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

AUG 30 1993

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: ID# 000524-UAT. Review of limited field rotational study of MON 12000 [3-chloro-5-[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]-sulfonyl]-1-methyl-1H-pyrazole-4-carboxylic acid, methyl ester]. MRID# 428121-04. Barcode D192510. Case 023936. CBTS# 12079.

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8/30/93

TO: Joanne Miller, Product Manager
Registration Division (H7505C)

As part of the Section 3 registration of MON 12000, Monsanto has submitted this limited field crop rotation study. This study was performed at the request of the Agency based on results of confined crop rotation studies.

RECOMMENDATIONS

1. Assuming that the HED Metabolism Committee determines that MON 12000 metabolites need to be regulated, the presence of these residues in all rotational crops studied (except lettuce) indicates that extensive field trials will be required to set tolerances for each crop group (40 CFR 180.34 f9) for which crop rotation will be permitted.

2. We will make a final conclusion on the results reported for head lettuce after storage stability studies are submitted and

reviewed.

3. If the HED Metabolism Committee decides that only the parent compound needs to be regulated, extensive field trials and tolerances will not be required based on the absence of the parent compound in the confined studies and, with the exception of one forage sample, in the limited field studies.

CONCLUSIONS

1. Limited field crop rotation studies were performed at three U.S. sites. Two sites (IA, OH) were chosen for being major areas of production of the primary crop (corn). The CA site was chosen for having the same soil type used in the confined studies.

2. MON 12000 was applied to the primary crop at a rate of 0.22 lb. ai/A in a split (pre- + postemergence) application. The rate was 1.4X of the maximum rate used for setting the temporary tolerances (Memo G. Herndon, 11/12/92).

3. Rotational crops were planted approximately 3 months (winter wheat) or 12 months (spring wheat, soybeans, sugar beets and lettuce) after the preemergence MON 12000 application.

4. A new analytical method was developed for rotational crops. The method differed from the proposed enforcement method in being able to detect a minor metabolite (dCSA) which is not picked up in the enforcement method. This rotational crop method was judged to be inadequate for accurate quantitation of MON 12000 residues due to an interfering peak which was sometimes present in the check samples. The reported residue levels may thus be exaggerated, especially in soybean grain.

5. Significant residues of MON 12000 metabolites were found in winter wheat forage (up to 0.069 ppm), straw (up to 0.141 ppm), grain (up to 0.55 ppm); spring wheat forage (up to 0.078 ppm), straw (up to 0.028 ppm), grain (up to 0.023 ppm); soybean forage (up to 0.280 ppm), hay (up to 0.367 ppm), grain (up to 0.256 ppm); and sugar beet roots (up to 0.022 ppm). MON 12000 per se was found only in CA spring wheat forage (0.09 ppm).

6. Pending the results of ongoing storage stability studies, no residues were found in head lettuce (see conclusion 8).

7. The residue levels found in soybean were much higher than those found in the soil (up to 0.004 ppm) and were comparable to those reported for the primary crop.

8. Previous storage stability studies indicate that MON 12000 residues are stable in corn, wheat and soybean matrices for up to 508 days. The storage stability studies for the RACs used in this

study are in progress. We will make a final conclusion on the results on the results reported for lettuce after these studies have been submitted and reviewed.

BACKGROUND

MON 12000 is a new sulfonylurea herbicide developed by Monsanto to control broadleaf weeds and nutsedge in field corn, turf and other crops. The Agency has granted an EUP and temporary tolerances in corn for this compound. The temporary tolerances in corn are: 0.1 ppm in grain, 0.3 ppm in forage and 0.3 ppm in fodder.

The metabolism of MON 12000 has been studied in corn for both pre- and postemergence applications. The pathway of MON 12000 metabolism depends primarily on the application method. Following postemergence foliar application, there is very little translocation or metabolism of MON 12000. Preemergence application, however, results in extensive metabolism of MON 12000. The initial step is cleavage of the sulfonylurea linkage in the soil (see figure 1, copied from the registrants submission MRID 4213940-05). The pyrimidine ring is not readily translocated so that the pyrazole-derived metabolites account for over 90% of the MON 12000 residues. All significant residues found in wheat, radish, soybean and lettuce during the confined rotational crop studies were also pyrazole-derived. The preemergence pathway is the only one of concern for crop rotation studies since all residues are a result of uptake from the soil.

