tolerances of 0.01 ppm on soybean grain. Our (J. Abbotts, 7/19/91) review of the corresponding temporary tolerance petition, PP#0F3918, identified numerous deficiencies to be resolved for establishment of permanent tolerances. These deficiencies have been addressed in this review.

Permanent tolerances have been established for dimethenamid on corn grain, fodder and forage at 0.01 ppm in 40 CFR 180.464. Temporary tolerances on soybeans, forage and hay at 0.01 ppm expire 3/1/94.

## Summary of Deficiencies Remaining to be Resolved

Recovery data for 0.01 ppm fortifications in soybeans.

Storage stability data.

Recycled/Recyclable
Printed with Soy/Canola Ink on paper that contains at least 50% recycled fiber

**Conclusions:**

1. The nature of the residue in soybeans has been adequately understood. The metabolism in soybeans is similar to that in corn. The residue to be regulated is the parent compound.

2. The nature of the residue in livestock is adequately understood. Like the similar tolerance in corn, tolerances for dimethenamid in ruminants and poultry are not required for this use.

3. An analytical method, TDS No. BS2304, is available for enforcement of 0.01 ppm dimethenamid in corn. Additional recovery data are needed for fortifications at 0.01 ppm in soybeans, since these data are only available for a soybean processing study. Multiresidue data for FDA's protocols were forwarded to FDA 2/92. Analytical reference standards (SAN 582H) are available from Industrial Chemicals Repository, Research Triangle Park, NC.

4. Storage stability data are needed for soybean processed commodities and for soybeans stored up to 26 months before analyses.

5. Provided additional recovery data at 0.01 ppm and storage stability data are adequate, CBTS concludes that residues of dimethenamid in soybeans are not likely to exceed the proposed 0.01 ppm tolerance.

6. Provided storage stability data are adequate, CBTS concludes that residues of dimethenamid in soybean processed products are not likely to exceed the rac tolerance.

7. There are no CODEX, Canadian or Mexican limits for dimethenamid (F. Ives, 2/21/92).

**Recommendation:**

CBTS recommends against the proposed tolerance because of Conclusions 3 and 4.

cc: Circu, RF, PP#3F04177, Bradley
H7509C:CBTS:M Bradley:CM#2:Rm804:305-7324:09/16/93
RDI:RSQuick:09/28/93:RALoranger:09/28/93:DEdwards:09/28/93
B:\DIMETHEN.DYN

# INTERNATIONAL RESIDUE LIMIT STATUS

CHEMICAL Dimethenamid (SAN 582 H)

CODEX NO. _____

CODEX STATUS:

☑ No Codex Proposal Step 6 or above

Residue(if Step 8): _____

| Crop(s) | Limit (mg/kg) |
|---|---|
| | |

PROPOSED U.S. TOLERANCES:

Petition No. 9F04177

RCB Rewiewer M. Bradley

Residue: parent only

| Crop(s) | Limit (mg/kg) |
|---|---|
| soybeans | 0.01 |

CANADIAN LIMITS:

☑ No Canadian limit

Residue: _____

| Crop(s) | Limit (mg/kg) |
|---|---|
| | |

MEXICAN LIMITS:

☑ No Mexican limit

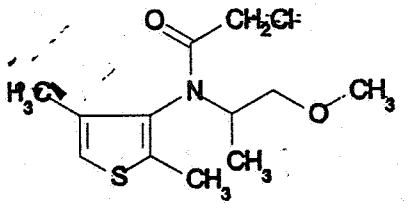
Residue: _____

| Crop(s) | Limit (mg/kg) |
|---|---|
| | |

NOTES:

From F. Ives 2/21/92

# DIMETHENAMID



Shaughnessy No. 129051

PP#3F04177 - Dimethenamid (SAN 582H) in Soybeans

(DP Barcodes D187839, D191922, and D193109)

MRIDs 42571601, 42571602, 42571603, 42606501, 42632801, and 42842501

Task 4

PETITIONER'S RESPONSE TO RESIDUE CHEMISTRY DATA REQUIREMENTS

## BACKGROUND

Sandoz Agro, Inc. is proposing a permanent tolerance for residues of dimethenamid in/on soybean grain at 0.01 ppm. Permanent tolerances have been established for residues of dimethenamid in/on corn fodder, forage, and grain at 0.01 ppm each [40 CFR §180.464]. Temporary tolerances for dimethenamid in/on soybeans and soybean forage and hay have been established at 0.01 ppm each (PP#1G3980); these tolerances expire 3/1/94. In a review of the petition for the temporary tolerances (CBTS No. 8000, DP Barcode D164289, J. Abbotts, 7/19/91), CBTS outlined the data that would be required to establish a permanent tolerance for soybean grain. Sandoz has submitted the following data which are reviewed in this document for adequacy in support of the present petition: an addendum to a soybean metabolism study (1992; MRID 42571601) originally submitted under MRID 41843801 and a corrected version of this addendum (1993; MRID 42842501); one volume of soybean field trial data (1992; MRID 42632801) pertaining to the reanalysis of samples from the 1990 growing season; one volume of soybean field trial data (1992; MRID 42606501) from a new field trial conducted in 1991; and a soybean processing study (1992; MRID 42571602). Sandoz also submitted a study pertaining to the determination of plant metabolites in rats (1992; MRID 42571603); these data are not reviewed here. MRID 42571601 will not be reviewed here since the petitioner submitted a corrected version of this document (MRID 42842501).

## DETAILED CONSIDERATIONS

### Product Chemistry

Product chemistry data relating to this petition have been reviewed (CBTS No. 8787, DP Barcode D169997, 7/29/92, M. Flood; CBTS No. 10761, DP Barcode D183774, 1/22/93, M. Flood; and CBTS No. 11323, DP Barcode D187725, 2/11/93, M. Flood). All product chemistry data requirements have been satisfied.

Proposed Use

The 7.5 lb ai/gal emulsifiable concentrate (EC) formulation (Frontier® Herbicide 7.5L; EPA Reg. No. 55947-140) is proposed for use on soybeans as a single or split broadcast soil preplant (surface or incorporated), preemergence, and early postemergence application at 0.75-1.46 lb ai/A depending on the soil texture and cation exchange capacity. Applications may be made using ground or aerial equipment in at least 2 gal/A of finished spray. The product may be tank mixed with other pesticides. The maximum proposed seasonal application rate is 1.5 lb ai/A. Applications through irrigation systems and the grazing or feeding of treated forage, hay, or straw to livestock are prohibited. Rotation to crops other than corn or soybeans prior to the spring after application is also prohibited, except that fall-seeded cereal crops may be planted 4 or more months after a spring application.

CBTS has previously approved the registrants' feeding/grazing restriction against soybean forage and hay (CBTS No. 8775, DP Barcode D170099, 1/6/92. R. Lascola).

Qualitative Nature of the Residue in Soybeans

The conclusions stated in the July 19, 1991 CBTS review of PP#1G3980 (CBTS No. 8000, J. Abbotts) regarding soybean metabolism will each be reiterated below, with CBTS comments from the Detailed Considerations section of the review in brackets, followed by the Petitioner's Response.

Conclusion/Comment No. 1a from 7/19/91 CBTS review

For permanent tolerances, the nature of the residue in/on soybeans is not adequately understood, and additional characterization is required. At present, the petitioner has confirmed the identities of metabolites representing ca. 30% TRR in forage, 24% TRR in hay, and 26% TRR in seed (grain). Efforts should be made to identify all metabolites present at concentrations >0.05 ppm and/or 10% of TRR. Further efforts should also be taken to characterize compounds which the petitioner has identified as unknowns. [These include the unknowns detected by HPLC in the methylene chloride extract of hay (Metabolism Report, Figure 45), two unknowns described above in the acetone extract of hay, and the unknown in the methanol extract from hay (Metabolism Report, Figure 48).] Further efforts should also be taken to characterize peaks identified in the methanol:water extract procedure, or to demonstrate that these peaks consist of multiple components. [Unassigned peaks were present at retention times of ca. 20 minutes in hay, and at ca. 32 minutes in seed. HPLC analysis of a methanol:water extract of a 1990 seed sample showed several unidentified peaks.] In order to document the identification of metabolites, petitioner must provide chromatograms which provide confirmation. Assignment of permanent tolerances will also depend on an evaluation of the toxicological significance of metabolites.

Petitioner's Response to Conclusion/Comment No. 1a

The petitioner responded to Conclusion No. 1a by submitting supplemental data (1993; MRID 42842501) regarding the metabolism of [$^{14}$C]dimethenamid residues in soybean matrices. These data were generated from samples from the original metabolism study which had been stored frozen for 11 months in conjunction with a storage stability study. These samples will be referred to as the "11-month samples", and samples from the original metabolism study will be referred to as the "initial samples". The supplemental data included clearly-labeled HPLC and TLC chromatograms and data from NMR and mass spectrometry analyses. [*Note: All methods used to characterize/identify/confirm metabolites in extractable residues of soybean matrices are fully described in the original metabolism report and reiterated in the appendices of the present*



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*submission*]. In addition, the registrant presented a comprehensive chart cross-referencing the new tables and figures presented in the petitioner's response and the original metabolism study. The original metabolism study is re-summarized below.

