95/96

MRID 43615901

MEMORANDUM

SUBJECT: Monosodium methanearsonate (MSMA). List B Reregistration Case No. 2395. Guideline Ref. No. 171-4(d): Analytical methods for MSMA and cacodylic acid in beef (fat, liver, muscle and kidney) and milk. MRID No. 43615901. CBRS No. 15487. DP Barcode No. D214494.

FROM: Christina B. Swartz, Chemist
Reregistration Section II
Chemistry Branch II: Reregistration Support
Health Effects Division (7509C)

THRU: Susan V. Hummel, Acting Section Head
Reregistration Section II
Chemistry Branch II: Reregistration Support
Health Effects Division (7509C)

TO: Mark Wilhite (PM-51)
Reregistration Branch
Special Review and Reregistration Division (7508W)

On behalf of the MAA (MSMA/DMSA) Research Task Force Three, Chemical Consultants International, Inc. (CCII) has submitted a study pertaining to analytical methods for quantitation of MSMA and cacodylic acid in beef (fat, liver, kidney and muscle) and milk [MRID No. 43615901]. The performing laboratory was PTRL East, Inc. of Richmond Kentucky.

Tolerances are currently established for the selective post-emergence herbicide methanearsonic acid (calculated as As_2O_3) resulting from application of the disodium and monosodium salts of methanearsonic acid in or on cottonseed (0.7 ppm) and in or on citrus fruit (0.35 ppm) [40 CFR §180.289]. A tolerance of 0.9 ppm (expressed as As_2O_3) is established for residues of methanearsonic acid in cottonseed hulls from application of the disodium and monosodium salts of methanearsonic acid in the production of cotton [40 CFR §186.4050].

The methanearsonic acid salts comprise List B reregistration case no. 2395. A Phase 4 review was completed 3/28/91 (memo, C. Olinger, CBRS Nos. 6974, 7058, 7097, and 7215). The registrant submitted the required metabolism studies, and subsequently the HED Metabolism

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Committee determined that the residues to be regulated (i.e. those that should be included in the tolerance expression) are MSMA and cacodylic acid (CA). For convenience, residues will continue to be calculated and expressed as As_2O_3. The Committee concurred with the CBRS conclusion that residues in animal commodities can be classified under Category 3 of 40 CFR §180.6(a), i.e. there is no reasonable expectation of finite residues. [refer to the 01/26/95 C. Swartz memo]

The MSMA/DSMA metabolite cacodylic acid (CA) is used as a defoliant on cottonseed, and has tolerances for residues in cottonseed (2.8 ppm), cattle kidney and liver (1.4 ppm), and cattle meat byproducts (other than kidney and liver, 0.7 ppm) associated with its use [40 CFR §180.311]. Additional data pertaining to the magnitude of the residue in livestock [GLN 171-4(j)] are required to support reregistration of products containing cacodylic acid. Although analytical methods in animal commodities are not currently required to support reregistration of products containing MSMA/DSMA, these methods are reviewed herein, as they can be applied to cacodylic acid data requirements for reregistration.

Recommendation

Analytical methods for animal tissues and milk are not required to support uses of MSMA. However, such methods are required for cacodylic acid. The method may be acceptable for cacodylic acid. The registrant may wish to continue with further method development prior to initiation of an independent laboratory validation (ILV). However, no additional data are required, pending submission of a successful ILV.

Conclusions

1. MSMA and CA residues are extracted from beef liver, muscle, fat, kidney and milk via homogenization with water, derivatization of the residues with methylthioglycolate, followed by quantitation using GC/ECD.

2. The stated limits of quantitation are 0.02 for muscle (meat); 0.05 for liver, kidney and fat; and 0.01 for milk.

3. Individual working standards are derivatized, and analyzed using GC/ECD to generate standard curves. Quantitation of residues in samples is accomplished using linear regression of peak area vs. concentration of the standards. Sample standard curves demonstrated linearity over the range of standards analyzed.

4. Sample chromatograms were submitted, and were adequate. However, sample calculations and raw data were not included in the report.

