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

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OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM:

SUBJECT: PP 1G3980. SAN-582H Herbicide in or on Soybeans.
MRID Nos. 418438-01 through -04.
DEB No. 8000. DP Barcode No. D164289.

New Chemical Review. Evaluation of Analytical Methods and Residue Data.

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Sandoz Crop Protection Corporation is proposing the following temporary tolerances for residues of its herbicide SAN-582H, 2-chloro-N-[(1-methyl-2-methoxy)ethyl]-N-(2,4-dimethyl-thien-3-yl)-acetamide, parent compound only:

Soybean grain	0.01 ppm
Soybean forage	0.01 ppm
Soybean hay	0.01 ppm

No temporary or permanent tolerances have as yet been established for this chemical. The petitioner has submitted other proposals for a temporary tolerance on corn (PP 0G3892) and for a permanent tolerance on corn (PP 0F3918). An EUP for soybeans with a crop destruct clause was granted in May 1991, and the company is now submitting data for an EUP with associated temporary tolerances.

The proposed EUP permits the use of up to 466 gallons (3495 lb ai) of SAN-582H 7.5L on up to 4660 acres of soybeans over a two year period.

Conclusions

1a. For purposes of this temporary tolerance petition, the nature of the residue in or on soybeans is adequately understood. The residue to be regulated is parent SAN-582H. Residues of parent in or on soybean RACs are present at or below the limits of detection.

For permanent tolerances, the nature of the residue in or on soybeans is not adequately understood, and additional characterization is required. At present, petitioner has confirmed the identities of metabolites representing about 30 percent of the residue in forage, 24 percent of the residue in hay, and 26 percent of the residue in seed (grain).

Efforts should be made to identify all metabolites present at concentrations greater than 0.05 ppm and/or 10% of the total radiocarbon residue. Further efforts should be taken to characterize compounds which petitioner has identified as unknowns. 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. In order to document the identification of metabolites, petitioner must provide chromatograms which provide confirmation. Further details are contained in this memo, section "Comments, Nature of the Residue in Plants." Assignment of permanent tolerances will also depend on an evaluation of the toxicological significance of metabolites.

1b. Pending more detailed analysis, the metabolism of SAN-582H in or on soybeans may be different from metabolism in or 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.

2a. Metabolism of the residue in ruminants was reviewed for the petition for temporary tolerances in or on corn, and CBTS concluded that for purposes of a temporary tolerance, the nature of the residue in ruminants is adequately understood. The residue to be regulated is parent SAN-582H. Due to the low predicted total residues in ruminant tissue and milk, it is not necessary that temporary tolerances be established for these commodities. 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).

2b. Metabolism of the residue in poultry was reviewed for the petition for temporary tolerances in or on corn, and CBTS concluded that for purposes of a temporary tolerance, the nature of the residue in poultry is adequately understood. The residue to be regulated is parent SAN-582H. Due to the low predicted total residues in poultry tissues and eggs, it is not necessary that temporary tolerances be established for these commodities. 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).

3a. 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 residues 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.

3b. 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.

3c. A previous review determined that the analytical method for parent SAN-582H only has undergone successful independent laboratory validation on corn and soybeans. CBTS has recommended that the method be validated for corn and soybeans at EPA laboratories (PP 0G3892, M.T. Flood, 6/11/91). For the purposes of this temporary tolerance, this method is sufficient to detect SAN-582H, the residue to be regulated.

For permanent tolerances, the nature of the residue in plants and animals must be adequately understood, 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.

4. Residue field trials were carried out in nine states. SAN-582H was present at or below the limits of detection of the analytical method, but the oxalamide and sulfonate metabolites were detected in some samples. The analytical methods used were those determined to be inadequate in conclusions 3a and 3b above. However, for the purpose of temporary tolerances, conclusion 1a based on the radiolabel metabolism study indicates that SAN-582H, the residue to be regulated, would not be expected to exceed the proposed tolerances.

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 or on soybeans.

5. No data were submitted on storage stability of SAN-582H or its metabolites in soybeans. For the purposes of temporary tolerances, data from the radiolabel plant metabolism study are sufficient to indicate that residues of SAN-582H are not detectable in or on soybeans. For permanent tolerances, storage stability data must cover the longest storage time between sampling and analysis for residue field trials. Storage stability data will be necessary for other components of the residue to be regulated once these other components have been determined.

6. For the purposes of temporary tolerances, residue data on soybean processed commodities are not required. 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 the purposes of 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. Adequate storage stability data must be submitted. Residues must also be analyzed on soybean grain dust as a processed commodity (Overview of Residue Chemistry Guidelines, 10/10/89, Attachment 2).

7. 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.

Recommendation

CBRS has no objection to the establishment of the proposed temporary tolerances for SAN-582H in or on soybean RACs, for a two year period. The method for enforcement purposes is the method indicated in Conclusion 3c above, subject to validation by EPA laboratories. Analytical reference standards of SAN-582H should be provided by petitioner to the Pesticides and Industrial Chemicals Repository, Research Triangle Park, NC.

For permanent tolerances, the petitioner must better determine the nature of the residue in plants and animals (Conclusions 1a, 2a, 2b), develop analytical methods for additional components of the residue, if necessary (Conclusion 3c), have the analytical method validated by an independent laboratory (Conclusion 3c), determine recoveries of the residue to be regulated under FDA's multiresidue protocols (Conclusion 3c), submit additional residue data as appropriate (Conclusion 4), submit adequate storage stability data (Conclusion 5), submit additional processing study data or reanalyze existing samples, as appropriate (Conclusion 6), and submit animal feeding studies if necessary (Conclusion 7).

We recommend that a copy of this review be sent to the petitioner.