In the original report, [3-thienyl-$^{14}$C]dimethenamid (specific activity, 15,400 dpm/$\mu$g; formulated as the 720 EC formulation) was soil-applied at field equivalent rates of 1.5 and 3 lb ai/A (1 and 2x the maximum proposed rate) one day after soybean planting. In the 1988 test, soybean commodities were harvested at the following days posttreatment: (i) forage, 49 days; hay and immature seed, 100 days; (iii) leaves, 113 days; and (iv) straw, mature seed, and roots, 118 days. In the 1990 test, soybean commodities were harvested at the following days posttreatment: (i) forage, 42 days; hay, 100 days; and (iii) straw and mature seed, 128 days. Samples were frozen on dry ice immediately after harvest, then stored at -20 C until analyzed. Total radioactive residues (TRR) were determined by LSS following combustion. In the 1988 samples, the TRR were: (i) 2.02-3.72 ppm in forage; (ii) 1.86-2.94 ppm in hay; (iii) 0.09-0.20 ppm in immature seed; (iv) 2.12-5.12 ppm in leaves; (v) 1.22-2.37 ppm in straw; and (vi) 0.195-0.48 ppm in seed. In the 1990 samples, TRR were: (i) 0.30-0.60 in forage; (ii) 0.91-2.28 ppm in hay; (iii) 0.89-1.71 ppm in straw; and (iv) 0.13-0.27 ppm in seed.

The petitioner provided no information on sample storage intervals. Based on the dates on submitted HPLC chromatograms and the sample harvest dates of July-September 1988, samples were stored for up to 44 months between harvest and analysis.

Extraction

Two different extraction methods were used; both methods were fully described in the original metabolism report. In one method, soybean matrices were extracted with 98% methanol. The extracts were then freeze-dried and stored frozen until analyzed. This method was designated the One-step Procedure.

The second method involved a sequential extraction with hexane (seed only), methylene chloride, acetone, and water. Solids were further fractionated by sequential acid and base hydrolyses; hydrolysates were extracted with ethyl acetate. This method was designated the Sequential Procedure.

Samples from the 1988 test were extracted using the Sequential Procedure and the One-step Procedure; 1990 samples were extracted using only the One-step Procedure.

Metabolite characterization/identification

Extracts of soybean matrices were analyzed using one or more of the following HPLC systems:

System I: A C-18 column using a mobile phase consisting of methanol:0.5% trifluoroacetic acid (TFAA) in water, 20:80 (v:v) changing to 65:35 (v:v) and returning to 20:80 (v:v) over a period of 60 minutes; detection by UV at 238 nm;

System II: A C-18 column using an isocratic mobile phase consisting of methanol:0.1% TFAA in water (50:50, v:v); detector not specified;

System III: A C-18/anion exchange column using a mobile phase consisting of methanol:0.005 N sulfuric acid (25:75, v:v) changing to methanol:water (75:25, v:v) over a period of 40 minutes; detector not specified;



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System IV: A C-18 column using a mobile phase consisting of methanol:0.5% TFAA in water, 80:20 (v:v) changing to 90:10 (v:v) over 10 minutes; detection by UV at 235 nm and by radioassay; and

System V: A C-18 column using a mobile phase consisting of methanol:0.5% TFAA in water, 20:80 (v:v) changing to 80:20 (v:v) over 60 minutes; detection by UV at 235 nm and by radioassay.

Extracts were analyzed by one-dimensional TLC on silica gel plates using two or more of the following solvent systems having different polarities:

System I: ethyl acetate:toluene:concentrated formic acid:water (87:3:5:5, v:v:v:v);

System II: 1-butanol:glacial acetic acid:water (60:15:25, :v:v:v);

System III: ethyl acetate:isopropanol:formic acid:water (60:30:5:5, v:v:v:v);

System IV: ethyl acetate:toluene (60:40, v:v);

System V: ethyl acetate:toluene:formic acid (60:40:5, v:v:v); and

System VI: ethyl acetate:toluene:formic acid (60:40:2, v:v:v).

One-Step Procedure extracts: The 98% methanolic extracts from 11-month soybean forage and seed samples, obtained from plants treated at the 1x rate, were analyzed by one-dimensional TLC. Metabolites were tentatively identified by comparison of the retention times with those of the following reference standards: dimethenamid and its sulfonate, thioglycolic acid (TGA), thiolactic acid (TLA), TGA-sulfoxide (STGA), TLA-sulfoxide (STLA), oxalamide, cysteine, glutathione, and homoglutathione conjugates. The major radioactive bands were scraped from the TLC plates and reanalyzed by TLC and/or HPLC. Metabolites were then identified and confirmed using at least two different TLC solvent systems (described above) having different polarities, and by HPLC system I.

Forage: The petitioner characterized/identified 46.88% TRR (0.951 ppm) in 11-month samples. Metabolites identified in forage extracts were sulfonate (13.4% TRR, 0.240 ppm), STLA (5.25% TRR, 0.0942 ppm), STGA (10.96% TRR, 0.196 ppm), oxalamide (8.02% TRR, 0.1443 ppm), TLA (2.66% TRR, 0.0476 ppm), TGA (1.52% TRR, 0.0273 ppm) and M11 (0.67% TRR, 0.0119 ppm). Two unknowns, a "low $R_f$" component and M9, collectively accounted for 4.29% TRR (0.077 ppm). As with the TLC analysis of the initial sample extract, five diffuse zones of radioactivity were detected: two zones collectively accounted for 6.28% TRR (0.1127 ppm); three additional zones each accounted for <0.01% TRR. The five diffuse zones were not scraped and reanalyzed by TLC.

Each TLC band representing an identified metabolite was scraped from the plate and reanalyzed by TLC, which revealed the presence of 1-3 components in each band. Metabolite bands identified in the extract were resolved into the following components after reanalysis of each band: (i) the sulfonate band was resolved into sulfonate and an unspecified "low $R_f$ " component (2.95% TRR, 0.0529 ppm); (ii) the STLA band was resolved into STLA and STGA; (iii) the STGA band was resolved into STGA and oxalamide; (iv) the oxalamide/TLA band was resolved into oxalamide and TLA; and (v) the TGA/M11 band was resolved into TGA, M9, and M11. TLC and HPLC chromatograms were provided for each analysis. All extractable radioactive residues present at >0.01 ppm were characterized/identified.

The distribution of radioactivity into metabolites identified in methanol extracts obtained from soybean forage using the One-step Procedure are presented in Table 1; these samples were obtained from plants treated at the 1x rate in 1988.

Seed: The petitioner characterized/identified 23.82% TRR (0.0470 ppm) in 11-month samples. Metabolites identified in seed extracts were sulfonate (3.44% TRR, 0.0067 ppm), STLA (2.93% TRR, 0.00575 ppm), STGA (5.71% TRR, 0.0112 ppm), oxalamide/TLA (collectively 3.17% TRR, 0.00621 ppm), and TGA/M11 (collectively 2.94% TRR, 0.0058 ppm). Six unspecified zones of radioactivity collectively accounted for 7.99% TRR (0.0114 ppm); each unidentified component (including each diffuse zone) was <0.01 ppm. The petitioner did not scrape and reanalyze any discrete metabolite band that was present at <0.01 ppm. Reanalysis of the TGA/M11 band failed to resolve the two metabolites into separate components.

The distribution of radioactivity into metabolites identified in methanol extracts obtained from soybean seed using the One-step Procedure are presented in Table 2; these samples were obtained from plants treated at the 1x rate in 1988.

Metabolite profiles obtained from the 11-month forage and seed sample extracts (prior to reanalysis of discrete metabolite bands) were found to be comparable with metabolite profiles presented in the original metabolism report. TLC and HPLC chromatograms were presented for each analysis. All extractable radioactive residues present at >0.01 ppm were characterized/identified.

The petitioner stated that HPLC analyses were useful only in qualitative metabolite characterization because soybean extracts were viscous and required excessive dilution, and because of high levels of background noise that reduced the sensitivity of the detector. The petitioner further indicated that the relatively low level of metabolites present in the extracts, coupled with high HPLC background noise, may have resulted in a number of "unidentified peaks" cited in the Agency review of the original metabolism report. HPLC chromatograms were provided to demonstrate that the following suspected HPLC peaks detected in 98% methanol extracts were not significantly different from background noise: (i) peak with retention time (rt) of 20 minutes in 1988 hay; (ii) peak with rt of 32 minutes in 1988 seed; and (iii) numerous strong peaks in 1990 seed.

The petitioner stated that samples from the 1988 study only were used to generate these supplemental metabolism data because these samples had the greater TRR levels. The petitioner noted that extracts of both 1988 and 1990 forage and seed samples (using the One-step Procedure) demonstrated similar TLC profiles.

Hay: No new data were submitted for hay using the One-step Procedure. The petitioner stated that forage was used as a representative substrate for all soybean foliage matrices for the following reasons: (i) it had the largest amount of extractable residues for all foliage matrices; (ii) fewer co-extracted interfering substances were present permitting easier analyses by TLC and HPLC; and (iii) soybean forage is more frequently used as an animal feed than soybean hay.

