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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Reregistration of Alachlor: Animal Metabolism Data; Chemical No. 90501; Branch No. 11162; DP Barcode No.: D186613; MRID Nos: 42594901 through 42594904

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Attached is the review of residue chemistry data submitted by Monsanto in response to requirements outlined in the Reregistration Standard Update (7/91). This information was reviewed by Acurex Corporation under supervision of CBRS, HED. The data assessment has undergone secondary review in the branch and has been revised to reflect branch policies.

The animal metabolism data submitted are adequate and no additional data are required. The information in these studies will be presented to the HED Metabolism Committee for a determination of the residues to be regulated. Radiovalidation of the analytical method using tissues from the metabolism study remains outstanding.

If you need additional input please advise.

Attachment 1: Review of Alachlor Residue Chemistry Data

cc: (with Attachment 1): CLOlinger (CBRS), Reg. Std. File, SF, Acurex, Circu., RF

ATTACHMENT I

ALACHLOR
(Chemical Code 090501)
(CBRS No. 11162; DP Barcode D186613)

TASK 3

Registrant's Response to Residue Chemistry Data Requirements

April 14, 1993

Contract No. 68-DO-0142

Submitted to:

U.S. Environmental Protection Agency
Arlington, VA 22202

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ALACHLOR

(Chemical Code 090501)

(CBRS No. 11162; DP Barcode D186613)

REGISTRANT'S RESPONSE TO RESIDUE CHEMISTRY REQUIREMENTS

Task 3

BACKGROUND

The Alachlor Reregistration Standard Update (7/91) required data depicting the metabolism of ring-labeled [^{14}C]alachlor in ruminants and poultry. The Update also required that representative samples from the metabolism studies be analyzed using accepted enforcement methods. Monsanto Agricultural Company submitted protocols for goat and hen metabolism studies that were reviewed by N. Dodd (CB No. 7816, 7/16/91). Because the parent, alachlor, is not found in feed items, the registrant proposed individually feeding two [^{14}C]metabolites, which are representative of the 2,6-diethylaniline and the 2-(1-hydroxyethyl)-6-ethylaniline classes of plant metabolites, to goats and hens at 100x the maximum expected dietary burden. The Agency concluded that the protocols were adequate; the proposed [^{14}C]metabolites adequately represent the two classes of plant metabolites and the feeding of parent would not be necessary.

Monsanto subsequently submitted data (1992; MRIDs 42594901 through 42594904) depicting the metabolism of the two representative alachlor plant [^{14}C]metabolites in lactating goats and laying hens. These submissions are reviewed here to determine their adequacy in fulfilling residue chemistry data requirements. The Conclusions and Recommendations stated herein pertain only to animal metabolism requirements and residue analytical methodology.

The qualitative nature of alachlor residues in plants is adequately understood. Alachlor and its metabolites containing the 2,6-diethylaniline moiety (2,6-DEA) or the 2-(1-hydroxy ethyl)-6-ethylaniline moiety (2,6-HEEA) are the residues of concern. Tolerances for residues in or on raw agricultural commodities are currently expressed in terms of alachlor (2-chloro-2',6'-diethyl-N-(methoxymethyl) acetanilide) and its metabolites calculated as alachlor (40 CFR §180.249).

Methods I(a), I(b), and II are currently available in PAM, Vol. II, but do not recover HEEA yielding residues. An HPLC method, described in the registrant's report no. MSL-5718, which employs oxidative coulometric electrochemical detection of both DEA and HEEA producing residues, has undergone successful Agency validation, and has been submitted to FDA for inclusion in PAM, Vol. II as Method III (W. Chin, CB No. 4299, 12/1/88). The FDA Pestrack database (PAM Vol. I Appendix) dated 11/90 indicates that alachlor is completely recovered through Multiresidue Protocols D and E. In addition, multiresidue

testing of five alachlor metabolites has been submitted and forwarded to FDA (M. Flood, CBTS No. 9358/CBRS No. 9628, 8/11/92).

There are no Codex MRLs, and therefore no compatibility issues with U.S. tolerances.

CONCLUSIONS/RECOMMENDATIONS

1. The submitted goat metabolism study (1992; MRIDs 42594901 and 42594903) is adequate. Goats were fed either a [^{14}C]Alcohol metabolite, N-[(2,6-diethyl)phenyl]-N-methoxymethyl-2-hydroxyacetamide, or a [^{14}C]Sulfone metabolite, N-{[(2-ethyl-6-(1-hydroxyethyl)]phenyl}-N-methoxymethyl-2-(methylsulfonyl)acetamide, which are representative of the 2,6-DEA and 2,6-HEEA classes of plant metabolites, respectively. Radioactive residues in the goat fed the [^{14}C]Alcohol metabolite at 321 ppm (145x) for 3 consecutive days ranged from 0.040 ppm in muscle to 0.537 ppm in liver. The maximum level of residues in milk was 0.468 ppm in the Day-3 pm sample. Radioactive residues in the goat fed the [^{14}C]Sulfone metabolite at 1,077 ppm (490x) for 3 consecutive days ranged from 0.158 ppm in fat to 4.25 ppm in kidney. The maximum level of residues in milk was 1.038 ppm in the Day-3 pm sample. Radioactive residues from both goats consisted of numerous related metabolites each accounting for 0.2-33.6% of the TRR. Aniline containing metabolites identified in the goat fed the [^{14}C]Alcohol metabolite accounted for 36% of the TRR in milk, 10% of the TRR in liver, 45% of the TRR in kidney, 12% of the TRR in muscle, and 25% of the TRR in fat. Aniline containing metabolites identified in the goat fed the [^{14}C]Sulfone metabolite accounted for 58% of the TRR in milk, 56% of the TRR in liver, 65% of the TRR in kidney, 54% of the TRR in muscle, and 52% of the TRR in fat. Both the [^{14}C]Alcohol and [^{14}C]Sulfone test substances were metabolized in a similar manner in goat. The test substances underwent loss of the methoxymethyl group, hydroxylation of ethyl side-chain(s) usually at the benzylic position, and glucuronidation of the hydroxyl groups.

2. The submitted hen metabolism study (1992; MRIDs 42594902 and 42594904) is adequate. Radioactive residues in hens fed the [^{14}C]Alcohol metabolite at 115 ppm (550x) for 3 consecutive days ranged from 0.078 ppm in muscle to 0.559 ppm in liver. Residues in egg yolks and whites increased throughout the dosing period to 0.272 and 0.186 ppm, respectively. Radioactive residues in hens fed the [^{14}C]Sulfone metabolite at 84 ppm (400x) for 3 consecutive days ranged from 0.015 ppm in fat to 0.237 ppm in liver. Residues in egg yolks and whites increased throughout the dosing period to 0.286 and 0.379 ppm, respectively. Aniline containing metabolites identified in hens fed the [^{14}C]Alcohol metabolite accounted for 32% of the TRR in egg yolks, 49% of the TRR in egg whites, 9% of the TRR in liver, 8-13% of the TRR in muscle, and 63-71% of the TRR in skin and fat. Aniline containing metabolites identified in hens fed the [^{14}C]Sulfone metabolite accounted for 39% of the TRR in egg yolks, 28% of the TRR in egg whites, 7% of the TRR in liver, 20-

27% of the TRR in muscle, and 15% of the TRR in skin and fat. With one exception, both the [^{14}C]Alcohol and [^{14}C]Sulfone test substances were metabolized in a similar manner, and the metabolite pathways were similar to those in goats. The test substances underwent loss of the methoxymethyl group, hydroxylation of ethyl side-chain(s) usually at the benzylic position, and glucuronidation of the hydroxyl groups. In hen fat and skin, the Alcohol metabolite also formed fatty acid esters.

3. The registrant must validate the enforcement analytical method using representative samples from the metabolism study to ascertain that the method will adequately recover all residues of concern. If residues other than those included in the 2,6-DEA and 2,6-HEEA classes of metabolites are deemed of toxicological concern, then additional methodology for determining these residues will be required.

DETAILED CONSIDERATIONS

Qualitative Nature of the Residue in Animals

Ruminants. Monsanto submitted data (1992; MRIDs 42594901 and 42594903) pertaining to the metabolism of two alachlor [^{14}C]metabolites in lactating goats. The in-life portion of the goat study (MRID 42594901) was conducted by PTRL East Inc., Richmond, KY, and the analytical portion of the study (MRID 42594903) was conducted by the Agricultural Group of Monsanto, St. Louis, MO. The [^{14}C]Alcohol and [^{14}C]Sulfone metabolites are representative of the 2,6-DEA an the 2,6-HEEA classes of plant metabolites, respectively. Both test substances were ^{14}C-labeled in the phenyl ring and ^{13}C-labeled in a methylene group. The [^{14}C]Alcohol metabolite had a radiochemical purity of 96.5% and a specific activity of 5.5 mCi/mmol (48,426 dpm/μg). The [^{14}C]Sulfone metabolite was a mixture of distereomeric isomers (Sulfone-A and Sulfone-B) that together had a radiochemical purity of 98% and a specific activity of 7.4 mCi/mmol (49,902 dpm/μg).

