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

JAN 24 1991

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: PP#0G3892. SAN-582H Herbicide in/on Field Corn.
MRID #'s 415965-54 through -57 (this petition), 416624-20, -21 (PP#0F3918).
DEB # 7183.

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 the combined residues of its herbicide SAN-582H, 2-chloro-N-[(1-methyl-2-methoxy)ethyl]-N-(2,4-dimethylthien-3-yl)-acetamide, and its oxalamide metabolite, N[(1-methyl-2-methoxy)ethyl]-N-(2,4-dimethylthienyl)-oxalamide:

Corn Forage	0.01 ppm
Corn Silage	0.01
Corn Grain	0.01
Corn Stover	0.01

No temporary or permanent tolerances have as yet been established.

The proposed EUP permits the use of up to 600 gallons (4,500 lbs ai) of SAN-582H 7.5L on up to 6360 acres of field corn over a two year period.

Conclusions

1a. The nature of the residue in corn grain, fodder, forage and silage is adequately understood for purposes of this temporary tolerance petition. The residue to be regulated is parent SAN-582H and its oxalamide metabolite.

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 given solvent are indeed unique so their concentrations should not be added to corresponding concentrations 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.

1b. Because of the low total levels of radioactivity in grain, further characterization is not required for a permanent tolerance.

2a. The nature of the residue in ruminants is adequately understood for purposes of this temporary tolerance petition. The residue to be regulated is parent SAN-582H. However, 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. 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.

2b. The nature of the residue in poultry is adequately understood for purposes of this temporary tolerance petition. The residue to be regulated is parent SAN-582H. However, 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. 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 registrant 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.

3. The analytical method submitted for SAN-582H and its oxalamide metabolite in corn is inadequate for purposes of this temporary tolerance petition. Recoveries and standard deviations are unacceptably high. Independent laboratory validation produced unsatisfactory recoveries at the 0.01 and 0.05 ppm fortification levels, and chromatogram peaks at these levels were not well defined. The petitioner must develop an analytical method for SAN-582H and the oxalamide metabolite which produces acceptable recoveries. The method must then undergo independent laboratory validation.

Use of diazomethane for methylation of oxalamide is not recommended. If a safer reagent cannot be found, documentation must be provided supporting the need for its use.

For permanent tolerances, the adequacy of the analytical methods cannot be determined until the nature of the residue in plants and animals is understood.

Once the nature of the residue is understood, for permanent tolerances recoveries must be obtained under FDA's multiresidue protocols. Analytical reference standards must be submitted to the Repository. The recent (1/8/91) new chemical screen for the permanent tolerance petition, PP#0F3918, failed on account of these deficiencies.

4. Storage stability data have been developed for SAN - 582H for three months only. Additional data are not needed for purposes of this temporary tolerance petition. Conclusions concerning the magnitude of the potential residue are based on the radiolabeled study. For permanent tolerances, 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. Based on the three month data, it is not certain that quantitative conclusions will be possible at the end of the projected study period. Storage stability data will be necessary for other components of the residue to be regulated once these have been determined.

5. Field trials were carried out in six states. Neither SAN-582H nor the oxalamide metabolite was detected in any sample analyzed. Additional analyses will not be required for purposes of this temporary tolerance petition. However, 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 tolerances in any event.

6. 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.

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 residue in corn have been determined.

Recommendation

CBTS recommends against the proposed temporary tolerances for reasons given in Conclusions 1a and 3: The registrant must revise the analytical method for SAN-582H and its oxalamide metabolite to produce acceptable recoveries. Supporting chromatograms should be submitted. The revised method must undergo independent laboratory validation.

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

A copy of this memorandum should be sent to the registrant.

Detailed Considerations

Manufacture and Formulation

The manufacturing process and product chemistry data have been reviewed by Dynamac Corporation (11/21/90) 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.

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% inerts. SAN 582H contains 7.5 lbs. active ingredient (ai) per gallon.

The structure of SAN-582H is given in Figure 1 (page 6).

Proposed Use

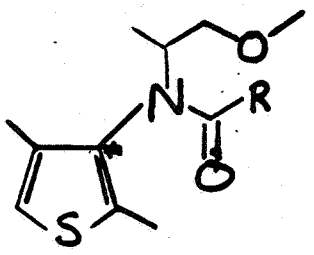
SAN 582H may be applied preplant surface, preplant incorporated, preemergence and/or early postemergence up to 4-

leaf corn. The herbicide may be ground or air applied at levels up to 1.6 pints per acre (1.5 lbs ai/A), which is the seasonal maximum level.

Do not graze or feed treated corn to livestock for at least 60 days following application.

Do not rotate to crops other than field corn for 12 months after application.

Figure 1



SAN-582H R = CH_2Cl

OXALAMIDE METABOLITE R = CO_2H

Nature of the Residue

Plants: Metabolism of SAN-582H in corn is discussed in the following report:

"Uptake, Translocation and Metabolism of the Herbicide SAN-582H in Corn;" Y.H. Atallah, P.A. Moore and T.R. Bade; July 18, 1990. Lab Project No. 414105-14 (MRID # 415965-54).