Detailed Considerations

Manufacture and Formulation

The manufacturing process and product chemistry data have been reviewed by Dynamac Corporation and undergone secondary review in CBTS. Product chemistry data gaps have been identified. However, for purposes of this temporary tolerance petition, there

should be no residue chemistry problems. (PP 0G3892, M.T. Flood, 1/24/91)

SAN 582H 7.5L Herbicide contains 78.5% 2-chloro-N-[(1-methyl-2-methoxy)ethyl]-N-(2,4-dimethyl-thien-3-yl)-acetamide, 7.1% related compounds (also considered as active ingredients), and 14.4% inert ingredients. SAN 582H contains 7.5 lb active ingredient (ai) per gallon.

The structure of SAN-582H, along with those of related compounds, is shown in Figure I, at the end of this memo.

Proposed Use

SAN 582H may be applied preplant surface, preplant incorporated, preemergence and/or early postemergence up to the unifoliate leaf stage of soybeans. The herbicide may be applied with ground or aerial equipment at levels up to 1.5 pints (1.4 lb ai) per acre, which is the seasonal maximum level.

Do not graze or feed treated soybean foliage to livestock for at least 60 days following application. Do not rotate to crops other than field corn or soybeans prior to the spring after application.

Nature of the Residue in Plants

Metabolism of SAN-582H in soybeans is discussed in the report:

"Uptake, Translocation, and Metabolism of the Herbicide SAN-582H in Soybean," March 22, 1991, Sandoz Crop Protection Corporation Report No. 414105-19 (MRID No. 418438-01) (designated below as Metabolism Report).

SAN-582H, radiolabeled in ^{14}C at the 3-thienyl position (ring carbon bonded to nitrogen) was added to unlabeled compound to give a resulting specific activity of 1.54×10^4 dpm/μg before use. Inert ingredients for the 720 EC formulation were added and methanol was added to a specified volume. Aliquots of this solution were diluted with water and applied to 1.0 square meter soil surface. Soil was treated the day after planting. Application rates were 1.5 lb ai/acre (maximum label rate) and 3.0 lb ai/acre (exaggerated rate).

Soybean RAC samples (forage, hay, seed--identical to grain) were collected. Forage samples were removed 49 days post treatment. At 100 days post treatment, separate samples of hay and immature seed were taken. At 118 days post treatment, separate samples of straw and seed were taken; root samples were also taken. Samples were frozen immediately after collection, shipped from the field

site in dry ice, and stored at -20°C until analyzed. Forage was harvested in July 1988, normal harvest was carried out in September 1988, and samples were analyzed from November 1989 to March 1991. An additional set of samples was harvested in 1990, but details on these samples were generally lacking.

Total radiocarbon in plant tissues was determined by combustion followed by scintillation counting. Total radiocarbon in plants is summarized in Table 1. Samples were analyzed for immature seed and straw, which are not RACs, for the purpose of isolation and identification of metabolites.

Table 1. Radiocarbon in Soybeans Grown in Treated Soil.

1988 Data				1990 Data			
Appl. Rate	**Plant Part**	**DAT***	**Avg. ppm****	**Appl. Rate**	**Plant Part**	**DAT***	**Avg. ppm****
1.5 lb ai/A	Forage	49	2.16	1.5 lb ai/A	Forage	42	0.30
	Hay	100	1.86		Hay	100	0.91
	Imm. Seed	100	0.09		Imm. Seed		NR
	Leaves	113	2.12		Leaves		NR
	Straw	118	1.22		Straw	128	0.89
	Seed	118	0.24		Seed	128	0.13
	Roots	118	2.64		Roots		NR
3.0 lb ai/A	Forage	49	3.72	3.0 lb ai/A	Forage	42	0.60
	Hay	100	2.94		Hay	100	2.28
	Imm. Seed	100	0.20		Imm. Seed		NR
	Leaves	113	5.12		Leaves		NR
	Straw	118	2.37		Straw	128	1.71
	Seed	118	0.48		Seed	128	0.27
	Roots	118	5.08		Roots		NR

Table notes:
*DAT = days after treatment
**Avg. ppm = average μg equivalent of ^{14}C-SAN-582H/fresh weight in g from multiple replicates
Imm. seed = immature seed,
Leaves were collected from the soil surface
NR = not reported

Further characterization was achieved on the 1988 samples using the extraction scheme shown in Figure II (end of this memo). The distribution of radioactivity with extraction is indicated in Table 2.

Table 2. Extraction of SAN-582H in Soybean RACs.

RAC	% Extractable						Not Extracted	Sum
	Hexane	Methylene Chloride	Acetone	MeOH	Acid-Released			
					Ethyl Acetate	Aqueous		
Forage	NA	7.9	44.5	18.8	5.3	5.8	5.5	87.8
Hay	NA	5.8	40.0	22.4	10.4	6.9	8.9	94.4
Seed	5.1	11.3	6.7	22.6	26.7	14.3	9.8	96.5

Table notes:
Hexane is used only for seeds to remove lipid content;
NA = not applicable.
MeOH = methanol.
Data were obtained from an application rate of 1.5 lb ai/acre.

The characterization of metabolites in each RAC is described below. Structures of known SAN-582H metabolites are shown in Figure I and Table III (end of this memo). The oxalamide (Figure I) is a known metabolite in soil, but may also be a plant metabolite.

Forage. Methylene chloride extracted 7.9% of the radioactivity in the sample. The major well-defined band by TLC was detected at a position corresponding to the oxalamide. However, extraction and subsequent HPLC analysis showed that only 14% of the radioactivity in this band was oxalamide, with the balance associated with coextracted materials. A major diffused band was extracted from TLC and found to have a multitude of peaks (about 9) upon HPLC analysis. The sulfoxide of thiolactic acid and sulfonate were also detected in the methylene chloride extract at levels below 0.01 ppm.

The acetone extract contained about 45% of the radioactivity in the forage sample. Three metabolites were isolated by TLC and confirmed by HPLC. These were sulfoxide of thiolactic acid, sulfonate, and oxalamide.

