UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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EXPEDITE

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OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: PP#0F3918. SAN-582H (Frontier®/Dimethenamid) in/on Field Corn. Evaluation of Analytical Methods and Residue Data. New Chemical Review.

DP Barcode: D178417. MRID #s 423103-01, 422895-02 through 422895-08. CBTS # 9880.

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This review is being expedited at the request of the Registration Division. The due date is 7/31/92.

Background

CBTS' 1/24/91 review of the corresponding temporary tolerance petition for SAN-582H herbicide in/on field corn -- PP#0G3892 -- identified numerous deficiencies with respect to permanent tolerances. Sandoz subsequently submitted a new analytical method for parent SAN-582H, and the method is now undergoing EPA method validation. The present submission, dated 4/15/92, is a response to our review and consists of metabolism, crop field trial data, and a processing study.

The following permanent tolerances are proposed for SAN-

582H:

Corn forage	0.01 ppm
Corn silage	0.01 ppm
Corn grain	0.01 ppm
Corn stover	0.01 ppm

The CAS name for SAN 582H is acetamide, 2-chloro-N-(2,4-dimethyl-3-thienyl)-N-(2-methoxy-1-methylethyl). The ISO proposed common name for SAN-582H is dimethenamid; the trade name is Frontier®.

Summary of Deficiencies Remaining to Be Resolved

---- Nature of Residue in Corn -- further characterization of components of methanol soluble fraction from forage.

---- Nature of Residue in Ruminants -- further characterization of the residue in liver.

---- Magnitude of Residue in Corn -- residue data on two corn metabolites in the absence of data obtained using common moiety analytical method.

---- Processing Study -- Analyses for any metabolites to be regulated.

---- Ruminant Feeding Study -- Requirement dependent on plant and animal metabolism.

---- Revised Section F -- Tolerances for grain, forage and fodder.

Conclusions

1a. The nature of the residue in corn plants is not as yet adequately understood. Additional characterization of the very polar components of the methanol soluble fraction (A5) from forage is necessary. Refer to discussion in text of this memo, page 13.

From data submitted thus far, about 20-30% of the residue has been identified. The principal metabolites are the sulfonate conjugate of SAN-582H and the sulfoxide of thiolactic acid conjugate of SAN-582H.

1b. Conclusions in this memo concerning the nature of the residue in corn plants as well as the need for residue data on two metabolites (Conclusion 5b) are provisional, pending review by the HED Metabolism

Committee.

2a. The nature of the residue in ruminants is not as yet adequately understood. Additional characterization of liver metabolites L15a, L15b and L15c is required -- at least to the point of demonstrating that they are not the same metabolite. Refer to our discussion in the text of this memo, pages 15-16.

From data submitted thus far, residue identification ranges from 18% (liver) to 39% (kidney). The principal metabolites are M7, formed from O-demethylation of parent; M22, a dimer formed from a probable mercaptan intermediate; and the cysteine conjugate.

2b. The nature of the residue in poultry is adequately understood for the proposed use due to the low concentration in the diet (0.02 ppm total residue in corn grain) and the levels of activity observed from an exaggerated dosing level (167 ppm, >8000X the expected dietary level). If future uses result in significant dietary increase of residues of SAN-582H, additional characterization of the residue in liver may be required.

From the submitted data, residue identification ranges from 0% (muscle, egg white) to 35% (fat). Parent SAN-582H 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.

2c. The conclusions in this memo concerning metabolism in ruminants and poultry are provisional, pending review by the HED Metabolism Committee.

3a. The residue analytical method for SAN-582H is now undergoing EPA method validation.

3b. To date, Sandoz has been unsuccessful in developing a common moiety analytical method. Sandoz's attempts to develop such a method are summarized in a concurrent memo.

3c. Enforcement analytical methods will have to be developed if any metabolites are to appear in the tolerance expression for SAN-582H. Such methods will have to undergo independent laboratory validation prior to EPA method validation. See Conclusion 5b.

3d. Sandoz has tested SAN-582H under FDA's multiresidue protocols. Recoveries must also be obtained for any

metabolite which is regulated.

4. Submitted storage stability data for SAN-582H demonstrate stability under frozen storage for periods up to 21 months. The oxalamide metabolite appears to be stable under frozen conditions for at least one year, but if this metabolite is to appear in the tolerance expression, samples must be reanalyzed using a method giving more reproducible recoveries.

   Comparative TLC analyses imply that the thioglycolic acid conjugate of SAN-582H, the oxalamide metabolite, the sulfoxide of thioglycolic acid conjugate and the sulfoxide of thiolactic acid conjugate are stable for periods up to 22 months. However, since the precision of the TLC method is not known, results are indicative only and cannot substitute for controlled storage stability studies, should they be necessary.

5a. SAN-582H was not detected (<0.01 ppm) in corn grain, forage and fodder from six field trials held in 1989, five trials held in 1990 and six trials held in 1991.

5b. Residue data for the sulfonate conjuate of SAN-582H and the sulfoxide of thiolactic acid conjugate of SAN-582H in corn grain, forage and fodder should be generated from field trials held in six states. Analyses should be supported by appropriate storage stability data. This requirement is made because of the absence of a suitable common moiety analytical method and is provisional, pending review by the HED Metabolism Committee.

   If these metabolites (or common moiety) are non-detected in residue samples, the appropriate tolerance will be for parent only, pending concurrence by the HED Metabolism Committee.

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

6. Residues of SAN-582H were non-detected in corn grain from plants treated at 5X the maximum use level. When this grain was wet and dry milled, the processed commodities failed to show detectable SAN-582H residues. We conclude that food/feed additive tolerances are not necessary for SAN-582H in corn processed commodities.

   Should it be necessary to regulate any metabolites of SAN-582H, processed samples will have to be analyzed

for these metabolites.

7. CBTS will make no final conclusion on the need for a residue transfer study in ruminants until the nature of the residue in corn and in ruminants is adequately understood. At this time, a residue transfer study in poultry is not necessary -- the metabolism in poultry is understood for this petition and the expected dietary contribution is very low.

8. CBTS generally considers that a tolerance for corn forage also applies to corn silage. Hence, there is not need for a separate tolerance on corn silage. Sandoz should submit a revised Section F in which tolerances of 0.01 ppm are proposed for corn grain, forage and fodder.

9. An International Residue Limit (IRL) status sheet is appended to this review. There are currently no IRL's for this compound. Hence, compatibility is not an issue.

Recommendation

CBTS recommends against the proposed tolerances for reasons given in Conclusions 1 (nature of residue in corn); 2a (nature of residue in ruminants); 5b (metabolite residue data and corresponding storage stability data); 6 (processing study for metabolites if they appear in the tolerance expression); 7 (possible feeding studies in ruminants); 8 (revised Section F).

CBTS recommends that the HED Metabolism Committee review the results and conclusions concerning plant and animal metabolism, including the need for residue data on two metabolites.

Detailed Considerations

Deficiencies listed in CBTS' 1/24/91 memo will be listed along with Sandoz's responses and CBTS' comments.

Deficiency #1a (Conclusion 1a from our 1/24/91 memo)

The nature of the residue in corn fodder, forage and silage is not adequately understood for permanent tolerances. Only 16.5% of the total radiocarbon residue was reportedly identified in fodder, 18.5% in forage and 20.5% in silage. At least some of these metabolites were only identified with TLC and one solvent system, and not all the reported percentages were supported by information given in the accompanying tables and discussion. We can conclude that SAN-582H, per se, is not a constituent of the residue in/on corn.

Efforts should be made to identify all metabolites present at concentrations greater than 0.05 ppm and/or 10% of the total radiocarbon residue. Because of the rather complicated extraction schema (Figure 2), the registrant should demonstrate that metabolites found at low concentrations in a gvien solvent are indeed unique so their concentrations should not be found in another solvent. Identification of metabolites should be based at a minimum on TLC in two solvent systems. If individual metabolites cannot be completely identified, it may still be possible to assign them to a given chemical class of compounds. Stronger

hydrolysis conditions may be necessary to achieve this limited characterization.

The registrant should explain the mass balance discrepancies in Table X, as noted on page 8 of this memo.

Sandoz Response

The response is contained in the following report:

"Uptake, Translocation and Metabolism of Herbicide SAN 582H in Corn," P. Moore, 4/1/92, Lab ID No. 414105-14A. (MRID # 422895-04)

Data were generated at Sandoz Agro Inc. in Des Plaines, IL.

A. The mass balance discrepancy on Table X in the original submission is due to sample loss during processing. Solids were combusted before hydrolysis and the hydrolysate and remaining solids were combusted. About 7.4 % of the total radioactivity was lost from the fodder sample which had been treated with 4.0 lbs ai/A. In contrast, Table XI, which includes fodder treated at 1.5 lbs ai/A, listed only the values of hydrolysate and remaining solids. Solids before hydrolysis were not combusted, and as a result complete mass balance seemed to occur. In other words the column listed as "solids" in Table X is not the same as the column listed as "solids" in Table XI. Revised tables have been submitted which clarify this point.

B. On page 9 of our memo, we noted that 41% of the total radioactive residue (TRR) from fodder treated at 4 lb ai/A could not be extracted with methanol solutions. Hydrolysis of this unextracted fraction apparently liberated N-2-(1-hydroxypropyl)-2,4-dimethylthienylamine, but no confirmation was reported. No other compound could be identified from this fraction.