One goat was dosed with the [^{14}C]Alcohol metabolite at a level of 321 ppm in the diet, which is equivalent to 145x the maximum theoretical dietary exposure based on the established tolerances for peanut and sorghum commodities (Table 1). Another goat was dosed with the [^{14}C]Sulfone metabolite at a level of 1,077 ppm in the diet, which is equivalent to 490x the maximum theoretical dietary exposure. A third goat was used as a control.

Table 1. Calculation of maximum dietary exposures for dairy cattle and laying hens.

	Tolerance (ppm)	% in Diet		Dietary exposure (ppm)	
		Cattle	Poultry	Cattle	Poultry
Peanut forage and hay	3.0	60	-	1.8	-
Sorghum fodder	1.0	40	-	0.4	-
Corn grain	0.2	-	50	-	0.10
Soybeans	0.2	-	35	-	0.07
Sunflower seeds	0.25	-	15	-	0.04
Total		100	100	2.2	0.21

Goats were dosed orally by capsule each morning for 3 consecutive days and were sacrificed within 24 hours of administering the final dose. Milk and urine were collected twice a day, and feces were collected daily. At sacrifice, samples of liver, kidneys, muscle, and fat were collected and were homogenized with dry ice. All samples were stored at approximately -20 °C until analysis. Samples were radioassayed within 1 month of sacrifice.

Samples of milk and liver from the control goat were fortified within 7 days of sacrifice with the [^{14}C]Alcohol and [^{14}C]Sulfone metabolites at approximately 2.5 ppm each. To assess storage stability of radioactive residues, fortified samples and treated samples of liver and milk were extracted as described below, and the extracts were analyzed by HPLC. Fortified samples were initially analyzed by HPLC within 2 months of sacrifice and were reanalyzed approximately 6 months later. Samples of milk and liver from the Alcohol and the Sulfone treated goats were initially analyzed within 39 days of sacrifice and were reanalyzed 3-7 months later. The registrant provided HPLC chromatograms of the initial and final analyses. HPLC chromatograms of the fortified samples indicated that the test substances were stable in storage at -20 °C for up to 6 months. Following 3-7 months of storage at -20 °C, the HPLC chromatograms of ^{14}C-residues in milk and liver samples were similar to chromatograms from the initial analyses. The submitted storage stability data adequately support the metabolism data. HPLC analyses of the actual residue samples were begun approximately 3 months after sacrifice and were completed within 7.5 months of sacrifice.

Total Radioactive Residues (TRR)

Radioactive residues in milk and urine samples were determined directly by liquid scintillation spectrometry (LSS). Radioactive residues in blood and solid fractions were determined by combustion to $^{14}CO_2$ and LSS. Milk and tissue samples were radioassayed in triplicate. Detection limits for both test substances were 0.002 ppm for liver and kidney, 0.003 ppm for muscle, 0.012 ppm for fat, and 0.001 ppm for milk. Total radioactive residues in milk and tissues from the treated goats are reported in Table 2. Sample calculations were provided.

For the [^{14}C]Alcohol metabolite, levels of radioactive residues were lowest in muscle (0.04 ppm) and highest in liver (0.537 ppm). In milk, the highest level of residues was detected in the Day-3 pm sample (0.468 ppm).

For the [^{14}C]Sulfone metabolite, levels of radioactive residues were lowest in fat (0.158 ppm) and highest in kidney (4.25 ppm). In milk, the highest level of residues was detected in the Day-3 pm sample (1.038 ppm).

Table 2. Total radioactive residues in milk and tissues from goats dosed orally for 3 consecutive days with either the [^{14}C]Alcohol metabolite at 321 ppm or the [^{14}C]Sulfone metabolite at 1,077 ppm.

	Total Radioactive Residues (ppm)[a]	
Matrix	[^{14}C]Alcohol Metabolite	[^{14}C]Sulfone Metabolite
Liver	0.537	3.370
Kidney	0.400	4.250
Muscle	0.040	0.403
Fat	0.055	0.158
Milk		
Day-1 pm	0.287	0.632
Day-2 am	0.045	0.350
Day-2 pm	0.396	0.830
Day-3 am	0.049	0.588
Day-3 pm	0.468	1.038
Day-4 am	0.081	0.684

[a]Values are the mean of triplicate analyses and are expressed in terms of the respective test substances.

Extraction and Hydrolysis of Residues

For the analysis of milk from Alcohol and Sulfone treated goats, 25% of the milk from each collection period was composited by goat, and the composited samples were analyzed by LSS. Levels of radioactive residues in composited milk samples from the [^{14}C]Alcohol and [^{14}C]Sulfone treated goats were 0.157 and 0.639 ppm, respectively.

Composite milk samples and homogenized liver, kidney, and muscle samples from both goats were extracted with acetonitrile (ACN)/water (1/1, v/v) and centrifuged. The distribution of ^{14}C-residues in milk and tissues are presented in Table 3. The supernatant fractions were concentrated and ultrafiltered using a 30,000 MW cutoff filter. The resulting filtrate fractions were analyzed by reverse-phase HPLC.

Fat samples from both goats were extracted with chloroform/MeOH/water (8/12/3, v/v/v) and were centrifuged. The resulting chloroform ($CHCl_3$) and MeOH/water fractions were filtered and analyzed by LSS (Table 3). The $CHCl_3$ fraction from fat of each goat was concentrated, redissolved in hexane, and partitioned with ACN. Each ACN fraction was concentrated and reconstituted in isopropyl alcohol/water (2/5, v/v), and the resulting emulsion was analyzed by HPLC. The aqueous fraction (27.1% TRR; 0.007 ppm) from the [^{14}C]Alcohol treated goat was not further analyzed. The aqueous fraction (69% TRR) from the [^{14}C]Sulfone treated goat was concentrated and ultrafiltered, and the filtrate was analyzed by HPLC.

Nonextractable liver solids from [^{14}C]Alcohol and [^{14}C]Sulfone treated goats were refluxed in ACN for 18 hrs. An additional 7.5% and 0.8% of the TRR was extracted from Alcohol and Sulfone liver solids, respectively. Another aliquot of nonextractable liver solids from each goat was enzyme hydrolyzed overnight using a protease (Pronase E) in 10 mM potassium phosphate (pH 7.4) at 37 °C. The resulting hydrolysates were centrifuged and analyzed by LSS (Table 3). Protease hydrolysis released an additional 44% and 12.5% of the TRR from [^{14}C]Alcohol and [^{14}C]Sulfone treated goats, respectively. The resulting supernatants were ultrafiltered, and the filtrates (Alcohol - 41.7% TRR; Sulfone - 9.8% TRR) were further analyzed by HPLC.

Nonextractable muscle solids (23.3% TRR; 0.007 ppm) from the Alcohol treated goat were also hydrolyzed with protease overnight at 37 °C and centrifuged. The resulting supernatant accounted for 18.3% of the TRR. The supernatant was ultrafiltered, and the aqueous filtrate was partitioned with ethyl acetate (EtOAc) and methylene chloride (DCM) at pH 7.4, 4, and 1. In each case, <1% of the TRR partitioned into the organic solvents. The Alcohol muscle hydrolysate was not further analyzed.

Characterization of Residues

Radioactive residues in solvent extracts from milk and tissues were characterized and quantified by reverse-phase HPLC. Reference standards were detected using a UV detector and ^{14}C-residues were detected by LSS of eluant fractions. Example chromatograms and sample calculations were provided.

HPLC analysis of soluble residues from the [^{14}C]Alcohol treated goat isolated 17 distinct fractions each accounting for 0.5-29.2% of the TRR in milk and tissues (Table 4). HPLC analysis of soluble residues from the [^{14}C]Sulfone treated goat isolated 26 distinct fractions each accounting for 0.2-33.6% of the TRR in milk and tissues (Table 5).

Table 3. Distribution of radioactive residues in milk and tissues from goats dosed orally for 3 consecutive days with either the [^{14}C]Alcohol metabolite at 321 ppm (145x) or the [^{14}C]Sulfone metabolite at 1,077 ppm (490x).

