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

JAN 23 1986

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: 524-316. Alachlor Special Review. Response to the Alachlor Registration Standard. Cattle Feeding Study (MSL-4464, 4373) Poultry Feeding Study (MSL-4620, 4514), and Swine Feeding Study (MSL-4620, 4515). [Accession Numbers 256625, 257273, 257272] [RCB Numbers 679, 1032]

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THRU: Charles L. Trichilo, Ph.D., Chief
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Monsanto Company has submitted cattle, poultry, and swine feeding studies entitled "Residue Determination of Alachlor Metabolites in Milk and Beef Tissues," (MSL-4373), "Residue Determination of Alachlor Metabolites in Eggs and Poultry Tissues" (MSL-4514), and "Residue Determination of Alachlor Metabolites in Hog Tissues" (MSL-4515). These studies are submitted in response to the Alachlor Registration Standard.

Tolerances are established for milk, eggs, and the meat and meat byproducts of cattle, goats, hogs, poultry, horses, and sheep at 0.02 ppm (40 CFR §180.249). These tolerances were set at the limit of detection on the basis of residue

studies detecting the parent and residues containing the 2,6-diethylaniline moiety only. The current submissions use methodology which analyze for the aforementioned residues and for metabolites containing the 2-ethyl-6-hydroxyethylaniline moiety discussed below. Here, the methodology to measure both kinds of metabolites is more sensitive (0.5 to 2 ppb limit of detection for each of the two classes of metabolite) and should allow reevaluation of alachlor tolerances for animal products after successful completion of a method tryout (MTO). In the present memorandum, the studies are reviewed for the purpose of estimating residue levels to be used to calculate dietary exposure for the Alachlor Special Review.

The alachlor plant metabolites used as dosing material in the cattle, poultry and swine feeding studies are listed in Table I and the structures are given in attachment 1. These are the same metabolites used as dosing material in the poultry and ruminant metabolism study (see M. Loftus, November 1, 1985, review of Monsanto Special Report MSL-3473, Accession No. 257285).

Table I

Alachlor Plant Metabolites* Used as Dosing Material in Cattle, Poultry, and Swine Studies

% Dose by Weight	
I - 40%	N-(Methoxymethyl)-N-[2-(1-hydroxyethyl)-6-ethylphenyl]-2-methylsulfonyl)acetamide; (hydroxyethyl methylsulfone metabolite of alachlor)
II - 15%	2-Hydroxy-N-(methoxymethyl)-N-(2,6-diethylphenyl) acetamide;(2-hydroxy analog of alachlor)
III - 15%	[(Methoxymethyl)(2,6-diethylphenyl)amino]oxoacetic acid, sodium salt; (oxanilic acid metabolite of alachlor)
IV - 15%	2-[(Methoxymethyl)(2,6-diethylphenyl)amino]-2-oxoethane sulfonic acid, sodium salt; (sulfonic acid metabolite of alachlor)
V - 15%	3-[Methoxymethyl)(2,6-diethylphenyl)amino-2-oxoethane-sulfinyl]-2-hydroxypropanoic acid, sodium salt; (sulfinyl lactic acid metabolite of alachlor)

* Structures given in Attachment 1.

In plants, the parent alachlor is not found, and plant metabolites can be separated into 2 general categories, those which contain the 2,6-diethylaniline moiety and those which contain the 2-ethyl-6-hydroxyethylaniline moiety or its sugar conjugate. Following high pressure acid hydrolysis (sample + strong HCl is heated to 150 °C in a capped tube), a technique used in the plant and now in the livestock metabolism studies to characterize residues, the former class of metabolites is converted to 2,6-ethylaniline (DEA) and the latter is converted to 2-ethylaniline (EA). In Table I, metabolites II to IV contain the 2,6-diethylaniline moiety and metabolite I contains the 2-(1-hydroxyethyl)-6-ethylaniline moiety. These metabolites are representative of those found in corn and soybeans, except for the omission of a metabolite representative of a sugar conjugate at the 2-(1-hydroxyethyl) site of the second class of metabolites (this type of sugar conjugate is also converted to EA following acid pressure hydrolysis).

In the ruminant metabolism study, the resulting residue was characterized in goat milk as yielding metabolites convertible to a 1:1 mixture of 2-ethylaniline (2-EA) and 2,6-diethylaniline (2,6-DEA) following acid pressure hydrolysis. In goat liver, several metabolites were found, but not further characterized. Residues in other goat tissues were not characterized, although residues in goat excreta were characterized and/or identified. The average ^{14}C activity found was < 3.5 ppb (muscle), < 3.9 ppb (fat), 14 ppb (liver), 11 ppb (kidney) and 4.9 ppb (milk).