Chlorosulfonamide acid (CSAA) is the primary pyrazole-derived metabolite in corn, accounting for 51-64% of the TRR. Minor metabolites include chlorosulfonamide ester (CSE, 2-7% of the TRR), N-demethyl chlorosulfonamide acid (dCSA, 2-7%), hydroxymethyl chlorosulfonamide (1-9%), N-demethyl chlorosulfonamide ester (0-4%), and N-conjugates of CSAA and CSE (0-14%). The enforcement method is based on hydrolysis of MON 12000 and measuring pyrazole-derived metabolites as methylated derivatives by GC. The metabolites detected by this method are MON 12000, CSAA and CSE.

The metabolic fate of MON 12000 in confined rotational crops is consistent with the corn metabolism study. The total residues ranged from 0.002-1.731 ppm. CSAA was the major metabolite, accounting for 34-67% of the TRR. dCSA was the most prevalent minor metabolite, accounting for up to 15% of the TRR. Based on these results, the Agency requested that limited field crop rotation studies be performed.

DETAILED CONSIDERATIONS

Test Chemical

Formulated MON 12000 (MON 12007) was applied as a wettable powder containing 25% active ingredient by weight. Assay of the test substance prior to shipment from Monsanto showed 25.3% MON 12000. Quality control samples returned from each site following application were 25.1-25.2% MON 12000, indicating that MON 12000 was stable during shipment and storage prior to application.

Test Sites

The three test sites chosen for this study were located in CA (Vacaville), IA (Colo) and OH (New Holland). The sites in IA and OH were chosen as representative areas where corn is a major crop. The CA site was chosen because it has sandy clay loam soil, similar to that used in the confined rotational crop study. The sizes for check and treated plots ranged from 0.25 acres (CA) to 0.56 acres (OH). Buffer areas between check and treated plots were 200-225 ft. Following harvest of the primary crop, each plot (check and treated) was divided up into five sections for rotational crops. The CA site was irrigated. No irrigation was used in IA and only lettuce was irrigated in OH.

Primary Crop

MON 12000 was applied both preplant and postemergence. The initial application was at the time of planting using preplant incorporation. MON 12000 was applied at a rate of 0.125 lb. ai/A as a tank mix with acetochlor (grass control) and MON 13900 (safener). A late postemergence application of MON 12000 was performed 1-2 months after planting, when the corn was 25-36 inches high. MON 12000 was applied at a rate 0.094 lb. ai/A without safener or acetochlor. The total MON 12000 applied was 0.22 lb. ai/A, which the registrant states is the proposed maximum use rate. However, CBTS has restricted the application rate to 0.094 lb. ai/A preplant plus 0.063 lb. ai/A postemergence for a maximum total of 0.157 lb. ai/A (Memo G. Herndon, 11/12/92). This limitation was accepted by the registrant for the EUP and temporary tolerances (Memo G. Herndon, 1/21/93). It should also be noted that the confined rotational crop study used an application rate of 0.19 lb. ai/A. Unless the registrant seeks to use the higher application rate for establishing permanent tolerances, the lower rate of 0.157 lb. ai/A should be used in any future crop rotation studies. The application rate used in this study (1.4X) may have lead to exaggerated residue levels as compared to what would be expected at the lower application rate.

Rotational Crops

Corn was harvested in the fall of 1990 and the check and treated

plots were each divided into five subplots. Preplant tillage was performed to a depth of 3-6 inches prior to planting of rotational crops. Winter wheat was planted in September and October, which was 109-167 DAT (days after the initial preplant MON 12000 application, average of 144 days). The following spring, sugar beets, lettuce, soybeans and spring wheat were planted. The DAT interval for these crops was 307-388 days (average of 354 days). The RACs sampled were in accordance with Subdivision O Table II and the techniques used were in accordance with the usual agricultural practices. Lettuce and sugar beets were not harvested at the CA site as a result of crop failure ascribed to the phytotoxic effects of MON 12000. Analysis of soil residues (Table 1) demonstrated that the CA site had the highest levels of MON 12000 (0.004 ppm), albeit only slightly higher than the IA site (0.003 ppm). The sampling techniques used for each RAC are summarized below. Check samples were always sampled before treated samples to avoid contamination. Samples were generally frozen within 1-4 hours of the beginning of the harvest.

