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

OCT 30 1992

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

**MEMORANDUM**

SUBJECT: SAN-582H Herbicide (Dimethanamid). Metabolism in Corn. Issues to Be Presented to the HED Metabolism Committee on 11/3/92.

FROM: Michael T. Flood, Ph.D., Chemist
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THROUGH: Elizabeth T. Haeberer, Section Chief
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Debra Edwards, for
10/30/92

TO: Metabolism Committee
Health Effects Division (H7509C)

In PP#0F3918 Sandoz Agro Inc. is proposing tolerances of 0.01 ppm for residues of SAN-582H, 2-chloro-N-[(1-methyl-2-methoxy)ethyl]-N-(2,4-dimethylthien-3-yl)-acetamide, in/on corn grain, forage and fodder. Temporary tolerances at 0.01 ppm have been established for residues of SAN-582H in/on these corn commodities as a result of PP#0G3892. Temporary tolerances of 0.01 ppm have also been established for SAN-582H in/on soybean grain, forage and hay as a result of PP#1G3980. There are no temporary tolerances for animal commodities.

In support of the temporary tolerances Sandoz submitted metabolism studies on corn and soybeans. Although these studies were acceptable for purposes of temporary tolerances, our reviews cited numerous deficiencies to be resolved before permanent tolerances could be established (PP#0G3892, M. Flood, memo of 1/24/91; PP#1G3980, memo of J. Abbotts, 7/19/91). Sandoz has responded in detail to our 1/24/91 review. In turn, their submission was reviewed in our 7/29/92 memo; and although certain deficiencies remain to be resolved, CBTS feels that our current knowledge of the metabolism of the herbicide in corn is sufficient to permit conclusions from the Metabolism Committee.

Corn Metabolism

SAN-582H labeled in the 3-thienyl position was applied to

soil one day after corn was planted. Samples were taken from 50 days after application (forage) to 130 days after application (grain, fodder). The herbicide is extensively metabolized, and parent was not found (≤0.01 ppm) in any of the samples. The residue was characterized by two totally independent methods. The first was a conventional fractionation scheme involving extractions with methanol solutions, methylene chloride, acid and base. A maximum of 19% of the total radioactive residue (TRR) could be identified (Table 1a). The second method -- done on only forage treated at 4.0 lbs ai/A (2.7X) -- was a single step extraction procedure employing counter-current chromatography (CCC). Using this procedure 32% to 42% of the TRR could be identified. We commented in our 7/29/92 memo that

> On one hand the fewer separation procedures of 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.

Results are given in the following tables. Identified metabolites and their concentrations are given in Table 1b. The following should be noted:

1. Identification of metabolites was made by TLC by comparison with standards. Identity of the major metabolites was confirmed by alternative techniques, such as HPLC, NMR and/or mass spectrometry.

2. TLC retention times of 38 model metabolites were compared with retention times observed in TLC's from extracts of treated corn racs.

3. Quantitation was made from TLC autoradiograms. However, the presence of coextractives apparently resulted in broadening of the TLC bands with the introduction of some uncertainty in the quantitative results. The CCC level reported for the sulfonate conjugate (Table 1b) was in actuality partially derived from the radioactivity of a multicomponent band of which the sulfonate was the chief component.

4. All the identified metabolites in corn result from displacment of Cl by sulfur compounds such as glutathione with the exception of soil metabolites oxalamide and M11. All identified metabolites in corn (and in animals) have the thienyl ring of parent.

Table 1a

Characteristics of SAN-582H Residues In Corn Plants
Treated at 1.5 lb ai/A (1X)

| | | | | Unidentified (%TRR) | | | |
|---|---|---|---|---|---|---|---|
| Crop Part | PHI(days) | $^{14}C$ Total (ppm) | Total Identified (%TRR) | Organosoluble (A2, A3, A4, B11) | Methanol (A5) | Water (A6) | Unextractable |
| Forage | 50 | 0.307 | 18.9 | 16.2 | 26.7 | 12.7 | 8.83 |
| Silage | 116 | 0.403 | 16.3 | 10.6 | 28.1 | 12.1 | 18.7 |
| Grain | 116 | 0.021 | ---- | ---- | ---- | ---- | 46.7 |
| Fodder | 130 | 0.504 | 12.2 | 8.08 | 8.20 | 22.8 | 37.1 |
| Grain | 130 | 0.022 | ---- | ---- | ---- | ---- | ---- |

* Letters (A2, A3, etc.) refer to extraction fractions. See Figure/attachment, extraction scheme.

