

Addendum #1 to Data Evaluation Record

MRID: 48822502 and 48822504

PC Code: 074801

OPPTS Guideline: 850.6100 Environmental Chemistry Method

Date: November 12, 2015

DER Study Title: ECM: (MRID 48822502) Brewin, S. 2012. TRIBUFOS: VALIDATION OF ANALYTICAL METHODOLOGY FOR THE DETERMINATION OF RESIDUES IN WATER. Huntingdon Life Sciences Project ID: BDG0129. Report prepared by Huntingdon Life Sciences Ltd., Eye Research Centre, Suffolk, England, sponsored and submitted by AMVAC Chemical Corporation, Newport Beach, California; 46 pages. Final report issued March 23, 2012.

ILV: (MRID 48822504) Spikings, E. 2012. TRIBUFOS: Independent Laboratory Validation of Methodology for the Determination of Residues of Tribufos in Water (Surface and Ground Water). Huntingdon Life Sciences Project ID: BDG0141. Report prepared by Huntingdon Life Sciences Ltd., Huntingdon Research Centre, Cambridgeshire, England, sponsored and submitted by AMVAC Chemical Corporation, Commerce, California; 46 pages. Final report issued April 11, 2012.

Changes Made: The classification of the DER was upgraded from Not Acceptable to **Acceptable** based on the review of additional information provided by the registrant. The registrant provided additional information to confirm that the ECMs and ILVs were conducted by separate laboratories, clarified how the LODs were established and discussed their appropriateness for meeting Agency needs, and specified the number of ILV trials used to confirm each of the ECMs.

Revised by: Cheryl Sutton _____ Date: 11/12/15 _____

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Literature Cited

Frelander, Dick. 2015. Tribufos: ECM and ILV Response to EPA DER Reviews. Amvac Reference Number: RAD-2015-023. Prepared and submitted by AMVAC Chemical Company, Newport Beach, CA. June 26, 2015.

Test Material: Tribufos

MRID: 48822502

Title: TRIBUFOS: VALIDATION OF ANALYTICAL METHODOLOGY FOR THE DETERMINATION OF RESIDUES IN WATER

MRID: 48822504

Title: TRIBUFOS: Independent Laboratory Validation of Methodology for the Determination of Residues of Tribufos in Water (Surface and Ground Water)

EPA PC Code: 074801

OCSPP Guideline: 850.6100

For CDM Smith

Primary Reviewer: Lynne Binari

Signature:



Date: 8/11/14

Secondary Reviewer: Lisa Muto

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Date: 8/11/14

QC/QA Manager: Joan Gaidos

Signature:



Date: 8/11/14

Analytical method for tribufos in water

Reports: ECM: EPA MRID No.: 48822502. Brewin, S. 2012. TRIBUFOS: VALIDATION OF ANALYTICAL METHODOLOGY FOR THE DETERMINATION OF RESIDUES IN WATER. Huntingdon Life Sciences Project ID: BDG0129. Report prepared by Huntingdon Life Sciences Ltd., Eye Research Centre, Suffolk, England, sponsored and submitted by AMVAC Chemical Corporation, Newport Beach, California; 46 pages. Final report issued March 23, 2012.
ILV: EPA MRID No. 48822504. Spikings, E. 2012. TRIBUFOS: Independent Laboratory Validation of Methodology for the Determination of Residues of Tribufos in Water (Surface and Ground Water). Huntingdon Life Sciences Project ID: BDG0141. Report prepared by Huntingdon Life Sciences Ltd., Huntingdon Research Centre, Cambridgeshire, England, sponsored and submitted by AMVAC Chemical Corporation, Commerce, California; 46 pages. Final report issued April 11, 2012.

Document No.: MRIDs 48822502 & 48822504

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. 46 of MRID 48822502). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided; an Authenticity Certification statement was not provided (pp. 2-4 and Appendix 4, p. 46 of MRID 48822502).
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. 46 of MRID 48822504). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided; an Authenticity Certification statement was not provided (pp. 2-4 and Appendix 2, p. 46 of MRID 48822504).