Sandoz responds that although the thienylamine was tentatively identified by TLC, the preliminary identification could not be confirmed. The metabolite has not been detected in the acid hydrolysate of any other corn RAC. There is reason to believe that it should not be present, because if it were present as a cysteine or glutathione conjugate, it would have been extracted into methanol. In any event, the TRR in the ethyl acetate extract of the hydrolysate was < 0.017 ppm for the maximum label rate; thus even if the above compound was present, it would be < 0.01 ppm since the TLC profile was diffuse, with no discrete bands.

An attempt was made to further characterize the solids remaining after 50% methanol extraction of corn fodder. An aliquot of solids was shaken overnight with DMSO according to the method reported by R. Huber and S. Otto (Bound Pesticide Residues in Plants, Proc. 5th Internat. Congress Pesticide Chem. 3: 357-362, 1983). When treated with ethanol, no precipitate formed

from the DMSO supernatant, indicating negligible amounts of starch present in fodder. No amino acids were present in the DMSO/ethanol (ninhydrin negative).

A second aliquot of solids was treated with 1N HCl for 3 hrs. at 140°C in a Parr digestion bomb. The dark black liquid hydrolysate was separated from remaining solids by filtration and extracted with ethyl acetate. The ethyl acetate fraction, 3.94% of TRR, contained no amino acids and showed a very broad band in two different TLC solvent systems. The acidic fraction was freeze dried and dissolved in methanol. TLC showed a very diffuse band. The acid hydrolysate amounted to 6.07% of TRR. The solids remaining after the Parr digestion were digested again in a Parr bomb with 10% NaOH for 3 hrs. at 140°C. After this hydrolysis, the remaining solids, crude cellulose, was combusted for total radiocarbon. The solids accounted for 0.50% of TRR. The alkaline hydrolysate was treated with concentrated HCl until the mixture reached pH 1 in order to precipitate lignin (R. Honeycutt and J.L. Alder, J. Agric. Food Chem. 23: 1097-1101, 1975). Radiocarbon associated with lignin amounted to 5.6% of TRR. The acidified hydrolysate, which amounted to 2.1% of TRR, was freeze dried, dissolved in methanol and subjected to TLC. The radiocarbon was diffuse. The extract was ninhydrin positive, indicating the presence of amino acids.

The registrant concludes that the bound residues were associated with lignin, cellulose, and apparently proteins and some conjugated metabolites. A flow chart describing the above-described procedure is given in Figure 54A, page 50. (Note that after extraction with 50% methanol, the remaining solids were split for separate experiments. However, the reported percents of TRR are based on the original sample, so to estimate the TRR in the entire unextracted part, the numbers in the preceding paragraph should be approximately doubled.)

C. Sandoz has responded in detail to our comments on pages 9 and 10 of our 1/24/91 memo. (We have appended a copy of pages 9 and 10 to this review as Attachment 1.) The labels given on the various fractions from Sandoz's extraction scheme (A1, A2, etc.) appear in the extraction diagram on page 9, which should be referred to.

The oxalamide metabolite was identified in the neutral methylene chloride extract A3 and quantitated at 1.39% of TRR (from fodder treated at 4.0 lb ai/A). Our concern was that identification was apparently made on the basis of a diffuse TLC band in one solvent system. According to Sandoz (Appendix 3, pg. 57), the radioactivity of the TLC plates was diffuse due to the interference of endogenous components. A more dilute sample would not have yielded a plate having sufficient radioactivity. "Nonetheless, the diffuse bands in general correspond to the Rf of the more discrete bands observed in forage. In addition, the

low level of activity in the latter two extracts [silage and fodder] had little contribution to the total amount of weak acids metabolites....(summation from neutral and acidic CH_2Cl_2 fractions)." Four metabolites and possibly a fifth were identified from the forage TLC. The registrant notes that the TRR values from the TLC radioscans for the 4 lb/acre RACS as reported in the labels generally represent average values for multiple RAC replicates, whereas the TLC radioscans shown in the figures represent only one replicate. This would account for the apparent discrepancies noted in the last paragraph of page 10 of our 1/24/91 memo. Appropriate extracts from the 1.5 lb/acre RACs were pooled together, so the values in the cross referenced tables and figures do correspond at this treatment level.

Additional characterization was performed on the acidified methylene chloride extract (A4) because metabolites of the neutral extract (A3) corresponded to those in A4, A4 had higher levels of metabolites, and interferences from endogenous materials were lower. Extracts from forage were chosen for various reasons. The TLC profiles from forage were similar to those from silage and fodder, the forage had the highest percentage of TRR in the extract, the TLC bands were the most discrete, and more bands could be observed.

Four major bands were observed in the TLC of the forage extract (ethyl acetate/toluene/formic acid/water:87/3/5/5). Metabolites M11 and the thioglycolic acid conjugate of SAN-582H were identified using TLC in two solvent systems. The two metabolites were also observed in extracts from treated soil and from corn seedlings. (Structures of these and the other metabolites are given in Attachment 2.) [redacted] thiolactic acid conjugate of SAN-582H, and the sulfoxide of the thiolactic acid conjugate were confirmed by HPLC. The sulfoxide of the thioglycolic acid conjugate of SAN-582H was identified by comparing NMR and MS spectra to those of the synthesized standard. Other metabolites also present were not identified.

The methanol soluble, freeze-dried aqueous fraction, A5, constituted 22.7% of TRR in fodder treated at 4 lb/A (see our 1/24/91 memo, page 10, paragraph 3). One band, 2.2% of TRR was tentatively identified as the glutathione conjugate. Sandoz has now provided evidence that this compound is the [redacted] conjugate by comparison with the same compound present in extracts from corn seedlings. HPLC, NMR and MS were used to identify the metabolite in corn seedlings. The presence of the glutathione conjugate was also indicated but could not be confirmed. Attempts to further characterize A5, particularly the band closest to the origin, were unsuccessful due to the large quantity of polar coextractives.

Additional attempts were made to characterize fraction A5. Sandoz applied the forage A5 (4 lb/A) fraction to HPLC. HPLC

fractions were collected and the three fractions containing major residue were then applied to TLC for further characterization. The fraction from forage was chosen for further workup because the A5 fraction was slightly cleaner, more forage was available and the total % TRR was greatest, and the TLC profile was qualitatively similar to that from fodder and silage. Each HPLC peak was shown to be composed of several metabolites in TLC. The sulfonate conjugate and probably the glutathione conjugate were present.

On page 12 of our 1/24/91 memo, we noted the presence of an intense unidentified band near the origin of the TLC from fraction A5 from forage. The radioactivity of this band constituted 13.9% of TRR. (Figure 10, page 112, and Table XXXI, page 92, of the first report.) Sandoz discusses the presence of this band on page 141 of the present report. Cleanup was unsuccessful because the radioactive band co-migrated with an intense brown pigment. This radiocarbon was rendered soluble in organic solvents after acetylation, which would be characteristic of sugars, known to be present in the matrix. As noted in the previous report β-glucosidase, α-glucosidase, sulfatase, or β-glucoronidase, when incubated with fraction A5, failed to render the radiocarbon organosoluble. According to Sandoz, this suggests that at least some of radiocarbon is irreversibly bound to the sugar plant matrix. Additionally, TLC of the methanol soluble CCC stationary phase for the 50% methanol extract of forage (see discussion below) showed that the polar band near the origin is smeared out over a broad range, suggesting numerous components (present report, Figure 96A). Further characterization is necessary. See our comments below.

The updated summary table for the metabolism of SAN-582H in corn at the maximum use level of 1.5 lb. ai/A is given in Tables 1a and 1b, below. (The tables correspond to Table 47A, page 166 of Sandoz's current submission.)

Table 1a

Characteristic of SAN-582H Residues in Corn Plants
Treated at 1.5 lb ai/A.

				Unidentified (%TRR)			
Crop Part	PHI(days)	^{14}C Total	Total Identified (%TRR)	Organosoluble (A2, A3, A4, B11)	Methanol (A5)	Water (A6)	Unextractable
Forage	50	0.307	18.89	16.2	26.7	12.7	8.83
Silage	116	0.403	16.34	10.6	28.1	12.1	18.7
Grain	116	0.021	N.D.	N.D.	N.D.	N.D.	46.7
Fodder	130	0.504	12.2	8.08	8.20	22.8	37.1
Grain	130	0.022	N.D.	N.D.	N.D.	N.D.	51.4

Table 1b

Residue Identified from Corn Plants Treated at 1.5 lb/acre with SAN-582H
%TRR (ppm)

Crop Part	PHI(days)	Oxalamide	Sulfoxide of Thiolactic Acid Conj.	Sulfoxide of Thio-glycolic Acid Conj.	Thiolactic Acid Conj.	Thioglycol-ic Acid Conj./M11/ other	Sulfonate Conjugate
Forage	50	3.58 (0.011)	1.60 (0.005)	1.66 (0.005)	2.28 (0.007)	3.71 (0.011)	6.06 (0.019)
Silage	116	0.57 (0.0023)	3.70 (0.015)	2.90 (0.012)	1.19 (0.005)	0.60 (0.002)	7.38 (0.03)
Grain	116	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Fodder	130	1.43 (0.007)	2.0 (0.010)	0.67 (0.003)	1.43 (0.007)	5.62 (0.028)	2.50 (0.013)
Grain	130	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

* Oxalamide and thiolactic acid were frequently scraped and analyzed together.