Sequential Procedure extracts: Briefly, fractions (methylene chloride, acetone, and water) from 11-month soybean forage samples obtained from plants treated at the 2x rate were analyzed by TLC. Each fraction was characterized by at least three different solvent systems (previously described) having different polarities. Based on the $R_f$s of the reference standards (previously described), corresponding TLC bands obtained using Solvent system I were scraped and reanalyzed by at least one other TLC solvent system as a metabolite confirmatory procedure. Chromatograms were presented for each analysis.

Forage: The petitioner characterized/identified 64.52% TRR (1.729 ppm). The parent compound, dimethenamid, was not detected. The metabolites TGA/M11 (collectively 2.49% TRR, 0.0667 ppm), oxalamide/TLA (collectively 19.5% TRR, 0.524 ppm), STGA (13.9% TRR, 0.371 ppm), STLA (7.90% TRR, 0.211 ppm), and sulfonate (14.1% TRR, 0.278 ppm) were distributed throughout the organosoluble extracts, with the majority detected in the acetone extract. An extremely polar component detected in acetone and methanol extracts accounted for 6.63% TRR (0.178 ppm). Reanalysis of discrete bands corresponding to TGA/M11 and oxalamide/TLA failed to resolve these bands into separate components.

The petitioner stated in the text of the submission that the TRR values obtained for forage fractions using the sequential method were "extrapolated down" to reflect the estimated TRR values for plants treated at a 1x rate; no explanation was given by the petitioner for conducting the extrapolation and no calculations were provided. The distribution of radioactivity into extractable fractions of forage using the Sequential Procedure are presented in Table 3; forage samples were obtained from plants treated at the 2x rate in 1988.

Seeds: 11-Month seed samples were not reanalyzed using the Sequential Procedure. Instead, the petitioner discussed previously reported and reviewed characterization/identification data presented in the original metabolism study. The petitioner stated that radioactive residues in seeds were highly polar components with HPLC peaks at or near background levels, and that characterization of extracts obtained from both extraction procedures gave similar HPLC chromatographic profiles. Sulfonate, STLA, STGA, and TGA/M11 (collectively 33.26% TRR) were identified in extracts obtained from both extraction procedures by TLC. The petitioner provided TLC and/or HPLC chromatograms for each reported analysis.

Hay: No new data were presented for the analysis of residues in hay, for the same reasons described above. Instead, the petitioner submitted a discussion regarding additional characterization/identification of unknown metabolites observed in extracts of hay (using the Sequential Procedure), from the original metabolism study. The discussion, which was not supported by any raw data, data summaries, or chromatograms for the hay analyses, is summarized below.

In methylene chloride extracts, a "diffuse zone" of unknown radioactivity observed for hay (TLC $R_f$ range, 0.67-0.89; HPLC rt, 30-31 minutes) and for forage (TLC $R_f$ range, 0.67-0.85) was further characterized by additional TLC solvent systems (not specified) followed by HPLC analysis (method not specified) of the scraped zone. These procedures tentatively identified the "diffuse zone" as TGA at 0.0652 ppm from 2x-treated hay samples (extrapolated to 0.0326 ppm for 1x-treated hay samples). Additional characterization of the unknown component in forage extracts using TLC solvent systems I, II, and IV indicated that the zone corresponded to the metabolites TGA/M11, which did not resolve as separate components. The petitioner infers that the residues observed in the hay extract are the same metabolites identified in the forage extracts.

In acetone extracts, two zones of radioactivity were observed for 2x-treated hay (TLC $R_f$ ranges, 0.37-0.48 and 0.45-0.58; HPLC rts of 4.7 and 28 minutes, respectively). The zone at $R_f$ 0.37-0.48 was incorrectly identified as a glutathione conjugate in the original report. Further characterization showed that the unknown component, initially eluted at 4.7 minutes, eluted with the solvent front using HPLC System IV. The unknown polar component accounted for 1.4% of TRR (0.046 ppm) in the initial 2x-treated hay sample (extrapolated to 0.023 ppm for 1x-treated hay samples). The second zone ($R_f$ 0.37-0.48; HPLC rt, 28 minutes) was identified as STGA based on TLC analyses of the initial forage sample extracts with TLC System II and of the extracts from 11-month forage samples with TLC Systems I, II, and III. Based on HPLC analyses, the second zone



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(now identified as STGA) accounted for 1.9% TRR (0.062 ppm) in 2x-treated hay (extrapolated to 0.031 ppm for 1x-treated hay).

In methanol extracts, three TLC zones of radioactivity were observed for 2x-treated hay (TLC $R_f$ ranges 0-0.15, 0.15-0.26, and 0.41-0.57). HPLC analysis of the 0.41-0.57 zone resolved two metabolites (rts of 4.5 and 28.3 minutes, respectively); analysis of the $R_f$ 0-0.15 zone also resolved a component with an rt of 4.5 minutes. The radioactivity in both 4.5 rt components was summed by the petitioner, who characterized this component (using TLC Systems I, II, and III) to be an unknown polar metabolite accounting for 0.8% TRR (0.025 ppm) in 2x-treated hay (extrapolated to 0.012 ppm for 1x-treated hay).

Further HPLC (method not specified) characterization of the $R_f$ 0.15-0.26 zone in hay methanol extracts revealed an unknown polar component accounting for 2.9% TRR (0.096 ppm) in 2x-treated hay (extrapolated to 0.048 ppm in 1x-treated hay).

The molecular structures for the metabolites identified in soybean forage and soybean seed are presented in Table 4.

Table 1. TLC characterization of 98% methanol extract of soybean forage (1988, 1.5 lb ai/A).

| Metabolite | Characterization of Entire Extract | | | | | | Further Characterization of Discrete TLC Bands | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Initial Sample Analysis [a] | | | 11-Month Sample Analysis | | | Scraped TLC Rf Range | Characterized Residues | Initial Sample | | 11-Month Sample | |
| | Rf Range | % TRR | ppm | Rf Range | % TRR | ppm | | | % TRR | ppm | % TRR | ppm |
| Sulfonate | -0.037-0.109 | 24.5 | 0.494 | -0.044-0.119 | 20.1 | 0.360 | 0.04-0.14 | Low Rf component | 3.59 | 0.0726 | 2.95 | 0.0529 |
| | | | | | | | | Sulfonate | 16.3 | 0.329 | 13.4 | 0.240 |
| STLA | 0.109-0.205 | 10.1 | 0.203 | 0.119-0.244 | 8.81 | 0.158 | 0.11-0.26 | STLA | 6.00 | 0.121 | 5.25 | 0.0942 |
| | | | | | | | | STGA | 2.51 | 0.0506 | 2.19 | 0.0393 |
| STGA | 0.205-0.351 | 15.3 | 0.309 | 0.244-0.359 | 11.7 | 0.210 | 0.25-0.41 | STGA | 11.5 | 0.231 | 8.77 | 0.157 |
| | | | | | | | | Oxalamide | 1.70 | 0.0343 | 1.30 | 0.0233 |
| Diffuse zone | 0.351-0.410 | 1.65 | 0.0334 | 0.359-0.431 | 3.32 | 0.0597 | N/A [b] | N/A | N/A | N/A | N/A | N/A |
| Oxalamide/TLA | 0.410-0.534 | 10.4 | 0.210 | 0.431-0.544 | 10.8 | 0.194 | 0.39-0.59 | Oxalamide | 6.45 | 0.130 | 6.72 | 0.121 |
| | | | | | | | | TLA | 2.55 | 0.0515 | 2.66 | 0.0476 |
| Diffuse zone | 0.534-0.602 | 2.34 | 0.0473 | 0.544-0.613 | 2.96 | 0.0530 | N/A | N/A | N/A | N/A | N/A | N/A |
| TGA/M11 | 0.602-0.708 | 4.13 | 0.0835 | 0.613-0.719 | 3.76 | 0.0674 | 0.63-0.77 | TGA | 1.67 | 0.0338 | 1.52 | 0.0273 |
| | | | | | | | | M9 | 1.48 | 0.0298 | 1.34 | 0.0241 |
| | | | | | | | | M11 | 0.73 | 0.0149 | 0.67 | 0.0119 |
| Diffuse zone | 0.708-0.814 | <0.01 | -- | 0.719-0.831 | <0.01 | -- | N/A | N/A | N/A | N/A | N/A | N/A |
| Diffuse zone | 0.814-0.919 | <0.01 | -- | 0.831-0.938 | <0.01 | -- | N/A | N/A | N/A | N/A | N/A | N/A |
| Diffuse zone | 0.919-1.00 | <0.01 | -- | 0.938-1.0 | <0.01 | -- | N/A | N/A | N/A | N/A | N/A | N/A |
| **Total characterized [c,d]** | -- | **<4.00** | **4.081** | -- | **<9.63** | -- | -- | -- | **9.09** | **0.183** | **4.40** | **0.190** |
| **Total identified [c]** | -- | **64.43** | **1.300** | -- | **55.17** | **0.989** | -- | -- | **49.41** | **0.996** | **42.48** | **0.762** |
| **Total [c,d]** | -- | **68.42** | **1.380** | -- | **61.48** | **1.102** | -- | -- | **58.50** | **1.179** | **46.88** | **0.951** |

[a] Values are from the original metabolism study and are reiterated in the present submission.
[b] N/A = not analyzed.
[c] Calculated by the study reviewer.
[d] All totals include diffuse zones, whether or not they were further characterized by TLC.