Classification: This analytical method is classified as **not acceptable**. An internal validation implementing major modifications to the ECM was not provided. 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

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

Executive Summary

This analytical method, Huntingdon Life Sciences BDG0129, is designed for the quantitative determination of tribufos in water using LC/MS/MS. The method is quantitative for tribufos at the stated LOQ of 0.1 µg/L. The LOQ is greater than the lowest toxicological level of concern in water. Major modifications were made to the ECM by the independent laboratory; however, an internal validation for the updated ECM implementing the ILV modifications was not provided. Additionally, 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	48822502	48822504		Water	23/03/2012	AMVAC Chemical Corporation	LC/MS/MS	0.1 µg/L

I. Principle of the Method

Water (5 mL) was combined with acetonitrile (2 mL), then loaded onto an reverse-phase solid-phase extraction (SPE) cartridge (Oasis HLB, 60 mg, 3 mL), preconditioned with acetonitrile (3 mL) and water (3 mL; Appendix 3, p. 44 of MRID 48822502). The loaded sample was rinsed with water:acetonitrile (70:30, v:v, 3 mL), dried under vacuum (*ca.* 30 seconds), then residues were eluted with acetonitrile (4.5 mL). The extract is brought to volume (5 mL) with acetonitrile (final matrix concentration 1 mL sample water / mL final extract).

Samples were analyzed for tribufos by HPLC (Acquity UPLC® BEH C₁₈, 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. 45 of MRID 48822502). Both quantitation (*m/z* 315→169) and confirmation (*m/z* 315→57) ion transitions were monitored for tribufos. Injection volume was 10 µL.

For the ILV, the water sample volume was doubled to 10 mL and the volume of acetonitrile (ACN) added prior to the SPE step was increased from 2 mL ACN / 5 mL of sample to 2.5 mL ACN / 5 mL of sample in order to achieve acceptable recovery at the low (LOQ) fortification level (pp. 13-14 of MRID 48822504).

The LOQ for tribufos was the same in the ECM and ILV at 0.1 µg/L (p. 15 of MRID 48822502; p. 18 of MRID 48822504). The LOD was 0.025 ng/mL, equivalent to 0.025 µg/L in the sample matrix for the ECM and 0.0125 µg/L for the ILV.

II. Recovery Findings

ECM (MRID 48822502): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD \leq 20%) for analysis of tribufos in surface and ground water (p. 15; Tables 3-6, pp. 20-23). Fortifications were performed at 0.1 $\mu\text{g/L}$ (LOQ) and 1 $\mu\text{g/L}$ (10x LOQ). Quantitation ion and confirmation ion results were comparable. The water matrices were characterized (p. 11).

ILV (MRID 4882504): Mean recoveries and RSDs were within guideline requirements for analysis of tribufos in surface and ground water (p. 17; Tables 3-6, pp. 21-24). Fortifications were performed at 0.1 $\mu\text{g/L}$ (LOQ) and 1 $\mu\text{g/L}$ (10x LOQ). Quantitation ion and confirmation ion results were comparable. The surface water was characterized (p. 12). The ground water was not characterized, but it was obtained from the same source (Anglian Water Denton Lodge Borehole 1) as the ground water used in the initial ECM (p. 12 of MRID 48822504; p. 11 of MRID 48822502). The number of trials conducted was not reported; the ILV only reported that the first validation attempt produced recovery below the acceptable level of 70-120% for the low (LOQ) fortification level (p. 17).

