121001

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

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SUBJECT: PP#0G2396 BAS 9052 H on soybeans. Evaluation of analytical methods and residue data.

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BASF Wyandotte Corporation, Agricultural Chemicals Division proposes that temporary tolerances for residues of the herbicide, 2-[1-(ethoxyimino)-butyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexan-1-one (BAS 9052H) be established as follows:

0.25 ppm in or on soybeans

0.05 ppm in or on eggs, milk and the meat, fat and meat byproducts of cattle, goats, hogs, horses, poultry and sheep.

This petition constitutes the first request for tolerances for residues of this chemical. The temporary tolerances are intended to be used in conjunction with Experimental Use Permit No. 7969-EUP-EU. The proposed experimental program will entail the application of 2400 lbs act to 70,530 acres of soybeans.

Conclusions

1. The nonphytotoxic oil concentrate which is to be added to the spray solution must be cleared under Sec. 180.1001 and should be listed on the product label.

2a. The metabolism of BAS 9052 H in soybeans has not been adequately delineated.

2b. Since the metabolism studies involved administration of the parent compound only, the metabolism of BAS 9052 H in animals is not considered to be adequately delineated.

3. Since the analytical method has not been validated for the metabolites of BAS 9052 H, we do not consider it adequate for enforcement of the proposed temporary tolerances.

4a. Since only one residue study reflects the proposed treatment and since the analytical method used to obtain residue data has been validated for the parent compound BAS 9052 H only, submitted residue data are not adequate to support the proposed temporary tolerance.

4b. We cannot conclude whether a food additive tolerance is needed for soybean processed fractions, since 1) the processed soybeans did not contain detectable residues and were harvested at a PHI of 97 days instead of the 70 proposed and 2) residues in the processed fractions were determined by a method validated for the parent compound only.

4c. Since only the parent compound BAS 9052 H was administered in the feeding studies, the studies are not adequate to support the proposed temporary tolerances for meat, milk, poultry and eggs.

Recommendations

For reasons listed in Conclusions 1, 2a, 2b, 3, 4a, 4b and 4c, we recommend against the proposed temporary tolerances.

For a favorable recommendation we will require:

1. Clearance of the nonphytotoxic oil concentrate under Sect 180.1001 and its listing on the label.

2. Elucidation of the structure of the metabolites MU, MU-1 and MU-2.

3. Metabolism/feeding studies involving administration to livestock of plant substrates containing weathered residues of BAS 9052 H.

4. Validation of the analytical method for the principal metabolites of BAS 9052 H: MSO (sulfoxide of BAS 9052H), MSO_2 (sulfone of BAS 9052), $MISO_2$, M2SO, $M2SO_2$, MU-1 its sulfoxide MU-2, its oxazole MU and sulfoxide of MU.

5. Residue data for soybeans, soybean processed fractions, meat, milk, poultry and eggs reflecting the proposed treatment and obtained by analytical method validated for the above principal metabolites of BAS-9052 H.

Detailed Considerations

Manufacture and Formulation

[redacted]

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

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As currently produced technical BAS 9052 H is >94.9% pure. [redacted] Since the maximum proposed application rate is 0.5 lb BAS 9052 H per acre, we would not expect a residue problem from these impurities.

The product for which registration is being sought is Poast Herbicide, an emulsifiable concentrate containing 20% BAS 9052H (1.35 lb act/gal).

The inert ingredients of Poast Herbicide include [redacted] are cleared under Sec. 180.1001(c).

Proposed Use

INERT INGREDIENT INFORMATION IS NOT INCLUDED

For the control of annual grasses in soybeans Poast Herbicide will be applied at the rate of 0.1-0.5 lb act/A in a minimum of 5 gals of water/A by air or in a minimum of 20 gals of water/A by ground equipment. For special grass problems a repeat application may be made within 2-4 weeks.

A nonphytotoxic oil concentrate is to be added to the spray solution at 1 pt to 1 qt. per acre for ground applications and 0.5 pt - 1 pt per acre for aerial applications. This additive which must be cleared under Sec 180.1001 should be listed on the product label.

There is a 70 day PHI. Treated fields are not to be grazed and treated soybean forage or hay is not to be fed to livestock.