Table 1- Soil residues (0-6 inch samples)

		Residues (ppm)		
Location	DAT[a]	MON 12000	3-CSAA	Total
CA	0	0.041	<0.009	0.041-0.050
	157[b]	0.006	0.022	0.028
	364[c]	0.004	<0.009	0.004-0.013
IA	0	0.022	<0.009	0.022-0.031
	109[b]	0.003	0.013	0.016
	310[c]	0.003	<0.009	0.030-0.012
OH	0	0.038	<0.009	0.038-0.047
	167[b]	<0.002	<0.009	0.000-0.011
	371[c]	<0.002	<0.009	0.000-0.011

[a]Days after preplant incorporation of M)N 12000 at a rate of 0.126 lb ai/A
[b]Time of winter wheat planting
[c]Time of lettuce planting

Wheat- Forage samples were clipped at random from the plots approximately 8 weeks (spring wheat) or 26 weeks (winter wheat) after planting, taking care to avoid soil contamination. For

grain, the entire plot was harvested and grain bagged directly from the hopper. Straw was collected after grain removal and run through a chopper/mulcher.

Lettuce- Heads were cut at the base and taken to a drop cloth. Half of the harvested plants were placed directly into residue bags with wrapper leaves intact. The other half of the plants had the wrapper leaves removed before bagging.

Sugar Beets- Roots were dug and taken to a clean drop cloth. Tops were cut off and bagged. Roots were brushed to remove loose soil and bagged.

Soybeans- Forage samples were cut at random approximately 8 weeks after planting. For hay samples, plants were cut at random, placed on a drop cloth and allowed to dry in the sun for 4 days (samples were moved indoors during the nights). For seed samples, mature plants were cut at random and run through a thresher.

Analytical Methods

The enforcement analytical method measures a methylated derivative of CSAA which represents the total of MON 12000, CSAA and CSE. Homogenized tissues are extracted with 40% acetonitrile/water. The aqueous extract is hydrolyzed, first with acid then with base. The hydrolysis procedure breaks the sulfonylurea bond in MON 12000, releasing CSE (fig. 1). The basic aqueous solution is acidified and extracted with 40% acetonitrile in methylene chloride. The organic layer is evaporated to dryness. The resulting residue is resuspended in acidic acetone/methanol and methylated with trimethylsilyldiazomethane. The resulting methyl derivative, DMCSE, is cleaned up and analyzed on GC/ECD. The reported limit of quantitation is 0.01 ppm.

A new analytical method was developed for the rotational crop studies. The purpose of the new method was to include dCSA in the analysis. This method differs from the enforcement method as follows: Instead of the hydrolysis step, the extract is treated with mild base. This procedure converts MON 12000 into a rearrangement ester, leaving the metabolites unreacted. The solution is acidified and extracted with 40% acetonitrile in ethyl acetate, partitioning the rearrangement ester and MON 12000 metabolites from the aqueous solution. The organic phase is then split- part is used to analyze the rearrangement ester directly on GC and part is used for methylation as described previously. The CSAA, dCSA and CSE residues are converted to DMCSE and quantitated by GC. The registrant does not explain why dCSA is not detected by the enforcement method but presumably this difference is a result of removal of the acid hydrolysis step (i.e., dCSA may be sensitive to acid hydrolysis). This method also has the advantage of measuring MON 12000 and its metabolites separately.

The lowest limit of method validation for the new method was 0.010 ppm for MON 12000, 0.018 ppm for CSAA and 0.019 for dCSA, expressed as parent equivalents. Each RAC analyzed was spiked with 0.01-0.10 ppm of MON 12000, CSAA or dCSA. The samples were analyzed and recoveries determined. The average recovery of MON 12000 was 100.9%; of CSAA, 88.1%; and of dCSA, 78.6%.

