

Test Material: Folpet

MRID: 49122712

Title: Harper, H. Folpet And Metabolites: Validation Of Methodology For The Determination Of Residues Of Folpet And Its Metabolites In Drinking Water

EPA PC Code: 081601

OCSPP Guideline: 850.6100

**For CDM Smith**

**Primary Reviewer:** Lisa Muto

**Signature:** 

**Date:** 6/9/14

**Secondary Reviewer:** Dan Hunt

**Signature:** 

**Date:** 6/9/14

**QC/QA Manager:** Joan Gaidos

**Signature:** 

**Date:** 6/9/14

**Analytical method for folpet and its transformation products phthalimide, phthalamic acid, phthalic acid, 2-cyanobenzoic acid and benzamide in drinking water**

**Reports:** ECM: EPA MRID No. 49122712. Harper, H. 2011. Folpet And Metabolites: Validation Of Methodology For The Determination Of Residues Of Folpet And Its Metabolites In Drinking Water. Huntingdon Life Sciences Study No.: LEB0046. Sponsor Reference No.: R-27683. Report prepared by Huntingdon Life Sciences Ltd., Cambridgeshire, England; sponsored and submitted by Makhteshim Chemical Works Ltd., Beer Sheva, Israel; 118 pages. Final report issued May 4, 2011.  
ILV: No ILV was provided.

**Document No.:** MRID 49122712

**Guideline:** 850.6100

**Statements:** ECM: The study was conducted in accordance with the UK GLP, EC Commission Directive 2004/10/EC, OECD and The UK Department of Health GLP, as well as SANCO/3029/99 rev. 4 and SANCO/825/00 rev. 7 (pp. 2B, 12; Annex 3, p. 96). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2A-3). A Certification of Authenticity statement was not provided.  
ILV: No ILV was provided.

**Classification:** This analytical method is classified as **Supplemental**. An ILV was not provided. LOQ was not calculated using acceptable methods. The matrix characterization and source of the drinking water were not reported. Reagent blanks were not included in the sample set.

**PC Code:** 081601

**Reviewer:** He Zhong, Ph.D.  
Biologist  
EPA/OPP/EFED

**Signature:**   
**Date:** 12-10-2014

**Executive Summary**

This analytical method, Sponsor Reference No.: R-27683, is designed for the quantitative determination of folpet and its transformation product phthalimide in pond water and sediment using GC/MS and for folpet transformation products phthalamic acid, phthalic acid, 2-cyanobenzoic acid and benzamide in drinking water using GC/MS or LC/MS/MS. The method is quantitative for folpet, phthalimide, 2-cyanobenzoic acid and benzamide at the stated LOQ of 0.1 µg/L and for phthalamic acid and phthalic acid at the stated LOQ of 2.0 µg/L. The lowest toxicological level of concern in water was not reported; however, the EPA Regional Screening Level for folpet is reported as 17 µg/L in residential tap water. Phthalimide, phthalamic acid, phthalic acid, 2-cyanobenzoic acid and benzamide were not listed in the database. An independent laboratory validation (ILV) was not provided.

**Table 1. Analytical Method Summary**

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
folpet	49122712	None provided		Water	05/04/2011	Makhteshim Chemical Works Ltd.	GC/MS	0.1 µg/L
folpet transformation product phthalimide							GC/MS	0.1 µg/L
folpet transformation products phthalamic acid and phthalic acid							LC/MS/MS	2.0 µg/L
folpet transformation products 2-cyanobenzoic acid and benzamide							LC/MS/MS	0.1 µg/L

## I. Principle of the Method

For folpet, fortified water samples (40 mL) were extracted with a mixture of sodium chloride (*ca.* 5 g), orthophosphoric acid (1 mL, 1 N) and toluene (5 mL) by shaking by hand for 1 minute (p. 19; Annex 2, pp. 90-92). The organic phase was removed and dried with anhydrous sodium sulphate. The aqueous phase was extracted again with 5 mL of toluene by shaking by hand for 1 minute. The combined extracts were mixed with 200  $\mu$ L of diglyme and diluted to 10 mL with toluene prior to analysis via gas chromatography with mass spectrometric detection (GC/MS). For phthalimide, fortified water samples (40 mL) were extracted with dichloromethane (5 mL) by shaking by hand for 1 minute. The organic phase was removed and dried with anhydrous sodium sulphate. The aqueous phase was extracted again with 5 mL of dichloromethane by shaking by hand for 1 minute. The combined extracts were mixed with 200  $\mu$ L of diglyme and diluted to 10 mL with dichloromethane prior to analysis via GC/MS. For phthalic acid, aliquots of the 10-mL fortified water samples were directly analyzed via liquid chromatography with tandem mass spectrometric detection (LC/MS/MS). For phthalamic acid and benzamide, fortified water samples (10 mL) were acidified with 100  $\mu$ L of 1M formic acid then directly analyzed via LC/MS/MS. For 2-cyanobenzoic acid, fortified water samples (40 mL) were acidified with 0.4 mL of 1M formic acid and purified using a pre-conditioned  $\text{NH}_2$  solid phase extraction (SPE) cartridge. The SPE cartridge was eluted with 6 mL of 10% ammonium in methanol. The eluate was reduced to dryness via rotary evaporation at *ca.* 40°C. The residue was reconstituted with 2 mL of 0.01M ammonium formate in methanol:water (20:80, v:v) via ultrasonication prior to analysis via LC/MS/MS.