Nature of the residue

Soybean plants were treated in the field with ^{14}C labeled BAS 9052H at the rate of 0.92 lb act/A. At one site (Alpha, N.J.) plants were treated at the 1-2 trifoliate stage and at another site (Greenville, M.S.) at the 3-4 trifoliate stage.

Initial ^{14}C activity in the whole plant (less roots) at both sites was 59 ppm as BAS 9052 H equivalent and fell to 0.2 ppm at Alpha after 69 days and 0.9 ppm at Greenville after 56 days.

Homogenized samples were extracted with two portions of 50% aqueous methanol. Extractability was 94% of the ^{14}C activity for the 6 day sample and 88% for the 41 day sample. Following evaporation of methanol the solution was extracted with dichloromethane (DCM Extraction I). The extracts from the 6, 19 and 41 day samples contained, respectively, 53%, 19% and 8% of the ^{14}C activity. Analysis of the ^{14}C-activity in the 19 day dichloromethane extract by reversed phase HPLC indicated the presence of MSO_2, M2SO and MSO_2 (See attachment) No BAS 9052 H was detected.

The ^{14}Cactivity in the dichloromethane fraction was separated into non-acidic and acidic fractions by extraction with 0.1N NaOH. In the 6 day sample the acidic metabolites were equivalent to 41% of the total activity and were identified as MSO and MSO_2.

The aqueous phase from the initial extraction with dichloromethane (see above: DCM Extraction I) was extracted at pH 2 with ethyl acetate. The activity in the ethyl acetate fraction increased from 11% of total activity at 6 days to 15% at 41 days. This activity was not identified. However, most of the ^{14}C activity (7% at 41 days) remained in hydrophilic forms not extractable with organic solvents. Passing the aqueous phase thru an Amberlite XAD-4 column, elution with methanol and subsequent digestion with pectinase cellulose and β-glucosidase released 33% of the total residues into ethyl acetate extract. The released metabolite was identified as MSO. The presence of MSO as the principal component of the residue was confirmed by refluxing the 41-day plants with 0.IN NaOH. Approximately 40% of the ^{14}C activity was extracted by that treatment. TLC indicated that the extract contained only MSO with a minor amount of MSO_2.

Soybean seeds from treated plants were harvested at 89 days and 110 days after treatment. Total ^{14}C activity in the seeds was 0.048 ppm and 0.52 ppm, respectively. The difference may be explained by a higher moisture content of the earlier harvested seeds.

The ^{14}C activity was distributed as follows: lipids 2%, aqueous insolubles 6%, proteins 8%, aqueous filtrate >78%.. Extraction of the aqueous filtrate at pH 7 with dichloromethane removed 29% of total ^{14}C activity. Subsequent adjustment of the pH to 2 and further extraction with dichloromethane removed an additional 38%. The remaining aqueous phase contained 12% of ^{14}C activity. The ^{14}C activity in the dichloromethane extract obtained at pH 2 appeared to be due to a metabolite designated MU-1 (23% of seed activity) which appeared to be an oxime since it reacted under Beckman rearrangement conditions to form MU a neutral product. MU-2, apparently a sulfoxide of MU-1 was also present (8% of seed activity).

The following metabolites, identified by adsorption and reverse phase chromatography, were detected in the dichloromethane extract obtained at pH 7: MU, 12% of seed ^{14}C activity; $M2SO_2$ (Beckman rearrangment product of MSO_2) 9% of seed ^{14}C activity, MSO_2 (sulphone of BAS 9052 H) 4% of ^{14}C activity; related sulfoxides (of MS, MU and M2S) 4%.

Enzymatic digestion of the radioactive residues which remained in the final aqueous phase of seed analysis procedure (12% of seed activity) showed that the conjugates which were found in the 41 day plants were not present in the seed. Instead, the residues formed a ^{14}C-osazone and had a molecular weight range commensurate with the carbohydrates of the seed.

In summary, residues in the whole plant consist of: MSO (sulfoxide of BAS 9052 H), which is mostly present in conjugated form and small amounts of MSO_2 (sulfone of BAS 9052H) M2SO and M_2SO. Residues in soybean seeds consist of: an unknown metabolite, MU-1; it sulfoxide, MU 2; its oxazole MU; $M2SO_2$ and a mixture of sulfoxides of MS, MU and M2S.