In the poultry metabolism study, 24 percent of the residue in eggs was characterized as metabolites containing either the DEA or HEEA moiety. Twelve percent of the residue in the eggs was characterized as other products including those containing the 2,6-(1-hydroxy-ethyl) aniline moiety, convertible to aniline. Sixty-four percent of the residue in the eggs was not characterized. The residue in poultry liver was found to consist of $\geq$ 30 percent products having a molecular weight > 10,000. The remaining residue was not characterized. The average ^{14}C activity found was 4.5 to 5.3 ppb (muscle), 54 ppb (liver), 17 ppb (kidney), and 30.4 ppb (eggs).

The above metabolism studies also indicated that the residue resulting in animal tissues, eggs, and milk may also be dependent on whether the plant metabolite was neutral or ionic. As can be seen from the structures shown in Attachment 1, metabolites III to V are ionic and the remaining are neutral, including the one containing the HEEA moiety. Thus, 55 percent neutral and 45 percent ionic metabolites were fed to the livestock.

It was concluded that the residue in ruminants and poultry is not adequately understood, primarily because residue in the tissues was either not characterized or only minimally

characterized and because a large percentage of the residue in eggs and milk was not characterized.

Essentially, the same analytical method was used for the cattle, poultry, and swine feeding studies. The method is essentially the same method as that submitted under Accession Number 255600 for milk and beef tissues, reviewed by M. Loftus on February 7, 1985.

The methodology consists of extraction of the sample with solvent, followed by centrifugation and evaporation of the extract. There are slight differences in the extraction procedure dependent on whether milk, eggs, fat, kidney, liver, or muscle is being analyzed. The extract is hydrolyzed in 50 percent NaOH and steam distilled into dilute acid. The acidic distillate is extracted with hexane, transferred to a second separatory funnel, and made basic. The DEA and HEEA are extracted from the distillate with methylene chloride and solvent exchanged into hexane. An aliquot of 4-fluoro-2,6-diethylaniline (FDEA) is added to the sample for calibration purposes and the FDEA, DEA, and HEEA are derivatized with heptafluorobutyric anhydride (HFBA) and quantified by capillary gas chromatography with mass spectrometric detection (GC-MS) using selected ion monitoring (SIM). Residues are reported as alachlor equivalents after appropriate calculations.

The methodology was validated at 0.5 ppb for DEA and HEEA producing metabolites in milk, muscle, and fat at either 1 to 2 ppb for liver and kidney. When the report on this method was originally submitted under Accession Number 255600, validation was at higher levels (1 to 2 ppb for milk, muscle, and fat). Here, background response for DEA was reduced by reducing contamination through the use of a laboratory with new equipment and separate glassware facilities. Most likely, then, these limits of detection will not be reached in an MTO.

Recovery data are given below in Table II. Recoveries were corrected for background controls and samples were corrected for the average recovery. Sample chromatograms of controls, fortified controls, and samples were provided for milk, eggs, and the various tissues.

Table II
Recovery Data for Eggs, Milk, and Tissues

	DEA Metabolites				
	Fort.(ppb)	%Recovery	(average %recovery)		
			Cattle	Poultry	Swine
milk	0.5 - 100	58 - 108	(73)		
eggs, muscle, fat	0.5 - 100	54 - 120	(74, 80)	(77, 72, 73)	(70, 73)
liver, kidney	2 - 500	58 - 103	(76, 82)	(83, 80)	(81, 81)

	HEEA Metabolites				
	Fort.(ppb)	%Recovery	(average %recovery)		
			Cattle	Poultry	Swine
milk	0.5 - 100	60 - 124	(92)		
eggs, muscle, fat	0.5 - 100	52 - 128	(81, 84)	(85, 78, 90)	(87, 82)
kidney	2 - 300	60 - 110	(85)	(79)	(86)
liver	1,2 - 500	54 - 114	(76)	(76)	(94)

Three out of four groups of four cows were dosed by capsule with, in terms of alachlor equivalents, 4.2 ppm (group I), 12.6 ppm (group II), and 42 ppm (group III) plant alachlor metabolites for 28 days. (Milligrams dosing material in capsule for a particular animal was determined on the basis of the previous week's feed intake by that animal).

