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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Special review of Alachlor. Analytical Methodology for Milk and Meat Tissues. Accession Number 255600 [RCB No.429]

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As part of the review of alachlor, Monsanto Company committed to develop and submit a residue method for animal products sensitive to 2 ppb. The present enforcement methodology for meat, milk, and eggs (Method II in PAM II) determines the parent and metabolites converted to 2,6-diethylaniline (DEA) by the hydrolysis step in the method. The sensitivity of the present enforcement method is 0.02 ppm for meat, milk, and eggs.

The metabolism of alachlor in animals is not understood due to the lack of pertinent data on this topic. Thus, the residue of concern in livestock and their products are not known at this time. Metabolism studies have been required by the Alachlor Registration Standard.

To develop the 2 ppb methodology for meat products, Monsanto has spiked samples with 5 alachlor metabolites listed below.

N-(Methoxymethyl)-N-[2-(1-hydroxyethyl)-6-ethylphenyl]-2-methylsulfonyl)acetamide; (hydroxyethyl methylsulfone metabolite of alachlor); CP 101394

2-Hydroxy-N-(methoxymethyl)-N-(2,6-diethylphenyl)acetamide; (2-hydroxy analog of alachlor); CP 51214

2-[(Methoxymethyl)(2,6-diethylphenyl)amino]-2-oxoethane sulfonic acid, sodium salt; (sulfonic acid metabolite of alachlor); CP 108065

[(Methoxymethyl)(2,6-diethylphenyl)amino]oxoacetic acid, sodium salt; (oxanilic acid metabolite of alalchlor); CP 108064

3-[Methoxymethyl)(2,6-diethylphenyl)amino-2-oxoethanesulfinyl]-2-hydroxypropanoic acid, sodium salt;(sulfinyl lactic acid metabolite of alachlor); CP 108267

Apparently, Monsanto chose these alachlor metabolites on the basis of recent plant metabolism studies in corn and soybeans. (PP#'s OF2348, 3F2832/Acc#. 251375; 4/23/84 review of M. Kovacs). These studies showed that the metabolites of concern consisted of 2 types: those converted to DEA and those converted to 2-ethylaniline (EA) following strong acid hydrolysis (called high pressure acid hydrolysis in Method II in PAM II). The major difference between metabolites converted to DEA and those converted to EA upon strong acid hydrolysis is that for the former metabolites, the ethyl side chains on the aniline are not substituted (i.e., metabolites contain the DEA moiety), whereas for the latter, one of the ethyl side chains is hydroxylated at the 1 position (i.e., metabolites contain the 2-(1-hydroxyethyl)-6-ethylaniline moiety, HEEA).

Of the 5 alachlor metabolites listed above, one (CP 101394) contains the HEEA moiety and the remaining contain the DEA moiety. In the solutions used for fortification of meat and milk samples, the molar ratio of the metabolite containing the HEEA moiety to those containing the DEA moiety was 1:1. Samples were not spiked with the parent. However, residues of the parent are not found in plants and thus secondary residues of the parent would not expected in animals. In addition, methodology which determines metabolites containing the DEA moiety would also determine the parent.

The analytical procedure determines residues of alachlor metabolites as derivatized DEA or HEEA. The hydrolysis step in this analytical method is less vigorous than that used in the aforementioned enforcement method and plant metabolism studies, and does not convert metabolites containing the HEEA moiety to EA, but rather converts these metabolites to HEEA.

The methodology consists of extraction of the sample with aqueous acetonitrile, followed by centrifugation and evaporation of the extract. There are slight differences in the extraction procedure dependent on whether milk, fat, kidney, liver or muscle are being analyzed. The extract is hydrolyzed in 50% 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 selective ion monitoring (SIM). Residues are reported as alachlor equivalents after appropriate calculations.

Monsanto obtained linear calibration curves for derivatized DEA and HEEA over the range of <0.5 - 30 ppb. Recoveries were reported for the total fortification range, but they were not reported for the various individual fortification levels. Recovery data for milk and tissues are given below.