Radiolabeled SAN-582H, labeled in the 3-thienyl position (Figure 1) was added to unlabeled compound to give a resulting specific activity of 1.52×10^4 dpm/ug, or 1.89 mCi/mmole. Inerts for the 720 EC formulation were then added and methanol added to a set volume. Aliquots of this solution were diluted with water and each applied to 1.0 m^2 soil surface. Soil was

treated the day after planting. Application rates corresponded to 1.5 lbs ai/A, the maximum label rate, and 4.0 lbs ai/A. According to the study protocol, previous field studies have shown that this latter application rate is the maximum tolerated rate.

Corn thinning samples were taken 19 days after treatment; forage samples were harvested at 50 days; silage samples were taken at 116 days, with grain and cobs kept as separate samples; and mature corn was harvested at 130 days. Samples were frozen immediately after collection, shipped from the field site in dry ice and stored at -20°C until analysis. Forage was harvested in July, 1988, and normal harvest occurred in October, 1988. Samples were analyzed from November, 1989 through March, 1990.

Total radiocarbon in plant tissues was determined by combustion followed by scintillation counting. Total radiocarbon in plants is given in Tables 1a and 1b.

Table 1a

Total Radiocarbon in Corn Plants
Grown in Soil Treated at 1.5 lbs ai/A

Sample Type	PHI	µg ^{14}C-SAN-582H Equivalents per Gram Fresh Weight
Thinnings	19	0.46
Forage	50	0.31
Silage	116	0.40
Cobs	116	0.012
	130	0.020
Grain	116	0.021
	130	0.023
Fodder	130	0.50
Roots	130	0.47

Table 1b See page 69 original report

Total Radiocarbon in Corn Plants
Grown on Soil Treated at 4 lbs ai/A

Sample Type	PHI	µg ^{14}C-SAN-582H Equivalents per Gram Fresh Weight
Thinnings	19	1.78
Forage	50	~~1.05~~ 0.752
Silage	116	1.12
Cobs	116	0.04
	130	0.06
Grain	116	0.05
	130	0.06
Fodder	130	1.60
Roots	130	1.69

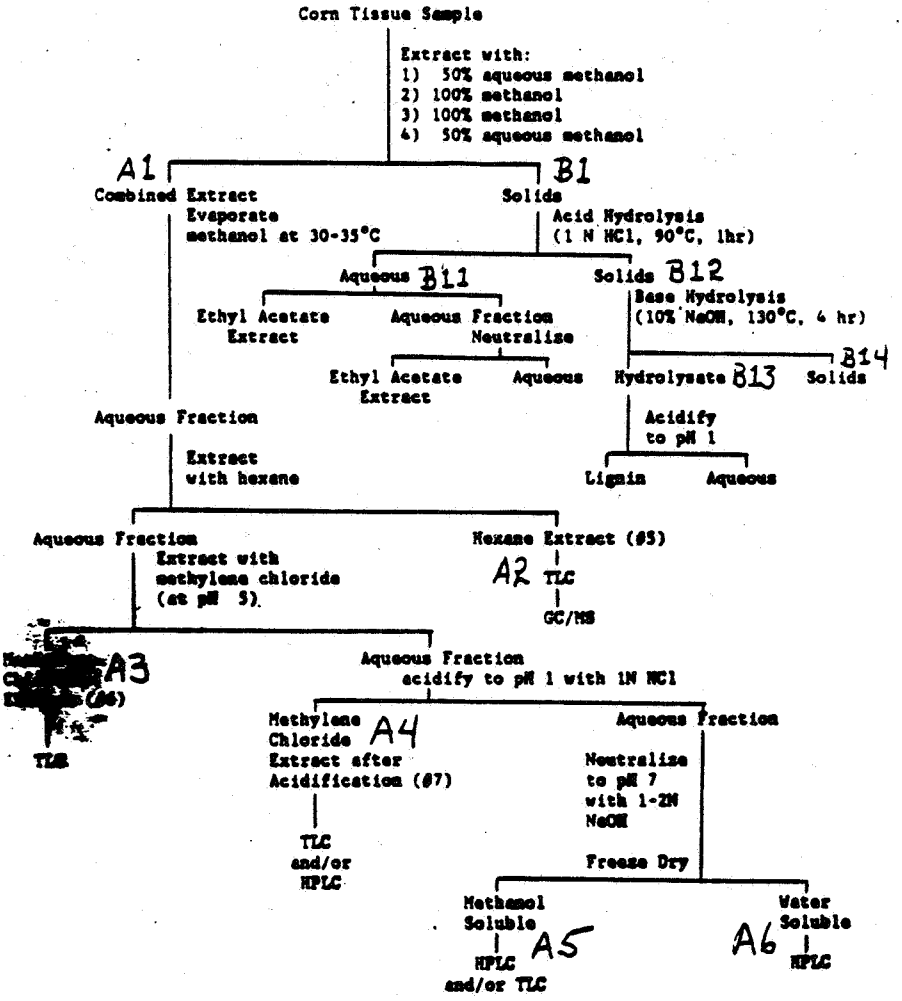
Further characterization was achieved using the extraction schema given in Figure 2 (next page).