The fourth group served as a control. Three out of four cows in each group were sacrificed on day 28 and the fourth was sacrificed after a 28-day withdrawal on day 57. The samples were not pooled.

Results of the cattle feeding study are given in Table III, for cattle sacrificed on day 29. Plots of residue versus dose are approximately linear, particularly when cow 12, fed at 42 ppm, is not included. (Cow 12 yielded higher residues which Monsanto attributes to poor health resulting in higher doses from lower feed intake and, for liver and kidney, the spilling of rumen contents on liver and kidney tissue. Residues in all tissues and milk were highest for cow 12 with kidney and liver tissues being much higher. (Table III does not include data for cow 12 in kidney and liver tissue). The residue versus dose plots for HEEA metabolites are steeper than those for DEA metabolites. Only milk had a y-intercept of zero.

The results of the cattle feeding study in Table III and the ppb ^{14}C activity found in the goat metabolism study are comparable. Generally, the feeding study showed residue levels 50 percent of that expected from the goat metabolism study.

Table III

Results of Dairy Cattle Feeding Study

Tissue	Dose, ppm[a]	PPB DEA[a] range (Avg)	PPB HEEA[a] range (Avg)	Total, ppb max (Avg)
Muscle	42	1.4-2.8 (2.0)[b]	3.6-13 (6.9)[b]	15.8 (8.9)
	12.6	< 0.5-0.8 (0.6)	1.0-2.0 (1.5)	2.8 (2.1)
	4.2	< 0.5 (< 0.5)	0.5-1.1 (0.7)	1.6 (1.2)
Fat	42	1.9-4.6 (3.0)[b]	2.6-9.4 (5.2)[b]	14.0 (8.2)
	12.6	0.7-2.1 (1.4)	0.9-2.4 (1.7)	4.5 (3.1)
	4.2	0.6-1.0 (0.8)	0.7-1.5 (1.1)	2.5 (1.9)
Liver	42	11 -15 (13)[c]	53 -54 (54)[c]	69 (67)
	12.6	4.8-6.5 (5.6)	6.4-10.3 (8.0)	16.8 (13.6)
	4.2	< 2.0-3.6 (3.2)	3.1-6.8 (4.6)	10.4 (7.8)
Kidney	42	27 -31 (29)[c]	34 -40 (37)[c]	71 (66)
	12.6	7.5-20 (16)	9.0-21 (16)	41 (32)
	4.2	3.1-6.2 (4.5)	3.3-5.4 (4.2)	11.6 (8.7)
Milk	42	1.8-6.7 (3.5)[b]	4.1-21.0 (8.7)[b]	27.7 (12.2)
	12.6	0.5-1.6 (1.2)	1.1-3.7 (2.9)	5.3 (4.1)
	4.2	< 0.5-0.9 (< 0.5)	0.5-1.6 (1.0)	2.5 (1.5)

[a] Expressed as alachlor equivalents.
[b] Includes cow 12, even though cow 12 had poor health.
[c] Does not include cow 12 because contents of rumen spilled on kidney and liver during necropsy.

For depurated cattle, sacrificed on day 57, detectable residues were not found in cattle tissues. In the milk, detectable residues were not found by day 35.

Below a maximum dietary intake has been calculated for beef and dairy cattle, both before and after correction for the percent crop treated, from the maximum residue found in the feed (assuming feeding of peanut forage and hay has been restricted).

Table IV
Maximum Dietary Intake for Beef Cattle

Feed	% on diet	Max. ppm	ppm in diet assuming 100% treated feed	% of feed treated	ppm in diet corrected for % treated
Soybean forage	20	2.6	0.52	26	0.135
Sorghum forage	25	1	0.25	8	0.02
Soybean meal	25	0.4	0.10	26	0.026
Corn grain	30	0.08	0.02	32	0.008
			0.89 ppm		0.19 ppm

Table V
Maximum Dietary Intake for Dairy Cattle

Feed	% on diet	Max. ppm	ppm in diet assuming 100% treated feed	% of feed treated	ppm in diet corrected for % treated
Soybean forage	40	2.6	1.04	26	0.27
Sorghum forage	10	1	0.1	8	0.008
Soybean meal	25	0.4	0.10	26	0.026
Corn grain	25	0.08	0.02	32	0.006
			1.26 ppm		0.31 ppm

Using the residue data in Table III for average total residue and the dietary intakes in Tables IV and V, we calculate the following residue levels for beef tissues and milk:

Table V A
Estimated Residue Levels (ppb)

	Assuming 100% crop treated	Corrected for % crop treated
Muscle	0.3	0.05
Fat	0.4	0.09
Liver	1.7	0.4
Kidney	1.8	0.4
Milk	0.3	0.07

The above residue levels in Table V A are to be used to calculate dietary exposure and are not necessarily those to be used for reevaluation of tolerances.