Table 1b

Residue Identified from Corn Plants Treated at 1.5 lb ai/A with SAN-582H
Residue Identified from Corn Forage Treated at 4.0 lb ai/A Using CCC Method -- Normalized to 1.5 lb ai/A

%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) |
| Forage CCC | 50 | 6.8 (0.019) | 10.2 (0.028) | 5.7 (0.016) | 6.8 (0.019) | 4.7 (0.013) | <15.8 (<0.044) |
| 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 | ---- | ---- | ---- | ---- | ---- | ---- |
| 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 | ---- | ---- | ---- | ---- | ---- | ---- |

* Oxalamide and thiolactic conjugate coeluted in TLC from CCC procedure.

## Residue Data

Sandoz was asked to submit residue data on the two plant metabolites found at highest concentration: the sulfonate conjugate of SAN-582H and the sulfoxide of thiolactic acid conjugate of SAN-582H. However, the company has been unable to develop a method for the latter metabolite having a sensitivity below 1 ppm. Residue data were submitted on October 15 for the sulfonate conjugate and have not been formally reviewed. The method for sulfonate conjugate can only be successfully validated at levels ≥ 0.2 ppm in corn forage. Examination of submitted

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chromatograms allows us to affirm that the metabolite is not present at a level greater than 0.05 ppm in corn forage. Although the claimed sensitivity of the analytical method is 0.01 ppm, a peak having the retention time of the sulfonate conjugate was observed in both treated samples and controls. Sandoz has presented preliminary evidence that this peak is from an interference, not the sulfonate conjugate. Residue data already submitted demonstrate the absence (≤0.01 ppm) of SAN-582H and the oxalamide metabolite -- apparently a soil metabolite -- in treated plants.

Common Moiety Analytical Method

Sandoz has heretofore been unable to develop a common moiety analytical method, i.e., a method which can quantitate a class of metabolites. Various acid and base hydrolysis procedures were attempted but failed to yield any common moiety. Reaction with Raney Nickel in the hope of removing sulfur-containing segments of various conjugates was unsuccessful. Oxidation and other procedures were also tried unsuccessfully. These attempts have been summarized in our 7/28/92 memo. We conclude that development of a common moiety method is unlikely.

Metabolism in Ruminants and Poultry

Ruminants.

$^{14}$C-SAN 582H was fed to one lactating goat for four consecutive days at an estimated dietary concentration of 223 ppm. If it is assumed that the total radioactive residue found in corn forage -- 0.31 ppm in SAN 582 equivalents -- were SAN-582H, per se, then the dietary exaggeration factor in the goat metabolism study was 720. (We assume that 25% of the diet of dairy cattle is corn forage, which consists of about 75% water. On a dry weight basis the concentration in the diet would be 0.31 ppm.)

The metabolic profile for ruminants is given in Table 2

Table 2

Metabolism Profile for Ruminants

| | | | Identified Metabolites - Percent of TRR | | | | |
|---|---|---|---|---|---|---|---|
| | Total Radioactive Residue (ppm SAN equivalents) | Radioactive Residue Corrected for Dose Exaggeration | M7 | M17 | M22 | Cysteine Conjugate | Glutathione Conjugate |
| Kidney | 9.9 | 0.014 | 24.1 | 9.0 | | 1.0 | 5.0 |
| Liver | 16.6 | 0.023 | | 2.7 | 6.1 | 7.2 | 2.2 |
| Muscle | 0.97 | 0.001 | | 11.42 | | 14.2 | 8.3 |
| Fat | 0.97 | 0.001 | 24.2 | 5.4 | | | 2.1 |
| Milk | 0.98 | 0.001 | | 5.2 | | 11.2 | 7.9 |

Based on these results, it is apparent that a metabolite present at 0.05 ppm in corn forage would not produce residues of any one metabolite in meat or milk in excess of 0.001 ppm.