The methanol extract contained about 20% of the total radioactivity in the sample. TLC and HPLC analysis of the methanol extract showed a pattern similar to that of the acetone extract. The major metabolites present in the methanol were the oxalamide, sulfonate, and sulfoxide of thiolactic acid.

The only acid released metabolite resolved in the TLC analysis of samples was the sulfoxide of thiolactic acid. About 2% of the radioactivity in the sample was incorporated in the biomass of lignin and a total of about 6% was unextractable after acid hydrolysis.

For all the forage samples, oxalamide was the most abundant metabolite, accounting for about 16.8% of radioactivity. Sulfonate, present in both the acetone and methanol extracts, accounted for about 7% of the radioactivity. The sulfoxide of thiolactic acid was present in amounts similar to that of the sulfonate but was largely present in the methanol extract. About 20 other entities containing radioactivity were detected in the forage samples. No parent SAN-582H was detected in any of the extraction samples.

Hay. Methylene chloride extracted 5.8% of the radioactivity in hay. None of the metabolites isolated on TLC plates were confirmed by HPLC except for the sulfoxide of thiolactic acid conjugate. HPLC analysis of compounds with TLC Rf range 0.67 to 0.89 showed a strong peak at retention time 30.7 min; this peak was classified as an unknown (Metabolism Report, Figure 45).

The majority of radioactivity (40%) in the sample was extracted in acetone. The major metabolites isolated by TLC and confirmed by HPLC were oxalamide, sulfonate, and sulfoxide of thiolactic acid. Two peaks on HPLC were classified as unknowns; these were present at concentrations of 0.07 and 0.03 ppm SAN-582H equivalents. In comparison, the oxalamide was present at 0.06 ppm, sulfonate at 0.20 ppm, and the sulfoxide of thiolactic acid at 0.12 ppm.

The methanol extract contained about 22% of the total radioactivity in hay. After analysis by TLC and HPLC, the major metabolites present in methanol were oxalamide, sulfonate, and sulfoxide of thiolactic acid. An unknown is also designated on HPLC with a retention time of about 28 min (Metabolism Report, Figure 48); this may be the same as one of the unknowns identified in the acetone extract (Metabolism Report, Figure 46).

About 10% of the total residue in hay was released by acid hydrolysis and extracted in ethyl acetate. The acid released metabolites were identified as oxalamide and the sulfoxide of thiolactic acid. About 5% of the total radioactivity in the sample was unextractable after acid hydrolysis, and about 1.5% of total radioactivity was incorporated in the biomass of lignin.

For all the hay samples, oxalamide accounted for about 5.3% of total radioactivity; sulfonate accounted for about 10.6%, and sulfoxides for about 7.8%. No parent SAN-582H was detected in any extract sample.

Seed. Hexane extracted about 5.1% of the radioactivity in mature seed. Methylene chloride extracted about 11.3% of the radioactivity in seed. The only significant metabolite in the methylene chloride extract was the sulfoxide of the thiolactic acid, present at close to 0.01 ppm. Total radioactivity extractable in acetone was less than 0.03 ppm. TLC analysis indicated the presence of the oxalamide, sulfonate, and sulfoxide of thiolactic acid, all present at less than 0.01 ppm.

The methanol extract contained about 20% of the radioactivity in hay. TLC analysis, followed by HPLC, identified the sulfonate and sulfoxide of thiolactic acid, each present at about 0.015 ppm in the samples from the exaggerated rate of application.

Acid-released material soluble in ethyl acetate represented about 25% of the radioactivity in seed. TLC analysis showed bands corresponding to the sulfoxide of thiolactic acid and another corresponding to the oxalamide.

For all seed samples, oxalamide accounted for 6.6% of total radioactivity; sulfonate for 7.5%; and sulfoxides for 11.7%. Parent SAN-582H was not detected in any extraction samples.

Overall concentrations of metabolites from the three RACs are reported in Table 3:

Table 3. Summary, SAN-582H Metabolites in Soybean RACs.

RAC	Total Radio-label, ppm	% of Total Radiocarbon Residue (ppm)			
		Parent	Oxalamide	Sulfonate	Sulfoxides
Forage	2.80	ND	16.8 (0.47)	7.0 (0.20)	6.0 (0.17)
Hay	2.62	ND	5.3 (0.14)	10.6 (0.28)	7.8 (0.20)
Seed	0.41	ND	6.6 (0.027)	7.5 (0.031)	11.7 (0.048)

Table notes:
ND for parent compound SAN-582H is reported as 0.003 ppm.
Values reported for total radiolabel represent maximum values detected.
Values reported for sulfoxides represent the total of sulfoxides of thiolactic acid and thioglycolic acid.
Data are reported for 1988 samples, at the 1.5 ai/acre application rate.

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Methanol:water extract. With the complex extraction scheme in Figure I, a matter of concern is that given metabolites might be distributed among several fractions, and their overall concentrations split accordingly. To address this matter, petitioner carried out extractions with methanol:water (98:2), then after filtering and clean-up, examined this total extract by TLC and HPLC. This procedure was generally able to extract 60-85% of radiocarbon, but with the 1990 seed sample, the procedure extracted only 33 percent of radiocarbon. Applicant submitted copies of TLC autoradiograms and HPLC chromatograms from the methanol:water extracts. Petitioner claimed that under this procedure, the major metabolites on soybean RACs were the oxalamide, the sulfonate, the sulfoxide of thiolactic acid, and the sulfoxide of thioglycolic acid. With this procedure, applicant claimed that up to 48% of the radiocarbon label is identified (in hay). However, applicant did not supply chromatograms which cross-reference TLC bands to HPLC peaks, or vice versa. For metabolites to be confirmed, petitioner must provide copies of TLC autoradiograms or HPLC chromatograms which confirm identity.