Percents of total radiocarbon in the various extracts from plant parts are give in Tables X and XI of the report. The registrant should better explain the mass balances. Taking fodder as an example, at the 1.5 lb ai/A use level (Table XI), 42.6% of the radiocarbon residue was not extracted by the methanol extractions (fraction B1, Figure 2). Acid hydrolysis of this solid fraction yielded an aqueous fraction (B11) and remaining solids (B12). Fraction B11 was subjected to ethyl acetate extractions. As indicated in Table XIII, the sum of these extractions [B11 (2.79% + 0.54% + 2.68%) + B12 (36.6%)] should equal the original solids value B1, and in fact it does. This is true for silage and forage also. However there is not a corresponding mass balance for the extractions from fodder (or forage or silage) treated at the 4.0 lbs ai/A level. In this case B11 + B12 does not equal B1.

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

metabolite was confirmed by two different TLC systems and HPLC. No supporting data have been submitted. For this and in all other relevant cases, HPLC chromatograms should be referred to the corresponding TLC and peaks labeled. HPLC chromatograms of standards should be at least referenced to allow verification. At this time, we are unable to confirm that 16.5% of TRR has been adequately identified, nor do we know what residue should be regulated for permanent tolerances. For purposes of this temporary tolerance petition, we can conclude that the nature of the residue in forage is adequately understood. The residue to be regulated is SAN-582H. See our discussion at the conclusion to this section (page 13).

and Oxalamide metab.

Assuming that the parent compound is metabolized into many compounds, the question arises as to the degree of characterization required. Our most recent discussion ("Overview of Residue Chemistry Guidelines," 10/10/89) assigns "trigger values" based on the total residue found, but also states that "efforts to characterize residues [to the 0.01 ppm level] should be made". If individual metabolites cannot be completely identified, it may still be possible to assign them to a given chemical class of compound. Stronger hydrolysis conditions may be necessary to achieve this limited characterization. [The registrant has tried unsuccessfully to generate a common moiety using acid hydrolysis, base hydrolysis and reaction with Raney Nickel (Appendix IV to the report).] Clearly, greater efforts should be made to identify individual metabolites present at higher concentrations. In this regard, a partition scheme such as that given in Figure 2 could artificially lower the concentrations of unidentified metabolites, for a given metabolite may be partially soluble in more than one extracting solvent. In characterization, the registrant should demonstrate that metabolites found at low concentrations in a given extract are indeed unique and that their concentrations should not be added to their corresponding concentrations found in another extract.

Forage

Because of the much lower PHI -- 50 days -- more radiocarbon was organosoluble. Unextractable radiocarbon (B1) amounted to 18.2% of TRR (at the 4.0 lbs ai/A application rate), methanol solubles from the freeze dried aqueous fraction (A5) comprised 33.1% of TRR and the two methylene chloride extracts (A3, A4) accounted for about 26.4% of TRR. TLC from fraction A5 showed that a large proportion of radiocarbon from this fraction had Rf values corresponding to the cysteine conjugate, GSH-conjugate and sulfonate of SAN-582H (page 47). Identification was tentatively confirmed by HPLC, but although HPLC chromatograms from forage samples have been submitted, we do not know which ones, if any, are relevant to identification and quantitation of the three metabolites, and a copy of the relevant TLC is not present.

These data should be submitted. Elsewhere (page 41, Figure 10 and Table XXXI), it is reported, based on TLC of A5, that 8.32% of TRR is due to the glutathione conjugate and 2.93% to the cysteine conjugate. However, a much more intense band (Rf 0.105-0.261) has not been identified, and all bands are so diffuse that they are probably composites of several metabolites.

TLC of neutral and acidic methylene chloride extracts (A3 and A4) indicated the presence of numerous metabolites at low concentrations. The highest concentration observed at the 4.0 lbs ai/A treatment level was 0.024 ppm -- for a band at the retention time of oxalamide. Also apparently identified at lower concentrations were the cysteine conjugate, the sulfoxide of thiolactic acid conjugate, the malonyl conjugate and the thiolactic acid conjugate. Identity of the oxalamide was confirmed with HPLC. Also mentioned is a "soil metabolite fraction '4'", whose presence was also confirmed by HPLC. HPLC may have confirmed the identity of the other species mentioned above, but this is not clear. Identification based solely on TLC with one solvent system is insufficient.

As reported in Summary Table 2, 18.5% of TRR in forage was identified. The metabolite present in greatest concentration was the GSH-conjugate, present at 8.3% of TRR. The oxalamide metabolite constituted 3.5% of TRR. Our comments concerning characterization of the residue in fodder apply also to forage.

Silage (PHI 116 days)

According to Table XXVI (page 87), which reports data from TLC, and Summary Table 2, the principal metabolite found was the GSH-conjugate, present at 9.8% of TRR. However the text (page 39) states that "HPLC analysis of these samples did not confirm the presence of this conjugate." As in the case of fodder and forage, SAN-582H appears to be metabolized into many minor constituents, few of which were identified.

Grain

Two grain samples were collected -- the first associated with silage (PHI 116 days), the second at normal harvest (PHI 130 days). Most of the radiocarbon (46-58%) could not be extracted. Radioactivity in the organic extracts from grain treated at the higher level was ≤0.01 µg/g. No individual metabolite was identified.