## Poultry

$^{14}$C-SAN 582H was fed to three hens for four consecutive days at a level equivalent to 167 ppm in the diet. Based on a diet of corn grain comprising 70% of the diet of laying hens, the exaggeration factor in the metabolism study was over 10,000. Correcting for such exaggeration, residue levels in poultry from the proposed use are expected to be nondetectable, even if the entire residue in corn grain were to consist of SAN-582H. The distribution of metabolites as percent of TRR is given in Table 3.

Table 3

Metabolism Profile for Laying Hens

| | | | Identified Compounds - Percent of TRR | | | |
|---|---|---|---|---|---|---|
| | Total Radioactive Residue (ppm SAN equivalents) | Radioactive Residue Corrected for Dose Exaggeration | SAN 582H | M3 | M8 | PL2088 |
| Liver | 8.33 | 0.0008 | | 5.1 | 7.8 | |
| Muscle | 0.58 | 0.00006 | | | | |
| Fat | 0.29 | 0.00003 | 34.9 | | | |
| Egg White | 0.30 | 0.00003 | | | | 7.8 |
| Egg Yolk | 0.62 | 0.00006 | | | | 10.1 |

**Questions to the Metabolism Committee:**

1. **Given that residues of parent SAN 582H are not detected in corn RACS at a detection level of 0.01 ppm, that the principal metabolite is not present at levels in excess of 0.05 ppm, and that development of a common moiety analytical method has not been successful, should the tolerance expression include only parent compound?**

2. **Given that neither residues of parent nor of any one metabolite are predicted to be present in meat, milk, poultry or eggs at levels greater than 0.001 ppm, is there concurrence that tolerances are not necessary for these commodities?**

Attachments: Figure: Corn Plant Extraction Scheme

Structures of Corn Metabolites.
Proposed Metabolic Pathway in Goat (Structures of Ruminant Metabolites).
Additional Poultry Metabolites

cc: PP#0F3918, D.McCall (Tox Branch II), RF, Circu., M.Flood, E. Haeberer.
H7509C:CBTS:Reviewer(MTF):CM#2:Rm800A:305-6362:typist(mtf):10/30/92.
RDI:BranchSeniorScientist:RALoranger:10/30/92.