Radioactive Residues from [^{14}C]Alcohol Metabolite

Matrix	Milk (0.146 ppm)[a]		Liver (0.504 ppm)		Kidney (0.489 ppm)		Muscle (0.031 ppm)		Fat (0.026 ppm)	
Fraction	%TRR[b]	PPM	%TRR	PPM	%TRR	PPM	%TRR	PPM	%TRR	PPM
ACN/Water[c]	(98.3)[d]	(0.143)	(42.3)	(0.213)	(96.0)	(0.470)	(76.7)	(0.024)	-[e]	-
$CHCl_3$	-	-	-	-	-	-	-	-	(68.1)[c]	(0.018)
MeOH/water	-	-	-	-	-	-	-	-	(27.1)	(0.007)
Solids	1.7	0.003	57.7	0.291	4.0	0.019	23.3	0.007	4.8	0.001
Proteolysate	-	-	(44.0)[c]	(0.222)	-	-	(18.3)	(0.006)	-	-
Solids	-	-	13.7	0.069	-	-	3.2	0.001	-	-
Total Extracted	98.3	0.143	86.3	0.435	96.0	0.470	95.0	0.030	95.2	0.025

Radioactive Residues from [^{14}C]Sulfone Metabolite

Matrix	Milk (0.629 ppm)		Liver (3.662 ppm)		Kidney (4.510 ppm)		Muscle (0.403 ppm)		Fat (0.161 ppm)	
Fraction	%TRR	PPM	%TRR	PPM	%TRR	PPM	%TRR	PPM	%TRR	PPM
ACN/Water[c]	(98.5)	(0.619)	(84.5)	(3.095)	(98.2)	(4.428)	(93.4)	(0.376)	-	-
$CHCl_3$	-	-	-	-	-	-	-	-	(29.0)[c]	(0.047)
MeOH/Water	-	-	-	-	-	-	-	-	(69.0)[c]	(0.111)
Solids	1.5	0.010	15.5	0.567	1.8	0.081	6.6	0.026	2.0	0.003
Proteolysate	-	-	(12.5)[c]	(0.458)	-	-	-	-	-	-
Solids	-	-	3.0	0.109	-	-	-	-	-	-
Total Extracted	98.5	0.619	97.0	3.553	98.2	4.428	93.4	0.376	98.0	0.158

[a]TRR in each matrix is the amount of radioactivity recovered (Extract+solids). [b]The registrant normalized values for the percent recovery. [c]Fractions analyzed by HPLC. [d]Values in parentheses were summed to calculate the total extracted. [e]Fraction not applicable to this matrix.

For further characterization of Alcohol (A-#) and Sulfone (S-#) HPLC fractions, preparative HPLC was used to isolate larger quantities of individual fractions from extracts of Alcohol treated goat liver, kidney, and milk, and Sulfone treated goat liver, kidney, muscle, and milk. In addition, extracts of Day-3 goat urine from Alcohol (1,380 ppm) and Sulfone (430 ppm) treated goats were used as sources for fractions, along with Alcohol treated hen excreta (90 ppm) from the poultry metabolism study discussed below. Extract fractions used for preparative HPLC were extracted and purified using the same procedures described above.

Fractions isolated by preparative HPLC were analyzed using a variety of mass spectrometry techniques (MS), high voltage electrophoresis (HVE), HPLC, and TLC. To provide further information about the structure of the aniline moiety in each metabolite, isolated metabolite fractions were also subjected to strong acid hydrolysis (SAH; 6 N HCl at 150 °C for 2 hrs) and analyzed by HPLC along with reference standards. Isolated metabolite fractions were also hydrolyzed with glucuronidase (sodium acetate buffer at pH 5 at 37 °C for 1.5 hrs) and analyzed by HPLC. The actual techniques used to characterize individual fractions and the source of the isolated fractions are presented in Table 6. Chemical names and structures of identified metabolites are presented in Figure 1.

Of the fractions derived from the [^{14}C]Alcohol treated goat, fraction A-23 was identified as the test substance. Fractions A-11 and A-12 were respectively identified as 1- and 2-hydroxyethyl metabolites of the test substance, and fraction A-18 was identified as an oxanilic acid metabolite. Fraction A-20 was identified as a metabolite lacking the methoxymethyl group of the test substance. Fractions A-21 and A-16 were identified as glucuronic acid conjugates of A-23 and A-20.

Of the fractions derived from the [^{14}C]Sulfone treated goat, fraction S-24 was identified as the Sulfone test substance. Fraction S-3 was identified as a related metabolite hydroxylated in the 1-position of both ethyl side chains, and S-16 was identified as a metabolite lacking the methoxymethyl group of test substance. Fractions S-15, S-20, and S-22 were identified as stereoisomers of a glucuronic acid conjugate of the test substance, and fractions S-4, S-6, and S-13 were identified as stereoisomers of the glucuronic acid conjugate of S-3. HPLC analysis of isolated S-6 also suggested that fraction S-5 is an interconvertable stereoisomer of S-6. Fraction S-11 was identified as the glucuronic acid conjugate of S-16.

To confirm the identity of HPLC fractions in each matrix, fractions from each matrix were isolated by HPLC and cochromatographed by TLC with the respective HPLC fractions, which had been previously identified.

Radioactive residues in the filtered proteolysate fractions from liver (Alcohol - 41.7% TRR; Sulfone - 9.8% TRR) were also characterized and quantified by reverse-phase HPLC. HPLC analysis of the liver proteolysate from the [^{14}C]Alcohol treated goat isolated 7 distinct fractions (AP-#) each accounting for 1.6-4.8% of the TRR (Table 4). However, the majority of radioactivity (24.9% TRR) was accounted for in numerous unresolved peaks. In the Sulfone liver proteolysate, the majority of radioactivity (6.9% TRR) was also accounted for

by minor unresolved peaks and two distinct HPLC fractions (SP-1 and SP-2), which accounted for 2.2 and 1% of the TRR (Table 5).

Liver proteolysate fractions AP-6 and AP-7 had the same HPLC retention times as fractions A-16 and A-20, respectively. Cochromatography of AP-7 and A-20 by TLC confirmed that both fractions were the same metabolite. However, analysis of AP-6 and A-16 by HVE indicated that these fractions contained different metabolites. Fraction AP-6 was not further analyzed. Fractions AP-1 through AP-5 did not cochromatograph with any of the identified HPLC fractions. SAH/HPLC analysis of these fractions also indicated that they were composed of numerous components, with the exception of AP-5. Fraction AP-5 (2.4% TRR) was converted solely to DEA by SAH. Acetylation and subsequent HPLC analysis of liver proteolysate fractions SP-1 and SP-2 indicated that these fractions were not discrete metabolites, but were composed of mixtures of metabolites; these fractions were not further analyzed.

Goat Metabolism Summary

The submitted goat metabolism study is adequate. Radioactive residues in the goat fed the [^{14}C]Alcohol metabolite at 321 ppm (145x) for 3 consecutive days ranged from 0.040 ppm in muscle to 0.537 ppm in liver. In milk, the highest level of residues was detected in the Day-3 pm sample (0.468 ppm). Radioactive residues in the goat fed the [^{14}C]Sulfone metabolite at 1,077 ppm (490x) for 3 consecutive days ranged from 0.158 ppm in fat to 4.25 ppm in kidney. In milk, the highest level of residues was detected in the Day-3 pm sample (1.038 ppm).

With the exceptions of liver and muscle from the Alcohol treated goat, >90% of the ^{14}C-residues in tissues and milk were soluble. Following enzymatic hydrolysis, >86% of the TRRs were extracted from liver and muscle.

Solvent extractable residues from both goats consisted of numerous Alcohol and Sulfone related metabolites each accounting for 0.2-33.6% of the TRR. Both the Alcohol and Sulfone test substances were metabolized in a similar manner in goat. Both test substances underwent loss of the methoxymethyl group, hydroxylation of the ethyl side-chain(s), usually at the benzylic position, and glucuronidation of the hydroxyl groups. All of the identified metabolites contain the aniline moiety with both ethyl side chains, which are either not hydroxylated, hydroxylated in one ethyl group, or hydroxylated in both ethyl groups.

Table 4. Characterization of HPLC fractions from milk and tissue extracts and liver proteolysate from a goat fed [^{14}C]Alcohol metabolite at 321 ppm (145x) for 3 consecutive days.