In this case because of the low total radiocarbon levels, further characterization is not required for permanent tolerances.

Short Term Exposure Studies with Corn Seedlings

Two experiments were performed. In the first, corn seedlings were grown in vermiculite for 2 weeks, then injected with ^{14}C-SAN-582H. Treated seedlings were held in a nutrient solution. One hour after harvest the major component was parent, at 87.7% of TRR.

Seedlings were grown for 21 days, after which time the roots were excised and the cuttings placed in a 5 ppm ^{14}C-SAN-582 solution containing calcium sulfate. The cuttings were kept in solution for 45 hours. TLC and HPLC of methanol soluble radiocarbon (60.6% of TRR) indicated that the cysteine conjugate and sulfonate were the two principal metabolites -- at 21.2% and 19.4%, respectively.

Conclusion

For purposes of this temporary tolerance petition, the nature of the residue in corn is adequately understood. The residue to be regulated is parent SAN-582H. The total radioactive residue in corn grain, the only possible human food item, was low. The levels found in ruminants and poultry after feeding at greatly exaggerated rates (see next section) suggest that levels in meat and milk will not be measurable from realistic dietary exposure. At this time we are unable to determine whether the oxalamide metabolite is a major component of the residue. Therefore, it is not advisable to include the oxalamide in the temporary tolerance expression.

Off. Conclusion

For permanent tolerances, additional characterization is required for forage and fodder, as outlined above.

Animals

Studies have been submitted for ruminants and poultry. Metabolism in ruminants is discussed in the following report:

"Metabolism of SAN 582H in a Lactating Goat," C.C. Yu and A.S. Guirguis, 2/7/90, Lab. Project No. 414105-6 (MRID # 41596[illegible]55).

^{14}C-SAN 582H, labeled in the 3-thienyl position, was fed in capsule form to one lactating goat for four consecutive days at a level of 8.92 mg/kg/day. The specific activity of the dose was 0.307 mCi/mmole. (Calculations appear in Appendix IV, pg. 106.) Based on the weight of the goat (46 kg) and an estimated food consumption of 1.6 kg/day per 40 kg goat, the concentration in the diet is calculated to be 223 ppm. A second goat served as a control. Milk samples were collected twice daily. Weighed samples of feces and measured volumes of urine specimens were

collected at 7, 24, 48 and 72 hrs. after initial dose. The animals were killed on Day 4, 7 hrs. after the last dose. The liver, kidneys and samples of thigh muscle, shoulder muscle and omental fat were collected, weighed and frozen immediately. Analysis of the samples occurred 8-15 months after samples were collected.

A total of about 36% of the radiocarbon was recovered in goat excreta after 72 hrs. Several samples were not collected, however, and the registrant estimates that the total should have been about 66%. Considerable amounts of radiocarbon would have remained in the GI tract. Total radiocarbon appeared to plateau in milk by 31 hrs. The highest level found in milk was 0.98 ppm at 55 hrs. Levels in tissue at 79 hrs. are given in Table 2.

Table 2

Total Radioactivity Levels in Goat Tissue
PPM SAN 582H Equivalents

Kidney	9.92
Fat	0.97
Muscle	0.97
Liver	16.62

Extraction schema for urine, feces, liver and kidney, milk, muscle, and fat are given in Figures 1-6 of the report.

Urine and Feces

SAN-582H was not detected in urine or feces. The following metabolites were characterized (Tables 3a and 3b). Identification was usually based on cochromatography in two or three solvent systems with further confirmation by HPLC. Structures of metabolites are given in Figure 4, attached.

Table 3a

Metabolites Found in Goat Urine

Metabolite	Percent of TRR
M3	1.8
M7	1.5
M17	3.9
Cysteine Conjugate	24.6
Glutathione Conjugate	6.7

Table 3b

Metabolites Found in Goat Feces

Metabolite	Percent of TRR
M3	<5.
M7	15.4
M17	5.1
Cysteine Conjugate	5.
Glutathione Conjugate	2.

Numerous other metabolites, none of which exceeded 7% of TRR in urine or 5% of TRR in feces, were observed but not identified.

Kidney

The extraction schema for liver and kidney is given in Figure 3 of the report. After an initial acetone extraction, additional metabolites were released by base and by acid. Extraction characteristics are given in Table VII of the report. Identified metabolites are given in Table 4:

Table 4

Identified metabolites in Kidney

Metabolite	Percent of TRR
M7	24.07
M17	8.96
Cysteine Conjugate	9.18

Glutathione Conjugate	5.2
Total =	47.41

M7, formed from parent by O-demethylation, was identified by TLC and HPLC, M17, the mercapturate conjugate of SAN-582H, and the GSH-conjugate were identified by TLC with two solvent systems. The cysteine conjugate was identified by TLC with one solvent system only. Confirmation is necessary for this metabolite to support a permanent tolerance petition. **(Additional requirements outlined in this section apply only to the permanent tolerance petition.)** Nine additional metabolites were separated by TLC. Concentrations ranged from 1.3 to 4.2% of TRR. A total of 81.6% of TRR could be ascribed to individual metabolites. An additional 11.2% remained in the aqueous phase and 14.1% remained unextractable. Total ^{14}C recovery exceeded 100%.

