LIST A (2)

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

JUN 26 1996

OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

**MEMORANDUM**

SUBJECT: **2,4-D.** (030001) Enforcement Analytical Method for Ruminant and Poultry Commodities. GDLN 171-4(d).
DP Barcode: D226556; CBRS No. 17267; MRID Nos.: 440165-01 and 440165-02; Case No. 0073

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THRU: Edward Zager, Branch Chief
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CBRS has been asked to review two separate (but similar) proposed enforcement methods for the determination of 2,4-dichlorophenoxy acetic acid (2,4-D) in meat, milk, poultry, and egg commodities. The submitted studies are entitled "Development and Validation of Analytical Methodology for the Quantitation of Residues of 2,4-Dichlorophenoxy Acetic Acid (2,4-D) in Beef Muscle, Liver, Kidney, Fat and Milk" and "Development and Validation of Analytical Methodology for the Quantitation of Residues of 2,4-Dichlorophenoxy Acetic Acid (2,4-D in Poultry Muscle, Liver, Fat and Eggs." The studies were sponsored by the Industry Task Force II on 2,4-D Research Data; the performing laboratory is PTRL East, Inc., in Richmond, KY.

## CONCLUSIONS AND RECOMMENDATIONS

1. The registrant has submitted two separate (but essentially comparable) proposed enforcement methods for determination of 2,4-D in ruminant and in poultry commodities. CBRS has reviewed the recovery data provided by the registrant and concludes that the methods are adequate provided that Conclusions 3 through 6 below are adequately addressed. Mean fortification recoveries for ruminant and poultry tissues range from 71.2% to 107.6%.

2. The registrant has also performed radiovalidation of the proposed enforcement method. Radiovalidation was performed on fat, kidney, and milk samples from the goat metabolism study and on eggs from the poultry metabolism study. 2,4-D recoveries were adequate, indicating that the proposed analytical method is acceptable.

3. A revised method which combines the two methods into a single method must be submitted. The single method should describe the specific methodological differences (e.g., different extraction solvents, etc) for each separate matrix. The method should also include a detailed description of the calculation procedure used to determine 2,4-D residues in the various matrices (see Conclusion #5). The registrant may also wish to alter the write-up such that instructions are more consistent with respect to tense. Once an adequate revised method has been received, CBRS will forward the method to EPA/Beltsville for a tolerance method validation.

4. The analytical method instructions should be modified to delete all references to the use of diazomethane as a derivatizing agent. Per CBRS policy, use of this derivatizing agent is generally not appropriate in an analytical enforcement method. The registrant in its write-up should refer only to the $BF_3$/MeOH derivatization method.

5. The registrant did not provide any sample calculations and the reviewer was unable to duplicate the registrant's calculated percent recoveries. The registrant should provide to the Agency complete raw data and sample calculations (including chromatograms showing peak areas, external standard linearity curves and associated data, standard curve calculations, etc.) for the following samples:

   - sample # 912C-73-5 (beef muscle) fortified at 0.20 ppm
   - sample # 912C-91-5 (milk) fortified at 0.10 ppm
   - sample # 912C-73-5 (beef muscle) fortified at 0.2 ppm



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6. Under the section entitled Quantitation of Recovery Samples (on p. 33 of the ruminant analytical method MRID), the registrant should remove instructions for adjusting recoveries for control values.

## DETAILED CONSIDERATIONS

The 1988 2,4-D Registration Standard required the registrant to submit validation data supporting analytical methods used to determine tolerances for 2,4-D in ruminant and poultry commodities. In 1993, the registrant (in a letter dated 7/22/93 from J.D. Connor to J. Coombs) indicated that Task Force scientist had reviewed the various methods available in PAM and found them unsuitable to measure residues of 2,4-D in poultry (or ruminants). The Task Force indicated that they would develop new methods for animal commodities, and that these new methods would be validated with samples from the ruminant and poultry metabolism studies.

### Test Matrices

The ruminant test matrices for these analyses were muscle, liver, kidney, fat and milk. The poultry test commodities were muscle, liver, fat, and eggs.

### Analytical Procedure

The registrant analyzed 5-gram samples from each of the beef and poultry matrices, except for milk and eggs for which 25-gram samples were used. Sample fortifications with 2,4-D were performed to verify recoveries at 0.05- 0.010-, 0.20- ppm for beef and poultry muscle, liver, and fat, at 0.05- 0.10-, and 0.20- ppm for beef kidney, and 0.01-, 0.05- and 0.10- ppm for eggs and milk.

Two methods (one for analysis of ruminant commodities and the other for analysis of poultry commodities) were submitted by the registrant. However, the methods are very similar or identical for comparable matrices and the registrant should combine these two methods in an appropriate manner into a single method (for both ruminant and poultry commodities) for incorporation into PAM.