We do not consider the nature of the residue in soybeans to be adequately delineated. In particular, the structure of the following metabolites of BAS 9052 H found in seeds): MU, MU-1 MU-2 will need to be elucidated. In addition, for a permanent tolerance the activity extracted from whole plants in the ETOAc extract will need to be identified.

In Animals

Four groups of rats; five male and five female rats in each group were administered ^{14}C labeled BAS 9052 H as follows: group A, 10 mg/kg (i.v.); group B, 10 mg/kg (oral); group C 10 mg/kg (oral); and group D 325 mg/kg (oral). The rats in group C were preconditioned on unlabeled BAS 9052 H for two weeks. Blood and excreta were collected for 2 days. Absorption, distribution and excretion of the radioactivity in rats were rapid. The major route of excretion was urine, with an average 78.5% of the administered activity being excreted via that route during the two days following administration. Approximately, 20.1% of the administered activity was excreted during the same period in feces. Less than 2% of the administered dose was associated with tissues. Highest levels were found in the liver reaching 0.6 ppm in groups A, B, and C and 13 ppm in group D. The major metabolites of BAS 9052 H in the rat excreta were identified as MSO, MISO, and M2SO. The metabolites in the tissues were not identified. After extraction of the livers with polar solvents approximately one half of the residues remained bound in groups A, B and C and about one third remained bound in group D.

Two lactating goats were fed an amount of ^{14}C-BAS 9052H (0.5 mg) equivalent to 1.25 ppm in thediet for eleven consecutive days.

Milk, urine, feces and blood samples were taken daily. Twenty four hours after the final dose the goats were sacrificed and tissue samples were removed for analysis.

All of the administered ^{14}C-activity was found in the urine and feces. None of the tissues showed levels of activity above the limit of quantitation of the instruments used (0.003 ppm). Chromatographic examination of the urine indicated that the major metabolites were MSO (38%), MSO (16%) and M2SO (15%).

The submitted metabolism studies are not considered adequate since only parent compound, BAS 9052 H was administered to animals, although essentially no residues of parent compound were present in treated soybean plants. For further consideration we will require metabolism studies involving administration to animals of plant substrates containing weathered residues of BAS 9052 H. In particular, we are interested in the metabolism of MU, MU-1, and MU-2.

Analytical Methodology

Residues of BAS 9052 H and its metabolites are extracted with MeOH/H_2O. The extract is cleaned up using an alkaline precipitation step. The coagulated mixture is filtered through Celite, the methanol is removed from the filtrate and the volume is adjusted with distilled water. Following addition of sodium hydroxide the aqueous solution is oxidized with H_2O_2. The petitioner claims that residues of BAS 9052 H and its metabolites are thereby converted to the common moiety 3-[2-(ethyl-sulfonyl)propyl] glutaric acid (see attachment for the reaction scheme).

The alkaline oxidation solution is acidified with concentrated hydrochloric acid, sodium chloride is added and the residues are partitioned into ethyl acetate. The ethyl acetate is dried over sodium sulfate and concentrated to near dryness. The substituted glutaric acid contained in the ethyl acetate is then converted to its dimethyl ester by refluxing for 30 minutes in a mixture of methanol; concentrated H_2SO_4 and Na_2SO_4. After the addition of water to the reaction mixture, the glutaric acid dimethyl ester is partitioned into dichloromethane which is cleaned up further by washing with saturated aqueous solution of sodium bicarbonate. The dichloromethane is dried over sodium sulfate and evaporated just to dryness. The residues are cleaned-up using various column chromatographic adsorbents. GLC equipped with flame photometric detector in the sulphur specific mode is used to determine the residues.

Validation data have been submitted for the parent compound only.

Recoveries from soybean seed and soybean seed processed fractions were 77 $\pm$ 14% at levels ranging from 0.05-5.0 ppm.

Recoveries from chicken tissues, (muscle and liver and kidney) and milk were 74 $\pm$ 10% at fortification levels ranging from 0.05-1.00 ppm.

Recoveries from beef tissues (muscle, liver and kidney) and milk were 74$\pm$ 10% at fortification levels ranging from 0.05 - 1.00 ppm.