The values that appear in the preceding table appear in Table 42A, except for the sulfonate conjugate, which was determined from TLC's given in Figures 79A-81A. The TLC bands that appear in Figures 79A-81A are very diffuse, so although the sulfonate conjugate may be present, there have to be large uncertainties in the reported values.

Similar percentages were obtained for racs treated at 4 lbs ai/A (Table 41A), except that only the sulfonate level in forage was estimated (Table 45A). Although a sulfonate standard was not run on the TLC plate, the table values imply that levels of the sulfonate conjugate in forage would be ≤5.61% of TRR. At this point we see no benefit in requiring a similar determination of the sulfonate in fodder.

D. Because it is apparent that SAN-582H is extensively metabolized to a number of compounds, each present at low enough concentration to preclude extensive characterization, we expressed concern that Sandoz's partition scheme "could artificially lower the concentrations of unidentified metabolites, for a given metabolite may be partially soluble in more than one extracting solvent."

In response, Sandoz developed a single step extraction procedure using counter-current chromatography (CCC). Forage treated at 4 lbs ai/A with radiolabeled SAN-582H was homogenized, mechanically shaken and sonicated in 50% methanol. The methanol solution extracted 87.8% of the radiocarbon. Combustion of the remaining solids accounted for 11.8% of TRR. After centrifugation, the methanol in the supernatant was evaporated and the remaining aqueous fraction was freeze-dried. The freeze-

dried residue was weighed out and aliquots solubilized in equal volumes of 1-butanol and 0.1 M NaCl (in water). The CCC system employed a mobile phase of 1-butanol (4 mL/min) and a liquid stationary phase of 0.1 M NaCl (immiscible in 1-butanol) in a teflon tubing coil, which was spun in a planetary motion at 1000 rpm. Eluting samples were collected as a function of time. Four fractions were collected. By use of this procedure, highly pigmented and polar plant coextractives should be removed into the stationary phase. Both mobile and stationary phases were analyzed by TLC.

The four mobile phase fractions were dried and brought up in methanol and then separately characterized by two different TLC solvent systems -- ethyl acetate/toluene/formic acid/water:87/3/5/5 and ethyl acetate/isopropanol/formic acid/water:80/30/5/5. As shown in Table 3, the major identified metabolites in these fractions were the sulfonate conjugate, the sulfoxide of thiolactic acid conjugate, the sulfoxide of thioglycolic acid conjugate, oxalamide, thiolactic acid conjugate, and the thioglycolic acid conjugate/M11/multicomponent band. Copies of chromatograms from two solvent systems are given in Fig. 93A (ethyl acetate/toluene/formic acid/water:87/3/5/5) and Fig. 94A (ethyl acetate/isopropanol/formic acid/water:60/30/5/5).

The stationary phase was dried and brought up in methanol and characterized with two different TLC solvent systems. The methanol soluble CCC stationary phase accounted for 20.3% of TRR (0.151 ppm). Metabolites identified were the sulfoxide of thiolactic acid conjugate, the sulfoxide of thioglycolic acid conjugate, the thioglycolic acid conjugate. The sulfonate conjugate remained near the origin in the first solvent system, and was possibly part of a very diffuse band extending to the origin in the second system. The remaining water soluble CCC stationary phase accounted for 0.15 ppm and could not be applied to TLC for characterization.

Mass balance from this procedure is shown in the following table. Four forage replicates were used.

Table 2

Mass Balance from Forage Treated at 4 lbs ai/A after
Extraction with 50% Methanol and Separated Using Counter-Current Chromatography

Total Radiocarbon 0.746µg/g

Methanol Extract 87.8% (0.655µg/g)

Counter-Current Chromatographic System Fractions

Mobil Phase Fractions 27.9% (0.208 µg/g)

Stationary Phase 46.9% (0.350 µg/g)
Methanol Soluble 20.3% (0.151 µg/g)
Water Soluble 19.8% (0.148 µg/g)

Unextractable Solids 11.8% (0.0882 µg/g)

Percent Recovery (from sum of mobile phase fractions, stationary phase fractions and unextractable solids)
80.0%

The TLC characteristics of both mobile and stationary phases are given in Table 3.

Table 3

TLC Characterization of 50% Methanol Extract from Forage (4 lb ai/A)
Cleaned-up by Counter Current Chromatography

Tentative I.D.	Mobile Phase (ppm)	Stationary Phase (ppm)	Total (4 lbs/A) (ppm)	% of TRR	Total (1.5 lbs/A) (ppm)
Sulfonate Conj.	0.0367	<0.0816	<0.118	<15.8	<0.044
Sulfoxide of Thiolactic Acid Conj.	0.0460	0.0302	0.0762	10.2	0.028
Sulfoxide of Thioglycolic Acid Conj.	0.0274	0.0151	0.0425	5.7	0.016
Unknown	0.011	----	0.0110	1.5	
Oxalamide/Thio-lactic Acid Conj	0.0342	0.0166	0.0508	6.8	0.019
Unknown	0.0194	----	0.0194	2.6	
Thioglycolic Acid Conj./M11/others	0.0274	0.0076	0.035	4.7	0.013

Values for 1.5 lbs/A were calculated from the corresponding 4.0 lbs/A values. Levels ascribed to sulfonate are in actuality due to a multicomponent band of which the sulfonate conjugate is a part.

Comment

Comparison of Tables 1b and 3 shows that percentages of TRR reported for each component differ significantly according to the extraction scheme. For example, the sulfoxide of thiolactic acid conjugate of SAN-582H constituted 1.60% of TRR of forage

extracted according to the earlier extraction procedure (Attachment 1), whereas in the 50% methanol forage extract cleaned up by CCC the same compound constituted 10.2% of TRR. Discounting the stationary 9phase contribution for the sulfonate conjugate in Table 3, 32% of TRR in forage was identified. The corresponding value from Table 1b was 19%. Do these differences result from the different extraction procedures? Or do they merely reflect analytical uncertainties? Probably both. On one hand the fewer separation procedures in the CCC method would likely reduce inevitable losses that occur in each step of any procedure. On the other hand, the first separation scheme probably produces superior resolution.

We are now confident of the identification of the metabolites that were isolated, but the fact remains that only a small fraction of the residue was identified. The two major metabolites appear to be the sulfonate conjugate of SAN-582H and the sulfoxide of thiolactic acid conjugate of SAN-582H, either of which could constitute ≤10% of the residue. More work is necessary to characterize the very polar components of the methanol soluble fraction (A5). One possibility would be to isolate the polar material by TLC and then reflux in acid. This approach has been useful with certain other pesticides. Mild acid hydrolysis (1N HCl/60 min/90°C) of the entire fraction was mentioned in the original submission (page 43). Referring to the unidentified band near the origin of the TLC from forage, discussed above, more stringent conditions could at least provide information on whether residues of the pesticide can be released from sugar components.

At this time we do not require further characterization of the unextractable solids.

Note on Degree of Characterization Required. As implicit in R. Schmitt's 7/25/89 guidance document for livestock metabolism studies, in which the "trigger" values were referred to a 10 ppm dosing level, radioactivity levels should not be divided by the degree of exaggeration (4x in this case) before "trigger values" are applied. (This will be made explicit in a forthcoming document on plant and livestock metabolism.) Secondly, degree of characterization is also a function of total residue identified. If, for example, 75% of the residue were identified, we would not be so stringent in requirements for characterization of the remaining 25%.

Summary of Plant Metabolism

The proposed metabolic pathway of SAN-582H in corn plants is given as Attachment 2. Most of the identified metabolites can be formed from oxidation, deamination, and/or decarboxylation of the cysteine conjugate of SAN-582H. Metabolites in which the thienyl ring is changed were not isolated from plants.

Regulatory Approach

From data thus far submitted, it appears that SAN-582H is extensively metabolized and no one metabolite is present in high concentrations. Since a common moiety analytical method apparently cannot be developed (see discussion below) and CBTS believes that no one metabolite is likely to be present at measurable levels, then the recommendation will be that the tolerance expression include parent only. However that we might be satisfied that no metabolite will be present at measurable levels, Sandoz should submit residue data on the two metabolites found in highest concentration in the metabolism study: the sulfonate conjugate of SAN-582H and the sulfoxide of thiolactic acid conjugate. Data from six field trials should be submitted. If previously treated corn plants are analyzed, additional storage stability data will have to be generated. Should it be necessary that these two metabolites appear in the tolerance expression, the analytical methods will have to undergo independent laboratory validation as well as EPA method validation.

At this time CBTS recommends that the HED Metabolism Committee review available metabolism data and CBTS' conclusions, including the requirement for residue data on the two metabolites.

Deficiency #2a (Conclusion #2a of our 1/24/91 memo)

For permanent tolerances, the nature of the residue in ruminants is not adequately understood. Percents of total radiocarbon residue identified in tissues and milk varied from 24.3% in milk to 47.4% in kidney. Attempts should be made to further characterize the residue, as specified in the text of this memo.

Sandoz Response

The following report has been submitted:

"SAN 582 H: Addendum to a Previous Goat Metabolism Study," C.C. Yu and A.S. Guirguis, 3/4/92, Laboratory Project ID No. 414105-6A. (MRID # 422895-02)

Sandoz has responded to our comments in several sections. We list these comments from our memo with Sandoz's response.