Copies of representative TLC chromatograms should be submitted. Given the magnitude of total residue in kidney, an unknown metabolite present at 1% of TRR would be present at a concentration of about 0.1 ppm. Therefore, further attempts must be made to identify the metabolites -- at least to the point of assigning them to a given chemical class.

Liver

Extraction characteristics are given in Table VIII of the report. Identified metabolites are given in the following table.

Table 5

Identified Metabolites in Liver

Metabolite	Percent of TRR
M22	12.01
M17	2.68
Cysteine Conjugate	7.21
Glutathione Conjugate	2.20
Total =	24.1

Of these four metabolites M17 was identified by TLC in one solvent system only. Confirmation of the identity of this metabolite is necessary.

Eleven unidentified metabolites were isolated. Five of these each constituted more than 5% of TRR: using the

nomenclature given in Table VIII, these are L1 (5.43%), L3 (7.08%), L4 (8.42%), L12 (12.99%) and L13 (7.84%). Of these, L3 and L4 were characterized using two TLC solvent systems. We do not understand the registrant's statement (page 16) that "each [unknown metabolite] accounted for less than 6% of liver radiocarbon (Table VIII)". Additional efforts should be made to identify these metabolites, particularly L4 and L12 which together comprise over 21% of TRR -- 3.5 ppm. In all, individual metabolites, identified and unidentified, constituted 80.6% of TRR. An additional 19.1% remained in the aqueous fraction and 3% could not be extracted. Further attempts should be made to characterize the residue remaining in the aqueous phase. If enzymes fail to release bound metabolites, more rigorous hydrolysis conditions might be employed.

Copies of relevant TLC chromatograms should be submitted.

The nature of the residue in liver is not adequately understood to support a permanent tolerance petition.

Muscle

The extraction schema for muscle is given in Figure 5 of the report (page 39). Over 85% of TRR could be extracted by acetone and methanol. Identified metabolites are given in the following table:

Table 6

Identified Metabolites in Muscle

Metabolite	Percent of TRR
M17	11.42
Cysteine Conjugate	14.21
Glutathione Conjugate	8.27
	Total = 33.9

The three metabolites were each characterized in two different TLC solvent systems.

Seven unidentified constituents were found in the methanol extract, including (using the registrant's nomenclature) M1 (8.52% of TRR), M2 (9.91%) and M10 (7.19%). Attempts should be made to characterize these three species -- at least to the point of identification of functional groups. No characterization of the acetone extract (13.5% of TRR) was reported. Some characterization is necessary -- at least to the point of demonstrating that this extract consists of numerous discrete

metabolites present at low concentrations.

Fat

The extraction schema for fat is given in Figure 6 (page 40) of the petition, and extraction characteristics are given in Table XI. Samples were extracted with hexane and then methanol. The residue remaining was then refluxed in 1 N NaOH. Three metabolites were identified: M7 (24.25% of TRR), M17 (5.43%) and GSH-Conj. (2.10%). M17 was characterized using only one TLC solvent system. Confirmation of the identity of this metabolite is necessary. Six unidentified metabolites, ranging in concentration from 2.96-6.81%, were found in the TLC from the methanol extract. Five of these were characterized in two solvent systems. At this time no further characterization of these metabolites is required. Unextractables and the final aqueous fraction comprised 15.5% and 9.25% of TRR, respectively. Attempts should be made to further release metabolites from these fractions, possibly using acid and/or enzymatic hydrolysis.

Milk

The extraction schema and extraction characteristics for milk are given in Figure 4 and Table IX, respectively, of the report. Identified metabolites include M17 (5.21% of TRR), cysteine conjugate (11.2%) and GSH-conjugate (7.90%). Seven additional metabolites were observed, ranging from 0.78% to 8.90% ("MK2"). The total percentage of isolated metabolites is 52.8%. Characterization of M17 in an additional TLC solvent system (or by a different analytical method) is required. We note that TLC retention times of metabolite "MK2" in two solvent systems are identical to those of metabolite "L4" in liver and very close to "M2" in muscle. The registrant should further characterize this metabolite. Additional radioactivity was found in a "protein and fat precipitate" (Fig. 4, 11.88%) and in unextractables (10.49%). Attempts should be made to release metabolites in these two fractions.

Summary

SAN-5[redacted] is apparently metabolized through conjugation with glutathione with subsequent formation of the cysteine conjugate, the mercapturate conjugate (M17), and dimerization of a mercaptan intermediate (M22). Metabolites M3 and M7 are formed from parent by dechlorination and O-demethylation, respectively.

As noted above, characterization is incomplete for purposes of permanent tolerances. If it is not possible to completely identify isolated metabolites, the registrant should attempt to provide some structural information. Identification solely through TLC requires at least two different solvent systems.

For this temporary tolerance petition, we conclude that the nature of the residue is adequately understood. The dose level used in the radiolabeled study is about 450 times the maximum expected in corn forage from considerations of the radiolabeled plant study. Corn forage can constitute 30% of the diet of beef cattle. Since the total residue found in forage at the 1x feeding level was 0.40 ppm, the predicted concentration in the diet is 0.12 ppm, or 0.48 ppm on a dry weight basis. The predicted total residue in liver would be less than 0.04 ppm and levels in other tissues and milk would be much lower.

