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

5/23/86

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: 524-316 - Alachlor Special Review and Response to the Alachlor Registration Standard - Analytical Methodology for Meat, Milk, and Eggs - (No Accession Number) (RCB #449)

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This Monsanto submission addresses deficiencies in analytical methodology for alachlor residues in meat, milk, and eggs which had been cited in a February 27, 1985 letter from R.J. Taylor (EPA) to Frank Serdy (Monsanto). The deficiencies cited in the February 27, 1985 EPA letter were based on the February 27, 1985 review of Accession Number 255600 (RCB #429) by M. Loftus.

The present enforcement methodology for meat, milk, and eggs (Method II in PAM II) determines the parent and metabolites containing the 2,6-diethylaniline (DEA) moiety as DEA. The sensitivity of the present enforcement method is 0.02 ppm for meat, milk and eggs. As part of the review of alachlor, Monsanto Company committed to develop and submit a residue method for animal products sensitive to 2 ppb. In addition, the Alachlor Registration Standard required additional methodology because the present enforcement method does not determine metabolites containing the 2-(1-hydroxyethyl), 6-ethylaniline (HEEA) moiety.

In response, on November 20, 1984. Monsanto submitted to the Agency analytical methodology for animal products (Accession Number 255600: February 7, 1985 review of M. Loftus) 2/27/85 letter from EPA to Monsanto). 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 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 selective ion monitoring (SIM). Residues are reported as alachlor equivalents after appropriate calculations. This method was used for their cattle, poultry, and swine feeding studies (Accession Numbers 256625, 265273, and 257272; January 23, 1986 review of M. Loftus).

The present submission specifically addresses deficiencies in analytical methodology for meat, milk and eggs cited in the February 27, 1985 letter. Each deficiency, the Monsanto response, and RCB comments and conclusions are given below.

Deficiency 1

Since adequate livestock metabolism studies are not available, the residues of concern in animal products are not known and the efficiency of the various components (free and bound) cannot be determined.

Monsanto Response

Since the M. Loftus February 7, 1985 review of analytical methodology, Monsanto submitted additional goat and hen metabolism studies using 14C-labeled alachlor metabolites. They assume that livestock metabolism is now adequately delineated and wish to know if that is a valid assumption.

RCB Comments and Conclusions

This deficiency has not been resolved. The livestock metabolism studies in question were reviewed by M. Loftus in a November 1, 1985 memorandum to R. Taylor. In this review it

was concluded that the metabolism of alachlor in ruminants and poultry is not adequately understood. The major deficiency with the livestock metabolism studies is that large portions of the residue were not chemically characterized in the tissues.

The outstanding questions on livestock metabolism are given in detail in the Conclusions and Recommendations of the November 1, 1985 memorandum. Monsanto should be forwarded either the November 1, 1985 memorandum in its entirety or its Conclusions and Recommendations.

Deficiency 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.

Monsanto Response

This question was answered in the submissions for livestock feeding studies (Monsanto reports MSL-4464, 4373, 4620, 4514, 4620, 4515; Accession Numbers 256625, 257273, 257272; January 23, 1986 review of M. Loftus to R. Taylor). In these studies, sensitivity was improved as compared to the sensitivity in Accession Number 255600 (February 27, 1985 review of M. Loftus) because background response for DEA was reduced by employing a new laboratory and equipment without DEA contamination. The LOD's and LOV's are given in the table below.

LIMIT OF DETECTION FOR ALACHLOR RESIDUES IN MILK, EGGS, AND ANIMAL TISSUES (PPB)

Matrix	LOD for DEA	LOV for DEA	LOD for HEEA	LOV for HEEA
Milk	0.15	0.5	0.16	0.5
Beef Muscle	0.19	0.5	0.31	0.5
Beef Fat	0.34	0.5	0.22	0.5
Beef Liver	2.20	2.0	*	2.0
Beef Kidney	1.60	2.0	1.02	2.0
Eggs	0.20	0.5	0.11	0.5
Chicken Muscle	0.17	0.5	0.19	0.5
Chicken Fat	0.43	0.5	0.04	0.5
Chicken Liver	0.65	1.0	0.42	1.0
Chicken Kidney	0.86	1.0	0.61	1.0
Pork Muscle	0.32	0.5	0.36	0.5
Pork Fat	0.42	0.5	0.19	0.5
Pork Liver	1.46	2.0	*	?.0
Pork Kidney	1.11	2.0	1.43	2.0

* LOD's for HEEA in beef and pork liver were artificially low because an adjacent matrix peak interfered with quantitation of low HEEA levels. Most small, 0.1-0.2 ppb, apparent HEEA peaks in control samples were not resolved from the larger matrix peak, and resulted in "not found" values.

RCB Comments and Conclusions

This deficiency is resolved.

Deficiency 3

The discussion of the validation results is unclear.

Monsanto Response

In this submission, Monsanto defines the limit of validation (sensitivity) of the method. They explain the limit of validation (LOV) in terms of the limit of detection (LOD) and limit of quantitation (LOQ), defined in Anal. Chem., 1983, 55, 2210-2218 as:

$$LOD = \text{mean} + 3\sigma$$
$$LOQ = \text{mean} + 10\sigma$$

Monsanto defines the LOV as the lowest level of demonstrated consistent recoveries and indicates that the LOV was always between the LOD and the LOQ, and usually near the LOD. The Anal. Chem. article defines the concentration between 3 and 10 as the region of less certain quantitation. They indicated that the LOD's and LOV's were reported because of the need to drive validation/detection limits as low as possible.

RCB Comments and Conclusions

This deficiency is resolved.

Deficiency 4

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

Monsanto Response

They refer the Agency to the aforementioned submissions for livestock feeding studies where the chromatograms were provided and reviewed in the 1/23/86 memorandum of M. Loftus to R. Taylor.

RCB Comments and Conclusions

This deficiency is resolved.

Deficiency 5

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.

Monsanto Response

They refer the Agency to the aforementioned livestock feeding studies reviewed is the 1/23/86 memorandum of M. Loftus to R. Taylor.

RCB Comments and Conclusions

This deficiency is resolved.

Recommendation

The registrant has not satisfied the analytical methodology data requirement for meat, milk, and eggs because of outstanding questions on livestock metabolism outlined in detail in the November 1, 1985 memorandum of M. Loftus to V. Walters. 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.

cc: Loftus, Hummel, S.R. File (Loftus), Griffith, Alachlor S.F., RS File, PMSD/ISB, Circu.
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