HPLC analysis of the methanol:water extract from forage showed three strong peaks, which were assigned to the oxalamide, the sulfonate, and the sulfoxides of thiolactic and thioglycolic acids (Metabolism Report, Figure 59). HPLC analysis of the methanol:water extract from hay indicated three strong peaks. These were assigned to the oxalamide, the sulfonate, and the sulfoxides of thiolactic and thioglycolic acids. An additional peak, at a retention time of about 20 min and about as strong as the oxalamide peak, was not identified (Metabolism Report, Figure 60). HPLC analysis of the methanol:water extract from seed showed a single strong peak, of magnitude comparable to that of the oxalamide peak in hay (Metabolism Report, Figures 60 and 61). This peak from seed extract was not identified (Figure 61); further characterization efforts seem appropriate.

Comments, Nature of the Residue in Plants

For purposes of this temporary tolerance petition, the nature of the residue in or on soybeans is adequately understood. The residue to be regulated is parent SAN-582H. The radioactive residue of parent in soybean seeds, the only likely human food item, was at or below the limits of detection. CBTS previously concluded that parent SAN-582H is the residue to be regulated for a temporary tolerance in or on corn (PP 0G3892, M.T. Flood, 1/24/91). The levels found in ruminants and poultry after feeding at exaggerated rates (PP 0G3892, Ibid.) suggest that levels of parent in meat and milk will not be measurable from realistic dietary exposure.

For permanent tolerances, the nature of the residue in or on soybeans is not adequately understood, and additional

characterization is required. Current Branch policy is that "efforts to characterize residues" to the 0.01 ppm level should be made (Overview of Residue Chemistry Guidelines, 10/10/89). Review of the petition for SAN-582H in or on corn concluded that efforts should be made to identify all metabolites present at concentrations greater than 0.05 ppm and/or 10% of the total radiocarbon residue (PP 0G3892, M.T. Flood, 1/24/91).

At present, petitioner has confirmed the identities of metabolites representing about 30 percent of the residue in forage, 24 percent of the residue in hay, and 26 percent of the residue in seed. Further efforts should be taken to characterize unknown compounds already identified: 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).

Unknowns were also identified in the HPLC analysis of methanol:water extracts: Unassigned peaks were present at retention times of about 20 min with the extract from hay (Metabolism Report, Figure 60) and about 32 min with the extract from seed (Figure 61). HPLC analysis of the methanol:water extract from a 1990 seed sample showed several more strong peaks (Figure 68). Further efforts should be made to characterize peaks identified in the methanol:water extract, to determine if they are caused by single compounds or if they sub-divide upon further analysis. As indicated above, identification of metabolites using the methanol:water extraction procedure must be supported by submission of chromatograms cross-referencing TLC bands and HPLC peaks.

Pending more detailed analysis, metabolism of SAN-582H in or on soybeans may be different from metabolism in or on corn. In corn grain, no metabolite comprised more than 2% of 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.

Nature of the Residue in Animals

Petitioner has previously submitted data on the nature of the residue in ruminants. CBTS found that for the purposes of a

temporary tolerance, the nature of the residue in ruminants is adequately understood. The residue to be regulated is parent SAN-582H. Due to the low predicted total residues in ruminant tissue and milk, it is not necessary that temporary tolerances be established for these commodities. 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).

Petitioner has previously submitted data on the nature of the residue in poultry. CBTS found that for the purposes of a temporary tolerance, the nature of the residue in poultry is adequately understood. The residue to be regulated is parent SAN-582H. Due to the low predicted total residues in poultry tissues and eggs, it is not necessary that temporary tolerances be established for these commodities. 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).

Analytical Method

Parent and Oxalamide. The method used to determine residues from field trial samples was submitted as Appendix IV to the residue data from the 1989 season (MRID No. 418438-03, Vol. 3 of 3) and as Appendix IV to the residue data from the 1990 season (MRID No. 418438-04, Vol. 2 of 2). The submitted method is:

"Determination of SAN-582H and its Oxalamide Metabolite in Soybean Forage, Hay, Grain, and Straw," Method AM-0850-0291-0, 2/25/91, Sandoz Crop Protection Corporation.

With this method, plant samples are finely chopped; grain samples are milled to a fine particle size. Samples are extracted with methanol:water (98:2), and an aliquot is partitioned with 1:1 methylene chloride:ethyl ether. The organic phase is concentrated and the oxalamide is converted to its methyl ester derivative with diazomethane. SAN-582H and the oxalamide derivative are cleaned up with a C18 column and quantitated by gas chromatography using an HP-1 capillary column and a mass selective detector (MSD).

The method was validated with samples of soybean forage, grain, hay, and straw from field test sites, fortified with 0.1 ppm each of SAN-582H and the oxalamide. Recoveries for SAN-582H ranged from 8.3 to 252 percent. Recoveries for the oxalamide ranged from 7.9 to 211 percent. By the petitioner's calculations, the average recovery for SAN-582H was 127 percent, with a standard deviation of 52 percent; the average recovery for the oxalamide was 112 percent with a standard deviation of 48 percent.

Petitioner submitted "representative" GC/MSD chromatograms for each fortified crop sample; these chromatograms in several cases showed high backgrounds and relatively small peaks, which may explain the considerable variability in recovery and detection. By the company's own estimation, the limits of detection of this method are 0.02 ppm for the parent and oxalamide. Registrant submitted no other data to indicate that the method can accurately detect residues at concentrations lower than 0.1 ppm.

Sulfonate. Petitioner also submitted a method for determination of the sulfonate metabolite of SAN-582H. With this method, the crop sample is extracted with methanol:water. An aliquot is concentrated to the water layer, the water is washed with methylene chloride, and methanol is added back to the water. This mixture is applied to a strong cation exchange (SCX) column in series with a strong anion exchange (SAX) column. The sulfonate is retained on the SAX column, and the SCX column is discarded. The sulfonate is eluted with a methanol:aqueous sodium carbonate solution. The eluent is then analyzed by HPLC using a C18 column and detection by UV absorbance.