For analysis of ruminant muscle tissue, a 5 gram sample is homogenized with acidified acetonitrile, with the analyte subsequently partitioned into diethyl ether and then into a 0.1% NaOH solution. The extract is acidified following rotary evaporation and put through C8 and C18 solid phase cleanup. The extract is eluted from the SPE columns with methyl-t-butyl ether (MTBE) which is subsequently concentrated; the analyte is then derivatized with boron trifluoride in methanol with the resulting methyl ester of 2,4-D partitioned into hexane for



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analysis by GC/ECD on a DB-1 (60m, 0.32 mm, 5 um film) non-polar capillary column. Analysis of beef fat is similar to that of muscle tissue except that the fat is homogenized in hexane and 2,4-D is extracted from the homogenate with 0.1% NaOH solution. This basic extract is acidified and partitioned against diethyl ether.

Ruminant liver tissue (5 gram sample) is subjected to a one-hour reflux in 2N HCl. The aqueous hydrolyzed extract is diluted with ACN and is subsequently cleaned up on a Florisil column to remove matrix interferences. The resultant extract is diluted with 1% NaOH, acidified following rotary evaporation, and partitioned into a solution of 10% EtAc in hexane, the organic phase is passed through a neutral alumina column and the analyte eluted with a solution of MeOH in NaOH. The extract is acidified and partitioned with MTBE, with the MTBE layer concentrated and the analyte methylated with $BF_3$/MeOH. For beef kidney, the procedure is similar to that for beef liver, except that the acetonitrile extract is subjected to a Florisil column cleanup. Analysis of milk (25 gram sample) is similar to that for beef liver.

The extraction scheme for poultry tissue and eggs is comparable to that of the corresponding beef matrices and is not further described in this review.

Calibration and Standard Curve Information

For calibration, a minimum of 4 calibration standards ranging from 0.01- or 0.05- $\mu$g/mL to 3 $\mu$g/mL (corresponding to 0.002- or 0.01- ppm to 0.6 ppm) were injected, and a linear regression curve using peak area vs. standard concentration generated.

The registrant did not report an estimated LOD or LOQ for 2,4-D for any of the matrices. CBRS inspection of the chromatograms and fortification recoveries indicate that the LOQ for beef tissues is ca. 0.05 ppm while that for milk is ca. 0.01 ppm. For poultry samples, corresponding values for tissues and eggs are 0.05 ppm and 0.01 ppm, respectively.

Fortification Recovery

The registrant completed a number of method recovery trials in a variety of ruminant and poultry commodities. Table 1 presents the method recoveries calculated in the tested matrices for 2,4-D.

Radiovalidation of Beef Fat, Beef Kidney, and Milk Methodologies

The registrant also performed radiovalidation of the proposed ruminant commodity enforcement method. Tissues from a previous metabolism study (ABC Report #40630, "Metabolism of Uniformly $^{14}$C-Ring Labeled 2,4-Dichlorophenoxyacetic Acid in Lactating Goats", April 22, 1993, MRID No. 42749701). The results of this radiovalidation are presented in Table 2. CBRS finds the radiovalidation to be adequate and concludes that the requirement for radiovalidation of the proposed method to be satisfied, since adequate recoveries were



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demonstrated.

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Chemistry Branch makes the foll wing comments with respect to the submitted methods.

- As indicated abov , the two methods should be combined into a single method which describes th specific differences (e.g., different extraction solvents, etc) for each matrix. The registrant may also wish to alter the write-up such that instructions are co sistently given in the active (and not passive) voice.

- The analytical met od instructions should be modified to delete all references to the use of diazom thane as a derivatizing agent. Per CBRS policy, use of this derivatizing agent is generally not appropriate in an analytical enforcement method if another methylating agent is suitable. The registrant in its write-up should refer only t the $BF_3$/MeOH derivatization method.

- The registrant did not provide any sample calculations and the reviewer was unable to duplicat the registrant's calculated percent recoveries: this is most likely because on one (of presumably many) example linearity curve was provided (see Fig re 7 of registrant's ruminant submission) and this did not coincide with (i.e., was not calculated from) the peak areas shown on the example external standard hromatograms (see Figures 8 through 13 of the ruminant MRID). The reg trant should provide to the Agency complete raw data and sample calculatio s (including chromatograms showing peak areas, external standard linearity urves and associated data, standard curve calculations, etc.) for sample # 912 -73-5 corresponding to beef muscle fortified at 0.20 ppm (derivatized with b oron trifluoride), for sample # 912C-91-5 (corresponding to milk fortified at 0. 0 ppm and derivatized with boron trifluoride), and for sample 912C-73-5 (corres onding to beef muscle fortified at 0.2 ppm, derivatized with boron trifluoride).

- Under Quantitatio of Recovery Samples, the registrant (on p. 33 of the ruminant analytical method MRID) states

> If the peak is observed in the control sample and the area for this peak is less than he peak area for the lowest standard analyzed, then subtract the control peak area from each recovery peak area before determining t e ug/mL for the recovery extract.
>
> If the peak a ea for the control sample is greater than the peak area observed in t e lowest standard analyzed, then determine the ug/mL value in the control extract and subtract this value from the value determined in the recovery samples.