Poultry

Sixty hens in three groups of twenty hens were orally dosed by capsule for 28 days. To reduce the number of birds sacrificed on a given day, each treatment group was equally divided and entered into the test period on a successive Tuesday and Thursday. The daily dose was 4 ppm (group I), 12 ppm (group II), or 40 ppm (group III). The dose was calculated based on bird dietary intake of approximately 110 g feed/bird/day. We calculate that bird fed at 1X received 550 mg/metabolites/day. In addition, a group of 40 hens were kept as a control. On day 29, half the hens were sacrificed and on day 57, the remaining hens were sacrificed. Eggs and tissues were pooled by treatment group, day entered test period, and day of sacrifice, e.g., hen tissues in group I, entered into test period on Tuesday and sacrificed on day 29 were pooled.

Results of the poultry feeding study are given in Table VI for hens sacrificed on day 29. Plots of residue versus dose are approximately linear. However, only eggs had a y-intercept close to zero. The results of the poultry feeding study and the ppb ^{14}C activity from the poultry metabolism are generally comparable. The feeding study showed residue levels > 50 percent of that expected on the basis of total ^{14}C activity, except for liver and kidney. For liver and kidney, the feeding study showed residue levels 10 to 30 percent of the level expected from the poultry metabolism study.

Table VI
Results of Poultry Feeding Study[a]

		Alachlor Residue Producing		
Tissue	Dose, ppm	DEA, ppb Maximum (Avg[b])	HEEA, ppb Maximum (Avg[b])	Total, ppb maximum (Avg[b])
Muscle	40	1.7 (1.5)	2.2 (1.8)	3.9 (3.3)
	12	0.5 (0.5)	0.5 (0.5)	1.0 (1.0)
	4	< 0.5 (< 0.5)	0.5 (0.5)	1.0 (1.0)
Fat	40	1.7 (1.7)	0.5 (0.5)	2.2 (2.2)
	12	0.8 (0.8)	< 0.5 (< 0.5)	1.3 (1.3)
	4	< 0.5 (< 0.5)	< 0.5 (< 0.5)	< 1.0 (< 1.0)
Liver	40	4.8 (4.8)	3.2 (2.8)	8.0 (7.6)
	12	1.6 (1.5)	< 1.0 (<.1.0)	2.6 (2.5)
	4	1.1 (1.1)	< 1.0 (< 1.0)	2.1 (2.1)

Table VI
Results of Poultry Feeding Study[a] (cont'd)

Tissue	Dose, ppm	Alachlor Residue Producing DEA, ppb maximum (Avg[b])	HEEA, ppb maximum (Avg[b])	Total, ppb maximum (Avg[b])
kidney	40	26 (18)	6.1 (4.2)	32 (22.2)
	12	2.4 (2.4)	< 1.0 (< 1.0)	3.4 (3.4)
	4	1.0 (1.0)	< 1.0 (< 1.0)	2.0 (2.0)
eggs	40	7.9 (7.5[c])	67 (60[c])	75 (675[c])
	12	2.3 (2.2[c])	20 (19[c])	22.3 (21.2[c])
	4	1.0 (0.8[c])	7.8 (6.1[c])	8.8 (6.9[c])

a/ Expressed as alachlor equivalents.
b/ Average for samples sacrificed on day 29, except for eggs.
c/ Average for days 8 to 28.

For the depurated hens, the residue in eggs decreased to 1 ppb after a 7-day withdrawal (day 35) and was nondetectable after a 14-day withdrawal, except for detectable residue on day 57 in group III. Monsanto ascribes the residue found on day 57 in eggs (0.7 ppb) to contamination. Detectable residue was also found in composite muscle and fat samples of group III hens (up to 0.8 ppb) and Monsanto indicates that contamination is also suspected. However, they do not offer reason why contamination was suspected.

Below, a maximum dietary intake has been calculated for poultry, both before and after correction for percent crop treated, from the maximum residue found in the feed.