The sulfonate is present as two diastereomers, and the HPLC chromatography partially separates them into two peaks, at an approximate ratio of 3:2. Petitioner supplied an HPLC chromatogram indicating that sulfonate in a fortified soybean forage sample can be detected at 0.1 ppm (MRID No. 418438-04, Report 414108-11, Figure 6, p. 402). However, an unfortified control soybean hay sample produced high background and interfering peaks where the sulfonate would be detected (Ibid., Figure 8, p. 404); and an unfortified soybean grain sample also produced interfering peaks (Ibid., Figure 14, p. 410). Chromatograms were submitted for samples of hay fortified at 0.5 ppm (Ibid., Figure 9, p. 405), and grain fortified at 0.5 ppm (Ibid., Figure 15, p. 411). No chromatograms were supplied to indicate detection in fortified samples at concentrations lower than 0.1 ppm in forage or lower than 0.5 ppm in hay or grain. Petitioner also reported that with some soybean samples, recoveries had to be calculated using one of the disastereomers because of interfering background in the vicinity of the second diastereomer (Ibid., p. 24).

Parent Only. Petitioner has submitted as part of PP 0G3892, validation by an independent laboratory of an analytical method for the parent compound SAN 582H only. This validation is reported in:

"Confirmatory Method Trial of the Residue Method 'A Method for the Determination of Residues of SAN-582H in Corn and Soil Samples'," 3/22/91, MRID No. 418239-02.

With this method, plant samples were extracted with methanol:water (95:5). The extract is cleaned up using a C18

column, followed by a silica gel column. The final eluate is evaporated to dryness, the residue is dissolved in 3 ml of toluene and the resulting solution is injected for gas chromatography analysis using a capillary column with a thermionic nitrogen specific detector. This method was validated with samples from corn and soybeans. The limit of detection was 0.01 ppm, and submitted chromatograms for fortified samples showed well resolved peaks. CBTS concluded that this method has undergone successful independent laboratory validation and recommended that the method be validated for corn and soybeans at EPA laboratories (PP 0G3892, M.T. Flood, 6/11/91).

Comment, Analytical Method

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 residues at concentrations lower than 0.1 ppm.

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 in 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, submitted as part of petition PP 0G3892, has undergone successful independent laboratory validation on corn and soybeans. CBTS has recommended that the method be validated for corn and soybeans at EPA laboratories. For the purposes of this temporary tolerance, this method is sufficient to detect SAN-582H, the residue to be regulated.

For permanent tolerances, the nature of the residue in plants and animals must be adequately understood, and 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.

Magnitude of the Residue

Residue Field Trials. Residue field trials carried out during the 1989 season are described in the document:

"Analysis of Soybean Samples for SAN-582H and its Oxalamide and Sulfonate Metabolites (1989 Season)," Sandoz Crop Protection Corporation, March 13, 1991, Report No. 414108-12 (MRID No. 418438-03).

During the 1989 season, field trials were conducted in six states (Georgia, Arkansas, Louisiana, Missouri, Minnesota, and Iowa) representing major soybean production regions of the United States. Two different SAN-582H formulations, 6.0EC (emulsifiable concentrate) and 7.5L (liquid) were applied separately at each site. These formulations contain 6 lb ai/gallon and 7.5 lb ai/gallon, respectively. Three application types were used for each formulation: preplant shallow incorporation, preemergence, and early postemergence at the unifoliate stage; application was by ground equipment. Application rate was 1.5 lb ai/acre, the intended maximum label use rate for SAN-582H on soybean, in all sites except in Arkansas, where a rate of 1.25 lb ai/acre was used.

Forage samples were collected at the V6 (6 trifoliates) stage of growth. Fresh hay samples were taken at or prior to senescence. Dry grain and straw samples were collected at the normal harvest interval. All samples were frozen within 24 hr of collection, shipped to a storage facility, and stored at -20°F or below.

Control and treatment samples for forage, hay, grain (identical to seed), and straw were analyzed for SAN-582H and its oxalamide metabolite. Selected samples were analyzed for the sulfonate metabolite as well. Single samples were analyzed for each formulation (EC or L), and for each type of application (preemergence, preplant incorporated, postemergence), for a total of 6 samples at each site for each RAC. Chemical analysis of residues was conducted by Sandoz Corporation. Forage samples were not collected in the Arkansas trial because soybeans had started blooming by the time the samples were to be collected. Results are summarized for soybean RACs in Table 4:

Table 4. Summary, SAN-582H Residues in Soybeans, 1989 Trials.

Site	Sample	Residue Range, ppm		
		Parent	Oxalamide	Sulfonate
AR	Hay	ND-0.03	ND-0.02	ND-0.21
	Grain	ND	ND	
GA	Forage	ND	ND-0.28	0.18-0.55
	Hay	ND	ND-0.02	ND
	Grain	ND	ND	
IA	Forage	ND	ND-0.04	ND
	Hay	ND	ND	ND
	Grain	ND	ND	ND
LA	Forage	ND	0.06-0.15	ND-0.42
	Hay	ND	ND-0.06	0.20-0.34
	Grain	ND	ND-0.02	
MN	Forage	ND	ND-0.03	ND-0.05
	Hay	ND	ND	ND
	Grain	ND	ND	
MO	Forage	ND	ND	0.05
	Hay	ND	ND	
	Grain	ND	ND	

Table notes:
ND = 0.02 ppm for parent SAN-582H and the oxalamide;
for the sulfonate, ND = 0.05 ppm for forage and grain, 0.10 for hay.
The single AR sample which showed detectable levels of SAN-582H showed ND when reanalyzed.
Sulfonate was measured only on selected samples.

