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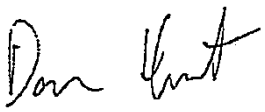
|---|---|
| Test Material: | Folpet |
| MRID: | 49122710 |
| Title: | Harper, H. Folpet: Method Validation For The Determination Of Folpet And Degradates In Pond Water And Sediment |
| EPA PC Code: | 081601 |
| OCSPP Guideline: | 850.6100 |

**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, phthalimic acid, phthalic acid, 2-cyanobenzoic acid and benzamide in pond water and sediment**

**Reports:** ECM: EPA MRID No. 49122710. Harper, H. 2009. Folpet: Method Validation For The Determination Of Folpet And Degradates In Pond Water And Sediment. Huntingdon Life Sciences Project Identity: MAK0978. Sponsor Reference No.: R-25157. Report prepared by Huntingdon Life Sciences Ltd., Cambridgeshire, England; sponsored and submitted by Makhteshim Chemical Works Ltd., Beer Sheva, Israel; 77 pages. Final report issued September 10, 2009.
ILV: No ILV was provided.

**Document No.:** MRID 49122710

**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 (p. 2B; Annex 2, pp. 75-76). 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. The fortifications of phthalimide, phthalamic acid and phthalic acid were not performed at 10×LOQ in the pond sediment or pond water and sediment. LOQ and LOD were not calculated using acceptable methods. The matrix characterization and source of the pond water and soil were not reported. Sample chromatograms were not provided for all fortification levels.

**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-25157, 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 pond water and sediment using LC/MS/MS. The method is quantitative for folpet at the stated LOQ of 1 µg/L for water and 5 ng/g for sediment; phthalimide at the stated LOQ of 0.5 µg/L for water and 20 ng/g for sediment; phthalamic acid and phthalic acid at the stated LOQ of 2.5 µg/L for water and 20 ng/g for sediment; and 2-cyanobenzoic acid and benzamide at the stated LOQ of 0.5 µg/L for water and 5 ng/g for sediment. The lowest toxicological level of concern in water was not reported; however, the EPA Regional Screening Levels for folpet are reported as 17 µg/L in residential tap water and 140

mg/kg in residential soil. 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 | 49122710 | None provided | | Pond water and sediment | 09/10/2009 | Makhteshim Chemical Works Ltd. | GC/MS | water – 1 µg/L sediment - 5 ng/g |
| folpet transformation product phthalimide | | | | | | | GC/MS | water – 0.5 µg/L sediment - 20 ng/g |
| folpet transformation products phthalamic acid and phthalic acid | | | | | | | LC/MS/MS | water – 2.5 µg/L sediment - 20 ng/g |
| folpet transformation products 2-cyanobenzoic acid and benzamide | | | | | | | LC/MS/MS | water - 0.5 µg/L sediment - 5 ng/g |

## I. Principle of the Method

Pond water extraction:

For folpet, water samples (25 mL) were extracted with a mixture of saturated sodium chloride solution (5 mL), orthophosphoric acid (1 mL, 1 N) and toluene (5 mL) by shaking by hand for 1 minute (pp. 21, 24). 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 µL of diglyme and diluted to 10 mL with toluene prior to analysis via gas chromatography with mass spectrometric detection (GC/MS). For phthalimide, water samples (25 mL) were extracted with dichloromethane (5 mL) by shaking by hand for 1 minute (pp. 21, 25). The organic phase was removed and dried with anhydrous sodium sulphate. A 1-mL aliquot of the dried organic phase was diluted to 2 mL with 2% diglyme in toluene prior to analysis via GC/MS. For phthalamic acid, phthalic acid and benzamide, 1-mL aliquots of the aqueous phase were directly analyzed via liquid chromatography with tandem mass spectrometric detection (LC/MS/MS). For 2-cyanobenzoic acid, a 20-mL aliquot of the aqueous phase was acidified with 200 µL of 1M formic acid and purified using a pre-conditioned $NH_2$ solid phase extraction (SPE) cartridge. The SPE cartridge was eluted with 6 mL of 10% ammonium in methanol. The eluate was reduced to *ca*. 0.5 mL via rotary evaporation at *ca*. 40°C. The residue was transferred to a polypropylene tube with acetone rinsing of the former flask. The volume was reduced to *ca*. 0.5 mL via rotary evaporation at *ca*. 40°C. The residue was reconstituted with water to a final volume of 2 mL prior to analysis via LC/MS/MS.