Excreta. About 38% of TRR in urine and 32% of TRR in feces were previously identified. We did not require additional characterization. Sandoz has submitted representative chromatograms and further characterized the residue. Small quantities of the sulfoxide of thioglycolic acid conjugate were found in urine and feces -- 2.5% in urine, 2.9% in feces.

Kidney. We requested confirmation of the cysteine conjugate of SAN-582H. This metabolite, present at 9.18% of TRR, was identified using TLC with only one solvent system. We requested

copies of representative chromatograms. We required further attempts to identify the remaining metabolites -- at least to the point of assigning them to a given chemical class.

Samples were extracted as previously reported, but additional extraction steps were added. After extraction with methanol, a saturated ammonium sulfate solution was added to the methanol extract and centrifuged to separate the protein. The supernatant was reduced to about 1 mL under nitrogen and centrifuged to separate the fat. Four mL of water was added to the protein and fat precipitate from kidney tissue and the resulting solution/mixture partitioned with acetonitrile. After refluxing with acid and base, an attempt to release conjugates was made using β-glucuronidase and sulfatase. The resulting mixture was partitioned with ethyl acetate. The aqueous fraction remaining was sequentially refluxed in 5 N NaOH and 5 N HCl and partitioned with ethyl acetate after each reflux. Results are given in Table V of the report.

As shown in Table V, an additional 1% was released by enzymes, 3% by base and 4% by acid. "At the 1-2x application rate each of these residues is calculated to be less than 0.002 ppm in kidney. Thus, further identification should not be required." We have already commented about this line of reasoning in the section on plant metabolism above. However, it is true that the dietary exaggeration factors in dosing ruminants and poultry greatly exceeded that for plants. No attempt was apparently made to identify any of the released metabolites by TLC.

Representative chromatograms have been submitted.

The cysteine conjugate was found to be present at about 1% of TRR when examined by TLC with a different solvent system. What had appeared as one band in two solvent systems was shown to be a composite of 4 compounds when a third system was used. One of these compounds was present at 4.11% of TRR (0.41 ppm).

Sandoz has therefore identified 39.5% of the residue in kidney.

Liver. Metabolites identified in liver constituted 24.1% of TRR. We required confirmation of the identity of M17, reported to be present at 2.68% of TRR but identified in one solvent system. We asked for additional characterization of five metabolites which each comprised over 5% of TRR -- particularly "L4" and "L12" which together comprised over 21% of TRR. We requested additional characterization of the aqueous phase, which constitued 19.1% of TRR.

Sandoz has reexamined the TLC plates with Rf's originally reported in Table VIII, page 31 of the original report. M22,

originally reported as comprising 12% of TRR is now 6.11% of TRR because the acid and base released metabolites originally identified as M22 were found to have a slightly different Rf value. No attempt was made to identify L4. L12 is now reported to be three different metabolites (L15a, L15b, L15c) depending on whether the species was found in the free extract, the acid-released extract or the base-released extract. Rf values in one solvent system were 0.50, 0.50 and 0.51 for free, acid-released and base-released metabolites, respectively. This division is arbitrary, and an Rf of 0.51 as opposed to 0.50 is probably not significant.

Identity of M17 was confirmed by TLC in three different solvent systems.

The aqueous and unextractable solids were treated with β-glucuronidase, sulfatase, 5N NaOH and 5N HCl (Figure 1 of the report). These treatments released an additional 1% by enzymes, 3% by base and 9% by acid. "Further identification of these fractions is not achieved because of low radioactivity in each fraction."

A total of 18.2% of TRR in the liver was identified.

Muscle. Previously, Sandoz reported 33.9% identification of TRR. The cysteine conjugate comprised 14.2%. We asked that additional characterization be attempted for metabolites M1 (8.52% of TRR), M2 (9.91%) and M10 (7.19%) -- at least to the point of identification of functional groups. We requested some characterization of the acetone extract (13.5% of TRR).

Sandoz responds that M1 was separated into 2 components (Mu1a and Mu1b) by a different TLC solvent system; M2 was further separated into 4 components (Mu2a, Mu2b, Mu2c, Mu2d) using a different TLC solvent system; and M10 was further separated into 3 components (Mu10a, Mu10b, Mu10c) in a different solvent system. Table VIII of the report shows that Mu1b was found at 8.07% of TRR.

The acetone extract was fractionated into aqueous and ethyl acetate-hexane (1:1) fractions. The water solubles were 6% of TRR; the organic fraction constituted 8% of TRR. Due to coextractives and low radioactivity further attempts to analyze these fractions by TLC were reportedly nonsuccessful.

Fat. We asked for confirmation of the identity of M17, which had been identified by TLC on the basis of one solvent system. We asked that Sandoz attempt to release metabolites from the unextractable fraction (15.5% of TRR) and the aqueous fraction (9.25% of TRR).

Sandoz has confirmed the identity of M17 in three solvent

systems. The "unextractable" fraction was dissolved in chloroform and partitioned with water. A total of 9.38% of TRR was found in the chloroform; 4.17% remained unextracted. The aqueous fraction was refluxed at 1N HCl for 1 hr. and extracted with ethyl acetate. The radioactivity was about evenly distributed between the aqueous and organic phases. Further separation by TLC was not successful.

A total of 31.8% of TRR has been identified.

Milk. We requested identification of metabolite M17 in a different TLC solvent system. We asked for further characterization of metabolite MK2. We asked that attempts be made to release metabolites in the "protein and fat precipitate" (11.88% of TRR) and in unextractables (10.49%).

M17 was characterized in an additional solvent system.

Metabolite MK2 was shown to be a composite of 4 metabolites when a different TLC solvent system was used. The major component comprised 3.09% of TRR.

The "protein and fat precipitate" was dissolved in water and partitioned with chloroform. Chloroform extractables comprised 3.19% of TRR; "soluble protein & fat" comprised 8.69%.

The unextractables (solids remaining after a 1 hr. reflux in 1N HCl) were refluxed in 1N NaOH for 1 hr and then extracted with ethyl acetate. The ethyl acetate fraction constituted 4.01% of TRR; the aqueous fraction constituted 5.54% and the solid residue constituted 5.7%.

A total of 24.3% of TRR in milk was identified.

Comment Additional evidence is necessary that liver metabolites L15a, L15b, and L15c are different compounds. Although one of the unidentified muscle metabolites, M1ub, was found to comprise 8.07% of TRR, we do not consider additional characterization to be necessary at this time, due to the relatively low total residue found in muscle (0.97 ppm) at the exaggerated dosing level (223 ppm -- about 750X the expected dietary level). Characterization of the residue in muscle, kidney, fat and milk is sufficient at this time.

Structures of ruminant metabolites are given in Attachment 3.

Deficiency #2b (Conclusion #2b of our 1/24/91 memo)

For permanent tolerances, the nature of the residue in poultry is not adequately understood. No metabolite was identified in eggs or muscle. Only 12.9% of the total radiocarbon residue was identified in liver and 26.2% in fat. The residue in poultry must be better characterized. Attempts should be made to characterize the water soluble fractions, which comprise 25-47% of the total radiocarbon residues. If

metabolites cannot be isolated directly by HPLC, the reigstrant may wish to employ enzymatic hydrolysis and then stronger acid/base hydrolysis. The principal metabolites in various tissues and eggs should be identified. If this proves to be impossible, salient structural characteristics should be determined.

Supporting TLC's and HPLC's should be submitted.

Sandoz Response

The following report has been submitted:

"SAN 582 H: Addendum to a Previous Hen Metabolism Study," C.C. Yu and D.A. Nietschmann, 3/4/92, Laboratory Project ID No. 414105-7A. (MRID # 422895-03)

Sandoz has responded to our comments in several sections. We list these comments from our memo with Sandoz's response.

In addition to samples from the original study, which had been maintained at -20°C, additional samples were obtained by dosing three other hens at 10 mg/kg/day with ^{14}C-SAN-582H having specific activity of 1.2 mCi/mmole. The protocol for this study is dated 4/3/91 and appears in the report as Appendix I.

As requested in our 1/24/91 memo, dates of sacrifice amd dates of analyses were submitted for the original study done in December, 1987 and the more recent one, done in April, 1991.

Excreta.

An additional three metabolites were identified in excreta: [redacted] sulfonate conjugate, 0.5%), M30 (sulfoxide of thiolactic acid conjugate, 3.5%) and M32 (thioglycolic acid conjugate, 1%). These species were identified by TLC cochromatography using at least two solvent systems. A total of 37.1% of TRR has been identified in excreta. The principal metabolite is M3, present at 10.0% of TRR. SAN-582H was found to be present at 2.03%.

Liver. The radioactive residue in the liver constituted 8.33 ppm from a dose level of 167 ppm. The residue in muscle, fat, egg white and egg yolk did not exceed 0.7 ppm. Three metabolites L14, L18, and L20 were each found at about 8% of TRR in liver. We asked for additional characterization of these three metabolites. We asked for additional characterization of the water soluble fraction, which constituted 24.8% of TRR.

Sandoz isolated metabolite band L14 and showed using a different solvent system that L14 consists of 7 components, none of which exceeds 2.6% of TRR. Additional work on L18 or L20 is not described.

