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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Evaluation of Alachlor Method Validation
2 Alachlor metabolites in peanuts and peanut hay

FROM: Susan V. Hummel, Chemist
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THRU: Charles L. Trichilo, Ph.D., Chief
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TO: Robert Taylor/Vickie Walters, PM#25
Herbicide Fungicide Branch
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Residue Chemistry Branch has been informed by Environmental Chemistry Laboratory (ECL), Chemical Operations Branch (COB), Benefits and Use Division (BUD) of the completion of the requested method validation. The method validation was reported by Han Tai by memorandum dated February 1, 1988.

A method validation was requested for two representative alachlor metabolites on peanuts and peanut hay (See S. Hummel memo of 6/2/87). The method validation was requested for the Monsanto Company method titled, "Analytical Method for the Determination of 2,6-Diethylaniline (DEA) and 2-(1-Methoxyethyl)-6-Ethylaniline (MEEA)-Yielding Alachlor Metabolites in Peanut Hay, Vines, Hulls, and Nutmeats," Appendix C of Monsanto Report Nos. MSL-5718 and MSL-5646 (Accession No. 263022), Author and date not given; Monsanto submission dated May, 1986, in response to the Alachlor Registration Standard. (Note: Monsanto Report No. 5646 was incorrectly identified by Monsanto as MSL-4636 at the time of submission.)

Tolerances have been established for alachlor and its metabolites in or on peanuts and other commodities. Alachlor [2-chloro-2',6'-diethyl-N-(methoxymethyl) acetanilide] is the active ingredient in LASSO Herbicide. Alachlor metabolites include those containing the diethylaniline (DEA) moiety and those containing the hydroxyethylethylaniline (HEEA) moiety. The two representative metabolites used for the method validation were:

DEA metabolite: sodium salt of 2-[(2,6-diethylphenyl)(methoxy-methyl)amino]-2-oxo-ethane sulfonic acid (tertiary amide sulfonic acid metabolite, containing the 2,6-DEA moiety)

Monsanto Code # CP 108065

EPA Code No. E 0033

HEEA metabolite: N-[2(1-hydroxyethyl)-6-ethylphenyl]-N-(methoxymethyl)-2-(methylsulfonyl) acetamide (hydroxyethyl tertiary amide sulfone metabolite, containing the 2,6-HEEA moiety)

Monsanto Code No. CP 101394

EPA Code No. E 0032

The method also requires standards of 2,6-diethylaniline (DEA, EPA Code No. E0346) and 2-(1-hydroxyethyl)-6-ethylaniline (HEEA, EPA Code No. E0705). 2,6-Diethylaniline is also available from Aldrich.

Method Summary

The method determines alachlor and its metabolites containing the DEA and HEEA moieties. Present PAM II methods determine alachlor and its metabolites containing the DEA moiety (PAM II Methods I(a) and 1(b) or alachlor, per se (PAM II method II). A similar method was used by Monsanto for analysis of sunflower seed and cottonseed commodities.

This analytical method is different from previously submitted methods. It does not require the use of the custom made glassware used in other methods submitted by Monsanto in response to the Registration Standard and in the PAM II methods. Methoxy- ethylethylaniline (MEEA) is produced from HEEA. The DEA and MEEA are determined by HPLC with oxidative coulometric electrochemical detection (OCED). No derivatization is required.

Samples are extracted with 20% water/acetonitrile. The extract is vacuum filtered, washed, and the solvent evaporated to near dryness. The extract is hydrolyzed in base under pressure at 155C to produce DEA and HEEA. The sample is cooled at room temperature for 1 hour. The DEA and HEEA are extracted with methylene chloride, and then with methanolic HCl. After separation, additional methanol is added, and the solution is allowed to sit overnight (for approximately 12 hours) to convert HEEA to MEEA (methoxyethylethylaniline). The pH of the aqueous/methanolic solution is then adjusted to 5-7. The volume of the methanol/water layer is adjusted with 50% methanol/water or HPLC mobile phase. The DEA and MEEA are then separated by reverse phase HPLC using a Zorbax C-8 column (4.6 mm x 15 cm) and 45:55 pH 4.8 acetate buffer/methanol (v/v). The detector is an

Oxidative Coulometric Electrochemical Detector - ESA Model 5100A Coulochem Detector with Model 5010 analytical cell and Model 5020 guard cell.