Table 2. Initial Validation Method Recoveries for Tribufos in Water

Matrix	Fortification Level ($\mu\text{g/L}$)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%) ¹	Relative Standard Deviation (%)
Surface (mere/lake) water	Quantitation ion					
	0.1 (LOQ)	5	75-83	79	3.6	4.6
	1	5	83-87	85	1.8	2.1
	Confirmation ion					
	0.1 (LOQ)	5	72-81	77	3.6	4.7
	1	5	83-86	85	1.3	1.5
Ground water	Quantitation ion					
	0.1 (LOQ)	5	78-81	80	1.5	1.9
	1	5	82-89	87	2.9	3.4
	Confirmation ion					
	0.1 (LOQ)	5	78-83	80	2.3	2.9
	1	5	82-90	86	3.0	3.6

Data were obtained from Tables 3-6, pp. 20-23 of MRID 48822502.

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).

Table 3. Independent Validation Method Recoveries for Tribufos in Water

Matrix	Fortification Level ($\mu\text{g/L}$)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Surface water	Quantitation ion					
	0.1 (LOQ)	5	62.2-75.2	71.7	5.43	7.6
	1	5	72.2-83.9	79.3	4.08	5.1
	Confirmation ion					
	0.1 (LOQ)	5	58.4-77.3	71.3	7.41	10.4
	1	5	69.1-80.3	76.5	4.62	6.1
Ground water	Quantitation ion					
	0.01 (LOQ)	5	65.2-79.9	75.5	5.87	7.8
	0.1	5	70.3-85.0	79.3	5.51	6.9
	Confirmation ion					
	0.1 (LOQ)	5	65.5-78.6	74.3	5.12	6.9
	1	5	70.7-86.5	78.9	5.78	7.3

Data were obtained from Tables 3-6, pp. 21-24 of MRID 48822504.

III. Method Characteristics

In the ECM and ILV, the LOQ value for tribufos in water was 0.1 $\mu\text{g/L}$ (p. 15 of MRID 48822502; p. 18 of MRID 48822504). The LOD was 0.025 ng/mL, equivalent to 0.025 $\mu\text{g/L}$ in the sample matrix for the ECM and 0.0125 $\mu\text{g/L}$ for the ILV. 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 $\mu\text{g/L}$
Limit of Detection (LOD)	0.025 ng/mL (0.025 $\mu\text{g/L}$ for ECM, 0.0125 $\mu\text{g/L}$ for ILV)
Linearity (calibration curve r and concentration range)	$r = 0.9997-0.9998^1$ (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 48822502; p. 18 of MRID 48822504.

1 ILV calibration curves were linear, $r = 0.9998-0.9999$, for concentration range of 0.025-2.0 ng/mL (see Tables 1-2, p. 20; Figures 1-2, pp. 25-26 of MRID 48822504). Reviewer-calculated calibration curves verified linearity for the ECM ($r^2 = 0.9993-0.9996$) and ILV ($r^2 = 0.9999$; 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 ILV doubled the sample volume taken through the extraction procedure to 10 mL and increased the volume of acetonitrile (ACN) added to the sample prior to the SPE step to 2.5 mL ACN / 5 mL of sample to achieve acceptable recovery at the low (LOQ) fortification level (pp. 13, 19 of MRID 48822504). An internal validation for the updated ECM implementing the increased sample volume size and extraction procedure modification was not provided.
2. 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 48822502). 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 48822502; p. 18 of MRID 48822504). Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples.
3. 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.
4. The number of ILV trials required to validate the ECM was not specified. The ILV did report that the first validation attempt produced recovery below the acceptable level of 70-120% for the low (LOQ) fortification level, and following investigation, the sample volume taken through the extraction procedure was doubled and the volume of acetonitrile added to the sample prior to the SPE step was increased (p. 17 of MRID 48822504).
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. 18 of MRID 48822504).
6. The ground water used in the ILV was not characterized; however, the water was obtained from the same source (Anglian Water Denton Lodge Borehole 1) as that used in the initial ECM and was characterized in that report (p. 12 of MRID 48822504; p. 11 of MRID 48822502).
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 48822502). 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	Surface water	1.82	1.89
Procedural recovery (%)			NA	96%
Mean stability		Ground water	1.93	1.95
Procedural recovery (%)			NA	100%

Data were obtained from Table 7, p. 24 of MRID 48822502. 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 (\equiv 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.