HPLC Fraction[a]	SAH Product[b]	Milk[c] %TRR	Milk[c] PPM[e]	Liver %TRR	Liver PPM	Kidney %TRR	Kidney PPM	Muscle %TRR	Muscle PPM	Fat[d] %TRR	Fat[d] PPM
A-1		3.7	0.005	6.8	0.034	3.0	0.015	10.6	0.003		
A-2	A	22.3	0.033			3.1	0.015	23.4	0.007		
A-3	A			2.4	0.012	2.2	0.011				
A-6						2.2	0.011				
A-7	UNK-1	8.9	0.013	0.5	0.003	2.1	0.010	6.5	0.002		
A-9								1.5	<0.001		
A-11	**EA**	**8.5**	**0.012**	**0.5**	**0.002**			**2.5**	**0.001**		
A-12	**EI**	**14.7**	**0.021**	**0.6**	**0.003**						
A-13	UNK-1			1.2	0.006	8.2	0.040	1.9	0.001		
A-14		3.1	0.005					1.5	<0.001		
A-16	**DEA**	**1.8**	**0.003**	**1.0/2.5**	**0.005/0.012**	**9.2**	**0.045**	**1.8**	**0.001**		
A-18	**DEA**					1.1	**0.005**				
A-19						2.0	0.010				
A-20/ AP-7	**DEA**			**0.9/2.6**	**0.005/0.013**	**1.3**	**0.006**				
A-21	**DEA**	**10.9**	**0.016**	**2.6**	**0.013**	**29.2**	**0.143**	**6.3**	**0.002**		
A-22				1.7	0.008					13.3	0.003
A-23	**DEA**			**2.1**	**0.011**	3.7	0.018	1.3	<0.001	**25.0**	**0.007**
AP-1				4.8	0.023						
AP-2	A, EA, DEA, others			2.1	0.010						
AP-3	A, EA, others			1.7	0.008						
AP-4				1.6	0.008						
AP-5	DEA			2.4	0.011						
AP-6				2.5	0.012						
Total Identified		35.9	**0.052**	**10.3**	**0.052**	**44.5**	**0.217**	**11.9**	**0.004**	**25.0**	**0.007**
Total Characterized		73.9	0.108	38.1	0.186	67.3	0.329	57.3	0.018	38.3	0.010

[a]HPLC fractions resulting from the feeding of [^{14}C]Alcohol metabolite are numbered in order of elution (fractions designated AP-# are from the liver proteolysate). Fractions in **bold** were identified. [b]Products of strong acid hydrolysis, A = aniline; Unk = unknown chemophore; EA = 2-ethylaniline; EI = 7-ethylindoline; and DEA = 2,6-diethylaniline. [c]Composite milk sample. [d]Data from analysis of the organic fat extract. [e]Expressed in equivalents of the [^{14}C]Alcohol metabolite.

Table 5. Characterization of HPLC fractions from milk and tissue extracts and liver proteolysate from a goat fed [^{14}C]Sulfone metabolite at 1,077 ppm (409x) for 3 consecutive days.

HPLC Fraction[a]	SAH product[b]	Milk[c]		Liver		Kidney		Muscle		Fat[d]	
		%TRR[e]	PPM	%TRR	PPM	%TRR	PPM	%TRR	PPM	%TRR	PPM
S-1						1.1	0.050				
S-2		2.1	0.013	1.2	0.045	1.8	0.081				
S-3	**A**	**3.0**	**0.019**	**1.3**	**0.046**	**3.6**	**0.164**	**2.6**	**0.011**	**1.5**	**0.002**
S-4	**A**			**4.8**	**0.175**	**10.5**	**0.476**	**2.6**	**0.011**	**4.7**	**0.008**
S-5	A			2.0	0.073	2.4	0.108	2.7	0.011		
S-6	**A**			**1.9**	**0.068**	**2.7**	**0.121**			**2.2**	**0.003**
S-7		3.5	0.022			1.3	0.060	2.0	0.008		
S-8						1.6	0.073				
S-9						1.6	0.073				
S-10		6.4	0.040			1.7	0.074	3.7	0.015	1.3	0.002
S-11	**EA**	**4.8**	**0.031**	**4.0**	**0.146**	**7.5**	**0.339**	**2.9**	**0.012**	**5.1**	**0.008**
S-12	UNK-1					0.8	0.037				
S-13	**A**			**3.2**	**0.119**	**4.7**	**0.212**			**2.0**	**0.003**
S-14						0.7	0.034				
S-15	**EA**	**4.1**	**0.026**	**2.4**	**0.088**	**8.5**	**0.382**	**2.6**	**0.010**	**7.2**	**0.012**
S-16	**EA**	**33.2**	**0.209**	**7.6**	**0.279**	**11.2**	**0.504**	**33.6**	**0.136**	**15.4**	**0.025**
S-17				1.7	0.062	2.2	0.098				
S-18		2.0	0.013	1.4	0.052	2.7	0.122	1.3	0.005	2.1	0.003
S-19	UNK-2	3.1	0.020	1.2	0.045	1.8	0.079	2.6	0.011		
S-20	**EA**	**3.8**	**0.024**	**22.1**	**0.810**	**11.6**	**0.521**	**3.3**	**0.013**	**7.9**	**0.013**
S-21				4.8	0.177	3.5	0.157				
S-22	**EA**	**4.2**	**0.026**	**7.1**	**0.261**	**4.1**	**0.184**	**2.4**	**0.010**		
S-23				1.2	0.045	0.2	0.011				
S-24	**EA**	**4.5**	**0.028**	**1.1**	**0.041**	**0.7**	**0.033**	**3.5**	**0.014**	**6.2**	**0.010**
S-25						0.4	0.016			2.1	0.003
S-26						0.3	0.012			1.3	0.002
SP-1/ SP-2[f]	EA			3.2	0.091						
Total Identified		**57.6**	**0.363**	**55.5**	**2.033**	**65.1**	**2.936**	**53.5**	**0.214**	**52.2**	**0.084**
Total Characterized		74.8	0.470	72.3	2.623	89.2	4.021	65.8	0.265	59.0	0.095

[a]HPLC fractions resulting from the feeding of [^{14}C]Sulfone metabolite are numbered in order of HPLC elution; fractions in **bold** were identified. [b]Products of strong acid hydrolysis, A = aniline; Unk = unknown chemophore; EA = 2-ethylaniline; and DEA = 2,6-diethylaniline. [c]Composite milk sample. [d]Combined data from HPLC analysis of the organic and aqueous fat extracts. [e]Expressed in equivalents of the [^{14}C]Sulfone metabolite. [f]Two HPLC fractions (0.2-2.2% TRR) from analysis of the liver proteolysate.

Table 6. Characterization procedures for Alcohol and Sulfone related metabolites isolated by HPLC.

HPLC Fraction[a]	Matrix[b]	Metabolite Characterization[c]	Identified[d]
A-1	hen excreta, goat liver and kidney	MS, SAH/HPLC, TLC, HVE, and HPLC	
A-2	milk	MS, SAH/HPLC, and HPLC	
A-3	hen excreta	TLC, HPLC, and SAH/HPLC	
A-7	hen excreta	MS, SAH/HPLC, TLC, and HPLC	
A-11	hen excreta	MS, SAH/HPLC, TLC, and HPLC, cochromatography with reference standard	✓
A-12	hen excreta	MS, SAH/HPLC, TLC, and HPLC	✓
A-13	hen excreta	MS, SAH/HPLC, HPLC, and TLC	
A-16	goat urine	MS, SAH/HPLC, TLC, HPLC, HVE, and HPLC analysis of glucuronidase hydrolysate	✓
A-18	hen excreta	MS, SAH/HPLC, TLC, HVE, and HPLC	✓
A-20	goat urine	MS, SAH/HPLC, TLC, and HPLC, cochromatography with reference standard	✓
A-21	goat urine	MS, SAH/HPLC, TLC, HVE, and HPLC analysis of glucuronidase hydrolysate	✓
A-23	goat urine	MS, SAH/HPLC, TLC, and HPLC, cochromatography with reference standard	✓
A-25	hen skin	MS, SAH/HPLC, HPLC of base hydrolysate, cochromatography with reference standard	✓
A-26	hen skin	MS, SAH/HPLC, HPLC of base hydrolysate, cochromatography with reference standard	✓
AP-1, -2, -3, and -5	goat liver	HPLC, and SAH/HPLC	
AP-6	goat liver	HPLC, SAH/HPLC, and HVE	
AP-7	goat liver	HPLC, TLC, SAH/HPLC, and cochromatography with A-20	✓
S-3	goat urine	MS, SAH/HPLC, TLC, HVE, and HPLC	✓
S-4	goat urine	MS, SAH/HPLC, TLC, and HPLC of glucuronidase hydrolysate	✓
S-6	goat urine	MS, SAH/HPLC, TLC, and HPLC of glucuronidase hydrolysate	✓
S-11	kidney	MS, SAH/HPLC, TLC, and HPLC of glucuronidase hydrolysate	✓
S-12	goat urine	MS, SAH/HPLC, TLC, and HPLC	
S-13	goat urine	MS, SAH/HPLC, TLC, and HPLC of glucuronidase hydrolysate	✓
S-15	goat urine	MS, SAH/HPLC, TLC, and HPLC of glucuronidase hydrolysate	✓

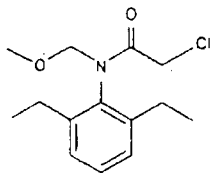
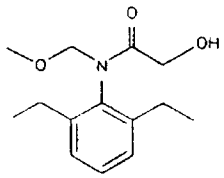
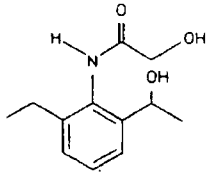
Continued

Table 6. (continued)