Additional residue field trials were carried out during the 1990 season and are described in the document:

"Analysis of Soybean Samples for SAN-582H and its Oxalamide and Sulfonate Metabolites (1990 Season)," Sandoz Crop Protection Corporation, March 13, 1991 Report No. 414108-12 (MRID. No. 418438-04).

During the 1990 season, field trials were conducted in five states (Arkansas, Illinois, Minnesota, North Carolina, and Ohio) representing key soybean production regions of the United States. The 7.5L formulation of SAN-582H was applied for these trials at an application rate of 1.5 lb ai/acre, the intended maximum label use rate on soybean. As before, three application types were used: preplant shallow incorporation, preemergence, and early postemergence at the unifoliate stage; application was by ground equipment. Samples of forage, hay, grain, and straw were collected as described for the 1989 field trials. Samples were frozen within 3 hr of collection, shipped to a storage facility, and stored at -20°F or below.

Control and treatment samples for forage, hay, grain, and straw were analyzed for SAN-582H and its oxalamide metabolite. Selected samples were analyzed for the sulfonate metabolite as well. Single samples were analyzed for each type of application, for a total of 3 samples at each site for each RAC. Results are summarized for soybean RACs in Table 5:

Table 5. Summary, SAN-582H Residues in Soybeans, 1990 Trials.

Site	Sample	Residue Range, ppm		
		Parent	Oxalamide	Sulfonate
AR	Forage	ND-0.03	0.02-0.06	
	Hay	ND	ND-0.03	
	Grain			
IL	Forage	ND	0.03-0.08	0.07
	Hay	ND	ND	
	Grain	ND	ND	ND
MN	Forage	ND	ND	
	Hay	ND	ND	
	Grain	ND	ND	
NC	Forage	ND	0.04-0.69	0.37
	Hay	ND	0.04-0.06	ND-0.26
	Grain	ND	ND	
OH	Forage	ND	ND	ND
	Hay	ND	ND	
	Grain	ND	ND	

Notes to Table 5:
ND = 0.02 for parent SAN-582H and the oxalamide;
for the sulfonate, ND = 0.05 ppm for forage and grain, 0.10 ppm for hay.
No grain samples were collected from the Arkansas site because the grower accidentally disked the site, destroying all soybeans. Sulfonate was measured only on selected samples.

When both trials are combined, the states where residue trials were conducted, plus their surrogate states (Residue Chemistry Overview, 10/10/89), represent about 79% of U.S. soybean production (Agricultural Statistics, U.S. Department of Agriculture, 1989).

Storage Stability Data. Petitioner submitted no storage stability data for SAN-582H or metabolites on soybeans. Petitioner referred to data indicating that SAN-582H and the oxalamide were stable for four months in frozen corn matrices; and were stable in soil for 12 months under frozen storage.

Soybean Processing Fractions. Residue data on processing fractions were described in the report:

"Residues of SAN-582H and its Oxalamide Metabolite in Soybean Processing Fractions," March 15, 1991, Sandoz Crop Protection Corporation Report No. 414108-13 (MRID No. 418438-02).

Field plots were treated preemergent with SAN-582H at 1.5 lb ai/acre (maximum intended label use rate), 4.5 lb ai/acre, or 7.5 lb ai/acre (5X). Soybeans were planted and grown to maturity. Soybean grain samples were harvested, boxed in the field, and shipped to a storage facility, and stored at -20°F or below. Samples from the 5X application rate were sent to the Food Protein Research and Development Center, Texas A&M University, College Station, Texas, where samples were processed using procedures that duplicated commercial processing procedures. Processing fractions were analyzed for SAN-582H and its oxalamide metabolite using the analytical method AM-0850-0291-0, described above.

The field application was conducted in 1988, the processing in 1989, and the analysis in 1991. Petitioner submitted no data on storage stability of SAN-582H in or on soybean RACs or processed commodities.

To validate the analytical method used in this report, petitioner submitted data on recoveries from fortified control samples. Data for soybean processed commodities are summarized in Table 6. Data were not presented for fortification levels lower than those shown in Table 6.

Table 6. Summary, Recoveries from Fortified Control Samples.

Sample Type	Fortification Level, ppm	Recoveries, %	
		SAN-582H	Oxalamide
Whole grain	0.20	127	80
Hulls	0.10	126	114
Meal	0.20	135	130
Crude Oil	0.05	145	146
	0.10	151	148
	0.10	124	55
Refined Oil	0.05	125	71
Soapstock	0.05	91	16
	0.10	121	36
	0.10	126	16

When treated soybean processing fractions were analyzed, no residues for SAN-582H or its oxalamide metabolite were found, at or above the limit of detection, which petitioner claimed is 0.02 ppm. No detectable residues were found on whole grain as well, so no concentration factors could be calculated for soybean processing fractions.

Comment, Magnitude of the Residue

The analytical methods used for residue field trials were the submitted methods described above for detection of the parent, oxalamide, and sulfonate, which are inadequate for temporary tolerances. However, for the purposes of temporary tolerances, the radiolabel plant metabolism study on soybeans indicates that SAN-582H, the residue to be regulated, would not be expected to exceed the proposed tolerances.

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 or on soybeans.

No data were submitted on storage stability of SAN-582H or its metabolites in soybeans. For the purposes of temporary

tolerances, data from the radiolabel plant metabolism study are sufficient to indicate that residues of SAN-582H are not detectable in or on soybeans. For permanent tolerances, storage stability data must cover the longest storage time between sampling and analysis for residue field trials. Storage stability data will be necessary for other components of the residue to be regulated once these other compounds have been determined.

For the purposes of temporary tolerances, residue data on soybean processed commodities are not required. 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 data submitted reported only samples 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.