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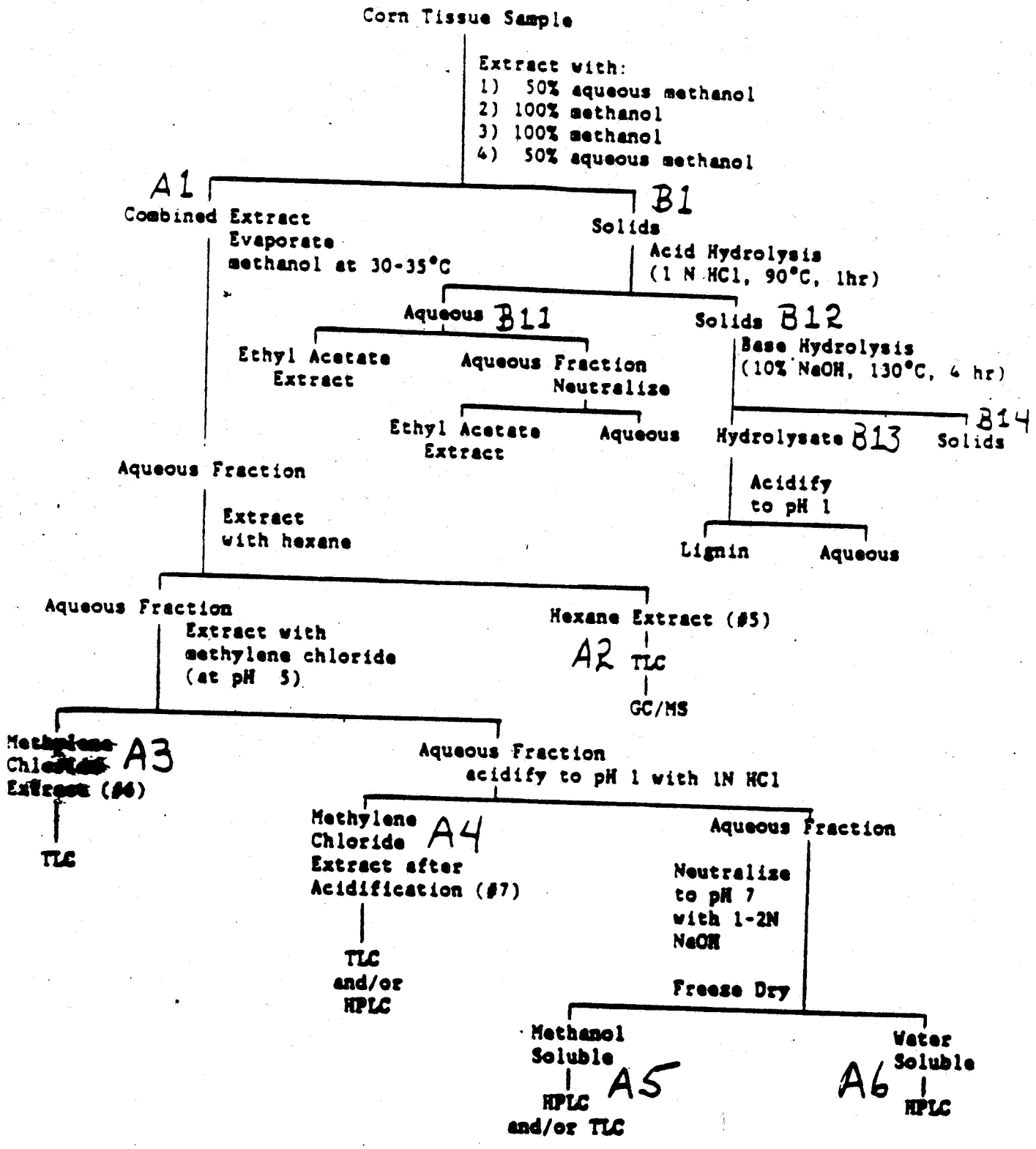
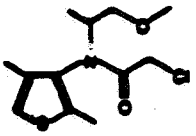
Figure 1. General extraction scheme for characterization of radiocarbon in corn samples from the SAN-582H corn metabolism study.



# DIMETHENAMID METABOLITES OF CORN



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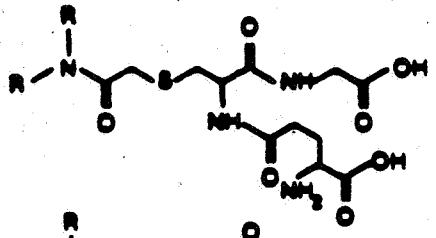
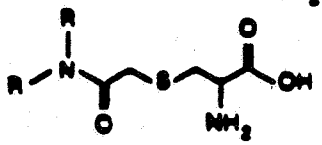
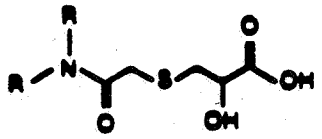