The aqueous fraction, resulting from methanol, acid and base extraction, was freeze dried, then refluxed for 4 hr. in

methanol, which extracted 16.5% of TRR. The remaining solids were refluxed for 2 hr. in 4N HCl and then partitioned with ethyl acetate. The ethyl acetate fraction constituted 5.4% of TRR; the aqueous fraction constituted 2.86% of TRR. The methanol extract was analyzed by TLC and resolved into 8 components ranging in concentration from 1% to 4% of TRR. These are reported as L22 to L29 in Table V.

Although additional characterization wás also requested for L18 and L20 in our earlier memo, upon reconsideration we conclude that for purposes of this petition characterization is sufficient. See our comments at the conclusion of this section.

Muscle. We requested additional characterization of the aqueous fraction, 36.2% of TRR, which remained after successive acid and base reflux.

Sandoz has characterized this fraction in a manner somewhat analogous to the aqueous fraction from liver. The methanol reflux of the freeze dried aqueous fraction comprised 14% of TRR. TLC showed a total of 10 components, each ranging from 1% to 2% of TRR (Table VI, Metabolites Mu13 to Mu22). 4N HCl released an additional 17% of TRR, which was separated into 8 components, none of which exceed 4% of TRR (Table VI, Metabolites Mu23 to Mu30). The remaining aqueous phase accounted for 5% of TRR.

No metabolite was identified in muscle. No metabolite was found at more than 7% of TRR.

At this time further characterization of the residue in muscle is not required.

Fat. Fat was previously extracted with hexane and methanol. SAN-582H, found in the methanol fraction, comprised 26.2% of TRR.

Sandoz has further characterized the hexane fraction and found an additional 8.5% SAN-582H. Unextractables, which constituted 21.3% of TRR, were further characterized by refluxing with 1N HCl, which released 7% of TRR. TLC analysis showed the presence of 5 components having 1%-3% of TRR. The remaining aqueous fraction was refluxed at pH 12 to release 4 additional metabolites ranging from 0.7% to 2.4% of TRR. After acid and base hydrolysis 7.68% remained in the aqueous phase.

At this time further characterization is not required.

Egg White. Previously, no metabolite was identified. Water solubles constituted 22.3% of TRR.

Sandoz has further characterized the water soluble fractions by incubation with β-glucuronidase and sulfatase enzymes, which released 19.5% of TRR. TLC separated this fraction into 8

components, one of which at 4.02% of TRR was identified as PL2088. The base-released metabolite EW5, reported previously, was shown to be PL2088 by TLC in two solvent systems. The total identified PL2088 is 7.8%. Metabolite EW6, present at 12.5% of TRR, is also identified as PL2088, but there seems to be no evidence besides TLC in one solvent system.

At this time further characterization is not required.

Egg Yolk. Previously, over 50% of TRR in yolk was acid or base released. An additional 46.9% of TRR remained in the aqueous phase.

Sandoz has further characterized TLC band "EY2", present at 10% of TRR using a different solvent system. The band was separated into 7 components ranging from 0.2% to 2% of TRR. "EY2b" at 2.1% of TRR has been identified as PL2088. Band "EY8", present at over 11% of TRR, which remained at the origin in the one solvent system used, was scraped from the plate and analyzed with a different solvent system. In this way EY8 was shown to consist of 7 components, none of which exceeded 2% of TRR.

The water soluble fraction was treated with β-glucuronidase and sulfatase, which together released 43% of TRR. TLC analysis showed the presence of 8 components, the major one of which (8%) was identified as PL2088 using two solvent systems.

At this time further characterization is not required.

Summary. The residue in poultry has been adequately characterized with the exception of two liver metabolites. However, we note that the total SAN-582H residue found in corn grain was only 0.02 ppm and the SAN-582H dosing level was 167 ppm referred to the daily diet. The exaggeration factor was therefore greater than 8000. We conclude that any particular metabolite -- even a major metabolite in liver -- will be unmeasurable. We therefore conclude that the nature of the residue in poultry is adequately understood. If in the future the SAN-582 residue in poultry feed items becomes significant, further characterization of the residue in liver may be required.

Sandoz's proposed metabolism pathways for SAN-582H in hen are given as Attachment 4. The diagram at least shows that SAN-582H is extensively metabolized. Other than parent, which was the principal constutent of the residue in fat, no one compound appears to comprise more than 10% of the residue in a given tissue.

As noted above, our conclusions concerning the nature of the residue in corn, ruminants and poultry are provisional, pending review by the HED Metabolism Committee.

Deficiency #3 (Conclusion #3 from our 1/24/91 memo)

The analytical method submitted for SAN-582H and its oxalamide metabolite in corn is inadequate....

An analytical method for parent SAN-582H -- BS-2304 -- has since been submitted and found acceptable by CBTS (PP#0G3892, memo of 6/11/91). The method is currently undergoing EPA Method Validation. We note that depending on the nature of the residue in plants and animals, a method may have to be developed for one or more of the metabolites.

Search for a Common Moiety

Because of extensive metabolism in plants, Sandoz has attempted to develop an analytical method for a common moiety. These attempts were summarized in Appendix 4 of the previous report (Sandoz Report No. 414105-14, MRID # 415965-54). Sandoz has now submitted an updated summary of these attempts (MRID # 423368-01), and CBTS is reviewing this submission in a concurrent memo. To date, Sandoz has been unable to develop a common moiety analytical method.

Deficiency #4 (Conclusion #4 from our 1/24/91 memo)

.....[Storage stability] data must be generated to cover the period from sampling to analysis for the residue field trials. Due to the uncertainties in the analytical method, it may be advisable to revise the method before continuing the study....Storage stability data will be necessary for other components of the residue to be regulated once these have been determined.

Sandoz Response

Sandoz has submitted the following report:

"Stability of SAN-582H and Its Metabolites in Stored Frozen Corn Samples QAU #90/05/16," T.R. Bade, 4/15/92, Lab. Project ID No. 414108-25. (MRID # 422895-08)

The report consists of two studies. In the first study, control corn matrices were fortified with 0.5 ppm SAN-582H and the oxalamide metabolite, analyzed on day zero, and reanalyzed at later dates. In the second study, radioactive metabolism samples were reanalyzed and compared with earlier analyses.

Fortified samples were analyzed at days 0, 90, 180 and 366 for SAN-582H and oxalamide using Method AM-0840-0790-0. As noted in our 1/24/90 memo, this method produces highly variable recoveries. Samples were analyzed at 21 months for SAN-582H using Method BS-2304.

Recoveries for oxalamide at 12 months showed a decline of only about 10%, but the standard deviations were so high (>30%) that we can only conclude that oxalamide residues are probably

stable. If the oxalamide metabolite is to appear in the tolerance expression, samples will have to be reanalyzed using a method producing more uniform recoveries.

Recoveries at 21 months for forage, silage, grain and fodder averaged 79.7%±10.9%. When corrected for method recovery using fortified check samples, this recovery becomes about 84%. We conclude that SAN-582H is stable over the time period of the study.

In an effort to assess the stability of metabolites, treated samples were reextracted and extraction profiles and certain metabolite concentrations compared. Samples were originally extracted 10/23/89; the later extraction occurred 9/18/91.

Radioactivity of various fractions did not change significantly over the 23 month period. Results for the acidic methylene chloride soluble fraction (A4) and the methanol soluble fraction (A5) are given in the following table. A more complete summary is given in Table V of the submission.

Table 4

Radioactivity Extracted from Corn Metabolism Samples

	Extractable Activity	
	1989/90	1991
Acidic Methylene Chloride Soluble		
Forage	0.0987 ppm	0.0985 ppm
Grain	0.003 ppm	0.00538 ppm
Fodder	0.089 ppm	0.078 ppm
Methanol Soluble		
Forage	0.193 ppm	0.2425 ppm
Grain	0.009 ppm	0.0116 ppm
Fodder	0.256 ppm	0.2934 ppm

The acidified methylene chloride extract was subjected to TLC and the results compared to the corresponding TLC taken in 1989. Results for selected metabolites identified in forage treated at 4 lbs ai/A are given in the following table. More complete data are reported in Tables VI (forage) and VII (fodder) of the submission.

Table 5

Comparative Concentrations of Metabolites Isolated by TLC from the CH2CL2/HCl Extract from Corn Forage Extracted on 11/20/89 and 9/24/91

	11/20/89 Analyses		9/24/91 Analyses	
Metabolite	PPM	%TRR	PPM	%TRR
TLA	0.0028	0.48	0.0092	1.58
Oxalamide	0.0172	2.92	0.0135	2.32
STGA	0.0081	1.38	0.0174	2.99
STLA	0.0104	1.77	0.0188	3.24

TGA = Thioglycolic Acid, STGA = Sulfoxide of TGA,
TLA = Thiolactic Acid, STLA = Sulfoxide of TLA.

The apparent increase in concentration of the metabolites could be due to incorporation of Ambis autoradiography, which would give more accurate and reproducible values than the previous method (location of radioactivity by X-ray film, scraping the spot from the plate, extraction of the silica gel, analysis of the extract by LSC). However, as the tables in the report indicate, a sligtly greater region of the plate was scanned in the later analysis. Agreement was better in the case of fodder.

CBTS Comment

Storage stability data are adequate to support the analyses for SAN-582H, per se. As noted, if the oxalamide metabolite is to appear in the tolerance expression, samples must be reanalyzed using a method giving more reproducible recoveries. Similarly, residue analyses for additional metabolites must be supported by adequate storage stability data.