The following poultry metabolism study was submitted:

"Metabolism of SAN-582H in Laying Hens," C.C. Yu and D.A. Nietschmann, 1/29/90, Laboratory ID No. 414105-7 (MRID # 415965-56).

^{14}C-SAN-582H, labeled in the 3-thienyl position, was fed in capsule form to three hens for four consecutive days at a dose rate of 10 mg/kg/day. The specific activity of ^{14}C-SAN 582H diluted with unlabeled compound was 0.62 mCi/mmole. Assuming that the daily feed consumption was 6% of the average body weight of 2.19 kg, 10 mg/kg is equivalent to 167 ppm in the diet (Appendix II, page 82). Three hens served as controls. Animals were killed 7 hours after the last dose.

Excreta from each hen was collected at 7, 24, 48, 72 hr. and at sacrifice. A total of 8 eggs were produced during the study. Fat, liver and muscle were collected after the birds were killed. Samples were immediately frozen after analysis. For permanent tolerances the registrant should submit the dates of sacrifice and dates of analysis.

Over 77% of the administered radiocarbon had been excreted at the time of sacrifice. Total recovery was 78.5%, which did not include radiocarbon in GI tracts at sacrifice.

Total residue levels in tissue and eggs at sacrifice are given in the following table. Residue levels did not reach a plateau in either egg whites or yolks.

Table 7

Residue Levels in Tissues and Eggs (79 hr.)

Liver	8.33 ppm
Breast Muscle	0.45
Leg Muscle	0.58
Fat	0.29

Egg White	0.30
Egg Yolk	0.62

Extraction procedures for excreta, liver, muscle, fat, egg yolk and egg white are given in Figures 1-6 of the report.

Excreta

Extraction characteristics are given in Table IV of the report. Methanol extracted 64.3% of TRR. Unchanged parent constituted 2.03% of TRR. Metabolite M3 (Fig. 4), dechlorinated SAN-582H, was found at the highest concentration, 10.03% of TRR. Fifteen metabolites were identified out of a total of 25. Identification was achieved using TLC in two solvent systems. In some cases MS and/or NMR provided further confirmation.

Liver

Duplicate liver samples were blended in water than centrifuged and extracts combined and evaporated. The dry residue was extracted with acetone, then methanol. Remaining solids were refluxed in 1N HCl for 1 hr., then extracted with ethyl acetate to recover acid-released metabolites. Remaining solids and the aqueous layer from the ethyl acetate extraction were refluxed at pH 12 for 1 hr. and extracted with ethyl acetate. A total of 72.5% of TRR was organosoluble after this treatment, an additional 24.8% remained in the aqueous phase, and 6.81% remained unextractable. No attempt was made to further characterize the non organosoluble residue. Extraction characteristics are given in Table V of the report. Metabolite M8 (Figure 4) was found at 7.8% of TRR, and Metabolite M3 was found at 5.14% of TRR. No other metabolite was identified, including three which were each present at about 8% of TRR (>0.6 ppm). Further characterization is needed.

Muscle

The extraction schema is identical to that for liver. A total of 53.0% was organosoluble, 36.2% remained in the final aqueous fraction (after successive acid and base refluxes) and 2.64% remained as solids.

Extraction characteristics are given in Table VI. The organosoluble fraction was shown by TLC to contain twelve metabolites, none of which was identified. The highest metabolite concentration was 7.0%, or 0.04 ppm. No attempt was apparently made to characterize the non-organosoluble residue. See our discussion, below.

Fat

Fat was extracted with hexane and methanol. The methanol fraction contained 65.5% of TRR, the hexane fraction contained 10.1% and solids constituted 21.3%.

Somewhat surprisingly, SAN-582H was the principal metabolite found -- 26.2% of TRR, 0.075 ppm. Eleven other metabolites were present at 0.9-8.6% of TRR. Neither the hexane fraction nor solids were further characterized. See our discussion, below.

Eggs

Extraction schema for yolk and white are given in Figures 5 and 6, respectively. Both procedures included acid and base hydrolysis steps. 75% of TRR in egg whites was organosoluble or could be made organosoluble by hydrolysis. Fourteen metabolites were isolated by TLC. The highest concentration of these was 9.4% (0.03 ppm). None was identified. Water solubles constituted 22.3% of TRR (0.066 ppm). No attempt was made to characterize the water solubles.

Almost all the organosoluble fraction in egg yolks was acid or base released -- 52.2% of TRR (0.32 ppm). An additional 46.9% remained in the aqueous phase after various extractions. No attempt was made to characterize this fraction. Eight metabolites in the organosoluble fraction were isolated by TLC. None was identified. The TLC band denoted "EY2" constituted 10.05% of TRR (0.06 ppm), but there could be more than one metabolite associated with this band, whose retention time is reported as "0.47-0.70". Activity remaining at the origin of the TLC plate comprised 11.1%. Again, it is not known whether or not this activity is due to one metabolite.

Copies of TLC's have not been submitted for this or any other of the poultry tissue studies. Relevant TLC's should be submitted.