Pond sediment extraction:

For folpet and phthalimide, sediment samples (2.5 g) were mixed with water (10 mL) then extracted with toluene (5 mL) by shaking by hand for 1 minute (pp. 21, 26). The toluene extracts were purified using an Envi-Carb solid phase extraction (SPE) cartridge with anhydrous sodium sulphate layered on top. The aqueous phase was extracted again with 5 mL of toluene, and the organic phase was also added to the Envi-Carb SPE cartridge. The eluates were mixed with 250 µL of diglyme and diluted to 12.5 mL with toluene prior to analysis via GC/MS. For phthalamic acid, phthalic acid, benzamide and 2-cyanobenzoic acid, the aqueous phase was diluted to 12.5 mL with water, then aliquots (*ca*. 3 mL) were filtered through a 0.2 µm syringe filter prior to analysis via LC/MS/MS.

Samples were analyzed for folpet and phthalimide by gas chromatography (Rxi-5ms, 30 m x 0.25 mm, 0.25-µm column) with mass spectrometry in negative ionization mode (p. 27). Ions were monitored for folpet (146 m/z) and phthalimide (146 m/z). Injection volume was 1 µL.

Samples were analyzed for phthalamic acid, phthalic acid, benzamide and 2-cyanobenzoic acid by liquid chromatography (Phenomonex Luna $C_8$, 15 cm x 2 mm i.d. 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 min. 100:0, 6-10 min. 0:100, 11-15 min. 100:0) with mass spectrometry (p. 28). Monitoring was performed with positive electrospray for benzamide and negative electrospray for phthalic acid, benzamide and 2-

cyanobenzoic acid. Ions were monitored for phthalamic acid (164→120 m/z), phthalic acid (165→121 m/z), benzamide (122→79 m/z) and 2-cyanobenzoic acid (146→102 m/z). Injection volume was 20 µL.

In the ECM, the LOQ values for folpet, phthalimide, phthalamic acid, phthalic acid, 2-cyanobenzoic acid and benzamide were 1 µg/L, 0.5 µg/L, 2.5 µg/L, 2.5 µg/L, 0.5 µg/L and 0.5 µg/L, respectively, for pond water and 5 ng/g, 20 ng/g, 20 ng/g, 20 ng/g, 5 ng/g and 5 ng/g, respectively, for pond sediment (pp. 10, 27-28). The LOD values for folpet, phthalimide, phthalamic acid, phthalic acid, 2-cyanobenzoic acid and benzamide were 0.2 µg/L, 0.2 µg/L, 1 µg/L, 1 µg/L, 0.1 µg/L and 0.25 µg/L, respectively, for pond water and 2.5 ng/g for all analytes for pond sediment. No ILV was provided.

## II. Recovery Findings

ECM (MRID 49122710): Mean recoveries and RSDs were within guideline requirements for analysis of folpet, phthalimide, phthalamic acid, phthalic acid, 2-cyanobenzoic acid and benzamide in pond water and sediment (p. 9). The pond water and sediment were not characterized; only the supplier was specified (Cambridge Environmental Assessments microcosm facilities; p. 19). 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 Pond Water and Sediment**

