Test Material: Tribufos

MRID: 48822501

Title: TRIBUFOS: VALIDATION OF ANALYTICAL METHODOLOGY FOR THE DETERMINATION OF RESIDUES IN SOIL AND SEDIMENT

MRID: 48822503

Title: TRIBUFOS: Independent Laboratory Validation of Methodology for the Determination of Residues of Tribufos in Soil (Sandy Loam and Clay Loam) and Sediment (Sandy Silt Loam)

EPA PC Code: 074801

OCSPP Guideline: 850.6100

For CDM Smith

Primary Reviewer: Lynne Binari **Signature:** Lynne Binari

Date: 8/11/14

Secondary Reviewer: Lisa Muto **Signature:** Lisa Muto

Date: 8/11/14

QC/QA Manager: Joan Gaidos **Signature:**

Date: 8/11/14

Analytical method for tribufos in soil and sediment

Reports: ECM: EPA MRID No.: 48822501. Brewin, S. 2012. TRIBUFOS: VALIDATION OF ANALYTICAL METHODOLOGY FOR THE DETERMINATION OF RESIDUES IN SOIL AND SEDIMENT. Huntingdon Life Sciences Project ID: BDG0137. Report prepared by Huntingdon Life Sciences Ltd., Eye Research Centre, Suffolk, England, sponsored and submitted by AMVAC Chemical Corporation, Newport Beach, California; 53 pages. Final report issued March 23, 2012.
ILV: EPA MRID No. 48822503. Brooks, S. 2012. TRIBUFOS: Independent Laboratory Validation of Methodology for the Determination of Residues of Tribufos in Soil (Sandy Loam and Clay Loam) and Sediment (Sandy Silt Loam). Huntingdon Life Sciences Project ID: BDG0142. Report prepared by Huntingdon Life Sciences Ltd., Huntingdon Research Centre, Cambridgeshire, England, sponsored and submitted by AMVAC Chemical Corporation, Commerce, California; 76 pages. Final report issued April 26, 2012.

Document No.: MRIDs 48822501 & 48822503

Guideline: 850.6100

Statements: ECM: The study was conducted in compliance with UK and OECD Good Laboratory Practice (GLP) standards, and EC Commission Directive 2004/10/EC (p. 3 and Appendix 4, p. 53 of MRID 48822501). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4 and Appendix 4, p. 53 of MRID 48822501). A statement of the authenticity of the study data was included with the quality assurance statement (p. 3 of MRID 48822501).
ILV: The study was performed in compliance with SANCO guidelines 3029/99 rev. 4 and 825/00 rev. 8.1, and conducted in compliance with UK and OECD GLP standards, and EC Commission Directive 2004/10/EC (p. 3 and Appendix 2, p. 76 of MRID 48822503). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4 and Appendix 2, p. 76 of MRID 48822503).

Classification: This analytical method is classified as **not acceptable**. Insufficient information was provided to establish that the ECM and ILV laboratories were two independent laboratory groups. The determinations of the LOQ and LOD were not based on scientifically acceptable procedures.

PC Code: 074801

Cheryl Sutton

Digitally signed by CHERYL SUTTON
DN: c=US, o=U.S. Government, ou=USEPA, ou=Staff, cn=CHERYL SUTTON, dnQualifier=0000017702
Date: 2014.12.09 10:07:08 -05'00'

Reviewer: Cheryl Sutton, Ph.D., Environmental Scientist **Date:** 11/25/14
U.S. EPA

Executive Summary

This analytical method, Huntingdon Life Sciences BDG0137, is designed for the quantitative determination of tribufos in soil and sediment using LC/MS/MS. The method is quantitative for tribufos at the stated LOQ of 0.01 mg/kg. The lowest toxicological level of concern in soil/sediment was not reported. No major modifications were made to the ECM by the independent laboratory; however, inadequate information was provided to conclusively determine that the ECM and ILV laboratories were two independent laboratory groups as defined by OCSPP guidelines.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Tribufos	48822501	48822503	EFED/ OPP	Soil & Sediment	23/03/2012	AMVAC Chemical Corporation	LC/MS/MS	0.01 mg/kg

I. Principle of the Method

Samples (10 g) of soil or sediment were extracted twice with 45 mL of hexane:acetone (95:5, v:v) plus anhydrous sulphate (*ca*. 5 g) by shaking (mechanical shaker, *ca*. 200 rpm) for *ca*. 30 minutes (Appendix 3, pp. 50-51 of MRID 48822501). Soil/sediment and extract were separated by centrifugation (*ca*. 3,500 rpm, *ca*. 3 minutes). Extracts were filtered (Whatman Number 1 filter paper), combined, and brought to 100 mL with hexane:acetone (95:5, v:v). An aliquot (1 mL) was treated with octanol (1 drop), taken to near dryness under nitrogen at *ca*. 30-40°C, then reconstituted in 10 mL acetonitrile (final matrix concentration 0.01 g soil/sediment / mL final extract) using ultrasonication.

