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

MAR 19 1996

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: 2,4-D. (030001) Independent Method Validation.
DP Barcode: D216962; CBRS No. 15807; MRID No.: 436911-01;
Case No. 0073.

FROM: David J. Miller, HSO, US Public Health Service *DM*
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THRU: Edward Zager, Chief *Edward Zager*
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CBRS has been requested to review the 2,4-D Industry Task Force II independent laboratory validation of a method for determination of 2,4-D in/on plants. The Industry Task Force II previously indicated in a letter dated 7/22/93 (and reviewed in W. Smith, 9/3/93, CBRS No. 12270, DP Barcode D193335) that the enforcement methods currently in PAM Vol. II are unsuitable for determining 2,4-D residues.

Subsequently, the Task Force submitted an analytical enforcement method for 2,4-D in plants (EN-CAS Method No. ENC-2/93) which was reviewed by CBRS and judged to be adequate (D. Miller, 1/29/96, CBRS No. 14004, DP Barcode D205346) pending a required independent laboratory validation per PR Notice 88-5.

In response, Task Force II submitted the present data (1995; MRID 43691101) pertaining to the required independent laboratory validation of Method ENC-2/93. These data are evaluated herein for their adequacy in fulfilling residue chemistry data requirements. The qualitative nature of the residue in plants is adequately understood. Acceptable wheat, lemon, and potato metabolism studies have been submitted. The HED Metabolism Committee (6/16/93) has concluded that the residue of concern is 2,4-D *per se* in plants and in animals.

Tolerances for plant commodities are currently expressed in terms of 2,4-D *per se* [40 CFR §180.142 (a through f, i, j, and k), §185.1450(a), and §186.1450]. Tolerances for animal commodities are expressed in terms of the combined residues of 2,4-D and/or its 2,4-dichlorophenol metabolite [40 CFR §180.142(h)].

Codex MRLs for residues of 2,4-D are expressed in terms of 2,4-D *per se*. The CODEX MRL and the U.S. tolerance expression are compatible for plant commodities only, pending incorporation of the HED Metabolism Committee recommendations into the tolerance expression for animal commodities. Issues regarding harmonization of U.S. tolerances with the Codex MRLs will be addressed when the registration eligibility of 2,4-D is determined.

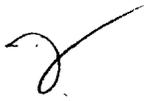
CONCLUSIONS AND RECOMMENDATIONS

1. The submitted data are adequate to satisfy the requirements for independent laboratory validation (PR Notice 88-5) of Method ENC-2/93. The data indicate that the method adequately recovers residues of 2,4-D from wheat grain, forage, and straw and confirm that the method is adequate for data collection and tolerance enforcement for residues in/on plant commodities.
2. An Agency method trial will be required for this method, and CBRS will request that EPA/Beltsville conduct this trial.

DETAILED CONSIDERATIONS

Task Force II submitted data (1995; MRID 436911-01) pertaining to the independent laboratory validation of an analytical method (EN CAS Method ENC-2/93) for the determination of 2,4-D in/on wheat straw, wheat grain, and wheat forage. The method was previously submitted to, and reviewed by, CBRS (D. Miller, 1/29/96, CBRS NO. 14004, DP Barcode D205346) which tentatively concluded that the method was adequate pending a required independent laboratory validation (ILV) per PR Notice 88-5. Independent laboratory analyses have now been conducted by Centre Analytical Laboratories (State College, PA) using wheat commodities (grain, hay, and straw) and the results submitted to CBRS for review. Chromatograms were provided for each wheat analysis.

Briefly, residues are extracted from plant matrices into 0.5 M KOH in ethanol:H₂O (EtOH, 1:1, v/v) and filtered. The resulting extract is refluxed for 1 hour in ~0.2 M HCl. Hydrolyzed residues are then cleaned-up using a C₁₈ solid phase extraction column by rinsing with water and hexane, and then eluting with hexane:ethyl acetate (EtOAc, 1:1, v/v).



Residues are then partitioned into 0.1 M Na₂HPO₄, acidified, and partitioned into diethyl ether (Et₂O). Residues are concentrated to dryness and then derivatized to the methyl ester with 14% boron trifluoride in methanol (MeOH). For samples of soybean (seed and forage), sugarcane, and rice straw, the derivatized sample is then oxidized with potassium permanganate. The derivatized residues from each matrix (except wheat forage and grain) are then partitioned into 25% toluene in hexane and cleaned-up using an Alumina column eluted with 25% toluene in hexane. For wheat forage and grain samples, the derivatized residues are extracted into toluene, diluted with hexane (toluene:hexane, 1:2, v/v), and cleaned-up using an Alumina column eluted with 35% toluene in hexane. Methylated residues in all matrices are determined by GC/ECD.

The time required for one analyst to run 11 samples was reported to be approximately 2 1/2 lab-days.

The above method was validated using control RAC samples of wheat (straw, grain, and forage). For method validation, 6 control samples of each matrix were fortified with 2,4-D at 0.01 and two control samples were fortified at 5 ppm (wheat grain) or 20 ppm (wheat straw and hay). Two control samples of each matrix were analyzed along with the fortified samples. Apparent residues of 2,4-D were <0.01 ppm in all control samples. Sample calculations and an adequate number of example chromatograms were provided. Results of the method validation are presented in Table 1.

These data indicate that the GC/ECD method ENC-2/93 is adequate for collecting data on residues of 2,4-D in/on raw agricultural commodities and has been independently validated. The method has a validated LOQ of ca. 0.01 ppm for each matrix.

Table 1. Procedural recoveries of 2,4-D using EN-CAS Method ENC-2/93.

Matrix	Fortification Level (ppm)	Percent Recovery	
		Mean ± SD	Range (No. Samples)
Wheat Grain	0.01	101 ± 12	78-116 (6)
	5.0 ^a	84.3 ± 1	83 (1), 86 (1)
Wheat Forage	0.01	79 ± 5	72-87 (6)
	20.0	90 ± 1	91 (1), 89(1)
Wheat Straw ^b	0.01	107 ± 5	101-116 (6)
	20.0	93 ± 7	86 (1), 99 (1)

^a Per the protocol (Protocol No. 94P-011-03), this fortification was to be made at 0.5 ppm and not 5.0 ppm.

^b No longer regulated as a RAC of wheat

In summary, the submitted data are adequate to satisfy the requirements for independent laboratory validation (PR Notice 88-5) of Method ENC-2/93. The data indicate that Method ENC-2/93 adequately recovers residues of 2,4-D from wheat grain and forage (and straw)

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and confirm that the method is adequate for data collection and tolerance enforcement for residues in/on plant commodities. CBRS will request that Beltsville conduct an Agency Tolerance Method Validation (TMV) on this method.

RDI: Pilot Team:3/3/96; RPerfetti:3/18/96;EZager:3/18/96.
cc: RF, SF, List A Rereg. F., Circ., DJM.

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