The registrant reported that soybean grain, unlike other RACs had significant interferences in the check samples when analyzing for CSAA + dCSA. Analysis of the soybean grain data indicates that the DMCSE peak in the treated samples is only 50% (or less) greater than the peak in the check samples. Analysis of the raw data and chromatograms reveals that soybean grain was the only RAC in which this interfering peak was found consistently in large amounts (0.181-0.216 ppm). Soybean forage samples contained the interfering compound in lesser amounts (0.02-0.09 ppm) and there were numerous instances where the peak was found in the samples from only one or two of the three locations. In one case the peak was seen only in the check samples (spring wheat straw from CA, 0.128 ppm) and not in the treated samples. The high levels and variable appearance of this interfering compound renders the quantitative aspects of this study questionable. However, the data can be used as a worst-case scenario (maximum possible residues). In some cases, especially soybean grain, the actual values for MON 12000 residues are probably lower (i.e., see Table 4). The uncorrected values will be used as the basis for the conclusions and recommendations.

As a result of this problem, the revised method is determined to be inadequate for accurate quantitation of MON 12000 in rotational crops. The method should be modified to either remove the interfering compound or to resolve it from DMCSE during the GC analysis. Future submission of rotational crop residue data should employ this modification or, alternatively, use the enforcement method.

Storage Stability

RACs were stored frozen prior to extraction. The time between extraction and analysis was 1-2 days. The storage time for wheat forage and grain was up to 16 months; wheat straw, up to 18 months; soybean forage, up to 18 months; soybean hay, up to 17 months; soybean grain, up to 16 months; sugar beet tops, up to 19 months; sugar beet roots, up to 15 months; and lettuce, up to 20 months. MON 12000 and CSAA have been shown to be stable in corn matrices for up to 441 days and stable in wheat and soybean RACs for up to 508 days. No new storage stability data was submitted with this study. However, the registrant reports that studies on the storage stability of MON 12000, CSAA and dCSA in the rotational crop RACs is currently in progress and that the results will be reported when completed.

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Results

Residues in Winter Wheat

Table 2 shows the residues of MON 12000 found in winter wheat which was planted 109-167 days after the preemergence treatment. No MON 12000 residues were detected but pyrazole-derived metabolites were observed in all matrices except forage from OH. The levels of MON 12000 metabolites ranged from <0.019-0.069 ppm in forage, from 0.060-0.141 ppm in straw and from 0.025-0.055 ppm in grain. The highest residue levels were observed at the CA site in all three RACs.

Table 2- Average residue levels in winter wheat

			Crop Age (d)	Residues (ppm)		
Sample	Locat.	DAT[a]		Mon[b]	Metab[c]	Total
Forage	CA	157	157	<0.010	0.069	0.069-0.079
	IA	109	229	<0.010	0.052	0.052-0.062
	OH	167	168	<0.010	<0.019	0.000-0.029
	AVERAGE	144	185	<0.010	0.047	0.040-0.057
Straw	CA	157	243	<0.010	0.141	0.141-0.151
	IA	109	294	<0.010	0.060	0.060-0.070
	OH	167	259	<0.010	0.079	0.079-0.089
	AVERAGE	144	185	<0.010	0.093	0.093-0.103
Grain	CA	157	243	<0.010	0.055	0.055-0.065
	IA	109	294	<0.010	0.025	0.025-0.035
	OH	167	259	<0.010	0.032	0.032-0.042
	AVERAGE	144	265	<0.010	0.037	0.037-0.047

[a]Days between preemergence treatment and planting of crop
[b]Mon 12000
[c]Mon 12000 metabolites 3-CSAA + dCSA

Residues in Spring Wheat

Table 3 shows the residues of MON 12000 found in spring wheat which was planted 323-364 days after the preemergence treatment. Forage from CA was the only RAC in this study which contained MON 12000

itself (0.09 ppm). MON 12000 metabolites were found in forage from CA and IA, straw from OH and grain from IA.