While the TLC results are indicative, they cannot substitute for controlled storage stability studies.

Deficiency #5 (Conclusion #5 from our 1/24/91 memo)

Field trials were carried out in six states....the submitted residue analyses are unsuitable to support permanent tolerances because the current analytical method is unacceptable. Either the samples must be reanalyzed using a revised method -- in which case appropriate storage stability data are necessary -- or new residue trials must be carried out with analyses by the revised method. Depending on the nature of the residue to be regulated, additional analyses and field trials may be required for permanent toelrances in any event.

Sandoz Response

The company has reanalyzed the corn samples from the 1989

field trial using the analytical method described in our 6/11/91 memo. The data appear in the following report:

> "Reanalysis of Corn Samples from the 1989 Season for SAN-582H Residue," N.C. Jimenez, 4/14/92, Lab Project ID No. 414108-20. (MRID # 422895-05)

Forage, silage, grain and fodder from six field trials held in NY, TX, IA, OR, IL and NE were reanalyzed using Sandoz method BS2304: "A Method for the Determination of Residues of SAN-582H in Corn and Soil Samples". As noted, the method was reviewed in our 6/11/91 memo. Percent recoveries from fortified control samples averaged 94±7% from forage, 88±15% from silage, 90±9% from grain and 84±11% from fodder. Fortification levels were 0.01 and 0.1 ppm. Samples were analyzed 24-29 months after sampling. The time period from extraction to final analysis did not exceed one week.

SAN-582H was not detected in any sample at a detection limit of 0.01 ppm.

Additional field trial data have been submitted from trials held during 1990 and 1991.

Data from the 1990 trials are present in the following report:

> "Analysis of Corn Samples from the 1990 Season for SAN-582H Residue", N.C. Jimenez, 4/14/92, Lab Project ID No. 414108-15. (MRID # 422895-06)

The 7.5L formulation of SAN-582H was applied in three application types -- preemergence (PRE), preplant incorporated (PPI) and early postemergence (PE). A single application of 1.5 lbs ai/A was made for all application types using small plot ground equipment. Five field trials were held in NE, NY, IL, OH and IA. Forage from corn treated PRE or PPI was harvested 60-71 days after application; forage treated PE was harvested 28-38 days after application. Silage from corn treated PRE or PPI was harvested 112-150 days after application; silage treated PE was harvested 84-116 days after application. Grain and fodder from corn treated PRE or PPI was harvested 157-164 days after application; grain and fodder treated PE was harvested 123-129 days after application.

Samples were analyzed 11-19 months after harvest using Sandoz method BS2304. Extraction occurred 1-8 days before final analyses. Percent recoveries averaged 97±10% from forage, 99±9% from silage, 88±8% from grain and 88±9% from fodder. Fortification levels were 0.01 and 0.1 ppm. SAN-582H was not detected in any sample. A residue of 0.015 ppm, detected in one forage sample from the Ohio trial, could not be confirmed using

GC/MS.

Data from the 1991 field trials are present in the following report:

"Analysis of Corn Samples from the 1991 Season for SAN-582H Residue," N.C. Jimenez, 4/14/92, Lab. Project ID No. 414108-21. (MRID # 423103-01)

SAN-582H 7.5L Herbicide was separately applied PRE, PPI, and PE to corn at 1.5 lb ai/A to corn grown in NC, CO, IN, OH, IL, and IA. Forage from corn treated PRE or PPI was harvested 56-63 days after application; forage from corn treated PE was harvested 28-46 days after application. Silage from corn treated PRE or PPI was harvested 104-143 days after application; silage from corn treated PE was harvested 83-125 days after application. Grain and fodder from corn treated PRE or PPI was harvested 125-161 days after application; grain from corn treated PE was harvested 104-129 days after application.

Analyses were carried out within 125 days after sampling. Sandoz should correct the dates for harvest/analysis of the Ohio samples. According to Table 3, fodder was analyzed on 9/7/91, but the date of harvest is given as 10/1/91. Samples were extracted 1-9 days before final analyses. Percent recoveries averaged 92±12% from forage, 84±10% from silage, 94±10% from grain and 81±11% from fodder. Control forage and silage samples were fortified at 0.01 or 0.1 ppm; control grain and fodder samples were fortified at 0.1 ppm. (As noted above, acceptable recoveries were obtained at the 0.01 ppm fortification level on grain and fodder from the 1990 trials.

SAN-582H was not detected in any sample.

As noted above, residue data for the sulfonate conjugate of SAN-582H and the sulfoxide of thiolactic acid conjugate of SAN-582H in corn grain, forage and fodder should be generated from field trials held in six states. Analyses should be supported by storage stability data. Alternatively, samples could be analyzed by a common moiety analytical method, should such a method become available. If these metabolites or common moiety are not detected in the corn samples, parent only will appear in the tolerance expression. The HED Metabolism Committee will review available plant and animal metabolism data as well as the need for additional residue data.

Deficiency #6 (Conclusion #6 from our 1/24/91 memo)

No corn processing study has been submitted. For permanent tolerances, such a study must be undertaken unless highly exaggerated rate data also show non detectable residues in corn grain.

Sandoz Response

The following report has been submitted:

"Magnitude of the Residue of SAN-582H in Corn Grain and Corn Processed Fractions," N.C. Jimenez, 4/14/92, Lab. Project ID No. 414108-27. (MRID # 422895-07)

Field plots, located in Ohio, were treated preemergent with SAN-582H 7.5L at rates of 1X, 3X and 5X the maximum use level of 1.5 lbs ai/A. Corn grain grown on the 5X plot as well as control grain were wet and dry processed into commercial fractions by the Food Protein Research and Development Center, Texas A&M University.

Samples were dry milled into grain dust of various sizes, grits (large, medium, small), coarse meal, meal, flour, hulls, solvent extracted presscake, crude oil, refined oil, refined bleached oil, deodorized oil, deodorized distillates and soapstock. Samples were wet milled into coarse gluten starch, gluten, starch, process water, hulls, solvent extracted presscake, crude oil, refined oil, soapstock, refined bleached oil, refined bleached deodorized oil, deodorized distillates. Complete details, including material balances, are given in the petition.

Grain was harvested 10/2/91, 4 1/2 months after application. Samples were processed between 10/7/91 and 12/19/91 and analyzed by Method BS2304 from January through March, 1992. Recoveries were obtained on each processed fraction at levels of 0.01-0.10 ppm. Percent recoveries averaged 87±15%.

SAN-582H was not detected in any sample at a level of 0.01 ppm, but several samples did show interferences and had to be reanalyzed using GC-MS.

We conclude that no food or feed additive tolerance is necessary for SAN-582H.

Should it be necessary to regulate any of the metabolites, processed samples will have to be analyzed for these metabolites.

Deficiency #7 (Conclusion #7 from our 1/24/91 memo)

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 residue in corn have been determined.

Sandoz Response and CBTS Comment

Sandoz's response is given in the administrative volume (Volume 1) of the present submission. Based on the goat and hen metabolism studies and the corn metabolism study, the company

argues that residues in animal tissues, milk and eggs will be below detection limits. For example, assuming that SAN-582H is present in corn forage at the level of total radioactivity - 0.33 ppm, a 500 kg cattle consuming 7.5 kg/day forage would ingest 0.00489 mg/kg/day. In the metabolism study, 8.92 mg/kg SAN-582H produced liver residues of 16.6 ppm. Using these numbers we predict that the maximum SAN-582H level in liver would be 0.008 ppm. In reality we would expect lower levels because the corn forage residue is comprised of numerous metabolites and the liver residue, obtained from dosing with SAN-582H, consists of numerous metabolites. (SAN-582H, per se, has not been detected in the corn residue or in liver.) Based on results from the goat metabolism study, levels in other tissues would be lower than those in liver. Similar arguments apply to poultry residues, but because corn grain is the major feed item in poultry and total residues in grain did not exceed 0.02 ppm, much lower levels would be expected.

The assumption inherent in this argument is that the SAN-582H residue in forage will have the same residue transfer characteristics in ruminants as SAN-582H, itself. The fact that identified metabolites in corn also appear in animals suggests that this assumption is correct. The sulfonate conjugate, apparently the major metabolite in corn forage, has been found in the excreta of hens.

CBTS will make no final conclusion on the need for residue transfer studies in ruminants until the nature of the residue in corn and in ruminants is adequately understood. Due to arguments presented in the section on poultry metabolism, above, CBTS concludes that a residue transfer study in poultry is not likely to produce measurable residues. Therefore such a study is not required at this time.

Other Considerations

An International Residue Limit (IRL) Status sheet is appended to this review. There are as yet no Codex, Canadian or Mexican IRL's for this compound. Hence, compatibility is not an issue.

Attachments:

1. Pages 9, 10 of our 1/24/91 memo for PP#0G3892.
2. Structures of Identified Plant Metabolites.
3. Structures of Identified Ruminant Metabolites.
4. Structures of Identified Poultry Metabolites.
5. International Residue Limit Status Sheet.