Samples were analyzed for tribufos by HPLC (Acquity UPLC® BEH C_{18}, 2.1 mm x 50 mm, 1.7 µm column) using a mobile phase of (A) water:methanol (90:10, v:v) + 0.01M ammonium formate + 0.1% formic acid and (B) methanol:formic acid (100:0.1, v:v) [percent A:B at 0-0.2 min. 30:70, 2.0-2.5 min. 5:95, 3-4 min. 30:70) with collision-induced dissociation (CID) MS/MS detection (ion spray positive) and selected reaction monitoring (SRM; p. 16, Appendix 3, p. 52 of MRID 48822501). Both quantitation (*m/z* 315→169) and confirmation (*m/z* 315→57) ion transitions were monitored for tribufos. Injection volume was 10 µL.

The LOQ and LOD for tribufos were the same in the ECM and ILV at 0.01 mg/kg and 0.025 ng/mL (equivalent to 0.0025 mg/kg in soil/sediment), respectively (Appendix 3, p. 52 of MRID 48822501; p. 18 of MRID 48822503).

II. Recovery Findings

ECM (MRID 48822501): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of tribufos in sandy clay and sand soils and sand sediment (pp. 11, 15; Tables 3-8, pp. 20-25). Fortifications were performed at 0.01 mg/kg (LOQ) and 0.1 mg/kg (10x LOQ). Quantitation ion and confirmation ion results were comparable. The soils were fully characterized, except soil moisture contents were not reported (p. 11).

ILV (MRID 4882503): Mean recoveries and RSDs were within guideline requirements for analysis of tribufos in clay loam and sandy loam soils and silt loam sediment (pp. 14, 21; Tables 7-12, pp. 27-32). Fortifications were performed at 0.01 mg/kg (LOQ) and 0.1 mg/kg (10x LOQ). Quantitation ion and confirmation ion results were comparable. The soils were fully characterized, except soil moisture contents were not reported (p. 14). The number of trials conducted was not reported.

Table 2. Initial Validation Method Recoveries for Tribufos in Soil and Sediment

Matrix	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)[1]	Relative Standard Deviation (%)
UK Sand sediment	Quantitation ion					
	0.01 (LOQ)	5	70-81	77	4.3	5.6
	0.1	5	74-82	78	4.0	5.1
	Confirmation ion					
	0.01 (LOQ)	5	73-82	78	3.8	4.8
	0.1	5	73-82	78	4.2	5.3
UK Sandy clay soil[2]	Quantitation ion					
	0.01 (LOQ)	5	73-83	77	5.1	6.6
	0.1	5	76-85	81	3.7	4.6
	Confirmation ion					
	0.01 (LOQ)	5	76-90	83	6.3	7.6
	0.1	5	77-85	81	3.5	4.4
UK Sand soil	Quantitation ion					
	0.01 (LOQ)	5	73-85	79	4.3	5.5
	0.1	5	79-85	81	2.3	2.8
	Confirmation ion					
	0.01 (LOQ)	5	77-86	79	3.8	4.8
	0.1	5	78-83	80	2.0	2.6

Data were obtained from Tables 3-8, pp. 20-25 of MRID 48822501.

[1] Standard deviations were reviewer-calculated from the data in the study report since the study author only reported means and RSDs (see DER Attachment 2).

[2] USDA classification sandy clay loam (p. 11).

Table 3. Independent Validation Method Recoveries for Tribufos in Soil and Sediment

Matrix[1]	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Sandy loam soil	Quantitation ion					
	0.01 (LOQ)	5	74.1-88.8	83.7	5.87	7.01
	0.1	5	78.3-89.1	85.3	4.35	5.10
	Confirmation ion					
	0.01 (LOQ)	5	84.5-97.0	89.3	5.27	5.90
	0.1	5	74.4-88.2	83.3	5.44	6.53
Clay loam soil	Quantitation ion					
	0.01 (LOQ)	5	85.5-95.0	90.3	4.09	4.53
	0.1	5	94.0-105.3	98.5	4.68	4.75
	Confirmation ion					
	0.01 (LOQ)	5	84.2-92.6	89.6	3.25	3.62
	0.1	5	91.9-103.2	97.7	4.42	4.52
Silt loam sediment	Quantitation ion					
	0.01 (LOQ)	5	79.1-88.6	83.9	4.35	5.18
	0.1	5	84.0-88.9	86.3	1.83	2.12
	Confirmation ion					
	0.01 (LOQ)	5	72.2-87.2	79.8	5.34	6.68
	0.1	5	83.7-90.7	87.2	2.64	3.03

Data were obtained from Tables 7-12, pp. 27-32 of MRID 48822503.