Table 3- Average residue levels in spring wheat

Sample	Locat.	DAT[a]	Crop Age (d)	Residues (ppm)		
				Mon[b]	Metab[c]	Total
Forage	CA	364	57	0.090	0.078	0.168
	IA	323	61	<0.010	0.065	0.065-0.075
	OH	343	57	<0.010	<0.019	0.000-0.029
	AVERAGE	343	58	0.037	0.054	0.078-0.091
Straw	CA	364	94	<0.010	<0.019	0.000-0.029
	IA	323	97	<0.010	<0.019	0.000-0.029
	OH	343	98	<0.010	0.028	0.028-0.038
	AVERAGE	343	96	<0.010	0.022	0.009-0.032
Grain	CA	364	94	<0.010	<0.019	0.000-0.029
	IA	323	97	<0.010	0.023	0.023-0.033
	OH	343	98	<0.010	<0.019	0.000-0.029
	AVERAGE	343	96	<0.010	0.020	0.008-0.030

[a]Days between preemergence treatment and planting of crop
[b]Mon 12000
[c]Mon 12000 metabolites 3-CSAA + dCSA

Residues in Soybeans

Table 4 shows the residues of MON 12000 found in soybeans which were planted 362-388 days after the preemergence treatment. No MON 12000 residues were detected, but all RACs had significant levels of MON 12000 metabolites. CA samples had the highest levels in forage (0.280 ppm) and hay (0.367 ppm) while IA samples had the highest levels in grain (0.305 ppm). As noted previously, the soybean grain values appear to be exaggerated by the presence of an interfering peak in the check samples.

Table 4- Average residue levels in soybeans

Sample	Locat.	DAT[a]	Crop Age (d)	Residues (ppm)- Mon[b]	Metab[c]	Total
Forage	CA	364	57	<0.010	0.280	0.280-0.290
	IA	362	44	<0.010	0.060	0.060-0.070
	OH	388	53	<0.010	0.062	0.062-0.072
	AVERAGE	371	51	<0.010	0.134	0.134-0.144
Hay	CA	364	97	<0.010	0.367	0.367-0.377
	IA	362	100	<0.010	0.119	0.119-0.129
	OH	388	115	<0.010	0.105	0.105-0.115
	AVERAGE	371	104	<0.010	0.197	0.197-0.207
Grain	CA	364	147	<0.010	0.256	0.256-0.266
	IA	362	130	<0.010	0.305	0.305-0.315
	OH	388	140	<0.010	0.241	0.241-0.251
	AVERAGE	371	139	<0.010	0.267	0.267-0.277
Grain[d]	CA	364	147	<0.010	0.075	0.075-0.085
	IA	362	130	<0.010	0.089	0.089-0.099
	OH	388	140	<0.010	0.037	0.037-0.047
	AVERAGE	371	139	<0.010	0.067	0.067-0.077

[a]Days between preemergence treatment and planting of crop
[b]Mon 12000
[c]Mon 12000 metabolites 3-CSAA + dCSA
[d]Signnificant interference was found in check samples when analyzing for MON 1200 metabolites. These are the values found if the check values are subtracted.

Residues in Lettuce Leaf

Table 5 shows the residues of MON 12000 found in lettuce which was planted 307-371 days after the preemergence treatment. No MON 12000 or metabolites were detected in lettuce samples. The CA crop failed, presumably due to the phytotoxic effects of MON 12000.

Table 5- Average residue levels in lettuce leaf

Sample	Locat.	DAT[a]	Crop Age (d)	Residues (ppm)- Mon[b]	Metab[c]	Total
w/ Wraps	CA	364	NC	NA	NA	NA
	IA	307	99	<0.010	<0.019	0.000-0.029
	OH	371	55	<0.010	<0.019	0.000-0.029
	AVERAGE	347	77	<0.010	<0.019	0.000-0.029
w/o Wraps	CA	364	NC	NA	NA	NA
	IA	307	99	<0.010	<0.019	0.000-0.029
	OH	371	55	<0.010	<0.019	0.000-0.029
	AVERAGE	347	77	<0.010	<0.019	0.000-0.029

[a]Days between preemergence treatment and planting of crop
[b]Mon 12000
[c]Mon 12000 metabolites 3-CSAA + dCSA
NC = No Crop

Residues in Sugar Beets

Table 6 shows the residues of MON 12000 found in sugar beets which were planted 307-388 days after the preemergence treatment. Significant metabolite residues were found only in IA beet roots (0.022 ppm). The CA crop failed, presumably due to the phytotoxic effects of MON 12000.