These instructions should be removed, as it is inappropriate to adjust test values for contamination of control samples.

cc: RF, SF, List A Rereg. File, Circ., DJM.
RDI: Pilot Team:6/20/96;RPerfetti:6/24/96;EZager:6/26/96.



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Table 1. 2,4-D Recc veries from Fortified Meat, Milk, Poultry, and Egg Samples.

| Commodity Class | Commodity | Fort ication L vel (p pm) | Recovery Data | | | |
|---|---|---|---|---|---|---|
| | | | No. of Samples | Recovery Range[a] | Mean Recovery | % RSD[b] |
| Ruminant | Muscle | ( .05 | 6 | 70.5-118.1% | 95.1% | 19.5% |
| | | ( .10 | 12 | 62.9-117.2% (2) | 92.1% | 20.9% |
| | | ( .20 | 3 | 68.1-102.6% | 85.1% | 20.3% |
| | Fat | ( .05 | 6 | 64.0-97.2% (1) | 85.0% | 13.8% |
| | | ( .10 | 3 | 66.0-76.9% (1) | 71.4% | 7.6% |
| | | ( .20 | 3 | 62.6-79.4% (1) | 71.2% | 11.8% |
| | Kidney | ( .05 | 5 | 60.8-101.0% (1) | 79.4% | 20.1% |
| | | ( .10 | 5 | 71.8-102.2% | 84.0% | 14.5% |
| | | ( .20 | 3 | 64.3-88.7% (1) | 77.8% | 15.9% |
| | Liver | ( .05 | 7 | 67.4-117.3% (1) | 93.0% | 19.0% |
| | | ( .10 | 7 | 63.5-104.5% (2) | 81.4% | 20.5% |
| | | ( .20 | 7 | 56.2-100.1% (3) | 78.2% | 20.6% |
| | Milk | ( .01 | 6 | 89.9%-112.7% | 101.4% | 8.6% |
| | | ( .05 | 3 | 101.1-117.6% | 107.6% | 8.1% |
| | | ( .10 | 3 | 91.4-118.8% | 104.4% | 13.2% |
| Poultry | Muscle | ( .05 | 8 | 76.2-116.6% | 97.5% | 15.5% |
| | | ( .10 | 6 | 71.4-115.1% | 88.0% | 19.6% |
| | | ( .20 | 4 | 70.7-86.0% | 81.0% | 8.6% |
| | Fat | ( .05 | 7 | 68.2-114.1% (1) | 96.9% | 16.8% |
| | | ( .10 | 4 | 78.3-108.8% | 93.5% | 18.2% |
| | | ( .20 | 4 | 86.5-108.2% | 98.9% | 10.9% |
| | Liver | ( .05 | 8 | 68.5-112.3% (1) | 91.4% | 18.1% |
| | | ( .10 | 4 | 76.3-88.4% | 83.7% | 6.2% |
| | | ( .20 | 4 | 71.7-106.9% | 84.9% | 19.6% |
| | Eggs | ( .01 | 8 | 85.4-127.6% (1) | 104.8% | 12.7% |
| | | ( .05 | 4 | 78.1-97.5% | 90.1% | 9.4% |
| | | ( .10 | 6 | 80.3-108.4% | 92.2% | 10.5% |

[a] Number in parentheses represent the number of samples which were outside the recovery range of 70-120%.

[b] Percent relative standard deviatio

Table 2. Comparison of 2,4-D Concentration in Metabolism Study Samples vs. Concentration Determined by Proposed Enforcement Method

| Matrix | 2,4-D Concentration | | Percent Relative to Reported Value |
|---|---|---|---|
| | Metabolism Study | Residue Methodology[a] | |
| Goat Fat | 0.04 ppm | 0.09 ppm<br>0.08 ppm<br>0.01 ppm[c]<br>0.01 ppm[c]<br>0.03 ppm[c]<br>0.04 ppm[c] | 100%[b] |
| Goat Kidney | 0.772 | 0.94<br>1.05 | 130%[b] |
| Goat Milk | 0.095 | 0.17 | 179% |
| Chicken Eggs | 0.004 | 0.005[c]<br>0.003[c]<br>0.005[c] | 100%[b] |

[a] Multiple values represent multiple duplicate analyses
[b] Average of multiple analyses
[c] These values are less than the LOQ of the proposed method

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|---|---|
| **Chemical:** | **2-4,D** |
| **PC Code:** | **030001** |
| **HED File Code** | **11200 Subject Files Chemistry** |
| **Memo Date:** | **06/26/96** |
| **File ID:** | **DPD226556** |
| **Accession Number:** | **412-05-0040** |

**HED Records Reference Center**
**10/29/2004**