Control values were all <0.05 ppm. A specificity study has been submitted. Of 179 compounds having a tolerance on either soybean seed, soybean seed process fractions, chicken tissues, beef tissues, milk or eggs 169 were tested. None interfered with the detection of BAS 9052H.

Soybeans treated with ^{14}C-BAS 9052 H at the rate of 1 kg/ha and containing 0.52 ppm of radioactive residues were analyzed by the above analytical method. A residue value of 0.10 ppm (19% of the total radioactive residue) was obtained. This compares with 17% of total radioactive residue in seeds identified in the plant metabolism studies as $M2SO_2$, MSO_2 and their corresponding sulfoxides. However, since no validation data have been submitted for these metabolites we cannot unequivocally conclude that they are determined by the above analytical method. In addition it is evident that the analytical method does not determine the heretofore unidentified metabolites of BAS 9052H such as MU, MU-1 and MU-2 which may constitute >41% of the residue in soybean seeds. These metabolites may also occur in meat and milk as a result of feeding of weathered residues of BAS 9052H to livestock.

Consequently, we do not consider the above analytical method adequate for enforcement of the proposed temporary tolerances. Adequate analytical methodology and validation data for MSO_2, $M2SO_2$ the corresponding sulfoxides and MU, MU-1 and MU-2 will be needed.

Residue Data

Preliminary storage stability data for BAS 9052 H have been submitted. There are no indications that significant losses of residue occurred from samples stored over a four month period at - 15 C. The petitioner indicates that further analyes of stored samples are scheduled.

Soybeans

Residue trials were conducted in 8 states (AK, IA, KS, LA, MI MS NC, TN). Up to two applications at rates ranging from 0.25 - 1 lb act/A were made; PHI's ranged from 62-133 days. However, only one study conducted in LA reflects the proposed two applications of 0.50 lb act/A and a 70 day PHI. Residues in that study were 0.05 ppm. Residues in other studies ranged up to 3.91 ppm at 62 days following two applications of 0.75 lb act/A. The petitioner claims that the 3.91 ppm value is aberrant and is due to contamination. The next highest residue value, 0.45 ppm was obtained at 82 days after two applications of 0.75 lb act/A.

The submitted residue data are not adequate to support the proposed temporary tolerance on soybeans. For further consideration additional residue data reflecting the maximum propoed use of 2 applications at 0.5 lb act/A and a 70 day PHI will be needed. Residue data must be obtained by analytical methods validated for the principal metabolites of BAS 9052 H which occur in soybean seeds (see nature of the residue).

Soybean processed fractions

Soybeans which received 1 application of 0.75 lb act/A or 2 applications of 0.50 lb act/A or 0.75 lb act/A were harvested at 111 and 97 days, respectively, and processed into meal, hulls and crude and deodorized oil. Residues of BAS 9052 H were less than 0.05 ppm in the harvest seed and all the processing fractions. Based on the above data, we cannot conclude that a food additive tolerance for soybean processed fractions is not necessary, since:

1) The harvested soybeans did not contain detectable residues.

2) Soybeans were harvested at 97 days vs the proposed PHI of 70 days.

3) Residue data were obtained by an analytical method validated for the parent compound only.

Meat, Milk, Poultry and Eggs

Lactating dairy cows, 3 in each group, were administered 0.6 or 50.0 ppm BAS 9052 H for thirty days. Milk was collected at various intervals during dosing. Tissue samples were collected at sacrifice.

No BAS 9052 H (<0.05 ppm) was retained in the muscle tissues of the cows dosed at either the 0.6 ppm or 50.0 ppm levels.

No BAS 9052 H (<0.05 ppm) was found in the liver tissue of the cows dosed at the 0.6 ppm level. Cows dosed at the 50.0 ppm level showed residues in the liver tissue ranging between 0.05-0.12 ppm.

No BAS 9052 H (<0.05 ppm) was found in the kidney tissue of the cows dosed at the 0.6 ppm level. Cows dosed at the 50.0 ppm level showed residues in the kidney tissue ranging between <0.05 ppm and 0.09 ppm.

No detectable residues of BAS 9052 H (<0.05 ppm) was found in the milk of the cows which received 0.6 ppm or 50.0 ppm at any sampling interval throughout the course of the study.