Comment

We realize that the dose level is highly exaggerated. Nevertheless, for permanent tolerances the residue in poultry must be better characterized. Attempts should be made to characterize the water soluble fractions, which comprise 25-47% of TRR. If metabolites cannot be isolated directly by HPLC, the registrant 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. Chemical treatment of the numerous metabolites could conceivably yield a common moiety.

For purposes of this temporary tolerance petition, the nature of the residue in poultry is adequately understood. The residue to be regulated is parent SAN-582H. Our discussion above for ruminants applies to poultry also. Only corn grain is a major poultry feed item, and levels in that rac are expected to be very low. Total residue levels in every poultry tissue are predicted to be < 0.01 ppm.

Analytical Method

The analytical method was submitted as Appendix IV to the field trial data report -- Lab Project No. 414108-5 (MRID # 415965-57): "Determination of SAN-582H and Its Oxalamide Metabolite in Corn Grain, Forage, Silage and Fodder," Analytical Method AM-0840-0790-0, K. Smith and T. Bade, 7/30/90.

Corn silage, fodder and forage samples are prepared by chopping and thorough mixing. Grain samples are milled to a fine particle size. Samples are then extracted with a 20:80:0.5 water/methanol/conc. HCl solution, and the extract partitioned against a 1:1 ethyl ether/methylene chloride solution. At this point both analytes are in the organic phase. The oxalamide is derivatized with diazomethane, and the residues are cleaned up using a C18 solid phase extraction column. The residues of SAN-582H and the methylated oxalamide are quantitated by gas chromatography using a mass selective detector. The reported limit of detection is 0.01 ppm for each of the two analytes.

Recoveries of SAN-582H and its oxalamide metabolite (as the methyl ester) are given in Table 8 (page 405 of the report).

Table 8

Recoveries of SAN 582H and Its Oxalamide Metabolite

RAC	Fortification Levels (ppm)	Percent Recovery (s.d., n) SAN-582	Oxalamide
Forage	0.2-0.5	140 (26.6, 7)	137 (26.4, 7)
Silage	0.1-0.5	138 (15.2, 4)	132 (15.9, 4)
Grain	0.1-0.5	166 (22.9, 4)	149 (29.5, 4)
Fodder	0.1-0.2	156 (34.8, 6)	130 (22.3, 6)

Comment

Recoveries and standard deviations are unacceptably high.

Recoveries were also obtained in conjunction with the residue trials. Average recoveries ranged from 124% to 148% -- again, unacceptably high. Recoveries were also obtained at the 0.01 ppm fortification levels for these samples. However, chromatograms were only submitted from samples fortified at 0.1 or 0.2 ppm.

Independent laboratory validation was not submitted as part of this temporary tolerance petition but is included in the recently submitted permanent tolerance petition, PP#0F3918:

> "Method Validation of SAN-582H and its Oxalamide Metabolite in Corn Grain and Forage," W. Brunk, 9/26/90, Biospherics Incorporated, Laboratory Project ID B 9005-CN1 (MRID # 416624-21).

Forage and grain were fortified at 0.01 and 0.05 ppm. Two validation sequences were attempted. In the first, recoveries from forage were 135±29% (n=6) for SAN-582H and 78±21% (n=6) for oxalamide; corresponding recoveries in grain were 104±14% (n=6) and 62±21% (n=6). In the second attempt, recoveries in forage were 195±22% (n=6) for SAN 582H and 131±44% (n=6) for oxalamide; corresponding recoveries from grain were 248±48% (n=6) and 100±18% (n=6). These latter data were "rejected" because of the high recoveries.

Submitted GC-MSD chromatograms suggest why recoveries were so high, for the peaks due to SAN-582H are very broad and barely discernible above background.

For this temporary tolerance petition, the registrant must revise the analytical method for SAN-582H and the oxalamide metabolite so that acceptable recoveries can be obtained at all fortification levels. Supporting chromatograms must be submitted. The revised method must undergo independent laboratory validation.

For the permanent tolerance petition, methods yielding acceptable recoveries must be developed for all components of the residue to be regulated -- as noted above -- and these methods must be confirmed by an independent laboratory.

Once the nature of the residue in plants is adequately understood, recoveries of the residue to be regulated must be obtained under FDA's multiresidue protocols. The recent (1/8/91) new chemical screen for the permanent tolerance petition, PP#0F3918, noted the absence of multiresidue method testing. Analytical reference standards have not been received at the Repository. The screen was therefore failed.

At this time an analytical method for residues of SAN-582H in animal tissues is not required.

Magnitude of the Residue

Residue data are given in the following report: "Analysis of Corn Samples for SAN-582H and Its Oxalamide Metabolite," N.C. Jimenez, 7/25/90, Lab. Project No. 414108-5 (MRID # 415965-57).

Storage Stability. Samples were frozen 2-5 hours after collection and remained frozen until analysis. Samples were taken from 6/1/89 to 10/30/89. Residue analysis occurred up to 13 months after harvest. Storage stability data for soil reportedly show stability of SAN-582H and its oxalamide metabolite for at least 12 months. "The stability in corn matrices would be expected to be similar and is in the process of being demonstrated." No data appear in the present petition, but limited (three month) storage stability data have been submitted in the permanent tolerance petition, PP#0F3918: Sandoz Project No. 414108-9 (MRID # 416624-20).