HPLC Fraction[a]	Matrix[b]	Metabolite Characterization[c]	Identified[d]
S-16	goat urine	MS, SAH/HPLC, TLC, and HPLC, cochromatography with reference standard	✓
S-19	goat urine	MS, SAH/HPLC, TLC, and HPLC	
S-20	goat urine	MS, SAH/HPLC, and HPLC of glucuronidase hydrolysate	✓
S-22	goat urine	MS, SAH/HPLC, and HPLC of glucuronidase hydrolysate	✓
S-24	goat urine	SAH/HPLC, TLC, and HPLC with a reference standard	✓
SP-1 and -2	liver	HPLC, SAH/HPLC, and acetylation/HPLC	

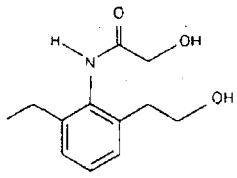
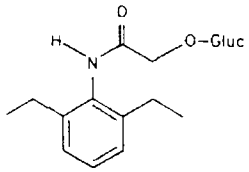
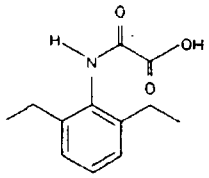
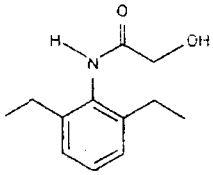
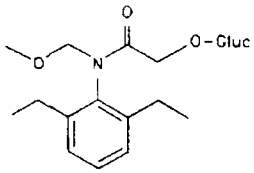
[a]Metabolite fractions isolated by HPLC. [b]Matrix from which fractions were isolated for characterization/identification. [c]Methods used for characterizing metabolite fractions. [d]The "✓" indicates which fractions were identified.

Figure 1. Chemical name and structure of alachlor, the Alcohol and Suflone metabolites and their metabolites identified in milk, eggs, and tissues of goats and hens.

Chemical names	Chemical Structure	Matrices[a]
Alachlor 2-chloro-2',6'-diethyl-N-(methoxymethyl)-acetanilide		
Alcohol metabolite (A-23) N-[(2,6-diethyl)phenyl]-N-methoxymethyl-2-hydroxyacetamide		Goat fat, kidney, liver, muscle, urine Hen liver, muscle, skin, fat, egg yolks
A-11 N-{[2-ethyl-6-(1-hydroxyethyl)]phenyl}-2-hydroxyacetamide		Goat liver, milk, muscle Hen liver, muscle, eggs, excreta

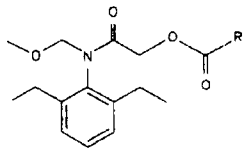
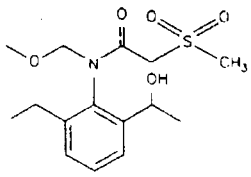
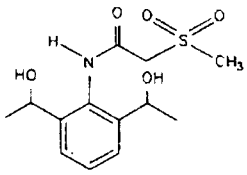
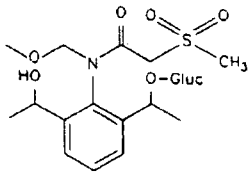
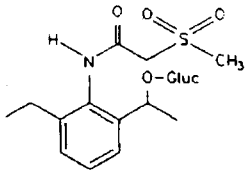
Continued

Figure 1. (continued)

Chemical names	Chemical Structure	Matrices[a]
A-12 N-{[2-ethyl-6-(2-hydroxyethyl)]phenyl}-2-hydroxyacetamide		Goat liver, milk Hen liver, muscle, eggs, excreta
A-16 Glucuronic acid (Gluc) conjugate of A-20		Goat kidney, liver, milk, muscle, urine Hen breast muscle, liver
A-18 N-[(2,6-diethyl)phenyl]-oxanilic acid		Goat kidney Hen excreta
A-20/AP-7 N-[(2,6-diethyl)phenyl]-2-hydroxyacetamide		Goat kidney, liver, urine Hen liver, eggs, breast muscle, skin
A-21 Glucuronic acid conjugate of the Alcohol metabolite (A-23)		Goat kidney, liver, milk, muscle, urine Hen breast muscle, liver

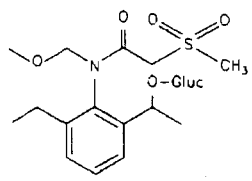
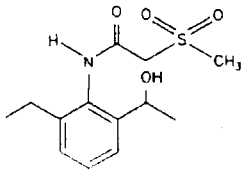
Continued

Figure 1. (continued)

Chemical names	Chemical Structure	Matrices[a]
A-25 and A-26 Linoleic (A-25) and oleic (A-26) acid esters of the Alcohol metabolite (A-23)	 A-25; R - $(CH_2)_4CH=CHCH_2CH=CH(CH_2)_4CH_3$ A-26; R - $(CH_2)_7CH=CH(CH_2)_7CH_3$	Hen fat, skin
Sulfone metabolite (S-24) N-{[2-ethyl-6-(1-hydroxyethyl)]phenyl}-N-methoxymethyl-2-(methyl-sulfone)acetamide		Goat fat, kidney, liver, milk, muscle Hen liver, muscle, skin, eggs
S-3 N-{[(2,6-(1-hydroxyethyl)]phenyl}-N-methoxymethyl-2-(methyl-sulfone)acetamide		Goat fat, kidney, liver, milk, muscle, urine Hen liver, muscle, eggs
S-4, S-6, and S-13 Stereoisomers of a glucuronic acid conjugate of S-3		Goat fat, kidney, muscle, liver, urine Hen liver, muscle, eggs
S-11 Glucuronic acid conjugate of S-16		Goat fat, kidney, liver, milk, muscle Hen .liver

Continued

Figure 1. (continued)

Chemical names	Chemical Structure	Matrices[a]
S-15, S-20, and S-22 Stereoisomers of a glucuronic acid conjugate of the Sulfone metabolite (S-24)		Goat kidney, liver, milk, muscle, fat, urine
S-16 N-{[2-ethyl-6-(1-hydroxyethyl)]phenyl}-2-(methyl-sulfone)acetamide		Goat fat, kidney, liver, milk, muscle, urine Hen liver, muscle, skin, eggs

Poultry. Monsanto submitted data (1992; MRIDs 42594902 and 42594904) pertaining to the metabolism of the [^{14}C]phenyl-labeled Alcohol and Sulfone metabolites of alachlor in laying hens. Both test substances were also ^{13}C-labeled in a methylene group. The [^{14}C]Alcohol metabolite had a radiochemical purity of 96.5% and a specific activity of 7.82 mCi/mmol (69,042 dpm/μg). The [^{14}C]Sulfone metabolite was a mixture of distereomeric isomers (Sulfone-A and Sulfone-B) that together had a radiochemical purity of 98.5% and a specific activity of 10.75 mCi/mmol (72,372 dpm/μg). The in-life portion of the hen study (MRID 42594902) was conducted by PTRL East Inc., Richmond, KY, and the analytical portion of the study (MRID 42594904) was conducted by the Agricultural Group of Monsanto, St. Louis, MO.

One group of five hens was dosed with the [^{14}C]Alcohol metabolite at a level of 115 ppm in the diet, which is equivalent to 550x the maximum theoretical dietary exposure based on the established tolerances for corn, soybean, and sunflower commodities (Table 1). A second group of five hens was dosed with the [^{14}C]Sulfone metabolite at a level of 84 ppm in the diet, which is equivalent to 400x the maximum theoretical dietary exposure. A third group of five hens was used as controls.

Hens were dosed orally by capsule each morning for 3 consecutive days. Eggs were collected twice a day and pooled by dose group. Excreta samples were collected daily and pooled by dose group. Hens were sacrificed within 24 hours of administering the final dose, and samples of liver, kidneys, breast and thigh muscle, fat, skin, and immature egg yolks were collected and composited

by dose group. Composited tissue samples were homogenized with dry ice. Eggs were separated into yolks and whites and were composited by dose group and collection period. All samples were stored at approximately -20 °C until analysis. Samples were radioassayed within 1 month of sacrifice.

Control samples of egg yolk and white were fortified approximately a week after sacrifice with the [^{14}C]Alcohol and [^{14}C]Sulfone test substances, respectively, at unspecified levels. To assess storage stability of radioactive residues, spiked samples and treated samples of liver and eggs were extracted as described below, and the extracts were analyzed by HPLC. Fortified control samples were initially analyzed by HPLC approximately 1 month after sacrifice and were reanalyzed approximately 6 months later. Samples of liver and egg whites from the Alcohol and the Sulfone treated hens were initially analyzed within 1 month of sacrifice and were reanalyzed 1.5-6.5 months later. The registrant provided HPLC chromatograms of the initial and later analyses.

HPLC chromatograms of the spiked samples indicated that the test substances were stable in storage at -20 °C for up to 6 months. Following 1.5-6.5 months of storage at -20 °C, the HPLC chromatograms of ^{14}C-residues in liver and egg white samples were similar to chromatograms from the initial analyses. The submitted storage stability data adequately support the metabolism data. HPLC analyses of the actual residue samples were begun approximately 3 months after sacrifice and were completed within 7 months of sacrifice.