Samples were analyzed for folpet and phthalimide by gas chromatography (Optima-17 or equivalent, 30 m x 0.25 mm, 0.5- $\mu$ m column) with mass spectrometry in negative ionization mode (p. 19; Annex 2, pp. 92-93). Confirmation of analytes was performed by a second GC/MS analysis (DB-5 or equivalent, 30 m x 0.25 mm, 0.25- $\mu$ m column). Ions were monitored for folpet (146 m/z) and phthalimide (147 m/z). Injection volume was 5  $\mu$ L (splitless) for both GC/MS analyses. The retention times of the two analytes differed in the two GC/MS analyses.

Samples were analyzed for benzamide and 2-cyanobenzoic acid by liquid chromatography (Acquity UPLC BEH  $\text{C}_{18}$ , 1.7  $\mu$ m, 50 x 2.1 mm column) using a gradient mobile phase of (A) water:methanol:formic acid (90:10:0.1, v:v:v) containing 0.01M ammonium formate and (B) 0.1% formic acid in methanol (time A:B; 0-0.2 min. 100:0, 2-2.5 min. 0:100, 3-4 min. 100:0) with mass spectrometry (p. 19; Annex 2, p. 94). Monitoring was performed with positive ionspray for benzamide and negative ionspray for 2-cyanobenzoic acid. Ions were monitored for benzamide (122 $\rightarrow$ 105 m/z quantitative, 122 $\rightarrow$ 77 m/z confirmation) and 2-cyanobenzoic acid (146 $\rightarrow$ 102 m/z quantitative, 192 $\rightarrow$ 146 m/z confirmation). Injection volume was 10  $\mu$ L.

Samples were analyzed for phthalic acid and phthalamic acid by liquid chromatography (Acquity UPLC BEH  $\text{C}_{18}$ , 1.7  $\mu$ m, 50 x 2.1 mm column) using a gradient mobile phase of (A) water:methanol:formic acid (90:10:0.1, v:v:v) containing 0.01M ammonium formate and (B) 0.1% formic acid in methanol (time A:B; 0-0.2 min. 100:0, 2-2.5 min. 5:95, 3-4 min. 100:0) with mass spectrometry (p. 19; Annex 2, p. 95). Monitoring was performed with positive ionspray for phthalamic acid and negative ionspray for phthalic acid. Ions were monitored for phthalamic acid

(166→149 m/z quantitative, 166→121 m/z confirmation) and phthalic acid (165→77 m/z quantitative, 165→121 m/z confirmation). Injection volume was 10 µL.

In the ECM, the LOQ values in the drinking water were 0.1 µg/L for folpet, phthalimide, 2-cyanobenzoic acid and benzamide and 2.0 µg/L for phthalamic acid and phthalic acid (p. 11). The LOD values were 0.05 µg/L for folpet, phthalimide, 2-cyanobenzoic acid and benzamide and 1 µg/L for phthalamic acid and phthalic acid. No ILV was provided.

## II. Recovery Findings

ECM (MRID 49122712): Mean recoveries and RSDs were within guideline requirements for analysis of folpet, phthalimide, phthalamic acid, phthalic acid, 2-cyanobenzoic acid and benzamide in drinking water (pp. 10-11). The drinking water was not characterized and only described as untreated, local drinking water (p. 18). Analytes were identified by GC/MS or LC/MS/MS.

ILV: No ILV was provided.