1 USDA soil classifications (p. 14 of MRID 48822503). The sediment, reported as a sandy silt loam, was classified as a silt loam by the reviewer using the USDA Soil Texture Triangle.

III. Method Characteristics

In the ECM and ILV, the LOQ and LOD values for tribufos in soil and sediment were 0.01 mg/kg and 0.025 ng/mL (equivalent to 0.0025 mg/kg), respectively (p. 15 of MRID 48822501; p. 22 of MRID 48822503). The LOQ was defined as the lowest fortification level at which acceptable recovery data were obtained. The LOD was defined as the concentration of the lowest calibration standard to yield a measurable chromatographic response, which also corresponded to the lowest concentration which produces a signal to noise ratio of ≥3.

Table 4. Method Characteristics

	Tribufos
Limit of Quantitation (LOQ)	0.01 mg/kg
Limit of Detection (LOD)	0.025 ng/mL (0.0025 mg/kg)
Linearity (calibration curve r and concentration range)	r = 0.9999[1] (0.025-2 ng/mL)
Repeatable	Yes
Reproducible	No[2]
Specific	Yes

Data were obtained from p. 15; Tables 1-2, p. 19 of MRID 48822501.

1 ILV calibration curves were linear, r = 0.9995-0.9999, for concentration range of 0.025-2.0 ng/mL (see Tables 1-6, pp. 24-26; Figures 1-6, pp. 33-38 of MRID 48822503). Reviewer-calculated calibration curves verified linearity for the ECM (r^2 = 0.9997-0.9998) and ILV (r^2 = 0.9989-0.9999; see DER Attachment 2).

2 Insufficient information was provided to establish that the ECM and ILV laboratories were two independent laboratory groups.

IV. Method Deficiencies and Reviewer's Comments

1. The determination of the LOQ and LOD were not based on scientifically acceptable procedures. The LOQ was defined as the lowest fortification level at which acceptable recovery data were obtained; no calculations were reported (p. 15 of MRID 48822501). The LOD was defined as the concentration of the lowest calibration standard to yield a measurable chromatographic response, which also corresponded to the lowest concentration which produces a signal to noise ratio of ≥3 (p. 15 of MRID 48822501; p. 22 of MRID 48822503). Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples. Additionally, the lowest toxicological level of concern in soil/sediment was not reported. An LOQ above toxicological levels of concern results in an unacceptable method classification.

2. The originating and validation laboratories belong to the same organization, Huntingdon Life Sciences Ltd. A statement specifying that the analysts and study director of the ILV were unfamiliar with the method was not provided.

3. The number of ILV trials required to validate the ECM was not specified.

4. The ILV implemented minor modifications to the LC/MS/MS instrument parameters and for the sediment extraction included a 10-second hand shaking step prior to the mechanical shaking extraction (pp. 17, 23 of MRID 48822503). The modifications were not a significant deviation from the original ECM.

5. It was reported for the ILV that a set of twelve samples (2 untreated samples, 5 samples fortified at the LOQ, and 5 samples fortified at 10x LOQ) was processed within a single working day, followed by an overnight LC/MS/MS analysis run (p. 22 of MRID 48822503).

6. For both the ECM and ILV, the soil/sediment matrices were fully characterized, except soil moisture contents were not reported.

7. As part of the ECM, a supplemental experiment showed that tribufos was stable in the final extracts when stored at approximately -20°C for seven days (p. 16 of MRID 48822501). The results are shown in Table 5 below.

Table 5. Final extract stability data

Sample type	Analyte	Matrix	Analyte detected (ng/mL)	
			Day 0	Day 7
Mean stability	Tribufos	Sand sediment	1.99	2.03
Procedural recovery (%)			NA	109%
Mean stability		Sandy clay soil	2.00	2.03
Procedural recovery (%)			NA	105%
Mean stability		Sand soil	2.03	2.06
Procedural recovery (%)			NA	109%

Data were obtained from Table 9, p. 26 of MRID 48822501. Stability data is the mean of two replicate samples.
NA = not applicable.
Samples were fortified with 2 ng of tribufos in 1 mL control final extract (≡ 2 ng analyte/mL final extract). Stability samples were analysed following initial fortification and after the storage period. Procedural recovery samples were fortified just prior to the second analysis (following storage of stability samples).

V. References

U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.

Attachment 1: Chemical Names and Structures

Tribufos

IUPAC Name: S,S,S-Tributyl phosphorotrithioate
CAS Name: Not reported.
CAS Number: 78-48-8
SMILES String: Not reported.