| Analyte | Fortification Level (µg/L) or (ng/g)* | Number of Tests | Recovery Range (%) | Mean Recovery (%) | Standard Deviation (%) | Relative Standard Deviation (%) |
|---|---|---|---|---|---|---|
| **Pond water** | | | | | | |
| Folpet | 1 (LOQ) | 5 | 88-103 | 95 | 6.7 | 7.0 |
| | 10 | 5 | 94-107 | 100 | 4.8 | 4.8 |
| | 1000 | 5 | 87-105 | 96 | 7.0 | 7.3 |
| Phthalimide | 0.5 (LOQ) | 5 | 82-94 | 88 | 5.1 | 5.8 |
| | 5 | 5 | 101-108 | 105 | 2.9 | 2.8 |
| | 500 | 5 | 89-110 | 99 | 9.1 | 9.2 |
| Phthalamic acid | 2.5 (LOQ) | 5 | 71-94 | 85 | 9.6 | 11.2 |
| | 5 | 5 | 76-110 | 101 | 14.2 | 14.1 |
| | 500 | 5 | 75-118 | 100 | 15.9 | 15.9 |
| Phthalic acid | 2.5 (LOQ) | 5 | 72-85 | 80 | 5.4 | 6.8 |
| | 5 | 5 | 91-104 | 100 | 5.4 | 5.4 |
| | 500 | 5 | 106-110 | 108 | 1.6 | 1.5 |
| 2-Cyanobenzoic acid | 0.5 (LOQ) | 5 | 80-106 | 93 | 12.0 | 12.9 |
| | 5 | 5 | 70-90 | 82 | 9.0 | 11.0 |
| | 500 | 5 | 102-112 | 105 | 4.2 | 3.9 |
| Benzamide | 0.5 (LOQ) | 5 | 70-72 | 71 | 1.1 | 1.5 |
| | 5 | 5 | 77-81 | 79 | 1.7 | 2.1 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | 500 | 5 | 82-86 | 84 | 1.9 | 2.2 |
| **Pond sediment** | | | | | | |
| Folpet | 5 (LOQ) | 5 | 88-98 | 92 | 4.8 | 5.2 |
| | 50 | 5 | 73-86 | 82 | 5.3 | 6.4 |
| | 500 | 5 | 87-92 | 89 | 1.9 | 2.2 |
| Phthalimide | 20 (LOQ) | 5 | 74-81 | 76 | 2.7 | 3.5 |
| | 50 | 5 | 74-75 | 74 | 0.4 | 0.6 |
| | 500 | 5 | 74-78 | 75 | 1.7 | 2.2 |
| Phthalamic acid | 20 (LOQ) | 5 | 105-111 | 108 | 2.4 | 2.2 |
| | 50 | 5 | 74-105 | 88 | 11.2 | 12.8 |
| | 500 | 5 | 77-86 | 81 | 4.0 | 5.0 |
| Phthalic acid | 20 (LOQ) | 5 | 91-110 | 101 | 7.7 | 7.6 |
| | 50 | 5 | 88-100 | 94 | 4.6 | 4.9 |
| | 500 | 5 | 96-103 | 99 | 2.6 | 2.6 |
| 2-Cyanobenzoic acid | 5 (LOQ) | 5 | 70-102 | 87 | 12.3 | 14.3 |
| | 50 | 5 | 76-105 | 97 | 12.2 | 12.5 |
| | 500 | 5 | 90-101 | 96 | 4.7 | 4.9 |
| Benzamide | 5 (LOQ) | 5 | 70-75 | 73 | 2.4 | 3.3 |
| | 50 | 5 | 71-79 | 74 | 3.1 | 4.2 |
| | 500 | 5 | 70-74 | 72 | 1.8 | 2.5 |

Data were obtained from p. 9; Tables 7-18, pp. 39-50 of the study report.
* Fortification units were µg/L for pond water and ng/g for pond sediment.