**Table 2. Initial Validation Method Recoveries for Folpet and Its Transformation Products in Drinking Water**

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
<b>Drinking water – Quantitative Method</b>						
Folpet	0.1 (LOQ)	5	102-107	105	1.9	1.8
	1.0	5	94-110	104	6.4	6.2
Phthalimide	0.1 (LOQ)	5	81-91	86	4.9	5.7
	1.0	5	85-94	91	3.4	3.8
Phthalic acid	2.0 (LOQ)	5	96-106	102	3.8	3.7
	20	5	93-97	95	1.5	1.6
Phthalamic acid	2.0 (LOQ)	5	76-97	85	8.6	10.1
	20	5	93-104	101	4.6	4.5
2-Cyanobenzoic acid	0.1 (LOQ)	5	71-93	85	9.3	10.9
	1.0	5	71-107	91	17.2	18.9
Benzamide	0.1 (LOQ)	5	95-101	97	2.3	2.3
	1.0	5	96-100	98	1.8	1.8
<b>Drinking water – Confirmatory Method</b>						
Folpet	0.1 (LOQ)	5	84-110	100	9.9	9.9
	1.0	5	104-108	106	1.8	1.7
Phthalimide	0.1 (LOQ)	5	76-88	81	5.1	6.3
	1.0	5	78-83	81	2.1	2.6
Phthalic acid	2.0 (LOQ)	5	88-107	99	7.2	7.3
	20	5	92-102	96	4.1	4.3

Phthalamic acid	2.0 (LOQ)	5	--*	--	--	--
	20	5	75-91	84	6.6	7.9
2-Cyanobenzoic acid	0.1 (LOQ)	5	93-105	100	4.3	4.4
	1.0	5	70-96	84	11.8	14.0
Benzamide	0.1 (LOQ)	5	97-106	102	3.6	3.5
	1.0	5	96-100	99	1.7	1.7

Data were obtained from pp. 10-11; Tables 1-12, pp. 26-37 of the study report.

\* The sensitivity of the confirmatory ion was not adequate to provide data for phthalamic acid at the LOQ.

**Table 3. Independent Validation Method Recoveries for Folpet and Its Transformation Products in Drinking Water**

Analyte	Fortification Level ( $\mu\text{g/L}$ ) or ( $\text{ng/g}$ )	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Folpet and its transformation products			No ILV provided.			
			No ILV provided.			

### III. Method Characteristics

In the ECM, the LOQ values in the drinking water were 0.1  $\mu\text{g/L}$  for folpet, phthalimide, 2-cyanobenzoic acid and benzamide and 2.0  $\mu\text{g/L}$  for phthalamic acid and phthalic acid (pp. 11, 21). The LOQ was defined as the lowest fortification level at which acceptable recovery data were obtained. No other justification or calculations were reported. The LOD values were 0.05  $\mu\text{g/L}$  for folpet, phthalimide, 2-cyanobenzoic acid and benzamide and 1  $\mu\text{g/L}$  for phthalamic acid and phthalic acid. The LOD was defined as the lowest calibration standard which demonstrated a signal to noise ratio greater than three. No ILV was provided.

**Table 4. Method Characteristics**

	Folpet	Phthalimide	Phthalic acid	Phthalamic acid	2-Cyano-benzoic acid	Benzamide
	<b>Drinking water</b>					
Limit of Quantitation (LOQ)	0.1 $\mu\text{g/L}$ (0.4 $\text{ng/mL}$ )	0.1 $\mu\text{g/L}$ (0.4 $\text{ng/mL}$ )	2.0 $\mu\text{g/L}$ (2 $\text{ng/mL}$ )	2.0 $\mu\text{g/L}$ (2 $\text{ng/mL}$ )	0.1 $\mu\text{g/L}$ (2 $\text{ng/mL}$ )	0.1 $\mu\text{g/L}$ (0.1 $\text{ng/mL}$ )
Limit of Detection (LOD)	0.05 $\mu\text{g/L}$					
Linearity (calibration curve $r^2$ and concentration range) <sup>1</sup>	$r^2 = 0.9976$ - 0.9985 (0-10 $\text{ng/mL}$ )	$r^2 = 0.9984$ - 0.9994 (0-10 $\text{ng/mL}$ )	$r^2 = 0.9992$ - 0.9996 (0-10 $\text{ng/mL}$ )	$r^2 = 0.9965$ - 0.9968 (0-10 $\text{ng/mL}$ )	$r^2 = 0.9992$ - 0.9996 (0-10 $\text{ng/mL}$ )	$r^2 = 0.9996$ - 0.9998 (0-10 $\text{ng/mL}$ )
Repeatable	Yes					
Reproducible	Could not be determined <sup>2</sup>					
Specific	Yes					

Data were obtained from p. 24, Tables 1-12, pp. 26-37 and Figures 1-12, pp. 40-51 of the study report.

<sup>1</sup> Calibration curves were provided for the quantitative and confirmatory ions. Reviewer-calculated calibration curves yielded similar linearity,  $r^2$  values of 0.9924-0.9998 for the quantitative ion and 0.9815-0.9996 for the confirmatory ion (see DER Attachment 2).

<sup>2</sup> No ILV was provided.