Table 6- Average residue levels in sugar beets

Sample	Locat.	DAT[a]	Crop Age (d)	Residues (ppm)- Mon[b]	Metab[c]	Total
Tops	CA	364	NC	NA	NA	NA
	IA	307	187	<0.010	<0.019	0.000-0.029
	OH	388	140	<0.010	<0.019	0.000-0.029
	AVERAGE	353	164	<0.010	<0.019	0.000-0.029
Roots	CA	364	NC	NA	NA	NA
	IA	307	187	<0.010	0.022	0.022-0.032
	OH	388	140	<0.010	<0.019	0.000-0.029
	AVERAGE	353	164	<0.010	0.020	0.011-0.030

[a]Days between preemergence treatment and planting of crop
[b]Mon 12000
[c]Mon 12000 metabolites 3-CSAA + dCSA
NC = No Crop

Discussion

Comparison of the results of this study with that of the confined crop rotation study (MRID# 423962-04, Table 7) shows that residues in the field study were lower than the confined study for wheat and lettuce. As observed in the confined study, soybeans in the field study effectively accumulated residues of MON 12000 metabolites. Forage residue levels were similar in both studies, straw residues were lower in the field study and grain residues were actually higher in the field trials, even after correcting for the check values. Residues in the root crops were similar in both studies (0.02 ppm). The high residue levels in soybean were similar to those observed on the primary crop (MRID# 421394-05, Table 8): 0.134-0.267 ppm in soybean vs. 0.035-0.231 ppm in corn. Assuming that the HED Metabolism Committee determines that MON 12000 metabolites need to be regulated, the ability of soybeans to accumulate MON 12000 metabolite residues and the presence of these residues in wheat and sugar beets indicate the need for extended field trials to set tolerances for crops which will be rotated after MON 12000 usage. Assuming that MON 12000 metabolites are shown to be stable during storage, head lettuce is excepted from this requirement since no detectable residues were observed in the field trials with this crop.

The registrant has recently proposed to change the tolerance

expression to include the parent compound **only** (Memo, G.F. Kramer 7/23/93). The HED Metabolism Committee will meet in September to determine whether MON 12000 metabolites will be included in the tolerance expression. If the HED Metabolism Committee decides that only the parent compound needs to be regulated, extensive field trials and tolerances will not be required based on the absence of the parent compound in the confined studies and, with the exception of one forage sample, in the limited field studies.

Table 7- Metabolite distribution in confined rotational crop study (365 DAT samples)

Sample	RAC	TRR (ppm)	% of TRR in Metabolites:			Total ppm in Metabolites[a]
			M2	M3	M6	
Soybean	Forage	0.188	77.6	ND	5.6	0.156
	Seed	0.076	53.3	ND	8.1	0.047
	Straw	0.559	58.6	ND	14.8	0.410
Wheat	Forage	0.100	75.2	ND	ND	0.075
	Grain	0.066	52.7	ND	10.6	0.042
	Straw	0.681	46.9	8.9	7.0	0.427
Radish	Top	0.047	70.4	ND	ND	0.033
	Root	0.022	73.2	ND	3.9	0.017
Lettuce	Leaf	0.011	42.4	ND	9.4	0.006

[a]Total of metabolites which are measured in the revised method:
M2 = Chlorosulfonamide acid (3-CSAA)
M3 = Chlorosulfonamide ester (CSE)
M6 = N-demethyl chlorosulfonamide acid (dCSA)

Table 8- Residues in corn after preemergence application of MON 1200

	MON 1200		Metabolites[b]		Total	
RAC	% TRR	ppm[a]	% TRR	ppm[a]	% TRR	ppm[a]
Forage	BLD	BLD	73.0	0.035	73.0	0.035
Silage	BLD	BLD	69.1	0.077	69.1	0.077
Fodder	BLD	BLD	60.8	0.231	60.8	0.231
Grain	1.5	0.002	58.6	0.058	60.1	0.060