Total Radioactive Residues (TRR)

Radioactive residues in samples were determined in triplicate by combustion to $^{14}CO_2$ and LSS. Detection limits for radioassays were $\leq$0.004 ppm for liver, kidney, muscle, skin, and egg whites, 0.011 ppm for egg yolks, and 0.006-0.007 ppm for fat and excreta. Total radioactive residues in eggs and tissues from the treated hens are reported in Table 7.

For the [^{14}C]Alcohol metabolite, levels of radioactive residues were lowest in muscle (0.078 ppm) and highest in liver (0.559 ppm). Radioactive residues in both egg yolks and whites increased throughout the dosing period to 0.176 and 0.186 ppm, respectively, at sacrifice. For the Sulfone metabolite, levels of radioactive residues were lowest in fat (0.015 ppm) and highest in liver (0.237 ppm). Radioactive residues in both egg yolks and whites increased throughout the dosing period to 0.286 and 0.379 ppm, respectively, at sacrifice.

Table 7. Total radioactive residues in eggs and tissues from hens dosed orally with either the [^{14}C]Alcohol metabolite at 115 ppm or the [^{14}C]Sulfone metabolite at 84 ppm for 3 consecutive days.

	Total Radioactive Residues (ppm)[a]	
Matrix	[^{14}C]Alcohol Metabolite	[^{14}C]Sulfone Metabolite
Liver	0.559	0.237
Kidney	0.275	0.206
Muscle		
breast	0.095	0.066
thigh	0.078	0.050
Skin	0.339	0.038
Fat	0.094	0.015
Eggs yolk		
Day-1	0.039	0.036
Day-2	0.054	0.176
Day-3	0.176	0.254
Sacrifice[b]	0.272	0.286
Egg white		
Day-1	0.105	0.174
Day-2	0.112	0.454
Day-3	0.186	0.379

[a]Values are the mean of triplicate analyses and are expressed in terms of the respective metabolites. [b]Immature yolks were collected from hens after sacrifice.

Extraction and Hydrolysis of Residues

For the analysis of egg yolks and whites from Alcohol and Sulfone treated hens, 25% of the egg yolks and whites from each collection period were composited by dose group, and the composited samples were analyzed by LSS. Levels of radioactive residues from the [^{14}C]Alcohol and [^{14}C]Sulfone treated hens were 0.166 and 0.230 ppm, respectively, in the composited yolk samples, and were 0.149 and 0.393 ppm, respectively, in the composited egg white samples.

Liver, muscle, and egg yolk and white samples were extracted 3 times with ACN/water (1/1, v/v) and centrifuged. The distribution ^{14}C-residues in eggs and tissues is presented in Table 8. The Supernatant fractions were concentrated and ultrafiltered using a 30,000 MW cutoff filter. The resulting filtrates were analyzed by reverse-phase HPLC.

Fat and skin samples from hens were extracted with $CHCl_3$/MeOH/water (8/12/3, v/v/v) and were centrifuged. The resulting MeOH/water fractions were radioassayed and not further analyzed. The $CHCl_3$ fractions were concentrated, redissolved in hexane, and partitioned with ACN (Table 8). Each ACN fraction was concentrated and reconstituted in isopropyl alcohol/water (2/5, v/v). The resulting fractions were analyzed by HPLC, with the exception of the Sulfone fat fraction which contained low levels of residues (0.005 ppm). Levels of ^{14}C-residues were $\leq$0.02 ppm in aqueous fractions from skin and fat and were not further analyzed.

Table 8. Distribution of radioactive residues in eggs and tissues from hens dosed orally for 3 consecutive days with either the [^{14}C]Alcohol metabolite at 115 ppm (550x) or the [^{14}C]Sulfone metabolite at 84 ppm (400x).

Radioactive Residues from [^{14}C]Alcohol Metabolite

Matrix	Egg yolks[a] (0.168 ppm)[b]		Egg whites[a] (0.153 ppm)		Liver (0.531 ppm)		Breast muscle (0.078 ppm)		Thigh muscle (0.080 ppm)		Skin (0.307 ppm)		Fat (0.084 ppm)	
Fraction	%TRR[c]	PPM	%TRR	PPM	%TRR	PPM	%TRR	PPM	%TRR	PPM	%TRR	PPM	%TRR	PPM
ACN/Water[d]	(71.4)[e]	(0.120)	(97.0)	(0.148)	(39.6)	(0.210)	(69.1)	(0.054)	(47.1)	(0.038)	-[f]	-	-	-
$CHCl_3$	-	-	-	-	-	-	-	-	-	-	(80.2)[d]	(0.246)	(83.0)[d]	(0.070)
MeOH/water	-	-	-	-	-	-	-	-	-	-	(6.5)	(0.020)	(13.4)	(0.011)
Solids	28.6	0.048	3.0	0.005	60.4	0.321	30.9	0.024	52.9	0.042	13.3	0.041	3.6	0.003
Proteolysate	(19.4)	(0.033)	-	-	(50.2)	(0.267)	(18.7)	(0.015)	(32.0)	(0.026)	(5.5)	(0.017)	-	-
Filtrate	19.0[g]	0.032	-	-	48.4[d]	0.257	18.5[g]	0.015	31.6[g]	0.026	5.5[g]	0.017	-	-
Solids	9.2	0.015	-	-	10.2	0.054	12.2	0.009	20.9	0.016	7.8	0.024	-	-
Total Extracted	90.8	0.153	97.0	0.148	89.8	0.477	87.8	0.069	79.1	0.064	92.2	0.283	96.4	0.081

Radioactive Residues from [^{14}C]Sulfone Metabolite

Matrix	Egg yolks[a] (0.228 ppm)		Egg whites[a] (0.394 ppm)		Liver (0.259 ppm)		Breast muscle (0.078 ppm)		Thigh muscle (0.049 ppm)		Skin (0.042 ppm)		Fat (0.012 ppm)	
Fraction	%TRR	PPM	%TRR	PPM	%TRR	PPM	%TRR	PPM	%TRR	PPM	%TRR	PPM	%TRR	PPM
ACN/Water[d]	(83.1)	(0.189)	(98.5)	0.388	(40.9)	(0.106)	(69.9)	(0.055)	(53.6)	(0.026)	-	-	-	-
$CHCl_3$	-	-	-	-	-	-	-	-	-	-	(23.2)[d]	(0.010)	(40.8)	(0.005)
MeOH/Water	-	-	-	-	-	-	-	-	-	-	(28.4)	(0.012)	(38.2)	(0.004)
Solids	16.9	0.039	1.5	0.006	59.1	0.153	30.1	0.023	46.4	0.023	48.4	0.020	21.0	0.003
Proteolysate	(11.3)	(0.026)	-	-	(48.8)	(0.126)	(24.4)	(0.019)	(37.1)	(0.018)	(32.1)	(0.014)	-	-
Filtrate	8.5[d]	0.020	-	-	40.3[d]	0.104	22.7[g]	0.018	33.2[g]	0.016	31.8[g]	0.014	-	-
Solids	5.6	0.013	-	-	10.3	0.027	5.7	0.004	9.3	0.005	16.3	0.006	-	-
Total Extracted	94.4	0.215	98.5	0.388	89.7	0.232	94.3	0.074	90.7	0.044	83.7	0.036	79.0	0.009

[a]Composited sample. [b]TRR in each matrix is the amount of radioactivity recovered (Extract+solids). [c]The registrant normalized values for the percent recovery. [d]Fractions analyzed by HPLC. [e]Values in parentheses were summed to calculate the total extracted. [f]Fraction not applicable to this matrix. [g]Fractions subjected to solvent partitioning.

Nonextractable solids from liver, egg yolks, skin, breast and thigh muscle from [^{14}C]Alcohol and [^{14}C]Sulfone treated hens were enzyme hydrolyzed using a protease (Pronase E) in 10 mM potassium phosphate (pH 7.4) at 37 °C for 24 hours. The resulting proteolysates were centrifuge and radioassayed (Table 8). Protease hydrolysis released an additional 5.5-48.4% of the TRR from yolks and tissues. The resulting supernatants were ultrafiltered (10,000 MW cutoff filter), and the filtrates from liver and egg yolk (Sulfone treated only) were concentrated and analyzed by HPLC. Filtrates from muscle, skin, and egg yolk (Alcohol treated) proteolysates were partitioned sequentially with DCM at pH 7.4, 12, and 1, and additional aliquots of these filtrates were partitioned sequentially with EtOAc at pH 7.4, 12, and 1. The registrant stated that the total organosoluble residues extracted from the filtrates comprised 8.1-28.4% (Alcohol-treated matrices) and 5.2-17.2% (Sulfone-treated matrices) of the radioactivity in the filtrates; these filtrate fractions were not further analyzed.

Characterization of Residues

Radioactive residues in solvent extracts from eggs and tissues were characterized and quantified by reverse-phase HPLC. Reference standards were detected using a UV detector at 254 nm and ^{14}C-residues were detected using a radiodetector or by LSS of eluant fractions. Example chromatograms and sample calculations were provided.