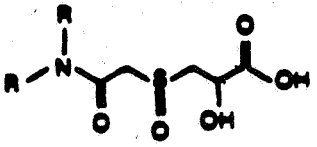
GLUTATHIONE



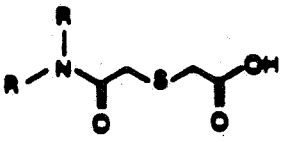
CYSTEINE



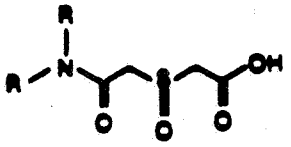
THIOLACTIC ACID



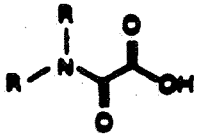
SULFOXIDE OF
THIOLACTIC ACID



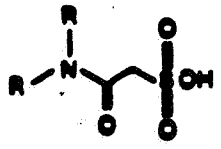
THIOGLYCOLIC ACID



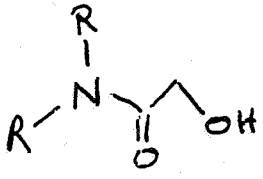
SULFOXIDE OF
THIOGLYCOLIC ACID



OXALAMIDE



SULFONATE



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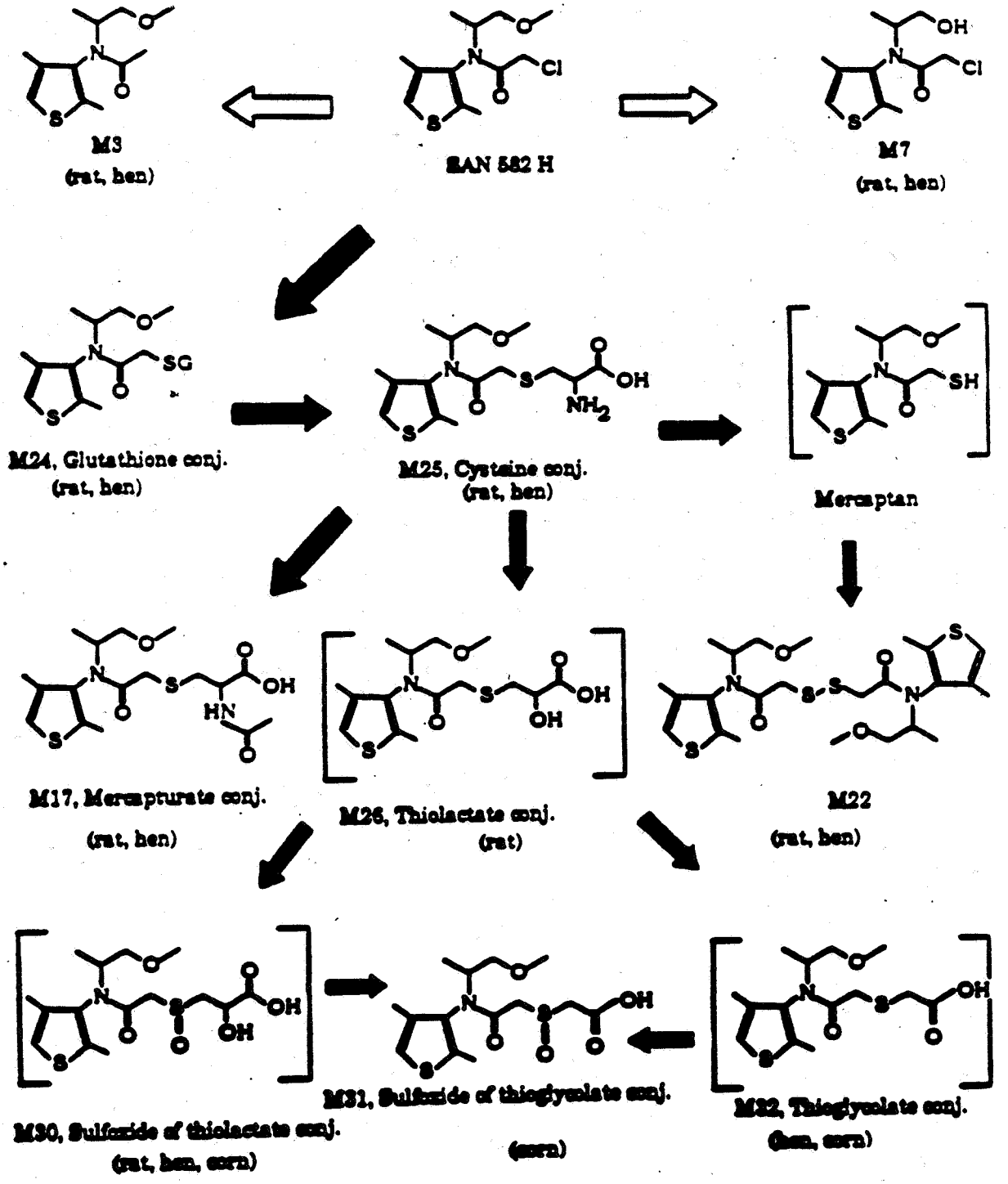
ATTACHMENT

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.

**Additional Metabolites Observed in Poultry**

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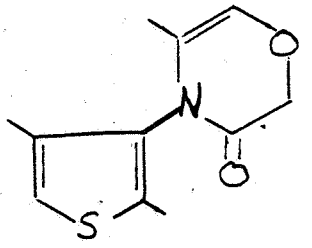


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