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

| Analyte | Fortification Level (µg/L) or (ng/g) | Number of Tests | Recovery Range (%) | Mean Recovery (%) | Standard Deviation (%) | Relative Standard Deviation (%) |
|---|---|---|---|---|---|---|
| Folpet and it transformation products | | No ILV provided. | | | | |
| | | No ILV provided. | | | | |

## III. Method Characteristics

In the ECM, the LOQ values for folpet, phthalimide, phthalamic acid, phthalic acid, 2-cyanobenzoic acid and benzamide were 1 µg/L, 0.5 µg/L, 2.5 µg/L, 2.5 µg/L, 0.5 µg/L and 0.5 µg/L, respectively, for pond water and 5 ng/g, 20 ng/g, 20 ng/g, 20 ng/g, 5 ng/g and 5 ng/g, respectively, for pond sediment (pp. 10, 27-28, 34). 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 for folpet, phthalimide, phthalamic acid, phthalic acid, 2-cyanobenzoic acid and benzamide were 0.2 µg/L, 0.2 µg/L, 1 µg/L, 1 µg/L, 0.1 µg/L and 0.25 µg/L, respectively, for pond water and 2.5 ng/g for all analytes for pond sediment. No calculation or comparison to background levels was reported.

**Table 4. Method Characteristics**

| | Folpet | Phthalimide | Phthalamic acid | Phthalic acid | 2-Cyanobenzoic acid | Benzamide |
|---|---|---|---|---|---|---|
| | **Pond water** | | | | | |
| Limit of Quantitation (LOQ) | 1 µg/L | 0.5 µg/L | 2.5 µg/L | 2.5 µg/L | 0.5 µg/L | 0.5 µg/L |
| Limit of Detection (LOD) | 0.2 µg/L | 0.2 µg/L | 1 µg/L | 1 µg/L | 0.1 µg/L | 0.25 µg/L |
| Linearity (calibration curve $r^2$ and concentration range) [1] | $r^2$ = 0.9983 (0-50 ng/mL) | $r^2$ = 0.9986 (0-50 ng/mL) | $r^2$ = 0.9992 (0.5-100 ng/mL) | $r^2$ = 0.9975 (0-50 ng/mL) | $r^2$ = 0.9993 (0.5-100 ng/mL) | $r^2$ = 0.9995 (0.25-100 ng/mL) |
| Repeatable | Yes | | | | | |
| Reproducible | Could not be determined[2] | | | | | |
| Specific | Yes | | | | | |
| | **Pond sediment** | | | | | |
| Limit of Quantitation (LOQ) | 5 ng/g | 20 ng/g | 20 ng/g | 20 ng/g | 5 ng/g | 5 ng/g |
| Limit of Detection (LOD) | 2.5 ng/g | 2.5 ng/g | 2.5 ng/g | 2.5 ng/g | 2.5 ng/g | 2.5 ng/g |
| Linearity (calibration curve $r^2$ and concentration range) [1] | $r^2$ = 0.9983 (0-50 ng/mL) | $r^2$ = 0.9986 (0-50 ng/mL) | $r^2$ = 0.9992 (0.5-100 ng/mL) | $r^2$ = 0.9975 (0-50 ng/mL) | $r^2$ = 0.9993 (0.5-100 ng/mL) | $r^2$ = 0.9995 (0.25-100 ng/mL) |
| Repeatable | Yes | | | | | |
| Reproducible | Could not be determined[2] | | | | | |
| Specific | Yes | | | | | |

Data were obtained from pp. 8, 10, 27-28 and Tables 1-6, pp. 36-38 of the study report.
1 Only one set of calibration data was provided for each analyte. The calibration data for each analyte was found to be linear, except for that of benzamide which was found to be quadratic (p. 8). Reviewer-calculated calibration curves yielded similar linearity, $r^2$ values of 0.9925-0.9987 (see DER Attachment 2).
2 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-25157, could not be validated.

2. In the ECM, fortifications of phthalamic acid and phthalic acid in the pond water and sediment and phthalimide in the pond sediment were not performed at 10×LOQ, as required by the OCSPP guidelines.