5. While average recoveries were generally acceptable (i.e. greater than 70%), CBRS notes that the recoveries were highly variable, and that significant numbers of recoveries were much

less than the 70% usually considered acceptable by the Agency. Recoveries obtained for cacodylic acid were better than those obtained for MSMA.

6. Analytical methods for animal tissues and milk are not required to support uses of MSMA. However, such methods are required for cacodylic acid. The method may be acceptable for cacodylic acid. The registrant may wish to continue with further method development prior to initiation of an independent laboratory validation (ILV). However, no additional data are required, pending submission of a successful ILV.

DETAILED CONSIDERATIONS

Description of the Method

The general principle of the method involves extraction of the residues via homogenization with water, derivatization of the residues with methylthioglycolate, followed by quantitation using gas chromatography and electroconductivity detection (GC/ECD).

Beef liver, muscle, and kidney

A 50-g sample of the beef matrix (liver, muscle or kidney) is homogenized with 50 mL water; after addition of 25 mL acetonitrile (ACN), the homogenate is shaken, centrifuged, and decanted. The centrifuge pellet is rinsed with ACN, shaken, and centrifuged 2 more times. The combined extract is adjusted to pH 12 using 10% NaOH, and then concentrated. The extract is transferred to a centrifuge tube using rinses with water and 2 ml 50% HCl. After centrifugation (including 2 rinses of the pellet) and filtration, the pH is adjusted to 10-12 using 10% NaOH, and the extract concentrated.

The concentrated extract is once again transferred to a centrifuge tube using rinses with water and 2 ml 50% HCl. The pH is adjusted (if necessary) to pH=2, and the extract centrifuged. The MSMA and CA residues in the extract are derivatized via vortexing of the supernatant with 1/2 ml methylthioglycolate. After 15 minutes have elapsed, the extract is vortexed again, and 5 mL hexane are added. The layers are allowed to separate, and, if necessary, the sample may be centrifuged again. Otherwise, an aliquot of the hexane layer is taken for analysis using GC/ECD.

Beef fat

A 50-g sample of the fat is homogenized with 100 mL hexane; subsequently, 100 mL water is added, and the mixture is shaken on a wrist-action shaker. The extract is centrifuged, and transferred to a separatory funnel. The water layer is transferred to a boiling flask, and the hexane layer is discarded. The emulsion layer is redissolved with fresh hexane, shaken, and partitioned with water 3 times. The combined aqueous extract is adjusted to pH 10-12 with 10% NaOH, concentrated, and adjusted to pH 2-3 using concentrated HCl. The extract is refluxed for 16-18 hours, and the residues derivatized as described above. The extract is vortexed with 5 mL hexane and centrifuged. After concentration,

a 1 mL aliquot is taken for analysis using GC/ECD.

Milk

A 250-g sample is shaken with 100 mL hexane, and the phases are allowed to separate. The aqueous layer is partitioned with hexane 3 more times, and the combined aqueous extract adjusted to pH 9-10 using 10% NaOH; after addition of anti-foam solution, the extract is concentrated. During transfer to a centrifuge tube, 3 mL of 50% HCl is added to the extract. After centrifugation, the extract is adjusted to pH 9-10 and concentrated. Prior to centrifugation, 3 mL of 50% HCl are added to the extract. After centrifugation, the pH of the extract is adjusted to pH 2, and the residues are derivatized as described above. After addition of 5 mL hexane, the extract is vortexed, and the layers are allowed to separate. The hexane layer may be viscous at this point, and centrifugation may be necessary. An aliquot of the hexane layer is taken for analysis using GC/MS.

Preparation of Standards

For the subject method validation study, individual MSMA and CA standards were prepared in HPLC-grade water; working standards of 7.5, 5, 2.5, 1.5, 0.5, .25, .10 and 0.05 µg/mL were used. No less than 4 standards were used to generate standard curves. For derivatization, 5 mL of a working standard are placed in a centrifuge tube with 30 ml water; 10-12 drops of concentrated HCl are added, followed by 0.5 ml methylthioglycolate. After vortexing, 5 mL hexane are added, the extract is vortexed, and the layers are allowed to separate. An aliquot is used for analysis.