Table VII
Maximum Dietary Intake for Poultry

Feed	% on diet	Max. ppm	ppm in diet assuming 100% treated	% treated	ppm in diet corrected for % treated
Soybean grain	50	0.2	0.1	26	0.026
Soybean meal	20	0.4	0.08	26	0.0208
Sunflower meal	15	0.2	0.03	2	0.0006
Corn grain	15	0.08	0.012	32	0.0038
			0.222 ppm		0.0512 ppm

Using the residue data in Table VI for average total residue and the dietary intakes in Table VII, we calculate the following residue levels for poultry tissues and eggs:

Table VII A

	Assuming 100% crop treated	corrected for % crop treated
Muscle	0.05 ppb	0.01 ppb
Fat	0.05 ppb	0.01 ppb
Liver	0.1 ppb	0.03 ppb
Kidney	0.1 ppb	0.03 ppb
Eggs	0.4 ppb	0.09 ppb

The above residue levels in Table VII A-are to be used to calculate dietary exposure and are not necessarily those to be used for reevaluation of tolerances.

Swine

Three out of four groups of four hogs were dosed via feed with, in terms of alachlor equivalents, 4, 12, and 40 ppm plant alachlor metabolites for 28 days. (Milligrams dosing material added to feed was based on the average daily consumption of all hogs during the previous week. Since males ate more then females, dose in ppm was slightly lower (by 0.1 ppm for males) The fourth group of four hogs served as a control. On day 29 one-half of the hogs were sacrificed and the remaining were sacrificed after a 28-day withdrawal on day 57.

Results of the swine feeding study are given in Table VIII for swine sacrificed on day 29. Correlation between dose and residue are good for swine muscle and kidney tissue, but poor for fat and liver tissue. In particular, residue containing the DEA moiety is 50 percent higher for the 1X dose compared to the 3X dose. Monsanto indicated that this may be due to sampling difficulties from too little omental fat. In plots of residue versus dose, a nonzero y-intercept was observed.

Table VIII
Results of Swine Feeding Study[a]

Tissue	Dose, ppm	DEA, ppb Maximum (Avg[b])	HEEA, ppb Maximum (Avg[b])	Total, ppb Maximum (Avg[b])
Muscle	40	1.0 (1.0)	4.0 (3.8)	5.0 (4.8)
	12	0.7 (0.6)	3.2 (2.5)	3.7 (3.1)
	4	0.5 (< 0.5)	1.0 (0.8)	1.5 (1.3)
Fat	40	2.6 (2.5)	2.0 (1.9)	4.6 (4.4)
	12	1.2 (0.9)	1.5 (1.1)	2.7 (2.0)
	4	1.8 (1.7)	0.9 (0.9)	2.7 (2.6)
Liver	40	6.9 (4.7)	7.7 (6.2)	14.6 (10.9)
	12	< 2.0 (< 2.0)	8.1 (6.1)	10.1 (8.1)
	4	< 2.0 (< 2.0)	2.2 (2.1)	4.2 (4.1)
Kidney	40	13 (8)	7.8 (6.6)	20.8 (14.6)
	12	2.6 (2.3)	6.5 (5.1)	9.1 (7.4)
	4	< 2.0 (< 2.0)	< 2.0 (< 2.0)	< 4.0 (N)

[a]/ Expressed as alachlor equivalents.
[b]/ Average for samples sacrificed on day 29.

For depurated hogs, sacrificed on day 57, detectable residue was not found in any of the tissues.

Below a dietary intake has been calculated for swine before and after correction for the percent crop treated from the maximum residue found in the feed.

Table IX
Maximum Dietary Intake for Swine

Feed	% on diet	Max. ppm	ppm in diet assuming 100% treated	% treated	ppm in diet corrected for % treated
Soybean meal	20	0.4	0.08	26	0.02
Soybean hulls	5	0.32	0.016	26	0.004
Sorghum forage	30	1	0.3	8	0.024
Sorghum grain	45	0.05	0.02	8	0.002
			0.42 ppm		0.050 ppm

The forcing of the dose response curve through zero by Monsanto compared to EPA's interpolation results in some differences for the residue estimates ranging up to a 5X difference for muscle, when comparing Monsanto's estimate to our estimate which includes percent crop treated. However, the largest difference represents only 0.01 ppb versus 0.05 ppb in muscle.