Table 2. TLC characterization of 98% methanol extract of soybean seed (1988, 1.5 lb ai/A).

| Tentative Identification | Characterization of Entire Extract | | | | | | Further Characterization of Individual Extractable Residues ≥0.01 ppm | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Initial Sample Analysis [a] | | | 11-Month Sample Analysis | | | | Initial Sample | | 11-Month Sample | |
| | TLC Rf Range | % TRR | ppm | TLC Rf Range | % TRR | ppm | Residue | % TRR | ppm | % TRR | ppm |
| ND [b] | -0.026-0.0395 | 2.84 | 0.0055 | -0.047-0.035 | 3.44 | 0.0067 | N/A [c] | N/A | N/A | N/A | N/A |
| Sulfonate | 0.0395-0.145 | 4.58 | 0.0089 | | | | N/A | N/A | N/A | N/A | N/A |
| ND | 0.145-0.211 | 2.38 | 0.0048 | 0.035-0.113 | 2.39 | 0.00469 | N/A | N/A | N/A | N/A | N/A |
| | | | | 0.113-0.195 | 2.43 | 0.000476 | | | | | |
| STLA | 0.211-0.289 | 4.19 | 0.0082 | 0.195-0.277 | 2.93 | 0.00575 | N/A | N/A | N/A | N/A | N/A |
| ND | 0.289-0.362 | 2.65 | 0.0052 | | | | N/A | N/A | N/A | N/A | N/A |
| STGA | 0.362-0.454 | 10.7 | 0.0210 | 0.277-0.362 | 10.3 | 0.0202 | STGA | 5.97 | 0.0116 | 5.71 | 0.0112 |
| ND | 0.454-0.526 | 2.07 | 0.0040 | 0.362-0.443 | 0.51 | 0.00099 | N/A | N/A | N/A | N/A | N/A |
| ND | 0.526-0.612 | 1.83 | 0.0036 | 0.443-0.552 | 2.26 | 0.00443 | N/A | N/A | N/A | N/A | N/A |
| ND | 0.612-0.704 | 3.57 | 0.0070 | | | | N/A | N/A | N/A | N/A | N/A |
| Oxalamide/TLA | 0.704-0.789 | 4.63 | 0.0090 | 0.552-0.607 | 3.17 | 0.00621 | N/A | N/A | N/A | N/A | N/A |
| TGA/M11 | 0.789-0.849 | 6.17 | 0.0120 | 0.607-0.689 | 5.94 | 0.0116 | TGA/M11 | 3.05 | 0.0060 | 2.94 | 0.0058 |
| ND | 0.849-0.914 | 1.45 | 0.0028 | 0.689-0.770 | <0.1 | 0.0001 | N/A | N/A | N/A | N/A | N/A |
| ND | 0.914-1.00 | 1.11 | 0.0022 | 0.770-1.0 | 0.3 | 0.0007 | N/A | N/A | N/A | N/A | N/A |
| **Total characterized [d,e]** | -- | **17.9** | **0.0351** | -- | **<7.99** | **0.0114** | -- | **17.90** | **0.0351** | **5.63** | **0.0114** |
| **Total identified [d]** | -- | **30.27** | **0.0591** | -- | **25.78** | **0.0505** | -- | **22.42** | **0.0437** | **18.19** | **0.0357** |
| **Total [d,e]** | -- | **48.17** | **0.0942** | -- | **<33.77** | **0.0618** | -- | **40.32** | **0.0788** | **23.82** | **0.0470** |

[a] Values are from the original metabolism study and are reiterated in the present submission.
[b] ND = Not determined due to low (≤0.01 ppm) levels of radioactivity.
[c] N/A = not applicable.
[d] Calculated by study reviewer.
[e] All totals include zones designated as ND, whether or not they were further characterized by TLC.

Table 3. Total radioactivity in fractions and metabolites extracted from soybean forage using the Sequential Extraction Procedure (1988, 3 lb ai/A).

| Metabolites | Extrapolated TRR [a,b] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Methylene Chloride | | Acetone | | Methanol | | Total Organic Extractable | | Characterization of scraped and reanalyzed bands [b] | |
| | % TRR | ppm | % TRR | ppm | % TRR | ppm | % TRR | ppm | % TRR | ppm |
| Extractable residues | 7.53 | 0.202 | 45.4 | 1.22 | 21.4 | 0.573 | 74.3 | 2.00 | N/A | N/A |
| Dimethenamid | ND | ND | ND | ND | ND | ND | ND | ND | N/A | N/A |
| TGA/M11 [d] | 3.27 | 0.0875 | 2.54 | 0.0681 | 0.492 | 0.0132 | 6.30 | 0.169 | 2.49 | 0.0667 |
| Oxalamide/TLA [d] | 1.83 | 0.0490 | 9.95 | 0.266 | 4.06 | 0.109 | 15.8 | 0.424 | 19.5 | 0.524 |
| STGA | 1.11 | 0.0296 | 8.90 | 0.238 | 5.00 | 0.134 | 15.0 | 0.402 | 13.9 | 0.371 |
| STLA | 0.6 | 0.0161 | 8.04 | 0.215 | 2.78 | 0.0744 | 11.4 | 0.306 | 7.90 | 0.211 |
| Sulfonate | 0.37 | 0.0099 | 12.4 | 0.333 | 5.13 | 0.137 | 17.9 | 0.480 | 14.1 | 0.378 |
| Extremely Polar Component | ND | ND | 3.36 | 0.090 | 3.87 | 0.104 | 7.23 | 0.194 | 6.63 | 0.178 |
| **Total characterized** * | **7.18** | **0.192** | **3.36** | **0.090** | **3.87** | **0.104** | **7.23** | **0.194** | **6.63** | **0.178** |
| **Total identified** * | **7.18** | **0.192** | **41.83** | **1.120** | **17.46** | **0.468** | **66.40** | **1.781** | **57.89** | **1.551** |
| **Total** * | **7.18** | **0.192** | **45.19** | **1.210** | **21.33** | **0.572** | **73.63** | **1.975** | **64.52** | **1.729** |

[a] The petitioner stated that the TRR values were "extrapolated down" to estimate the TRR values that would have been obtained using samples obtained from plants treated at the 1x level. No calculations were presented.

[b] Total forage TRR from the 2x treatment was 5.354 ppm (mean of four replicates).

[c] Values represent collective characterization of bands obtained by analyses of methylene chloride, acetone, and methanol extracts by solvent system I; the bands were scraped and reanalyzed using TLC solvent system VI.

[d] Components could not be resolved separately by TLC radioscan.

* Calculated by the study reviewer.

CBTS Comment

In summary, using the One-step Procedure, the petitioner characterized/identified 46.88% TRR (0.951 ppm) in forage extracts and 23.82% TRR (0.0470 ppm) seed extracts obtained from plants treated at the 1x rate and stored frozen for 11 months.

Metabolites identified and confirmed in forage extracts were sulfonate (13.4% TRR, 0.240 ppm), STLA (5.25% TRR, 0.0942 ppm), STGA (10.96% TRR, 0.1963 ppm), oxalamide (8.02% TRR, 0.1443 ppm), TLA (2.66% TRR, 0.0476 ppm), TGA (1.52% TRR, 0.0273 ppm) and M11 (0.67% TRR, 0.0119 ppm). Two unknowns, a"low $R_f$" component and M9, collectively accounted for 4.29% TRR (0.077 ppm). Five diffuse zones of radioactivity were detected: two zones collectively accounted for 6.28% TRR (0.1127 ppm); three additional zones each accounted for <0.01% TRR. Coeluting metabolites were resolved into separate components upon reanalysis of discrete TLC bands.

Metabolites identified and confirmed in seed extracts were sulfonate (3.44% TRR, 0.0067 ppm), STLA (2.93% TRR, 0.00575 ppm), STGA (5.71% TRR, 0.0112 ppm), oxalamide/TLA (collectively 3.17% TRR, 0.00621 ppm), and TGA/M11 (collectively 2.94% TRR, 0.0058 ppm). Six unspecified zones of radioactivity collectively accounted for 7.99% TRR (0.0114 ppm); each unspecified component was <0.01 ppm.

Using the Sequential Procedure, the petitioner characterized/identified 64.52% TRR (1.729 ppm) in forage extracts obtained from plants treated at the 1x rate and stored frozen for 11 months. The parent compound, dimethenamid, was not detected. The identified metabolites were TGA/M11 (collectively 2.49% TRR, 0.0667 ppm), oxalamide/TLA (collectively 19.5% TRR, 0.524 ppm), STGA (13.9% TRR, 0.371 ppm), STLA (7.90% TRR, 0.211 ppm), and sulfonate (14.1% TRR, 0.278 ppm). An extremely polar component detected in acetone and methanol extracts accounted for 6.63% of TRR (0.178 ppm). Coeluting metabolites were not resolved into separate components upon reanalysis.

No data were provided for the reanalysis of residues in seed extracts obtained using the Sequential Procedure. Instead, the petitioner discussed previously reviewed characterization/identification data presented in the original metabolism study. The petitioner stated that radioactive residues in seeds were highly polar components with HPLC peaks at or near background levels, and that characterization of extracts obtained from both extraction procedures gave similar HPLC chromatographic profiles. Sulfonate, STLA, and STGA were identified in extracts obtained from both extraction procedures by TLC. The petitioner provided TLC and/or HPLC chromatograms for each reported analysis.