[a]Normalized ppm, calculated for use rate which is 4X less than the exaggerated rate used in these trials
[b]Total of metabolites which are measured in the revised method
BLD = Below Limit of Detection (0.001 ppm)

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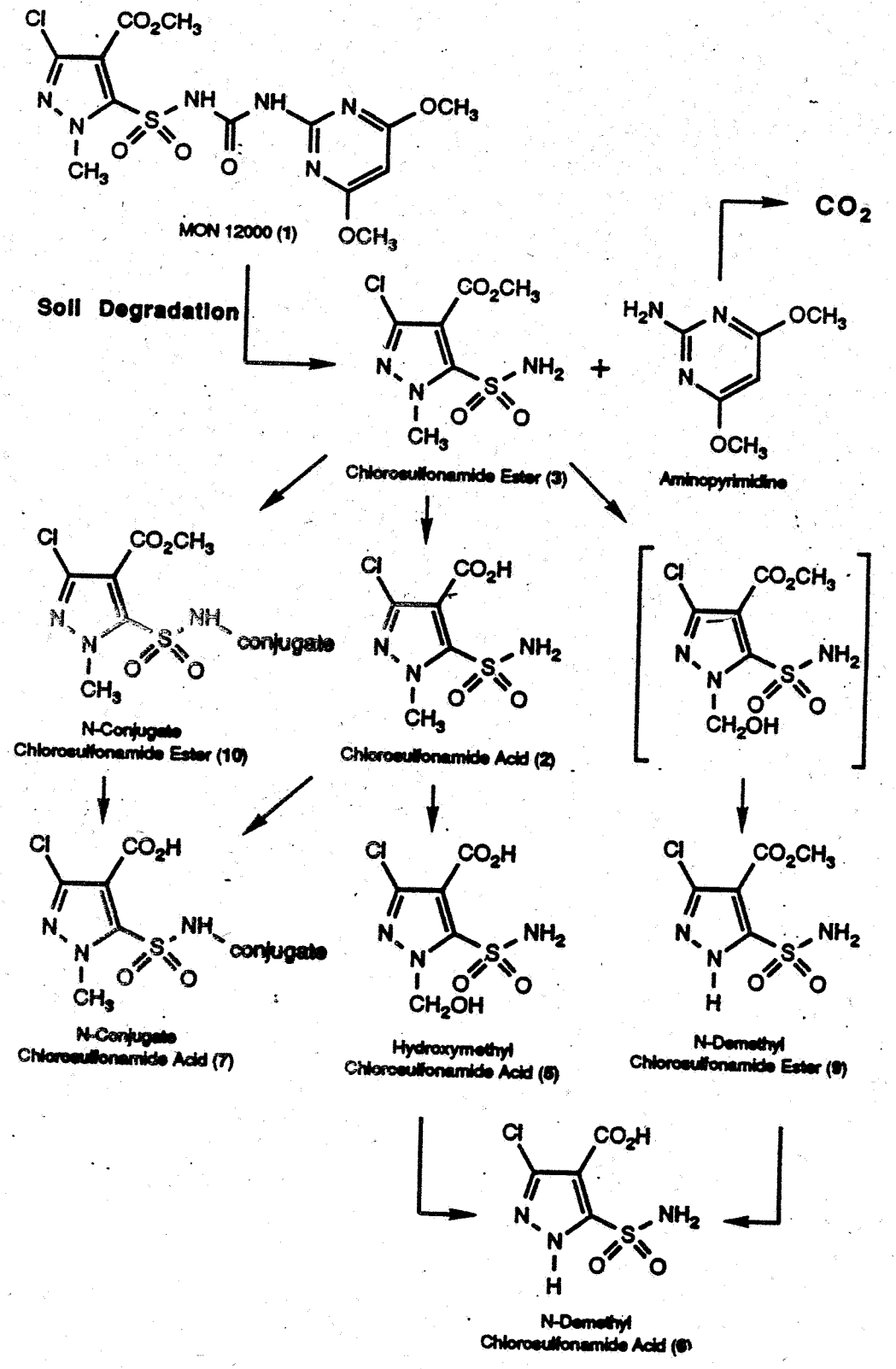
Figure 5. Metabolic Pathway of MON 12000 Following Preemergence Application



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