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The goat feeding/metabolism study was also discussed under the Nature of the Residue. When two lactating goats were administered ^{14}C labeled BAS 9052 H at 1.25 ppm for eleven days no ^{14}C activity <0.003 ppm was detected in the muscle tissue, liver, kidney or milk.

Laying hens were administered BAS 9052 H at 1.0 ppm, 10 ppm and 100.0 ppm for thirty days. Eggs were collected daily throughout the course of the study while tissue samples were collected 8, 15, 22, 30, 37 and 45 days after the administration of the initial dose. Residues in the muscle tissues from the three feeding levels were <0.05 ppm - 0.19 ppm, respectively. Residues in the liver were <0.05, <0.05-0.17 ppm and 0.23-0.47 ppm, respectively. Residues in the eggs were <0.05 ppm (0.22 ppm obtained at 17 days is considered aberrant), <0.05 ppm - 0.34 ppm and 0.05 ppm - 1.88 ppm, respectively.

The above feeding studies do indicate that the consumption of treated soybeans containing residues of BAS 9052 H per se at the proposed tolerance level would not result in detectable (<0.05 ppm) residues of BAS 9052 H in meat, milk, poultry or eggs. However, the studies are not adequate to support the proposed temporary tolerances, since most of the residue in treated soybean seeds consists of the metabolites of BAS 9052 H and not of the parent compound.

We will require feeding studies involving administration of plant substrates containing weathered residues of BAS 9052H. Animal tissues should be analyzed by methods validated for the principal metabolites of BAS 9052H.

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Reviewer
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TS-769:Reviewer: E.Zager:LDT:X77324:CM#2RM:810:Date:10/21/80
RDI:Section Head:RJH:Date:11/19/80