No new data were submitted for residues in hay using either extraction procedure. The petitioner stated that forage was used as a representative substrate for all soybean foliage matrices for the following reasons: (i) it had the largest amount of extractable residues for all foliage matrices; (ii) fewer co-extracted interfering substances were present permitting easier analyses by TLC and HPLC; and (iii) soybean forage is more frequently used as an animal feed than soybean hay.

However, the petitioner submitted a discussion regarding additional characterization/identification of unknown metabolites observed in extracts of 2x-treated hay (using the Sequential Procedure), from the original metabolism study. The discussion was not supported by any raw data, data summaries, or chromatograms for the reanalyzed hay extracts. In methylene chloride extracts, using a combination of TLC and HPLC methods, the petitioner identified an unknown residue as TGA/M11 (0.0625 ppm, %TRR not reported); metabolite standards did not resolve separately during the analyses. The residues were quantified in methylene chloride extracts of 2x-treated



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forage only, and identical zones observed in hay extracts were inferred to be the same components identified in forage. In acetone extracts, two zones of unidentified radioactivity were identified as STGA (1.9% TRR, 0.062 ppm) and an unknown polar metabolite (1.4% TRR 0.046 ppm) by a combination of TLC and HPLC methods. In methanol extracts, three radioactive TLC zones were resolved into two components by HPLC. These components were characterized as unknown polar metabolites accounting for 0.8% TRR (0.025 ppm) and 2.9% TRR (0.096 ppm)

The petitioner provided HPLC chromatograms to demonstrate that the following suspected HPLC peaks (cited in the previous review) detected in 98% methanol extracts were not significantly different from background noise: (i) peak with a retention time (rt) of 20 minutes in 1988 hay; (ii) peak with a rt of 32 minutes in 1988 seed; and (iii) numerous peaks in 1990 seed.

Conclusion/Comment No. 1b from 7/19/91 CBTS review

Pending more detailed analysis, the metabolism of dimethenamid in/on soybeans may be different from metabolism in/on corn. Some metabolites identified in corn RACs have not been identified in soybean RACs, and some metabolites identified in soybean RACs have not been identified in corn RACs. [In corn grain, no metabolite comprised more than 2% of the applied radiocarbon. In corn fodder, metabolites identified included the oxalamide, the sulfoxide of the cysteine conjugate, and the glutathione conjugate. In corn forage, the major metabolite identified was the glutathione conjugate; other metabolites included the oxalamide, the cysteine conjugate, the sulfoxide of thiolactic acid, the malonyl conjugate, and the thiolactic acid conjugate. In corn silage, the glutathione conjugate was identified by TLC but not confirmed by HPLC (PP 0G3892, M.T. Flood, 1/24/91). In contrast, the metabolites identified in soybean RACs were the oxalamide, the sulfonate, and the sulfoxides of thiolactic and thioglycolic acid; other metabolites observed in corn have not been identified in soybean RACs.]

Petitioner's Response to Conclusion/Comment No. 1b

The petitioner responded to Conclusion No. 1b by submitting data (1993; MRID 42842501) pertaining to the comparative metabolism of dimethenamid in soybean and corn seedlings.

In-life phase

Soybean and corn seedlings were grown in-house in soil treated with [$^{14}$C]dimethenamid at a rate equivalent to ca. 8 lb ai/A (ca. 5x the maximum proposed rate). The petitioner stated that seedlings were used for the study because they yielded extracts with fewer co-extracted interfering substances than did the mature RACs; furthermore, the RACs contained relatively low levels of $^{14}$C-residues. Corn and soybean seedlings were harvested after 2-3 weeks and 2-5 weeks, respectively. Seedlings were continually grown and harvested in the same treated soil over an unspecified period of months and then pooled. Storage intervals and conditions following plant harvest were not reported.

Extraction

Pooled samples of corn and soybean seedlings were extracted with 50% methanol and extracts were evaporated to remove methanol. The resulting aqueous fraction was sequentially partitioned with hexane and acidic methylene chloride (pH 1-2). The aqueous fraction was lyophilized and resolubilized sequentially in methanol and water. The methanol-soluble residues obtained from the lyophilized extract were designated as A5; the methylene chloride/HCl fraction was designated as A3&A4.

Metabolite characterization/identification

In the characterization/identification scheme presented below, no quantitative data (e.g., TRR or %TRR values) were reported. Instead, the petitioner presented qualitative data including autoradiographs of TLC plates, HPLC chromatograms, and scans from NMR and mass spectrometric analyses to support the identification and confirmation of dimethenamid metabolites in soybean and corn seedlings.

Soybean seedlings: Analysis of fractions A3&A4 and A5 using TLC systems I and III identified sulfonate, STLA, STGA, oxalamide/TLA, and TGA/M11 by comparison with the $R_f$s of the appropriate radiolabeled reference standards. These metabolites were confirmed by scraping the metabolite bands from the TLC plates and reanalyzing the residues by TLC, HPLC, NMR and/or GC/MS (previously described in the original metabolism report).

Corn seedlings: Analysis of fractions A3&A4 and A5 using TLC system I identified sulfonate, oxalamide/TLA, STGA, and STLA; TGA was tentatively identified, but not confirmed. Confirmatory analyses were conducted as described above for soybean seedlings.

The molecular structures for the metabolites identified in corn and soybean seedlings are presented in Table 4.

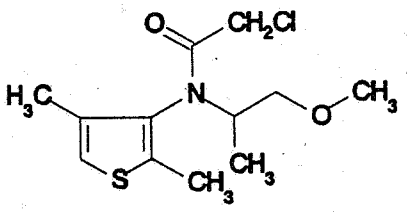
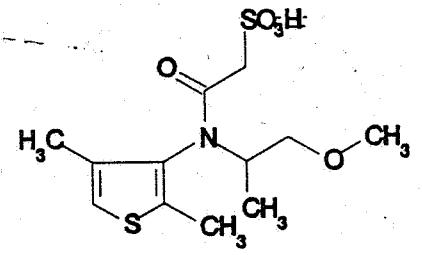
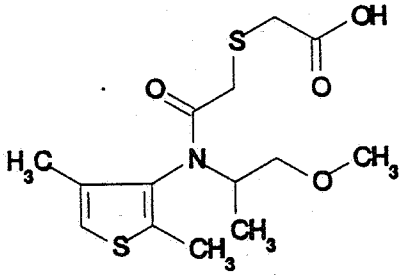
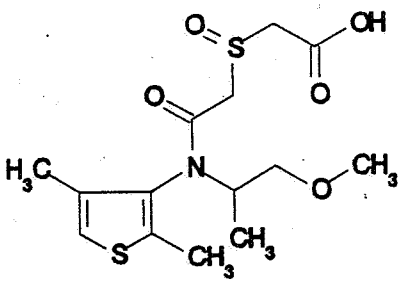
The petitioner also submitted data for a previously reviewed corn metabolism study (PP#0G3892, M. Flood, 1/24/91) for the purpose of demonstrating the similarities in the metabolic profiles for corn and soybean matrices. Corn forage was extracted with 50% methanol. Following evaporation of the methanol, the extract was lyophilized and cleaned by counter-current chromatography. The petitioner identified sulfonate, STLA, STGA, oxalamide/TLA, and TGA/M11; M9 was not detected in corn forage extracts. The corn forage metabolite profile is similar to the soybean forage metabolite profile except that M9 was detected only in soybean forage; however, M9 is only a minor metabolite (1.48% TRR. 0.0298 ppm).

The petitioner stated that the metabolic profile of dimethenamid in soybean is qualitatively identical to that of corn, but quantitatively different. Based on the available evidence, the petitioner proposed a metabolic pathway for dimethenamid in plants in which the parent is initially conjugated with glutathione or homoglutathione and then hydrolyzed to the cysteine conjugate. The parent also may be oxidized to oxalamide via M11. The cysteine conjugate may have three different fates: (i) oxidation to the sulfonate metabolite; (ii) deamination, decarboxylation, and oxidation to TGA, followed by oxidation to STGA; or (iii) deamination and oxidation to the TLA conjugate followed by oxidation to STLA (soybeans only). Cysteine, glutathione, and homoglutathione conjugates were proposed to be transient intermediates.

CBTS Comment

The conclusions of the 7/19/91 CBTS review regarding the metabolites identified in corn were based on incomplete data pertaining to the metabolism of dimethenamid in corn. The petitioner has since submitted additional corn metabolism data and CBTS has concluded that the nature of the dimethenamid residue in corn is adequately understood (CBTS No. 10763, M. Flood, 1/4/93). It was determined that dimethenamid is extensively metabolized in corn and that the sulfonate conjugate is the principal metabolite. Other metabolites identified in corn include the oxalamide, the thiolactic acid conjugate, the thioglycolic acid conjugate, the sulfoxide of the thiolactic acid conjugate, the sulfoxide of the thioglycolic acid conjugate, and M11. The presence of the glutathione conjugate in forage and fodder extracts was indicated but could not be confirmed.