HPLC analysis of soluble residues from the [^{14}C]Alcohol treated hens isolated 23 distinct fractions each accounting for 0.5-23.1% of the TRR in eggs and tissues (Table 9). HPLC analysis of soluble residues from the [^{14}C]Sulfone treated hens isolated 19 distinct fractions each accounting for 0.4-44.4% of the TRR in eggs and tissues (Table 10).

For further characterization of Alcohol- and Sulfone-related HPLC fractions, preparative HPLC was used to isolate larger quantities of individual fractions from extracts of Alcohol treated hen liver, yolks, and excreta, and Sulfone treated hen liver and egg whites. In addition, extracts of Day-3 goat urine from Alcohol and Sulfone treated goats were used as sources for HPLC fractions. Extract fractions used for preparative HPLC were extracted and purified using the same procedures described above.

HPLC fractions were analyzed using a variety of MS techniques, NMR, HVE, HPLC, and TLC. To provide further information about the structure of the aniline moiety in each metabolite, isolated fractions were also subjected to SAH and analyzed by HPLC. Isolated fractions were also hydrolyzed with glucuronidase (as described above), and the hydrolysates were analyzed by HPLC.

Alcohol- and Sulfone-related metabolites identified in eggs and tissues are listed in Tables 8 and 9, respectively, and are presented in Figure 1. The designation of HPLC fractions in the hen metabolism study corresponds to the same metabolite fractions as in the goat study. With a few exceptions, HPLC fractions found in the goat study were also found in the hen study. The identification and characterization of isolated fractions are presented above in

Table 6. To confirm the identity of HPLC fractions from egg and tissue extracts, fractions isolated from liver and eggs were cochromatographed by TLC with the respective HPLC fractions, which had been previously identified.

All of the Sulfone-related HPLC fractions from eggs and tissues were previously detected in the goat study. Alcohol-related HPLC fractions A-4, A-5, A-10, A-15, and A-17 were not previously found in the goat study; however, these fractions accounted for only minor portions (each 0.4-2.2% TRR) of the residues in liver and/or muscle. The principle difference between the studies was that fractions A-24, A-25, and A-26, which were not found in goat fat, together accounted for 70.3 and 74% of the TRR in poultry fat and skin, respectively. Fractions A-25 and A-26 were identified as fatty acid esters (Figure 1) of the [^{14}C]Alcohol metabolite based on their HPLC coelution with reference standards and MS analysis. In addition, HPLC analysis of hydrolyzed (1N KOH, 16 hrs) fraction A-26 indicated that this fraction was converted to the parent metabolite by hydrolysis. Fraction A-24, which consisted of a broad band of radioactivity leading up to fraction A-25, was not further characterized.

Radioactive residues in the filtered liver proteolysate fractions (Alcohol - 48.4% TRR; Sulfone - 40.3% TRR) were also characterized and quantified by reverse-phase HPLC. HPLC analysis of the liver proteolysate from [^{14}C]Alcohol treated hens isolated 7 distinct fractions (AP-#) each accounting for 1.5-9.5% of the TRR (Table 9), with the remainder of the radioactivity (20.4% TRR) distributed among minor unresolved peaks. In the Sulfone liver proteolysate, there were 3 distinct fractions each accounting for 1.7-7.9% of the TRR; however, the majority (27.9% TRR) of radioactivity was accounted for by minor unresolved peaks. The poultry liver proteolysate fractions corresponded to similar fractions found in goat liver proteolysates. None of the proteolysate fractions cochromatographed with any of the identified soluble metabolite fractions. The filtered proteolysate fraction from egg yolk (Sulfone treated, 8.5% TRR) was also characterized by HPLC although no quantitative data were presented. The chromatographic profile of the Sulfone treated egg yolk was similar to the profile of Sulfone treated liver proteolysate fractions.

Hen Metabolism Summary

The submitted hen metabolism study is adequate. Radioactive residues in hens fed the [^{14}C]Alcohol metabolite at 115 ppm (550x) for 3 consecutive days ranged from 0.078 ppm in muscle to 0.559 ppm in liver. Residues in egg yolks and whites increased throughout the dosing period to maximums of 0.272 and 0.186 ppm, respectively, on Day-3. Radioactive residues in hens fed the [^{14}C]Sulfone metabolite at 84 ppm (400x) for 3 consecutive days ranged from 0.015 ppm in fat to 0.237 ppm in kidney. Residues in egg yolks and whites increased throughout the dosing period to maximums of 0.286 and 0.379 ppm, respectively, on Day-3.

Solvent extraction and protease treatment released $\geq$79% of the ^{14}C-residues from tissues and eggs. Solvent extractable residues from both groups of hens consisted of numerous

metabolites (0.4-44.4% of the TRR, each) related to the 2,6-DEA and 2,6-HEEA classes of plant metabolites. As in the goat study, both the Alcohol and Sulfone test substances were metabolized in a similar manner. Both test substances underwent loss of the methoxymethyl group, hydroxylation of the ethyl side-chain(s) usually at the benzylic position and glucuronidation of the hydroxyl groups. The one exception to this was in hen skin and fat, in which the majority of residues were comprised of fatty acid esters of the Alcohol metabolite. All of the identified metabolites contained the aniline moiety with both ethyl side chains, which were either not hydroxylated, hydroxylated in one ethyl group, or hydroxylated in both ethyl groups.

Table 9. Characterization of HPLC fractions of egg and tissue extracts from hens fed the [^{14}C]Alcohol metabolite at 115 ppm (550x) for 3 consecutive days.

HPLC Fractions[a]	SAH Product[b]	Yolks[c]		Whites[c]		Liver		Breast muscle		Thigh muscle		Fat[d]		Skin[d]	
		%TRR	PPM[e]	%TRR	PPM	%TRR	PPM	%TRR	PPM	%TRR	PPM	%TRR	PPM	%TRR	PPM
A-1						1.7	0.009	9.3	0.009	3.7	0.003				
A-2	A	1.6	0.003			1.7	0.009	4.0	0.004	2.5	0.002				
A-3	A	1.8	0.003	5.0	0.008	1.0	0.005	4.0	0.004	2.5	0.002				
A-4						1.0	0.005	2.2	0.002						
A-5						0.7	0.004			1.0	0.001				
A-6						1.5	0.008	8.6	0.008	4.2	0.003				
A-7	UNK-1	3.1	0.005	6.1	0.009	2.1	0.011	3.1	0.003	3.0	0.002				
A-9						0.6	0.003	2.0	0.002	1.1	0.001				
A-10		2.4	0.004	3.0	0.005	0.6	0.003	1.5	0.001	1.8	0.001				
A-11	**EA**	**18.0**	**0.030**	**37.6**	**0.057**	**1.5**	**0.008**	**6.8**	**0.006**	**5.2**	**0.004**				
A-12	**EI**	**3.3**	**0.006**	**6.9**	**0.011**	**0.5**	**0.003**	**1.3**	**0.001**	**1.0**	**0.001**				
A-13	UNK-1			4.4	0.007	1.1	0.006	1.9	0.002	1.1	0.001				
A-14						0.8	0.004								
A-15								0.4	<0.001						
A-16	**DEA**					**1.6**	**0.008**	**0.8**	**0.001**						
A-17						0.6	0.003								
A-20	**DEA**	**6.6**	**0.011**	**4.4**	**0.007**	**0.5**	**0.003**	**0.5**	**<0.001**					0.4	0.001
A-21	**DEA**					**4.7**	**0.025**	**2.8**	**0.003**						
A-22						0.9	0.005					1.6	0.001		
A-23	**DEA**	**3.6**	**0.006**			**0.6**	**0.003**	**0.5**	**<0.001**	**2.0**	**0.002**	**7.1**	**0.006**	**4.3**	**0.013**
A-24												14.3	0.012	7.1	0.022
A-25	**DEA**											**38.1**	**0.032**	**44.4**	**0.136**
A-26	**DEA**											**17.9**	**0.015**	**22.5**	**0.069**
AP-1a - AP-4						18.1	0.093								
AP-5/AP-6	DEA					1.5/ 9.5	0.008/ 0.049								
Total Identified		**31.5**	**0.053**	**48.9**	**0.075**	**9.4**	**0.050**	**12.7**	**0.012**	**8.2**	**0.007**	**63.1**	**0.053**	**71.2**	**0.218**
Total Characterized		40.4	0.068	67.3	0.103	52.8	0.276	49.6	0.047	29.2	0.023	79.0	0.066	78.7	0.241

[a]HPLC fractions resulting from the feeding of [^{14}C]Alcohol metabolite are numbered in order of elution (fractions designated AP-# are from the liver proteolysate). Fractions in **bold** were identified. [b]Products of strong acid hydrolysis, A = aniline; Unk = unknown chemophore; EA = 2-ethylaniline; EI = 7-ethylindoline; and DEA = 2,6-diethylaniline. [c]Composite samples. [d]Data from HPLC analysis of organic extracts from fat and skin. [e]Expressed in equivalents of the [^{14}C]Alcohol metabolite. [f]Five peaks each accounting for 2.4-4.9% of the TRR.