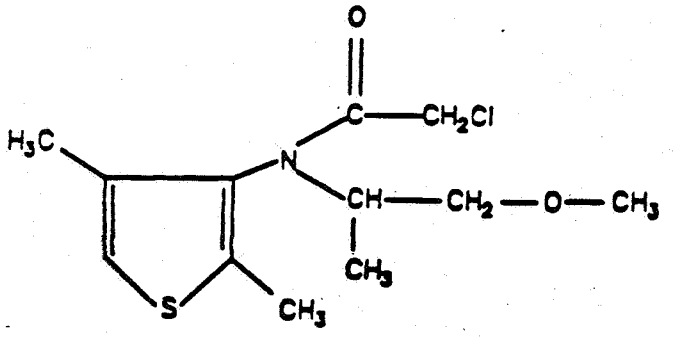
For the purposes of permanent tolerances, samples will have to be reanalyzed using an acceptable analytical method, or new trials will have to be conducted. In order to evaluate whether or not residues concentrate during processing, it may be necessary to analyze metabolites which are found on soybeans. 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. Adequate storage stability data must be submitted. Residues must also be analyzed on soybean grain dust as a processed commodity (Overview of Residue Chemistry Guidelines, 10/10/89, Attachment 2).

Meat, Milk, Poultry, Eggs

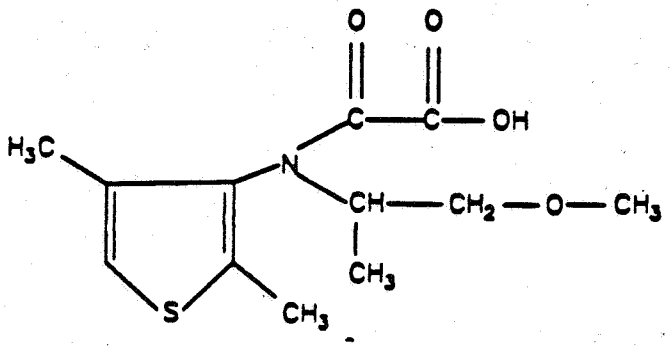
Results of feeding studies have not been submitted and are not necessary for temporary tolerances. For permanent tolerances the need for such studies will be assessed once the nature of the residue in plants and animals and the magnitude of the residue in soybeans have been determined.

cc:Circ, SAN-582H SF, RF, Abbotts, PIB/FOD (C. Furlow),
PP 0G3892, PP 1G3980
RDI:FBSuhre:7/18/91:EZager:7/18/91
H7509C:CBII-RS:JAbbotts:CM-2:Rm812a:557-8230:7/18/91

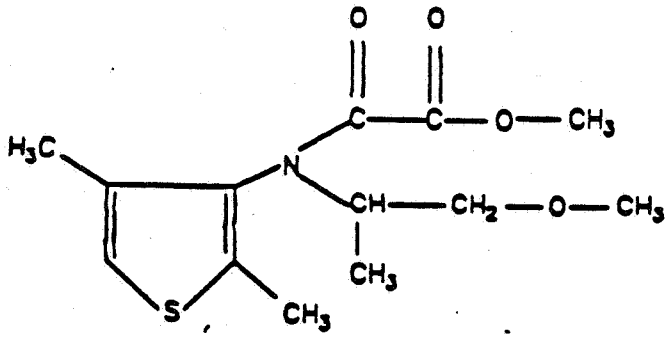
FIGURE 1. Chemical Structures and Names



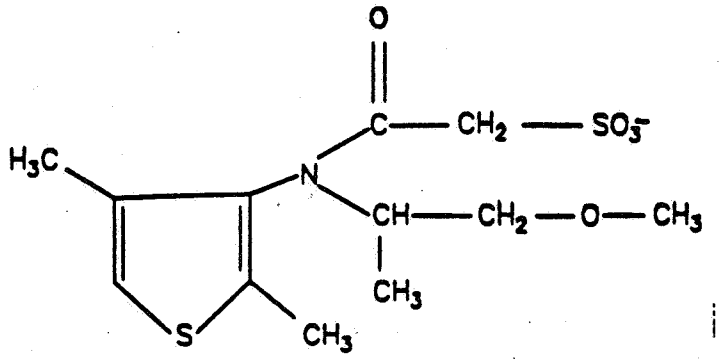
SAN-582H; 2-chloro-N{(1-methyl-2-methoxy)ethyl}-N-(2,4-dimethyl-thienyl)-acetamide