Table 4. Dimethenamid and its metabolites in soybeans and corn (MRID 42842501).

| Code | Chemical Name<br>Structure | Substrate<br>Common Name |
|---|---|---|
| I. | 2-chloro-N-(1-methyl-2-methoxyethyl)-N-(2,4-dimethyl-thien-3-yl) acetamide | |
| |  | SAN-582H, dimethenamid |
| II. | N-(2,4-dimethyl-3-thienyl)-N-(2-methoxy-1-methylethyl)-2-sulfonyl acetamide | |
| |  | soybean seedlings, forage, and seed<br>corn seedlings<br>sulfonate |
| III. | N-(2,4-dimethyl-3-thienyl)-N-(2-methoxy-1-methylethyl)carboxymethylene thionylacetamide | |
| |  | soybean forage and seed<br>thioglycolic acid conjugate, TGA |
| IV. | N-(2,4-dimethyl-3-thienyl)-N-(2-methoxy-1-methylethyl)carboxymethylene sulfinylacetamide | |
| |  | soybean seedlings, forage, and seed<br>corn seedlings<br>sulfoxide thioglycolic acid conjugate, STGA |



(continued)

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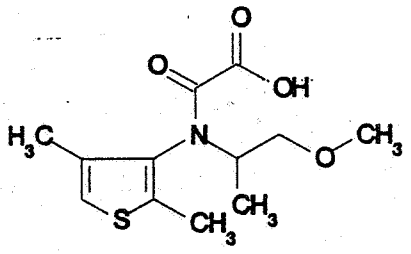
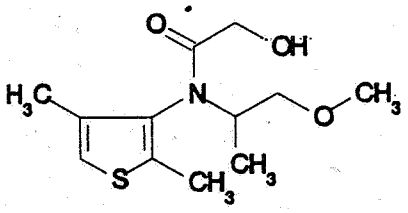
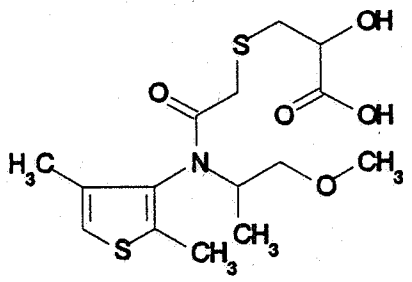
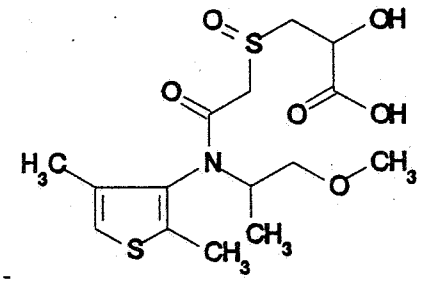
Table 4 *(continued)*.

| Code | Chemical Name<br>Structure | Substrate<br>Common Name |
|---|---|---|
| V. | N-(2,4-dimethyl-3-thienyl)-N-(2-methoxy-1-methylethyl)oxamic acid | |
| |  | soybean seedlings, forage, and seed<br>corn seedlings<br>**oxalamide** |
| VI. | N-(2,4-dimethyl-3-thienyl)-2-hydroxy-N-(2-methoxy-1-methylethyl) acetamide | |
| |  | soybean forage and seed<br>**M11** |
| VII. | N-(2,4-dimethyl-3-thienyl)-N-(2-methoxy-1-methylethyl)-2-carboxy-2-hydroxyethyl thionylacetamide | |
| |  | soybean seedlings, forage, and seed<br>corn seedlings<br>**thiolactic acid conjugate, TLA** |
| VIII. | N-(2,4-dimethyl-3-thienyl)-N-(2-methoxy-1-methylethyl)-2-carboxy-2-hydroxyethyl sulfinylacetamide | |
| |  | soybean seedlings, forage, fodder, and seed<br>corn seedlings<br>**sulfoxide of thiolactic acid conjugate, STLA** |



*(continued)*

Qualitative Nature of the Residue in Animals

Conclusion/Comment Nos. 2a and 2b from the 7/19/91 CBTS review

For permanent tolerances, the nature of the residue in ruminants is not adequately understood. Attempts should be made to further characterize the residue, as specified in the previous review (PP 0G3892, M.T. Flood, 1/24/91).

For permanent tolerances, the nature of the residue in poultry is not adequately understood. Attempts should be made to further characterize the residue, as specified in the previous review (PP 0G3892, M.T. Flood, 1/24/91).

Petitioner's Response to Conclusion/Comment Nos. 2a and 2b

The petitioner did not submit any new data for this topic, but instead presented a discussion of residue transfer in meat, milk, and poultry. The petitioner stated that residue transfer to animals is minimal and that all the major metabolites identified in corn and soybeans have been detected in at least one animal metabolism study (with rats, mice, chickens, or goats). The petitioner expects that no detectable radioactive residues would transfer to meat, milk, poultry, or eggs from feed containing residues at the proposed 0.01-ppm tolerance; the maximum total residue transfer was 1.36% of the dietary dose (in goat muscle). Based on TRR in soybeans from the 1988 study, the petitioner calculated that the maximum total residues in ruminant and poultry tissues would be 0.043 ppm and 0.0065 ppm in cattle liver and chicken liver, respectively; these calculations were based on feeding levels of 0.0228 and 0.0078 mg/kg/day for cattle and chickens, respectively.

CBTS Comment

CBTS has previously concluded that the nature of the residue in ruminants is adequately understood (CBTS No. 10763, M. Flood, 1/4/93) based on a metabolism study in which lactating goats were fed [$^{14}$C]dimethenamid at 223 ppm. Based on a diet consisting of 25% soybeans, the feeding level is much greater than 1000x the expected dietary burden. The parent compound is extensively metabolized in ruminants; no one compound is present at more than 10% of the total dimethenamid residue.

CBTS has also concluded that the nature of the residue in poultry is adequately understood (CBTS No. 9880, M. Flood, 7/29/92) based on a study in which laying hens were fed [$^{14}$C]dimethenamid at 167 ppm. Based on a diet consisting of 50% soybeans, the feeding level is much greater than 1000x the expected dietary level. The parent is the major constituent in poultry fat, but has not been identified in any other tissue. No other metabolite constituted 10% or more of the residue in any tissue.

The HED Metabolism Committee had concluded at the time when permanent tolerances for corn commodities were established that tolerances for dimethenamid in ruminants and poultry would not be required. The establishment of the proposed soybean grain tolerance is not expected to increase the maximum theoretical dietary burden of dimethenamid in animals.

Residue Analytical Methods

Conclusion/Comment Nos. 3a, 3b, and 3c from the 7/19/91 CBTS review

The submitted method to determine parent SAN-582H and oxalamide is inadequate for purposes of this temporary tolerance petition. Standard deviations of recoveries are unacceptably high. By petitioner's own assessment, the limits of detection with this method are 0.02 ppm, higher than the requested tolerance. Fortification samples show unacceptable deviation of recoveries at 0.1 ppm, and no other data were submitted to indicate that the method can detect residue at concentrations lower than 0.1 ppm.

Use of diazomethane, which is explosive and carcinogenic, for methylation of oxalamide is not recommended. If a safer reagent cannot be found, documentation must be provided supporting the need for using diazomethane.

On the basis of the data submitted, the method for determination of the sulfonate metabolite of SAN-582H must also be considered inadequate. Soybean RACs produce high backgrounds and/or interfering peaks at the positions where the sulfonate would be detected by HPLC. 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.

A previous review determined that the analytical method for parent SAN-582H only has undergone successful independent laboratory validation on corn and soybeans. For purposes of permanent tolerances, methods yielding acceptable recoveries must be developed for all components of the residue to be regulated, and these methods must be confirmed by an independent laboratory. Once the nature of the residue in plants and animals is adequately understood, recoveries of the residue to be regulated must be obtained under FDA's multiresidue protocols. Analytical reference standards must be provided to the Pesticides and Industrial Chemicals Repository, Research Triangle Park, NC.

Petitioner's Response to Conclusion/Comment No. 3c

The petitioner is proposing the use of the analytical method for parent only for the determination of dimethenamid in/on soybeans and their processed commodities. This method, designated as TDS No. BS2304 in this submission, has undergone successful independent laboratory validation with corn and soybeans and CBTS has requested that this method be validated for corn and soybeans at EPA laboratories (M. Flood, 9/24/92).

Briefly, subsamples (50-100 g) of milled soybeans were extracted twice with methanol:water (95:5, v:v). The extracts were combined and aliquots were cleaned by solid phase extraction on a pre-packed reversed-phase C-18 column, followed by chromatographic cleanup on a silica gel column using ethylacetate:cyclohexane (2:8, v:v) as the eluant. The extracts were then concentrated by rotary evaporation, dissolved in toluene, and injected onto a GC equipped with a thermionic nitrogen-specific detector (TSD); the limit of detection was stated to be 0.01 ppm.

Confirmatory analyses for dimethenamid were conducted using Method AM-0865-0791-0, a GC method using an HP-1 or HP-5 column and mass selective detection (MSD). This method can be used to determine residues of dimethenamid and oxalamide. Samples are extracted with methanol:concentrated sulfuric acid (100:3, v:v). The extracts are reduced in volume on a water bath at 95 C and then cleaned using solid-phase extraction on a C-18 Bond Elut column. The extract is reduced in volume, dissolved in toluene and injected onto the GC. The limit of detection is 0.01 ppm. Representative chromatograms of reference standards, control samples, fortified

control samples, and treated samples were included and sample calculations were presented for both methods.