#### IV. Method Deficiencies and Reviewer's Comments

1. An ILV report was not provided with the ECM; therefore, the method, Sponsor Reference No.: R-27683, could not be validated.
2. The LOQ was only defined as the lowest fortification level at which acceptable recovery data were obtained. The LOQ should not be defined by arbitrarily chosen limits. Additionally, the lowest toxicological level of concern in pond water and sediment was not reported; however, folpet is found in the EPA Regional Screening Level (<http://www.epa.gov/>). Screening levels for folpet are 17 µg/L in residential tap water. Those levels are greater than the LOQ defined by the ECM method. Phthalimide, phthalamic acid, phthalic acid, 2-cyanobenzoic acid and benzamide were not listed in the database. An LOQ above toxicological levels of concern results in an unacceptable method classification.
3. Matrix characterization and source of the drinking water were not characterized; the drinking water was only described as untreated and “from the local supply” (p. 18).
4. No reagent blank was included with the samples. A reagent blank is included in the minimum sample set recommended by the OCSPP 850.6100 guidelines.
5. The study author discussed the reason for the increase in LOQ fortification levels for phthalamic acid and phthalic acid from the original LOQ (0.1 µg/L; p. 24). For phthalic acid, the control samples of drinking water contained a significant amount of phthalic acid. For phthalamic acid, there were difficulties during extraction from water.
6. The study report specifically noted to avoid plastic during the extraction of phthalic acid and phthalamic acid wherever possible (Annex 2, pp. 90-92).
7. Data was reported for all analytes with both quantification and confirmation ions except phthalamic acid, for which sensitivity limitations did not allow the confirmation ion data to be collected (Table 8, p. 33). The reviewer noted that the LOQ and LOD of phthalamic acid for the confirmation method were listed as 20 µg/L and 5 µg/L, respectively in Table 8 of the study report. The reviewer assumed that this was a typographical error.
8. The extraction method which was used in this ECM for the extraction of folpet and its transformation products from drinking water differed from the water extraction methods which were reported in the submitted ECMs for folpet in tap water (MRID 49122709; Study No. IFU94002/01-FOL) and for folpet and its transformation products in pond water and sediment (MRID 49122710; Sponsor Reference No.: R-25157).

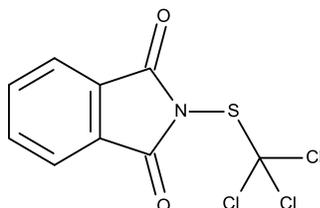
## **V. References**

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.

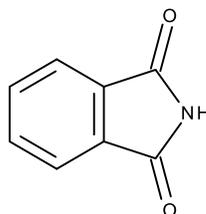
40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.

**Attachment 1: Chemical Names and Structures****Folpet, Folpan**

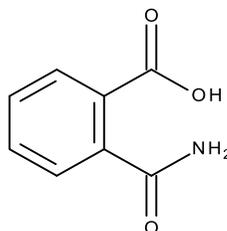
**IUPAC Name:** N-(Trichloromethylthio)phthalimide  
**CAS Name:** 2-[(Trichloromethyl)thio]-1H-isoindole-1,3(2H)-dione  
**CAS Number:** 133-07-3  
**SMILES String:** O=C(N(SC(Cl)(Cl)Cl)C(=O)c1cccc2)c12

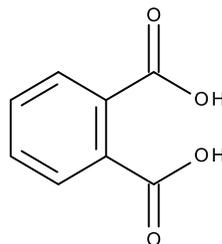
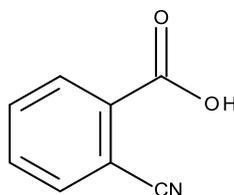
**Phthalimide**

**IUPAC Name:** Isoindole-1,3-dione  
**CAS Name:** 1,3-Dihydro-1,3-dioxoisoindole  
**CAS Number:** 85-41-6  
**SMILES String:** c1ccc2c(c1)C(=O)NC2=O

**Phthalamic acid**

**IUPAC Name:** 2-(Aminocarbonyl)-benzoic acid  
**CAS Name:** 2-(Aminocarbonyl)-benzoic acid  
**CAS Number:** 88-97-1  
**SMILES String:** O=C(O)c(c(ccc1)C(=O)N)c1



**Phthalic acid****IUPAC Name:** 1,2-Benzenedicarboxylic acid**CAS Name:** 1,2-Benzenedicarboxylic acid**CAS Number:** 88-99-3**SMILES String:** O=C(O)c1ccccc1C(=O)O**2-Cyanobenzoic acid****IUPAC Name:** 2-Cyanobenzoic acid**CAS Name:** Not reported**CAS Number:** 3839-22-3**SMILES String:** c1ccc(c1)C#NC(=O)O**Benzamide****IUPAC Name:** Benzamide**CAS Name:** Not reported**CAS Number:** 55-21-0**SMILES String:** O=C(N)c1ccccc1