Oxalamide; N{(1-methyl-2-methoxy)ethyl}-N-(2,4-dimethyl-thienyl)-oxalamide

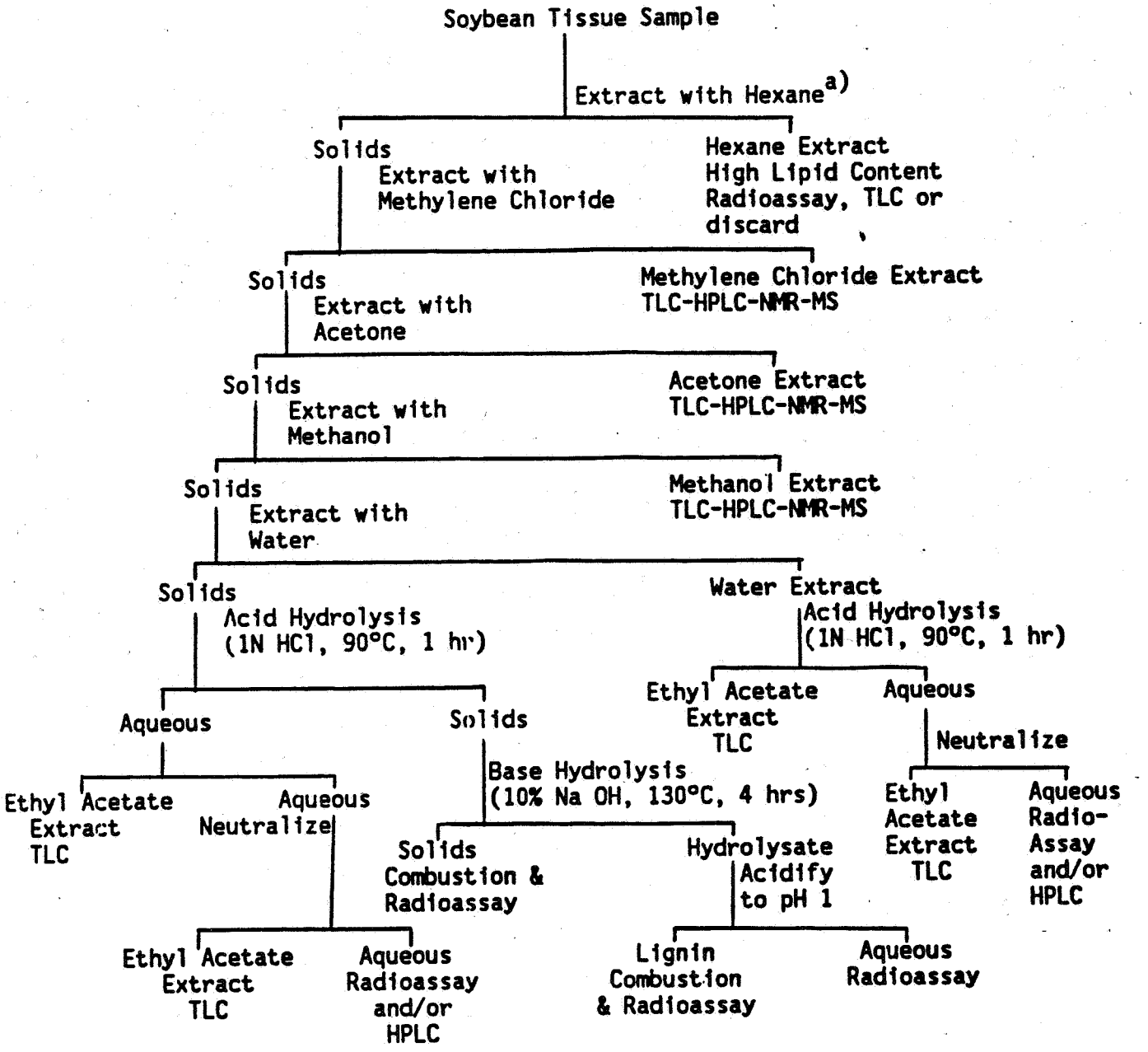


Oxalamide Methyl Ester; N{(1-methyl-2-methoxy)ethyl}-N-(2,4-dimethyl-thienyl)-oxalamide methyl ester



Sulfonate; N-{1-methyl-2-methoxy)ethyl}-N-(2,4-dimethyl-thienyl)-acetamide-2-sulfonate

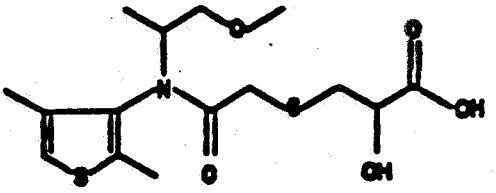
FIGURE II GENERAL EXTRACTION SCHEME FOR CHARACTERIZATION OF RADIOCARBON IN SOYBEAN SAMPLES FROM THE SAN-582H SOYBEAN FIELD METABOLISM STUDY.



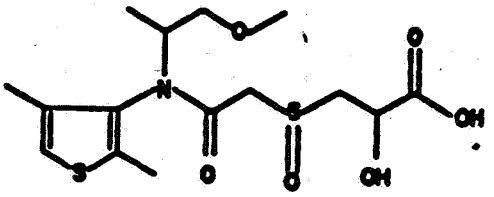
a) Hexane extract only for seeds to remove lipids, all other samples were extracted starting with methylene chloride.

Table III. Structures of model plant metabolites of SAN-582H

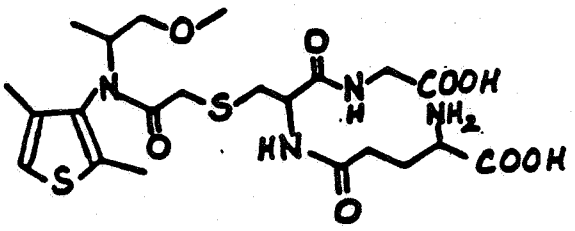
1. Thiolactic acid conjugate of SAN-582H



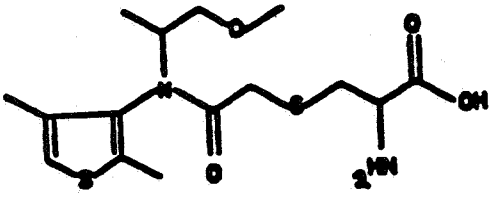
2. Sulfoxide of the thiolactic acid conjugate of SAN-582H



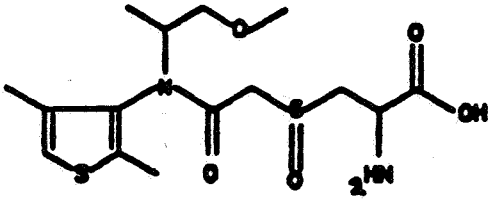
3. Glutathione conjugate of SAN-582H



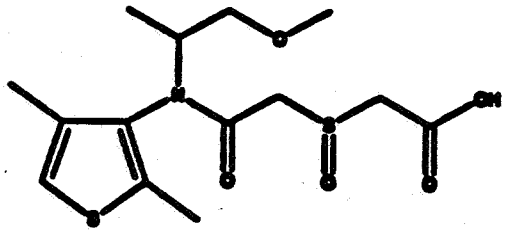
4. Cysteine conjugate of SAN-582H



5. Sulfoxide of the cysteine conjugate of SAN-582H



6.



Sulfoxide of thioglycolic acid