	DEA Metabolites	
	Fort., ppb	Recov., %(ave,%)
milk	2 - 100	60 - 82 (73)
tissues	2 - 100	56 - 94 (78)

	HEEA Metabolites	
	Fort., ppb	Recov., %(ave,%)
milk	0.5 - 100	75 - 111 (85)
liver, fat	1 - 100	53 - 92 (77)
muscle, kidney	2 - 100	63 - 107 (79)

Sample chromatograms of a milk blank and 1 ppb fortified milk sample were provided. No chromatograms were provided for tissues. The chromatograms of the milk blank show a small signal for DEA and a clean background for HEEA. Monsanto indicates in their report that, in general, the DEA background is higher than that of the HEEA background. They attribute the DEA background to laboratory contamination.

For metabolites containing the DEA moiety, the reported sensitivity of the method is 2.0 ppb for milk and tissue. For metabolites containing the HEEA moiety, the reported sensitivity is 0.5 ppb for milk, 1.0 ppb for fat and liver and 2.0 ppb for muscle and kidney. Monsanto indicates that the sensitivity of the method was limited by the presence of background responses in blanks.

Monsanto does not report method detectability except to say that the limit of validation (sensitivity) was in most cases the limit of detection as defined in Anal. Chem. 1983, 55, 2210. However, the Monsanto discussion of validation results does not seem to be in agreement with definitions given in the cited paper. Comparing the DEA chromatogram for a milk blank with that of a 1 ppb fortified milk sample, RCB notes that the relative size of the DEA signal (as compared to the signal of the internal standard) is much smaller for the blank than for the 1 ppb fortified sample. Comparison of these 2 chromatograms suggests that method detectability for DEA is lower than the reported sensitivity of 2 ppb. Monsanto should provide method detectability for each matrix. Since DEA and HEEA are determined as separate peaks, knowledge of method detectability may be necessary in order to determine whether total alachlor residues in meat products are less than 2 ppb.

Until adequate livestock metabolism studies are submitted, RCB cannot determine whether the metabolites used to spike the meat tissues and milk are representative of residues in animal products. Livestock metabolism studies are also used to elucidate the efficiency of the extraction of the various components (free and bound) of the residue so that extraction/residue release procedures can be developed as part of the analytical methods. Thus, until adequate livestock metabolism studies are submitted RCB cannot determine whether the analytical method described above is adequate for animal commodities. Depending on the outcome of these metabolism studies, it may be necessary to ascertain whether the total residue of concern in animal products is determined by this methodology.

In addition, representive chromatograms of blanks and fortified tissues should be submitted for all tissues and recoveries should be reported at each fortification level. Since DEA and HEEA are determined as separate peaks, method detectability should be reported so that one would be able to determine whether alachlor residues in animal products are less than 2 ppb.

Conclusions and Recommendations

1. Adequate livestock metabolism studies are not available at this time. Thus, the residues of concern in animal products are not known and the efficiency of the extraction of the various components (free and bound) cannot be determined. Until adequate livestock metabolism studies are submitted, RCB cannot determine whether the submitted analytical methodology is adequate for animal products. Depending on the outcome of livestock metabolism studies, it may be necessary to ascertain whether the total residue of concern in meat, milk, and eggs is determined by this methodology.

2. For metabolites containing the DEA moiety, the reported sensitivity of the method is 2.0 ppb for milk, muscle, fat, liver and kidney. For metabolites containing the HEEA moiety, the reported sensitivity is 0.5 ppb for milk, 1.0 ppb for fat and liver, and 2.0 ppb for muscle and kidney. Since DEA and HEEA are determined as separate chromatographic peaks, method detectability in each matrix should be reported so that it will be possible to determine whether total alachlor residues in animal products are less than 2 ppb.

   In addition, Monsanto should be informed that their discussion of validation results is unclear.

3. Representative chromatograms were only provided for milk. Representative chromatograms of blanks and fortified samples should be provided for all matrices.

4. Recoveries in milk and tissues were reported only for the fortification range. Recoveries should be reported for each individual fortification level in order to properly evaluate the methodology.

5. On the basis of conclusions 1, 2, 3 and 4, RCB can not determine whether the submitted methodology for animal products is adequate.

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