Untreated control samples of soybean commodities were fortified with dimethenamid at 0.02-0.20 ppm and analyzed by Method TDS No. BS2304. Analyses were conducted by the petitioner. Interfering peaks due to sample background were noted for three forage samples and one straw sample. Confirmatory analyses using GC/MSD indicated that residues of dimethenamid were nondetectable (<0.01 ppm) in these samples.

Determination of dimethenamid in soybean processed commodities also was conducted using Method TDS No. BS2304. The following modifications were performed: (i) for oils (crude, degummed, refined, and refined bleached), samples (10-20 g) were dissolved in pentane and partitioned three times with acetonitrile; the acetonitrile extracts were combined and concentrated by rotary evaporation. Water was added and the extracts were then analyzed according to Method TDS No. BS2304 beginning at the silica gel column cleanup step; and (ii) for lecithin and soapstock, samples (5-10 g) were dissolved in acetone and then pentane was added. Samples were then analyzed as described above for oils. Confirmatory analysis of soybean processed commodities was not performed because no interfering peaks were present.

Concurrent method recoveries of dimethenamid from field residue samples are presented in Table 5 and method recoveries from soybean processed commodities are presented in Table 6.

Table 5. Concurrent recoveries of dimethenamid from soybean grain, forage, hay, and straw fortified with dimethenamid at 0.02-0.20 ppm and analyzed by Method TDS No. BS2304.

| Soybean commodity | Number of samples | Fortification level (ppm) | Percent Recovery |
|---|---|---|---|
| **1990 (MRID 42632801)** | | | |
| Forage | 6 | 0.05-0.15 | 84-105<br>Average ± sd = 95 ± 8 [a] |
| Grain | 4 | 0.05-0.10 | 80-110<br>Average ± sd = 97 ± 13 |
| Hay | 5 | 0.02-0.10 | 86-108<br>Average ± sd = 94 ± 9 |
| Straw | 4 | 0.05-0.10 | 78-104<br>Average ± sd = 90 ± 11 |
| **1991 (MRID 42606501)** | | | |
| Forage | 7 | 0.10 | 87-113<br>Average ± sd = 99 ± 9 |
| Grain | 7 | 0.10 | 71-102<br>Average ± sd = 87 ± 11 |
| Hay | 7 | 0.10 | 73-106<br>Average ± sd = 88 ± 13 |
| Straw | 7 | 0.10 | 70-108<br>Average ± sd = 87 ± 13 |

[a] Calculated by the petitioner; sd = standard deviation.

Table 6. Concurrent recoveries of dimethenamid from soybean processed commodities fortified with dimethenamid at 0.01-0.10 ppm and analyzed by Method TDS No. BS2304.

| Soybean commodity | Fortification level (ppm) | Percent Recovery [a] |
|---|---|---|
| Whole grain | 0.01 | 86 |
| | 0.10 | 80 |
| Grain dust | 0.01 | 84 |
| Hulls | 0.01 | 83 |
| | 0.10 | 65 |
| Solvent-extracted meal | 0.01 | 68 |
| | 0.10 | 86 |
| Crude oil | 0.01 | 86 |
| Crude lecithin | 0.01 | 66 |
| Refined oil | 0.01 | 122 |
| Soapstock | 0.01 | 82 |

[a] Each recovery value represents one sample.

Storage Stability Data

Conclusion/Comment No. 5 from the 7/19/91 CBTS review

For permanent tolerances, storage stability data must cover the longest storage time between sampling and analysis for residue field trials and processing studies. Storage stability data will be necessary for other components of the residue to be regulated once these other components have been determined.

Petitioner's Response to Conclusion/Comment No. 5

No storage stability data were submitted to validate sample storage intervals and conditions for the soybean field residue and processing studies. The registrant cited previously submitted storage stability data for residues of dimethenamid in/on soybean matrices which have been reviewed (CBTS No. 10890, M. Flood, 12/16/92). These data indicated that residues of dimethenamid were stable in/on soybeans stored frozen (≤-12 C) for up to 16 months. Samples from the soybean residue field trials were stored frozen for 19.8-26.3 months (1990 samples) and 3.7-13.4 months (1991 samples) prior to analysis. Samples of soybeans and their processed fractions were stored frozen for 0.5-13.3 and 12-13.6 months, respectively, prior to analysis.

CBTS Comment

No data are available reflecting the storage stability of residues of dimethenamid in soybean processed commodities. In addition, samples of soybeans from the 1990 field residue trials were stored for up to 26 months prior to analysis; no data are available reflecting the storage stability of residues of dimethenamid in/on soybeans stored frozen for longer than 16 months.

Magnitude of the Residue in/on Soybeans

Conclusion/Comment No. 4 from the 7/19/91 CBTS review

For permanent tolerances, the nature of the residue in plants must be adequately understood, and analytical methods to detect residues to be regulated must be established. Either samples must be reanalyzed using a revised method, in which case appropriate storage stability data would be necessary, or new residue trials must be carried out with analyses by the revised method. In any event, additional field trials or residue analyses may be required depending on the nature of the residue in/on soybeans.

Petitioner's Response to Conclusion/Comment No. 4

The petitioner responded to Conclusion/Comment No. 4 by submitting residue data (1992; MRID 42632801) pertaining to the reanalysis of samples from field trials conducted in 1990 using Method TDS No. BS2304 as well as residue data (1992; MRID 42606501) from new field trials conducted in 1991. These data are presented below. We note that the 1990 field trials were described in the previous 7/19/91 CBTS review.

Fifteen tests were conducted in 1990 in AR(3), IL(3), MN(3), NC(3), and OH(3) and twenty-one tests were conducted in 1991 in GA(3), IN(3), KS(3), MD(3), MN(3), NE(3), and OH(3) in which soybeans received a single application of the 7.5 lb/gal EC (Frontier® Herbicide 7.5L) formulation at 1.5 lb ai/A made with three different types of application systems, preplant shallow incorporated (1- to 4-in. depth), broadcast preemergence, and broadcast early postemergence. Soybean commodities were harvested at the following intervals after application: 22-55 days (forage, at the 6 trifoliate stage of growth), 78-113 days (hay), and 93-158 days (grain and straw). The applied rate was 1x the maximum proposed seasonal rate. Applications were made using ground equipment with $CO_2$ tractor-mounted, hand-held, or backpack sprayer in 14.9-28.33 gal/A of finished spray and at pressures of 19-42 psi. The petitioner provided adequate raw data pertaining to the field portions of the tests. These raw data include field notes and/or reports on application, sprayer calibration, number of nozzles, nozzle spacing, harvest, plot size and maintenance, and equipment. Plot sizes ranged from 0.01 to 0.06 A.

Samples were stored in dry ice within 5 hours of collection, shipped frozen, and stored frozen at -40 to -3 C for 19.8-26.3 months (1990 samples) and 3.7-13.4 months (1991 samples) prior to analysis. Samples were analyzed using Method TDS No. BS2304.

Residues of dimethenamid were nondetectable (<0.01 ppm) in/on the following reanalyzed samples from the 1990 study and new samples from the 1991 study: 36 samples of forage, 33 samples of grain, 36 samples of hay, and 33 samples of straw. Apparent residues of dimethenamid were nondetectable (<0.01 ppm) in/on 13 samples of untreated forage, 11 samples of untreated grain, 12 samples of untreated hay, and 11 samples of untreated straw. Interfering peaks were noted in the GC/TSD chromatograms of the following soybean commodities: forage (10 treated and 3 control samples), hay (2 treated samples), and straw (3 treated and 1 control sample). Confirmatory analyses of these samples by GC/MSD indicated that residues of dimethenamid were nondetectable (<0.01 ppm) in each sample. The petitioner explained that the interfering peaks were due to sample background.

CBTS Comment

Geographic representation is adequate since the tests states of AR(5%), GA(1%), IL(18%), IN(9%), KS(2%), MD(1%), MN(9%), NE(4%), NC(2%), and OH(7%) and the neighboring states of IA(17%)



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and MO(6%) collectively accounted for ca. 80% of the 1990 U.S. soybean production (Agricultural Statistics 1991, USDA). The available data indicate that residues of dimethenamid were nondetectable (<0.01 ppm) in/on soybean commodities harvested 22-55 days (forage), 78-113 days (hay), and 93-158 days (grain and straw) following a single application of the 7.5 lb/gal EC (Frontier® Herbicide 7.5L) formulation at 1x the maximum proposed seasonal rate.

Magnitude of the Residue in Soybean Processed Fractions

Conclusion/Comment No. 6 from the 7/19/91 CBTS review

For the purposes of permanent tolerances, residue data submitted on soybean processed commodities are inadequate. Data on recoveries of fortified samples reinforce the defects of the method used. Recoveries of SAN-582H were unacceptably high for crude oil; recoveries of the oxalamide were unacceptably high for crude oil, and unacceptably low for crude oil (one sample) and soapstock. In addition, petitioner claimed a limit of detection of 0.02 ppm, but samples were fortified at levels several times higher. These data do not provide convincing evidence that concentration of SAN-582H or the oxalamide from whole grain to processing fractions could be detected if it occurred. In addition, no storage stability data were provided for SAN-582H on soybeans or soybean processed commodities.