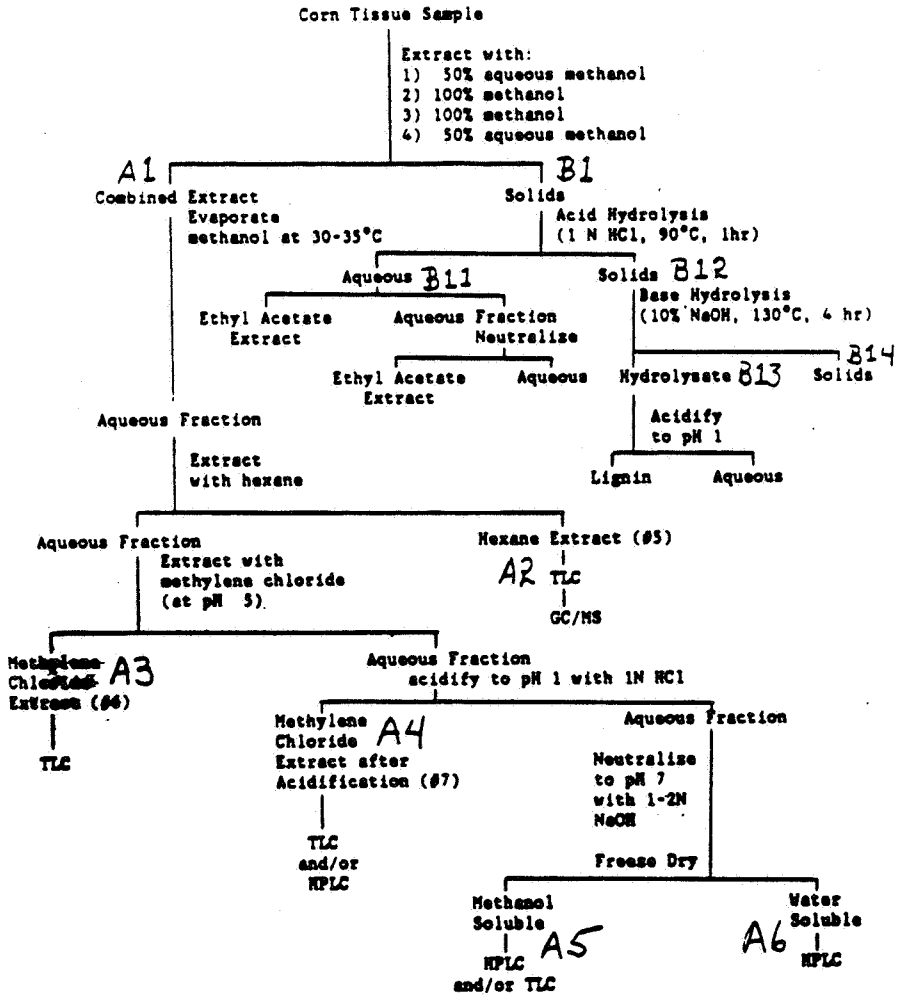
cc: RF, SF(SAN-582H), Circu., PP#0G3892, PP#0F3918, MikeFlood, E. Haeberer.

H7509C:CBTS:Reviewer(MTF):CM#2:Rm800A:305-6362:typist(mtf):7/28/92.
RDI:BranchSeniorScientist:RALoranger:7/27/92.

Fodder

Referring to the extraction schema, Figure 2 of this memo, and Table X of the report, we note that about 41% of TRR from fodder treated at 4 lb ai/A -- fraction B1 -- could not be extracted by successive methanol extractions. Hydrolyzed radiocarbon apparently liberated N-2-(1-hydroxypropyl)-2,4-dimethylthienylamine, but no confirmation or quantitation is reported. This compound was identified by MS as a product of acid hydrolysis of a cysteine conjugate standard. Otherwise, no compound was identified from fraction B1.

Figure 2 General extraction scheme for characterization of radiocarbon in corn samples from the SAN-582H corn metabolism study



Attempted identification of compounds centered on fractions A3, A4, A5 and A6. Attempts to characterize fraction A3 are not described in the text, but in Table XV one of two TLC bands is tentatively identified as the oxalamide metabolite, a soil metabolite, present at 1.39% of TRR (See Figure 1). This identification was apparently made on the basis of a diffuse TLC band in one solvent system.

The methylene chloride/acid extractable fraction (A4) from fodder treated at the higher level -- 7.88% of TRR -- could be separated into 4 TLC bands. A copy of the autoradiogram is given in Figure 4 of the report, and results are tabulated in Tables XVII and XVIII for treatment at 4.0 and 1.5 lb ai/A, respectively. Only the sulfoxide of the cysteine conjugate was identified from the 4 bands of Figure 4. However at the lower application rate, an additional 4 compounds were identified, including the oxalamide metabolite.

TLC of the methanol soluble, freeze-dried aqueous fraction, A5 -- 22.7% of TRR -- showed 8-9 zones or bands. The major band, corresponding to 6.6% of TRR (from fodder treated at 4.0 lbs ai/A), was not identified. One band, 2.2% of TRR, was identified as the glutathione conjugate (GSH-conj.) based on the retention time in one solvent system. (Structures of plant metabolites are given in Figure 3, attached.) However, this assignment is not supported by results from an acetone extraction on the freeze dried aqueous fraction (not shown in the extraction schema), which apparently showed no GSH conjugate. The residue from this acetone extract was incubated with β-glucuronidase, sulfatase, β-glucosidase and α-glucosidase. No hydrolysis to non polar compounds could be detected.

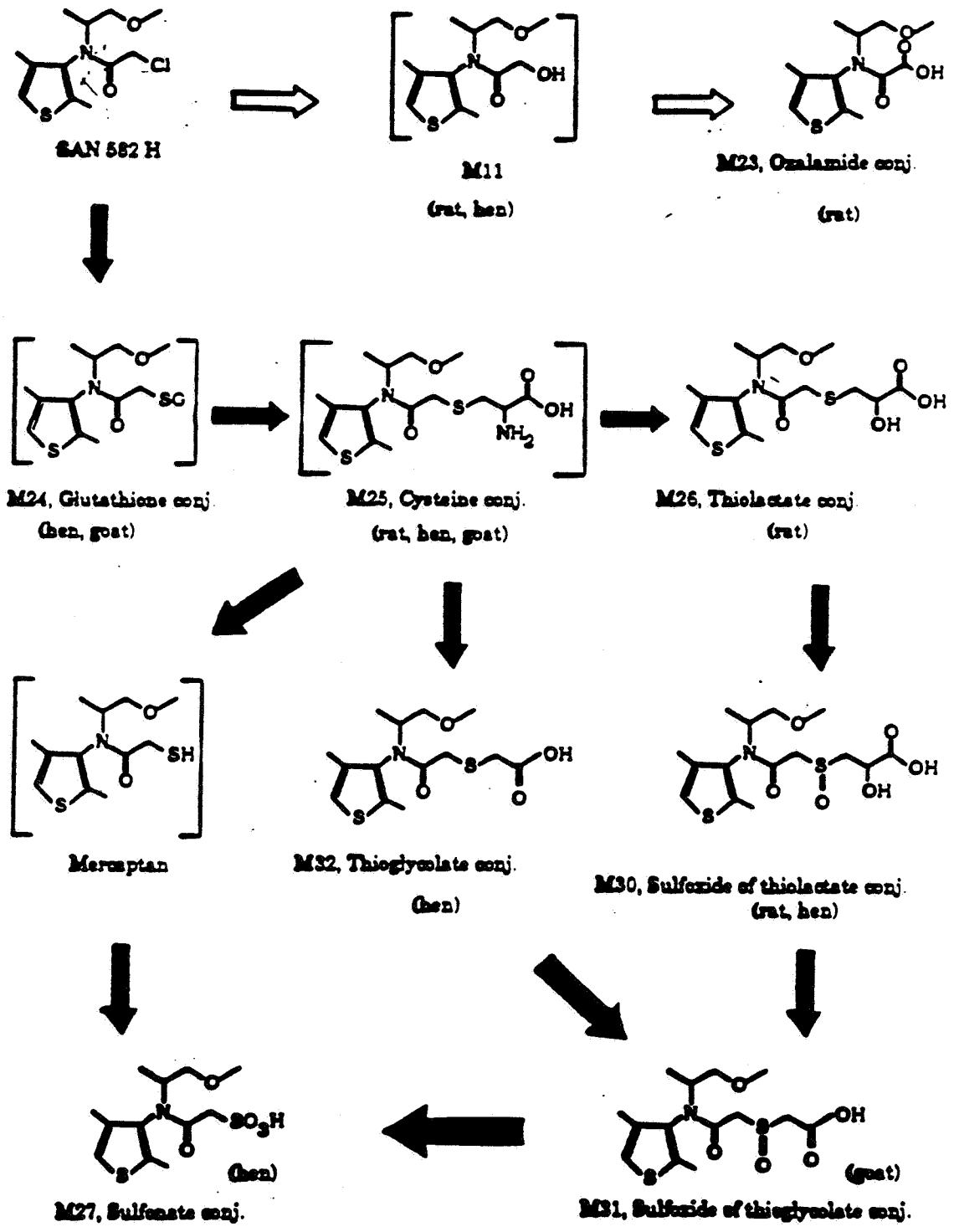
Fraction A6, the water soluble fraction from the freeze dried aqueous fraction, constituted 8.9% of TRR. As shown in Table XXXIII, 13-16 peaks were identified by HPLC, and none corresponded to concentrations greater than 0.01 ppm when referred to the 1.5 lb ai/A level.