3. The reported LOD in the ECM was not based on scientifically acceptable procedures as defined in 40 CFR Part 136. The LOQ was only defined as the lowest fortification level at which acceptable recovery data were obtained. The LOQ and LOD 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 and 140 mg/kg in residential soil. 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.

4. Matrix characterization and source of the pond water and sediment were not characterized; only the supplier was specified (Cambridge Environmental Assessments microcosm facilities; p. 19).

5. Sample chromatograms were not provided for the mid-level fortification (10×LOQ, 2×LOQ or 2.5×LOQ); chromatograms were only provided for the matrix blanks and spiked samples at the LOQ and highest fortification (1000×LOQ, 200×LOQ, 100×LOQ or 25×LOQ, depending on the matrix and analyte; Figures 1-14, pp. 53-66).

6. The study author discussed the reason for the difference in LOQ fortification levels for phthalimide in sediment and phthalamic acid and phthalic acid in water and sediment from the original LOQ (0.5 µg/L for water and 5 ng/g for sediment; pp. 10, 33). The control samples contained a significant amount (>30% of the original LOQ) of those analytes in the matrices indicated; therefore, it was necessary to increase the LOQ for the analytes in the indicated matrices so that the inferences would be <30% of the LOQ.

7. The water extraction method which was used in this ECM for the extraction of folpet and its transformation products in pond water differed from the water extraction methods which were reported in the submitted ECMs for folpet and its transformation products in drinking water (MRID 49122712; Sponsor Reference No.: R-27683) and for folpet in tap water (MRID 49122709; Study No. IFU94002/01-FOL).

8. The active substance percentage of folpet in the formulated product, Folpan 80 WDG, was determined to be 79.2% w:w using Method R-13553 (pp. 20, 35). The procedure of this different method which used HPLC/UV analysis was detailed in the study report (pp. 26, 29). This method was used to generate a certificate of analysis for Folpan 80 WDG (pp. 20, 35).

## V. References

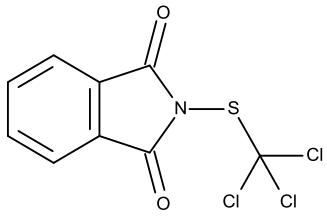
U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

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**

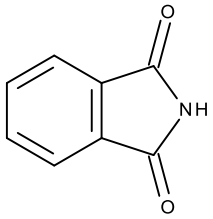
**Folpet, Folpan**

**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



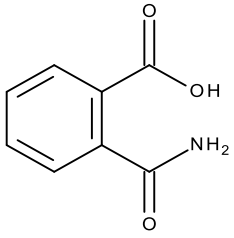

**Phthalimide**

**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



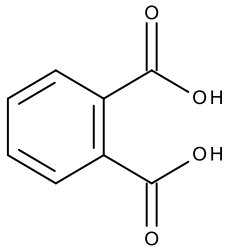

**Phthalamic acid**

**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



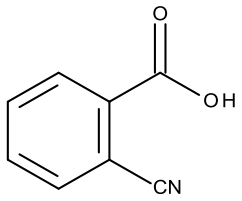

**Phthalic acid**

**IUPAC Name:** 1,2-Benzenedicarboxylic acid
**CAS Name:** 1,2-Benzenedicarboxylic acid
**CAS Number:** 88-99-3
**SMILES String:** O=C(O)c(c(ccc1)C(=O)O)c1




**2-Cyanobenzoic acid**

**IUPAC Name:** 2-Cyanobenzoic acid
**CAS Name:** Not reported
**CAS Number:** 3839-22-3
**SMILES String:** c1ccc(c(c1)C#N)C(=O)O




**Benzamide**

**IUPAC Name:** Benzamide
**CAS Name:** Not reported
**CAS Number:** 55-21-0
**SMILES String:** O=C(N)c(cccc1)c1