The limit of quantitation (LOQ) was found to be 0.02 ppm for MSMA and CA in beef muscle. In kidney, liver and fat, the LOQ was determined to be 0.05 ppm for both MSMA and CA. The LOQ in milk was determined to be 0.01 ppm. Standard curves showing peak area vs. concentration were submitted for both MSMA and CA, demonstrating linearity over the range of working standards.

Fortification

Control beef muscle, liver and fat were fortified with a mixed standard of MSMA and CA at 0.02, 0.05, 0.10 and 0.20 ppm. Milk was fortified with the mixed standard at 0.01, 0.05 and 0.10 ppm. All samples were fortified just prior to homogenization.

Results

The recoveries obtained from each beef matrix are shown below. Adequate sample chromatograms were included in the study report.

Recoveries of MSMA and CA From Fortified Beef Tissues and Milk.[1]

Matrix	Fort. (ppm)[2]	N[3]	MSMA % Recovery	CA % Recovery
Muscle	0.02	8	59.8-94.2 (74.3 ± 10.0)	59.5-105.6 (74.1 ± 16.4)
	0.05	4	41.0-96.3 (68.6 ± 24.2)	66.6-95.9 (82.3 ± 13.1)
	0.10	17	65.0-116.2 (80.6 ± 14.8)	71.2-94.1 (83.6 ± 7.6)
	0.20	4	53.0-123.9 (93.6 ± 29.7)	67.0-88.1 (80.2 ± 9.7)
Liver	0.05	9	56.5-117.0 (76.6 ± 19.1)	63.2-80.2 (70.9 ± 5.3)
	0.10	6	44.4-113.0 (89.2 ± 25.2)	67.2-76.2 (70.2 ± 3.2)
	0.20	6	86.2-117.7 (79.5 ± 38.4)	64.4-74.7 (70.7 ± 3.9)
Kidney	0.05	10	66.1-89.8 (92.6 ± 9.3)	64.9-117.1 (89.9 ± 16.7)
	0.10	5	62.1-92.5 (74.1 ± 10.6)	72.9-92.5 (81.8 ± 7.4)
	0.20	5	70.3-101.1 (89.4 ± 11.5)	70.5-83.2 (77.1 ± 5.2)
Fat	0.05	8	45.5-96.5 (71.7 ± 16.1)	68.7-120.6 (100.4 ± 16.2)
	0.10	6	54.6-95.0 (72.9 ± 15.4)	74.7-94.5 (83.9 ± 8.2)
	0.20	3	60.5-68.5 (64.9 ± 4.1)	81.0-92.7 (85.0 ± 6.7)
Milk	0.01	8	85.2-131.3 (109.7 ± 15.1)	46.0-88.7 (67.6 ± 12.8)
	0.05	4	65.1-114.0 (86.7 ± 20.8)	70.0-102.2 (86.9 ± 15.0)
	0.10	10	43.2-108.8 (75.7 ± 20.1)	60.5-94.9 (81.6 ± 10.8)

[1] Results show the range in % recovery, and the mean ± std. dev.

[2] The fortification level considered to be the LOQ for each matrix is shaded.

[3] N = the number of samples analyzed at each fortification level.

CBRS Comments

The registrant did not include sample calculations in the submission. When the method is subjected to an independent laboratory validation, such calculations should be included. Many of the recoveries were below the 70% usually considered to be acceptable to the Agency. Although the average recoveries were acceptable (with a few exceptions), a wide range in recoveries was obtained for many of the matrices. Recoveries for cacodylic acid were generally better than those for MSMA; however, analytical methods for animal matrices are not required to support reregistration of MSMA/DSMA.

The registrant may wish to continue method development prior to initiating an independent laboratory validation (ILV); however, no additional data are required pending submission of an acceptable ILV.

cc: CBSwartz: MSMA List B file: RF: SF: Circulation
7509C:CBRS:CBSwartz:CM#2:Rm 804F:703 305 5877:7 25 95
RDI:SVHummel:5 28 96 RBPerfetti:06/05/96 EZager:06/05/96