For permanent tolerances, samples will have to be reanalyzed using an acceptable analytical method, or new trials will have to be conducted. In order to evaluate whether or not residues concentrate during processing, it may be necessary to analyze metabolites found on soybeans. Determination of which metabolite(s) to analyze requires an understanding of the nature of the residue in soybeans and development of an acceptable analytical method. Residues must also be analyzed on soybean grain dust as a processed commodity.

Petitioner's Response to Conclusion/Comment No. 6

The petitioner responded to this deficiency by submitting data (1992; MRID 42571602) from a new soybean processing study. In a test conducted in OH during the 1991 season, soybeans were harvested at maturity following a single preemergence broadcast application of the 7.5 lb/gal EC (Frontier® Herbicide 7.5L) formulation at 7.5 lb ai/A (5x the maximum proposed seasonal application rate). The test plot size was 0.05 A (55 ft. x 40 ft.). Applications were made using ground equipment with a $CO_2$ hand-held sprayer in 20.19 gal/A of finished spray and at a pressure of 22 psi. The petitioner provided adequate raw data pertaining to the field portion of the tests.

Treated and untreated soybean samples were harvested 137 days after application. Samples were shipped in a freezer truck on the day of sampling to the Engineering Biosciences Research Center of Texas A&M University (College Station, TX) for processing. At the processing facility, soybeans treated at 5x were dried, cleaned by aspiration, screened, and processed by batch using a simulated industrial procedure. The processing resulted in grain dust, hulls, kernels, meal, crude oil, degummed oil, crude lecithin, refined oil, soapstock, refined bleached oil, refined bleached hydrogenated oil, and refined bleached hydrogenated deodorized oil. Adequate descriptions of the processing method and material balance were provided. The processed fractions were then shipped frozen to the analytical laboratory (Sandoz Agro, Inc., Des Plaines, IL). Untreated control and treated samples were analyzed for residues of dimethenamid using Method TDS No. BS2304. Samples of soybeans and their processed fractions were stored frozen for 0.5-13.3 and 12-13.6 months, respectively, prior to analysis.



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The treated soybean samples that were used for processing bore nondetectable (<0.01 ppm) residues of dimethenamid. After processing, residues of dimethenamid were also nondetectable (<0.01 ppm) in three samples of grain dust, and one sample each of hulls, solvent-extracted meal, crude oil, degummed oil, crude lecithin, refined oil, soapstock, and refined bleached oil. Apparent residues of dimethenamid were nondetectable (<0.01 ppm) in one sample of each untreated processed commodity.

CBTS Comment

The available processing data indicate that residues of dimethenamid were nondetectable (<0.01 ppm) in soybean hulls, meal, crude and refined oil, soapstock, and grain dust processed from soybeans bearing nondetectable (<0.01 ppm) residues of dimethenamid following treatment at 5x the maximum proposed seasonal rate. The maximum theoretical concentration factor for soybeans is 12x (Agency's Maximum Theoretical Concentration Factor Memorandum, dated 1/93).

Meat, Milk, Poultry, and Eggs

Conclusion/Comment No. 7 from the 7/19/91 CBTS review

Results of animal feeding studies have not been submitted and are not needed for temporary tolerances. For permanent tolerances, the need for such studies will be assessed once the nature of the residue in plants and animals and the magnitude of the residues in or on soybean commodities have been determined.

Petitioner's Response to Conclusion/Comment No. 7

The petitioner has not responded to this conclusion.

EPA MEMORANDA CITED IN THIS REVIEW

| | |
|---|---|
| CBTS No.: | 7183 |
| Subject: | PP#0G3892. SAN-582H Herbicide in/on Field Corn. New Chemical Review. Evaluation of Analytical Methods and Residue Data. |
| From: | M.T. Flood |
| To: | J. Miller |
| Dated: | 1/24/91 |

| | |
|---|---|
| CBTS No.: | 8000 |
| DP Barcode No.: | D164289 |
| Subject: | PP1G3980 - SAN-582H Herbicide in or on Soybeans. New Chemical Review. Evaluation of Analytical Methods and Residue Data. |
| From: | J. Abbotts |
| To: | S. Khattar and Toxicology Branch |
| Dated: | 7/19/91 |
| MRID(s): | 418438-01 through -04 |

| | |
|---|---|
| CBTS No.: | 8775 |
| DP Barcode No. | D170099 |
| Subject: | Feeding Restrictions for Soybeans; Response to Sandoz Letter of 10/9/91. |
| From: | R. Lascola |
| To: | J. Stone/C. Giles-Parker |

Dated: 1/6/92

CBTS No.: None
Subject: PP#0F3918 - SAN-582H in/on Field Corn. Petition Method Validation Request.
From: M.T. Flood
To: D.A. Marlow
Dated: 7/16/92

CBTS No.: 8787
DP Barcode No.: D169997
Subject: SAN582H: Product Chemistry Data Submitted to Support Registration.
From: M. Flood
To: C. Giles-Parker/J. Stone and A. Kocialski
Dated: 7/29/92
MRID(s): 41662401-41662405

CBTS No.: 9880
DP Barcode No.: D178417
Subject: PP#0F3918. SAN-582H (Frontier®/Dimethenamid) in/on Field Corn. Evaluation of Analytical Methods and Residue Data. New Chemical Review.
From: M. Flood
To: C. Giles-Parker/J. Stone and A. Kocialski
Dated: 7/29/92
MRID(s): 42289502-42289508 and 42310301

CBTS No.: None
Subject: PP#0F3918 - SAN-582H in/on Field Corn. Petition Method Validation Request.
From: M.T. Flood
To: D.A. Marlow
Dated: 9/24/92

CBTS No.: None
Subject: The Metabolism Committee Meeting Held on November 3, 1992: Plant and Animal Metabolism of SAN-582H.
From: M. Flood
To: The Metabolism Committee
Dated: 11/10/92

CBTS No.: 10890
DP Barcode No.: D184696
Subject: SAN-582H/Dimethenamid. Responses to CBTS Review of 7/29/92.
From: M.T. Flood
To: C. Giles-Parker/J. Stone and Toxicology Branch
Dated: 12/16/92
MRID(s): 42543601

CBTS No.: 10763
DP Barcode No.: D183772
Subject: SAN-582H (Dimethenamid/Frontier®). Metabolism and Residue Data. Submission dated 10/15/92.
From: M. Flood
To: C. Giles-Parker/J. Stone and Toxicology Branch

Dated: 1/4/93
MRID(s): 42516000, 42516003, 42516201, and 42516202

CBTS No.: 10761
DP Barcode No.: D183774
Subject: SAN582H: Product Chemistry Data Submitted to Support Registration.
From: M. Flood
To: C. Giles-Parker/J. Stone and Toxicology Branch II
Dated: 1/22/93
MRID(s): 42512900-42512912

CBTS No.: 11323
DP Barcode No.: D187725
Subject: PP#0F3918 -- SAN 582H (Dimethenamid). Product Chemistry Requirements. Amendments dated 2/28/93, 2/29/93.
From: M. Flood
To: C. Giles-Parker/J. Stone
Dated: 2/11/93
MRID(s): 42647501

MASTER RECORD IDENTIFICATION NUMBERS

The citations for the MRID documents referred to in this review are presented below.

42571602 Jimenez, N.C. (1992) Magnitude of the Residue of SAN-582H in Soybean Grain and Soybean Processed Fractions. Laboratory Project ID: 414108. Report No. 36. Unpublished study submitted by Sandoz Agro, Inc. 253 p.

42606501 Jimenez, N.C. (1992) Crop Residue Study with SAN-582H on Soybeans (1991 Season). Laboratory Project ID: 414108. Report No.: 31. Unpublished study submitted by Sandoz Agro, Inc. 613 p.

42632801 Jimenez, N.C. (1992) Reanalysis of Soybean Samples from the 1990 Season Crop Residue Study with SAN-582H. Laboratory Project ID: 414108. Report No. 35. Unpublished study submitted by Sandoz Agro, Inc. 416 p.

42842501 Moore, P. A. (1992) Response to the EPA's Concerns on The Soybean Metabolism of SAN-582H Study. SCPC Report No. 414105-19, MRID# 418438-01. Replacement for previously submitted study MRID 42571601. Unpublished study conducted by Sandoz Agro, Inc., Des Plaines, IL. 200 p.

References (not used):

[The following MRID was not reviewed because a corrected document was submitted (MRID 42842501).]

42571601 Moore, P. A. (1992) Response to the EPA's Concerns on The Soybean Metabolism of SAN-582H Study. SCPC Report No. 414105-19, MRID# 418438-01. Unpublished study conducted by Sandoz Agro, Inc., Des Plaines, IL. 200 p.

[The following MRID was not reviewed because it did not contain data that would be useful to satisfy residue chemistry data requirements.]

42571603 Yu, C.C.; Guirguis, A.S.; and Nietschmann. (1992) SAN 582H: Determination of the Presence of Plant Metabolites in Rat. Laboratory Project No. 414105. Report No. 28A. Unpublished study conducted by Sandoz Agro, Inc. 64 p.



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