An addendum to the method was supplied by Monsanto directly to BUD/COB (See letter of A. G. Hackett to D. Rains, 6/17/87). The modified adaptor specified in the method to attach the Wheaton flask to the rotary evaporator could be replaced by a connecting hose adaptor from Ace Glass fitted with a rubber stopper. Commercially available clamps may be used in place of the oil bath lift. The addendum also contained this caution,

"Be careful while handling the hot Wheaton pressure flasks; they are under pressure. Wear long protective gloves when removing the flasks from the oil bath and keep the hot flasks behind glass hood doors for at least 45 minutes after removal from the bath."

The method determines alachlor and its metabolites containing the DEA and HEEA moieties. Present PAM II methods determine alachlor and its DEA metabolites (PAM II Methods I(a) and I(b)) or alachlor, per se (PAM II Method II). A similar method was used by Monsanto for analysis of sunflower seed and cottonseed commodities.

RCB requested that the method be validated for alachlor metabolite residues in peanuts at 0.01, 0.10, and 0.50 ppm; and in peanut hay at 0.01, 0.20, and 2.0 ppm.

ECL/COB/BUD used the modifications in the Monsanto method addendum and made another small modification in the method. ECL also noted the special precaution given in the method addendum.

Recoveries were determined on duplicate samples. Recoveries ranged from 74 to 90% ($85 \pm 7\%$) for the DEA metabolite in peanuts, 87 to 97% ($91 \pm 3\%$) for the HEEA metabolite in peanuts; and from 72 to 97% ($82 \pm 10\%$) for the DEA metabolite in peanut hay, 70 to 88% ($82 \pm 8\%$) for the HEEA metabolite in peanut hay. The registrant's recoveries ranged from 67-120% for the DEA metabolite in peanuts, 67-101% for the HEEA metabolite in peanuts; and from 63-91% for the DEA metabolite in peanut hay, 59-102% for the HEEA metabolite in peanut hay.

The EPA Repository recently contacted RCB, indicating that an additional supply of the alachlor metabolites and DEA and HEEA was needed. (FTS 629-3951). We have contacted the registrant and requested a sufficient supply of the two alachlor metabolites listed above, and diethylaniline and hydroxyethylethylaniline, be sent to the EPA Repository (5-10 g each). Note that the code numbers for the alachlor metabolites and DEA and HEEA indicates that the repository supply is extremely limited.

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We note that Monsanto has recently submitted a draft of a different analytical method, proposed for enforcement. Formal submission of this method is expected shortly. Monsanto has validated their proposed enforcement method for corn grain, corn stover, corn forage, soybean grain, soybean forage, peanuts, peanut hulls, and peanut hay. The method has a limit of detection of 0.01 ppm for DEA metabolites, and 0.015 ppm for HEEA metabolites. Samples are extracted, hydrolyzed in base, and the DEA and HEEA steam distilled. The DEA and HEEA are extracted with methylene chloride, solvent exchanged into isooctane. Cleanup is by liquid solid chromatography (LSC) using a disposable aminosilica column. DEA and HEEA are collected in separate fractions. HEEA is derivitized with acetyl chloride. DEA and acetyl-HEEA are quantitated by GC (DC-200 column) with a nitrogen detector (NPD).

CONCLUSIONS

The method titled, "Analytical Method for the Determination of 2,6-Diethylaniline (DEA) and 2-(1-Methoxyethyl)-6-Ethylaniline (MEEA)-Yielding Alachlor Metabolites in Peanut Hay, Vines, Hulls, and Nutmeats," Appendix C of Monsanto Report Nos. MSL-5718 and MSL-5646 (Accession No. 263022), is suitable for enforcement on these commodities. A copy of the method is being sent to FDA for inclusion in PAM II as Method III.

The method determines alachlor and its metabolites containing the DEA and HEEA moieties. Present PAM II methods determine alachlor and its DEA metabolites (PAM II Methods I(a) and I(b)) or alachlor, per se (PAM II Method II). A similar method was used by Monsanto for analysis of sunflower seed and cottonseed commodities.

We note that Monsanto has submitted additional analytical methodology which they consider suitable for enforcement. This methodology should be subjected to a method validation, as well.

RECOMMENDATIONS

We recommend that this method be included in PAM II as Method III.

cc: RF, circu, S. Hummel, M. Bradley, R. Thompson, FDA, Alachlor Reg. Std File (Boodee), PM#25, K. Kissler, W. Bontoyan, MTO F., PMSD/ISB

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