Comment

According to Summary Table 2, page 21 of the report, 16.5% of the total radiocarbon residue (TRR) could be identified. However, we are unable to account for the percentages of constituents from the individual extractions. For example, the concentration of the oxalamide metabolite is listed in the table as 3.4% of TRR. Only 1.4% of TRR can be accounted for from Tables XVI and XVIII. Moreover, it appears that the majority of assignments were made on the basis of TLC in one solvent system. Submitted TLC autoradiograms generally show very broad bands. Identification on this basis alone is insufficient. At a minimum, two different solvent systems should be employed. According to Summary Table 2, the presence of the oxalamide

ATTACHMENT 2

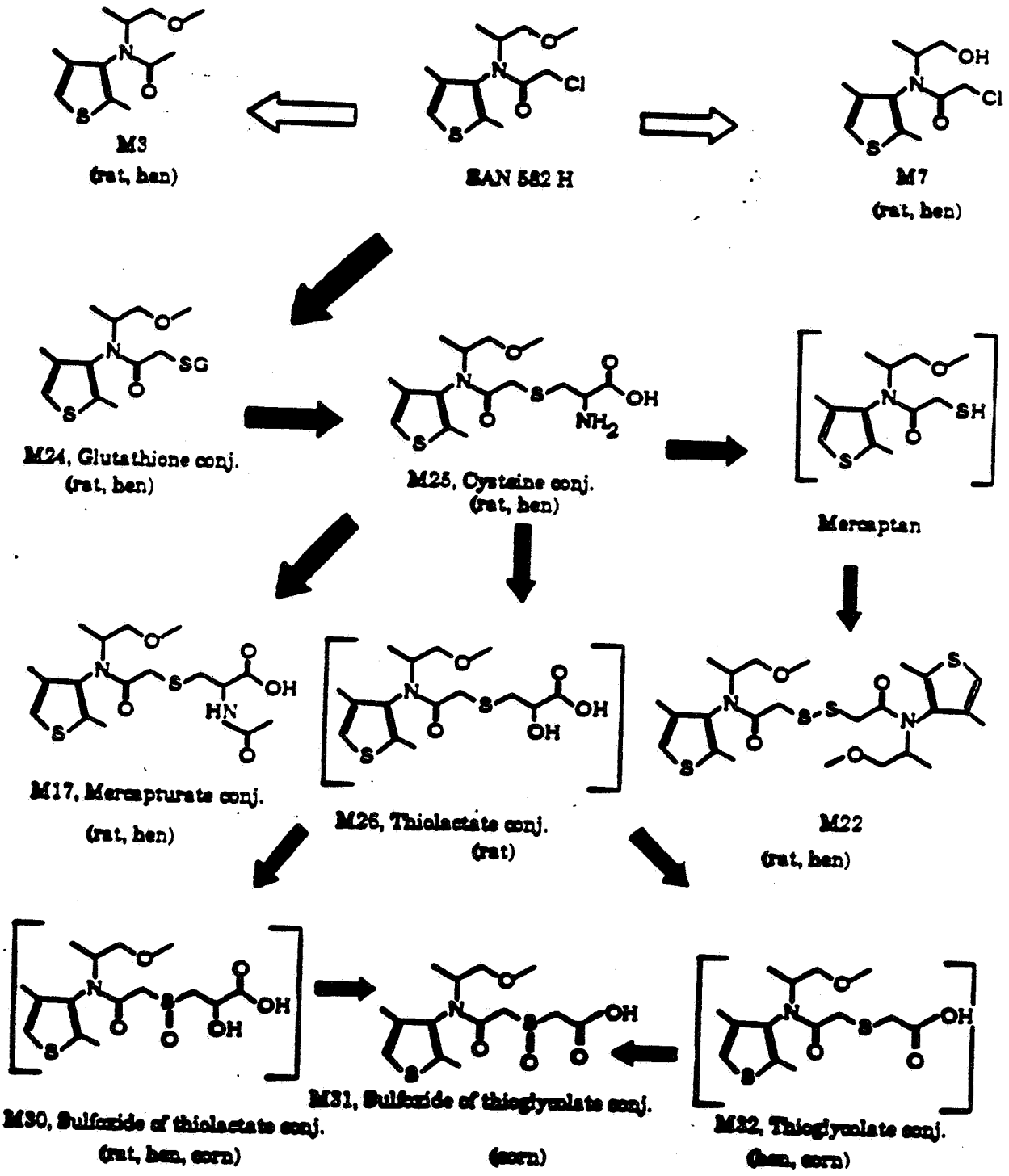
Proposed Metabolic pathways for SAN 582 H in Corn and Soil (parentheses indicate metabolites also in other species; brackets indicate suggested intermediates).



Note: Solid arrows indicate glutathione pathways.

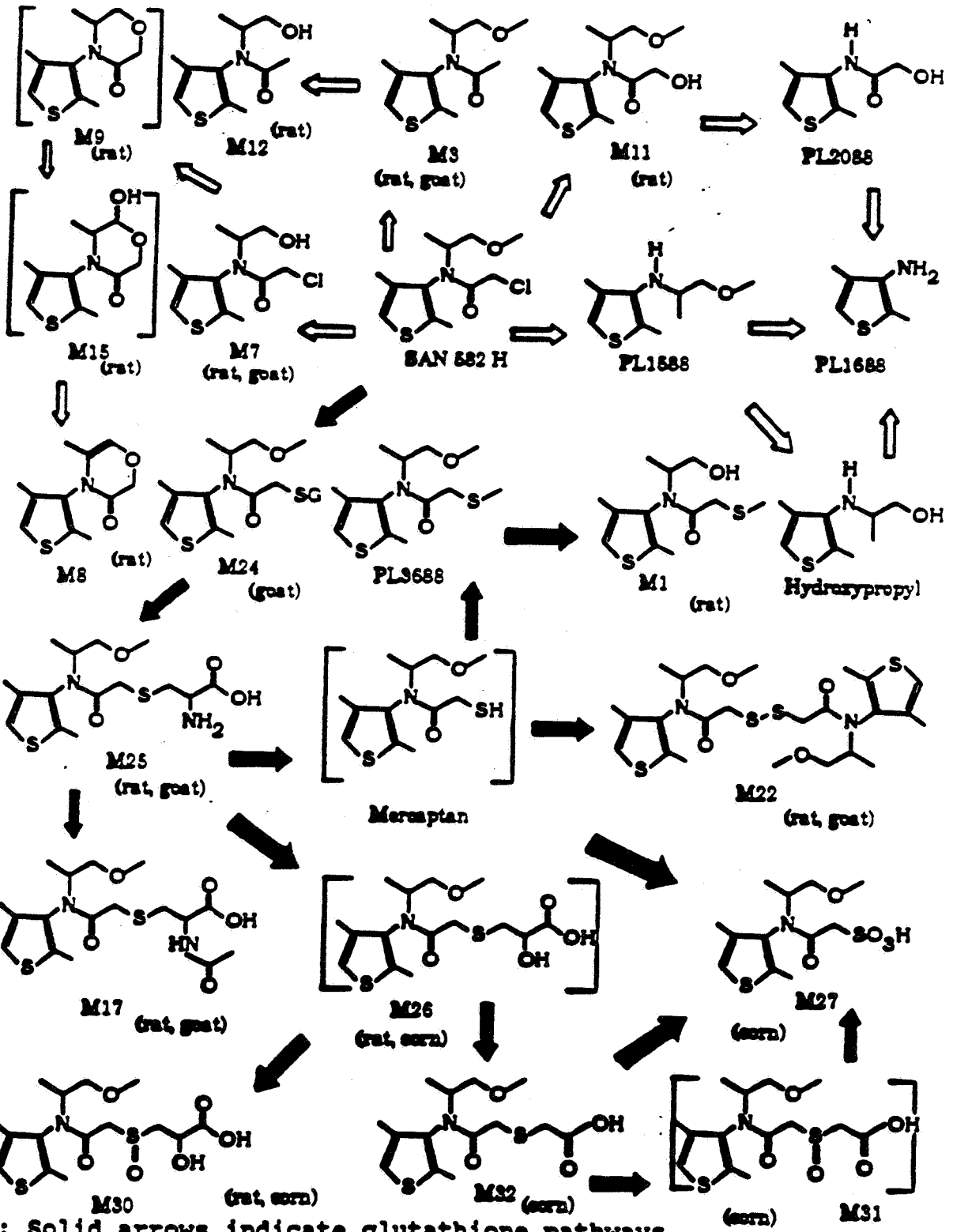
ATTACHMENT 3

Proposed Metabolic pathways for SAN 582 H in Goat (parentheses indicate metabolites also in other species; brackets indicate suggested intermediates).



Note: Solid arrows indicate glutathione pathways.

Proposed Metabolic pathways for SAN 582 H in Hen (parentheses indicate metabolites also in other species; brackets indicate suggested intermediates).



Note: Solid arrows indicate glutathione pathways.

ATTACHMENT 5

F. Lees 2/21/92

Attachment:

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INTERNATIONAL RESIDUE LIMIT STATUS

CHEMICAL SAN-582H / DIMETHENAMID (2-chloro-N-(2,4-dimethyl-3-thienyl)-N-(2-methoxy-1-methylethyl)

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. OF3918

DEB Reviewer F100D

Residue: & Parent

Crop(s)	Limit (mg/kg)
CORN	
FORAGE	0.01
GRAIN	0.01
FODDER	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

Form Revised 1989