There appears to be a significant decline in SAN-582H levels over three months in grain and fodder. However, the analytical data are quite variable. Percent recoveries from freshly spiked samples varied from 36-153%. Therefore no real conclusion is possible at this time. A similar situation occurs with oxalamide.

For this temporary tolerance petition, acceptable storage stability data are not necessary. Our conclusions concerning potential residues were based solely on the radiolabeled study. However, these data are clearly inadequate for the permanent tolerance petition. Data must be generated to cover the period from sampling to analysis for the residue field trials. Moreover, as noted above, there is a major problem with the analytical method, and it is far from certain that any quantitative conclusions will be possible even if the study is continued for the projected time period of three years. Finally, we note that once the nature of the residue in corn is understood, all components of the residue to be regulated must be tested for storage stability.

Residue Data. Residue trials were conducted in 1989 in six states: [redacted], NE, OR, IA, TX and NY. Two types of SAN-582H formulations were applied separately at each test site: the 6.0 EC (emulsifiable concentrate) and 7.5 L (liquid). Three application types -- preplant incorporated, preemergence and postemergence -- were used for each formulation. Postemergent application was a broadcast application over the top of the canopy when corn was at the 5-6 leaf stage. A rate of 1.5 lbs ai/A was used for all applications. This is the proposed maximum use level. Neither SAN-582H nor the oxalamide metabolite was detected in any sample analyzed.

Additional analyses will not be required for purposes of

this temporary tolerance petition. The metabolism study on corn strongly suggests that SAN-582H will not be a significant component of the residue. However, the submitted residue analyses will not support permanent tolerances because the 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. In any event additional field trials/residue analyses may be required depending on the nature of the residue in/on corn.

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

Meat, Milk, Poultry and 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 corn have been determined.

Attachments:

Attachment 1: Figure 3. Structures of Plant Metabolites.
Attachment 2: Figure 4. Identified Animal Metabolites.

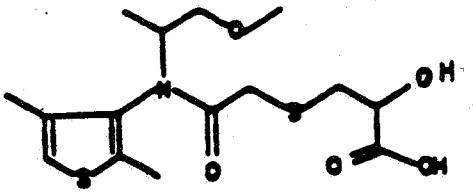
cc: SF (SAN 582H), RF, Circu., Reviewer (MikeFlood), C.Furlow(PIB/FOD), E.Haeberer, PP#0G3892, PP#0F3918.

RDI:SectionHead:E.T.Haeberer:1/17/91:BranchSeniorScientist: R.A.Loranger:1/17/91.

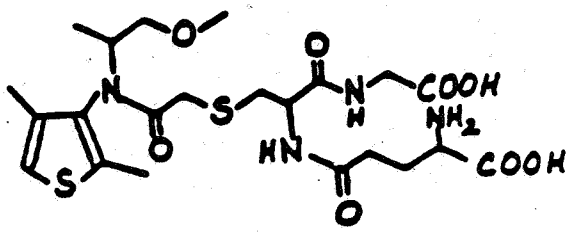
H7509C:CB[redacted]Reviewer(MTF):CM#2:Rm810:557-4362:typist(mtf): 1/18/91.

FIGURE 3 Structures model plant metabolites of SAN-582H

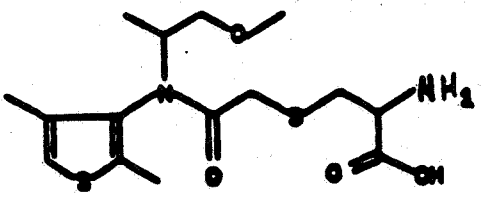
1. Thiolactic acid conjugate of SAN-582H



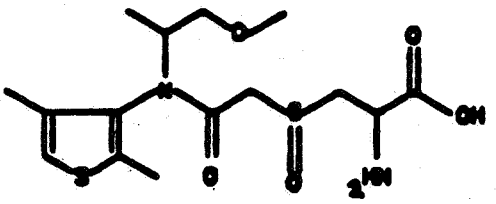
2. Glutathione conjugate of SAN-582H



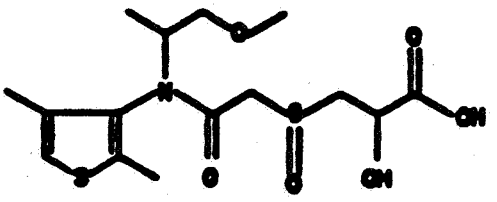
3. Cysteine conjugate of SAN-582H



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



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



6. N-malonyl conjugate of the cysteine conjugate of SAN-582H

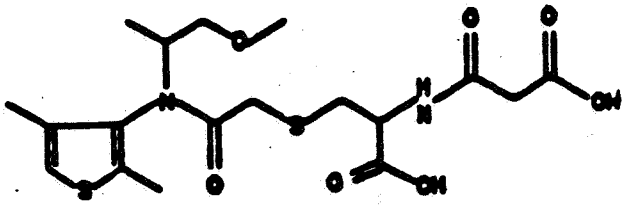


Figure 4. **Metabolites Identified in Ruminants and Poultry**