Conclusions and Recommendations

1. Since questions remain as to the nature of the residue in livestock (see November 1, 1985 memorandum of M. Loftus), we are not able to conclude that the requirement in the Registration Standard for residue data on the magnitude of the residue in cattle, goats, poultry, and eggs has been satisfied. If new classes of metabolites, in addition to those containing the DEA and HEEA moiety, are found in the livestock metabolism studies, either samples remaining from the present studies will need to be reanalyzed for the new class(es) of metabolites, or new studies will be required if intact samples are not available.

   In addition, to properly evaluate the feeding studies information on the time and conditions under which samples were stored and a storage stability study needs to be submitted.

2. The analytical methodology used in these feeding studies, is adequate to determine residues of alachlor and metabolites containing the DEA and HEEA moieties. However, dependent on the outcome of additional studies required to delineate the nature of the residue in livestock tissues, milk, and eggs, additional analytical methodology to measure any new classes of metabolites may be necessary. Thus, at this time we will not request an MTO.

3. For the purpose of calculation of alachlor dietary exposure for the alachlor special review, we tentatively estimate the following range of DEA + HEEA metabolite residue levels for livestock tissues, milk, and eggs, pending resolution of the metabolism questions discussed in Conclusion 1.

	Range(ppb)		
beef muscle	0.05	-	0.3
beef fat	0.09	-	0.4
beef liver	0.4	-	1.7
beef kidney	0.4	-	1.8
poultry muscle	0.01	-	0.05
poultry fat	0.01	-	0.05
poultry liver	0.03	-	0.1
poultry kidney	0.03	-	0.1
pork muscle	0.02	-	0.1
pork fat	0.03	-	0.3
pork liver	0.05	-	0.4
pork kidney	0.05	-	0.4
milk	0.07	-	0.3
eggs	0.09	-	0.4

The above lower estimates represent the average residue expected assuming that livestock were fed feed items containing alachlor residues reflecting the percent crop treated in the U.S.A. The above higher estimates represent the average residue expected assuming that livestock always were fed with treated feed (worst case). The latter would be expected, for example, when a grower treated all the feed crops with alachlor, and feeds his livestock with his treated feed. The above estimates are not to be used to establish new tolerances. Maximum residue levels assuming a worst case livestock dietary intake would be used for tolerance assessment instead of use of average residue levels. Also, before new tolerances would be established for animal products, a successful method try-out (MTO) would be necessary.

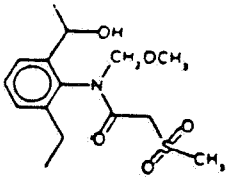
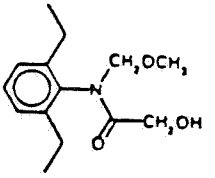
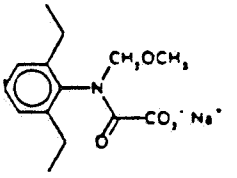
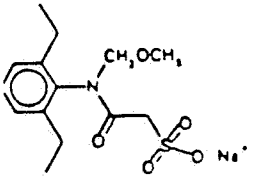
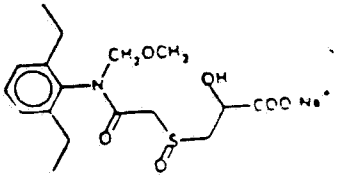
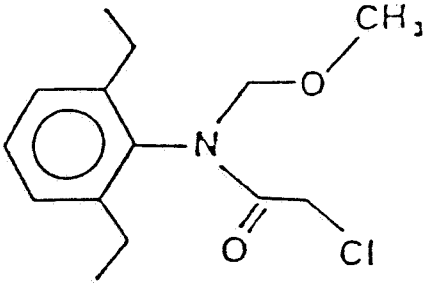
cc with Attachment: All copies

cc: Gary Burin (SIS), Mike McDavit (SRB), Loftus, Special Review File (see Loftus), Hummel, Griffith, Beusch, alachlor SF, RF, Circu

RDI:EZ:12/12/85:RDS:12/12/85
86223:Loftus:C.Disk:KENCO:12/20/85:TAR:LF
Edited by SVH 1/6/86 and 1/22/86

Attachment 1

Plant Metabolites of Alachlor Used to Dose
Lactating Goats and Hens
in
Goat and Hen Metabolism Study

Metabolite		
I	the hydroxyethyl, methylsulfone metabolite of alachlor	
II	2-hydroxy analog of alachlor	
III	oxanilic acid metabolite of alachlor	
IV	sulfonic acid metabolite of alachlor	
V	sulfinyl lactic acid metabolite of alachlor	
Parent	alachlor*	

* The parent is not found in plants.