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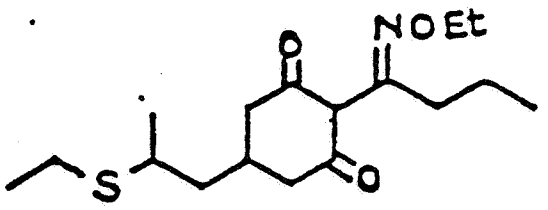
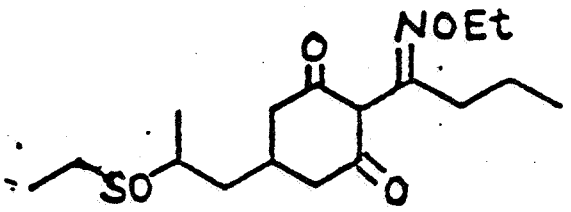
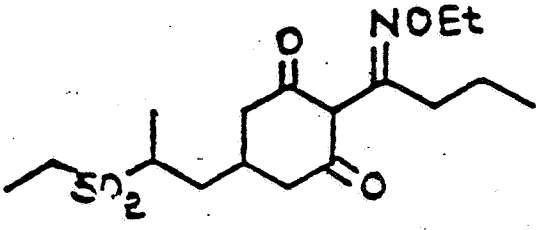
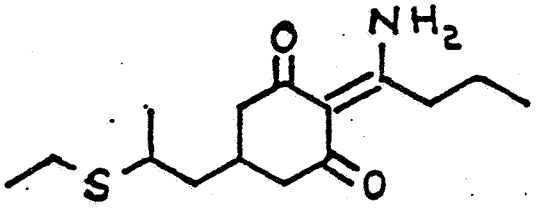
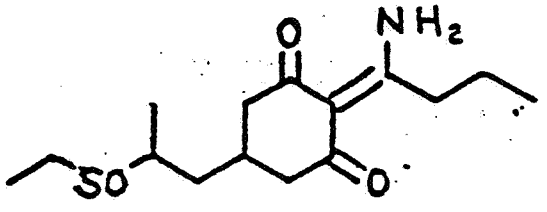
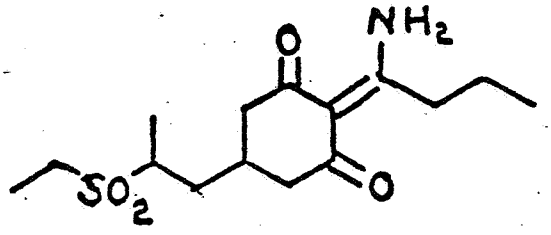
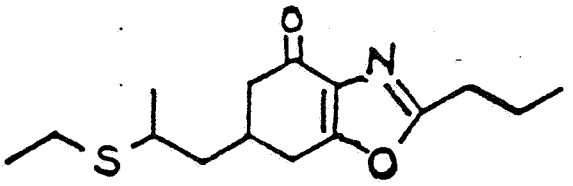
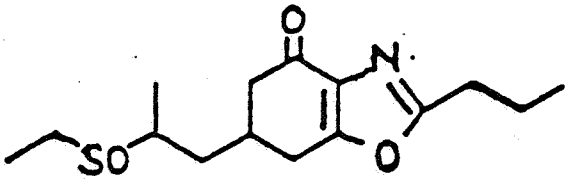
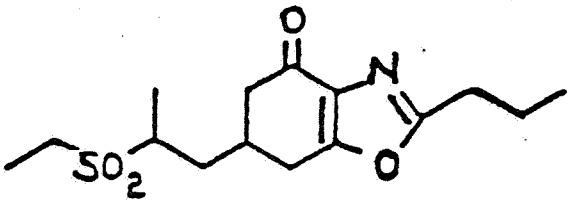
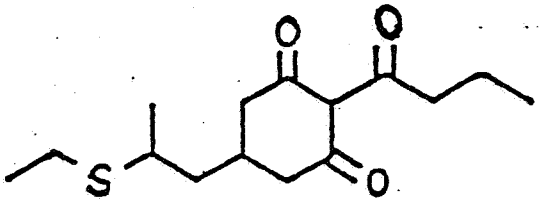
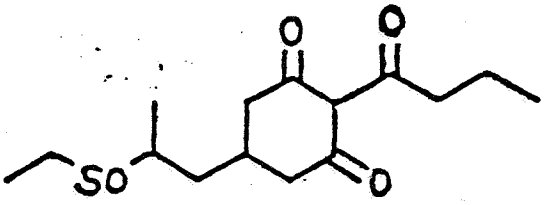
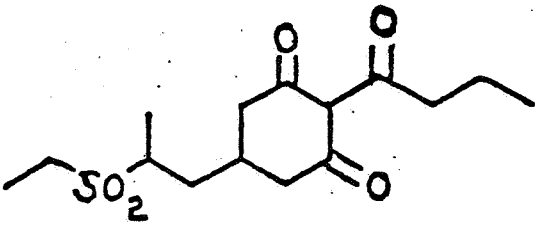
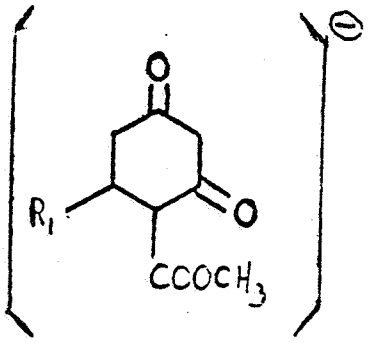
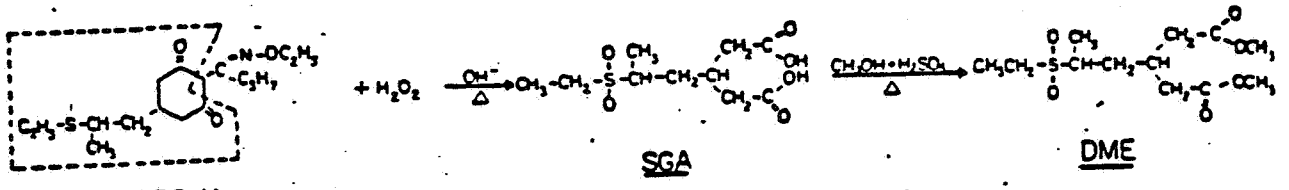
Glossary of Chemical Names and Their Structures

STRUCTURAL FORMULA	ABBREVIATION
	MS or BAS 9052 H
	MSO
	MSO_2
	MIS
	MISO
	$MISO_2$

	M2S
	M2SO
	$M2SO_2$
	M6S
	M6SO
	$M6SO_2$
 Na	MCD-Na

THE ANALYTICAL METHOD REACTION SCHEME



BAS 9052 H

SGA

DME