Table 10. Characterization of HPLC fractions of egg and tissue extracts from hens fed [^{14}C]Sulfone metabolite at 84 ppm (400x) for 3 consecutive days.

HPLC Fraction[a]	SAH Product[b]	Egg yolks[c]		Egg whites[c]		Liver		Breast muscle		Thigh muscle		Skin[d]	
		%TRR	PPM[e]	%TRR	PPM	%TRR	PPM	%TRR	PPM	%TRR	PPM	%TRR	PPM
S-1						2.7	0.007						
S-2						1.4	0.004	13.1	0.010	4.5	0.002		
S-3	**A**	**3.2**	**0.007**	**1.1**	**0.005**	**1.2**	**0.003**	**5.3**	**0.004**	**4.0**	**0.002**		
S-4	**A**	**1.5**	**0.003**	**1.6**	**0.006**			**3.9**	**0.003**				
S-5	A	2.6	0.006	1.7	0.007	1.6	0.004	5.7	0.004	4.9	0.002		
S-6	**A**							**13.4**	**0.010**	**6.8**	**0.003**		
S-7		3.1	0.007	2.7	0.011	1.8	0.005	4.6	0.004	3.9	0.002		
S-8								2.2	0.002	2.4	0.001		
S-10				3.8	0.015					1.1	0.001		
S-11	**EA**					**2.0**	**0.005**						
S-12	UNK-1					2.3	0.006						
S-13	**A**					**1.0**	**0.003**						
S-14						1.0	0.003			0.6	<0.001		
S-16	**EA**	**23.1**	**0.053**	**16.3**	**0.064**	**2.2**	**0.006**	**3.1**	**0.002**	**5.2**	**0.003**	**1.7**	**0.001**
S-18		3.2	0.007	4.1	0.024	1.2	0.003			0.7	<0.001		
S-19	UNK-2	5.3	0.012	4.0	0.023	1.1	0.003			1.2	0.001		
S-23		1.7	0.004	1.7	0.017			0.5	<0.001				
S-24		**10.7**	**0.024**	**9.2**	**0.079**	**0.6**	**0.002**	**1.4**	**0.001**	**3.8**	**0.002**	**12.9**	**0.005**
S-26								1.0	0.001				
SP-1						7.9	0.017						
SP-2						5.4	0.012						
SP-4						1.7	0.004						
Total Identified		**38.5**	**0.087**	**28.2**	**0.154**	**7.0**	**0.019**	**27.1**	**0.020**	**19.8**	**0.010**	**14.6**	**0.006**
Total Characterized		54.3	0.124	46.2	0.251	35.2	0.085	54.0	0.042	39.1	0.020	14.6	0.006

[a]HPLC fractions resulting from the feeding of [^{14}C]Sulfone metabolite are numbered in order of elution (fractions designated SP-# are from the liver proteolysate). Fractions in **bold** were identified. [b]Products of strong acid hydrolysis, A = aniline; Unk = unknown chemophore; EA = 2-ethylaniline; and DEA = 2,6-diethylaniline. [c]Composite samples. [d]Data from HPLC analysis of organic extract from skin. [e]Expressed in equivalents of the [^{14}C]Sulfone metabolite.

Radiovalidation of Enforcement Methodology

The registrant did not analyze representative samples from either metabolism study using enforcement methodology. The Agency (W. Chin, CB No. 4299, 12/1/88) has recommended the HPLC method in Monsanto's report MSL-5718 for inclusion in PAM Vol. II as Method III. In this method, residues are hydrolyzed using a strong base and detected by HPLC with a oxidative coulometric electrochemical detector as either DEA or HEEA. The registrant stated that metabolites that are hydroxylated at the 1-position of both ethyl side chains are not known to be detectable by Method III.

The registrant estimated possible theoretical recoveries of radioactive residues from tissues based on the identity of the metabolites and the results of the strong acid hydrolysis and HPLC analysis conducted on fractions from the metabolism studies. The registrant calculated the type and amounts of aniline chemophores that would theoretically be produced by strong acid or base hydrolysis of metabolism study matrices. The estimated recoveries by Method III of ^{14}C-residues that yield aniline chemophores are presented in Table 11. The data indicate residues characterized as containg an aniline moiety account for up to 37% of the TRR in tissues (excluding fat and skin), milk, and eggs, and may not be recovered by the proposed PAM Method III. If these additional aniline containing residues are determined to be of toxicological concern, then the registrant must develop a method(s) that will account for these residues. In addition, the registrant must analyze representative samples from the animal metabolism studies using current or proposed enforcement methodology.

Table 11. Theoretical recovery of ^{14}C-residues containing aniline from the goat and hen metabolism studies by PAM Method III.

Matrix	Goat Metabolism Studies			
	Treated with [^{14}C]Alcohol metabolite		Treated with [^{14}C]Sulfone metabolite	
	%TRR characterized[a] as aniline chemophores	%TRR[b] theor. reco. by PAM Method III	%TRR characterized[a] as aniline chemophores	%TRR[b] theor. reco. by PAM Method III
Liver	10.1	7.1	57.5	44.3
Kidney	49.8	44.5	57.6	43.6
Muscle	35.3	11.9	67.5	48.3
Fat	25.0	25	56.2	41.8
Milk	58.2	21.2	52.2	54.6
Matrix	Hen Metabolism Studies			
	Treated with [^{14}C]Alcohol metabolite		Treated with [^{14}C]Sulfone metabolite	
	%TRR characterized[a] as aniline chemophores	%TRR[b] theor. reco. by PAM Method III	%TRR characterized[a] as aniline chemophores	%TRR[b] theor. reco. by PAM Method III
Liver	12.1	8.9	8.6	4.8
Breast	20.7	11.4	34.8	4.5
Thigh	13.2	7.2	24.7	9.0
Skin/Fat	71.2/63.1	71.2/63.1	14.6	14.6
Yolks	34.9	28.2	41.1	33.8
Whites	53.9	42.0	29.9	25.5

[a]Based on the total amounts of aniline containing chemophores that would be theoretically produced by acid or base hydrolysis of goat and hen matrices. [b]Sum of metabolites theoretically producing DEA or 1-HEEA following strong base hydrolysis.

References

Citations for the MRID documents referenced in this review are presented below. Submissions reviewed in this document are indicated by shaded type.

42594901 Downs, J.; Marsh, D.; Krautter, G. (1992) The Metabolism of Two (carbon 14)-Labelled Alachlor Metabolites in Lactating Goats: Part 1: In-life Aspects and Quantification of (carbon 14)-Residues in Milk, Tissues, and Excreta: Lab Project Number: 601: 1409: MSL-12108. Unpublished study prepared by PTRL East, Inc. 82 p.

42594902 Downs, J.; Marsh, D.; Krautter, G. (1992) The Metabolism of Two (carbon 14)-Labelled Alachlor Metabolites in Laying Hens: Part 1: In-life Aspects and Quantification of (carbon 14)-Residues in Eggs, Tissues, and Excreta: Lab Project Number: 602: 1411: MSL-12197. Unpublished study prepared by PTRL East, Inc. 81 p.

42594903 Nadeau, R.; Flory, D.; Schneider, R.; et al. (1992) The Metabolism of Two (carbon 14)-Labeled Alachlor Metabolites in Lactating Goats: Part II: Quantitation Characterization, and Identification of the Radioactive Residues in Milk and Tissues: Lab Project Number: MSL-12416: 1145. Unpublished study prepared by Monsanto. 300 p.

42594904 Nadeau, R.; Flory, D.; Schneider, R.; et al. (1992) The Metabolism of Two (carbon 14)-Labeled Alachlor Metabolites in Laying Hens: Part II: Quantitation Characterization, and Identification of the Radioactive Residues in Eggs and Tissues: Lab Project Number: MSL-12417: 1145. Unpublished study prepared by Monsanto. 301 p.

Agency Memoranda

CB No.: 4299
Subject: Alachlor (Lasso®) In or On Sorghum Forage. Evaluation of Analytical Methods and Residue Data.
From: W. Chin
Dated: 12/1/88
MRID(s): none

CB No.: 7816
Subject: PP#0F2348. Alachlor. Protocols for Goat and Hen Metabolism Studies.
From: N. Dodd
Date 7/16/91
MRID(s): None.

CBRS No.: 9628
CBTS No.: 9358
Subject: Alachlor. Monsanto Response to Registration Standard.
From: M. Flood
Date 8/11/92
MRID(s): None

13544

R108344

Chemical:	**Alachlor**
PC Code:	**090501**
HED File Code	**13000 Tox Reviews**
Memo Date:	**06/01/93**
File ID:	**DPD186613**
Accession Number:	**412-05-0095**

HED Records Reference Center
06/03/2005