MATERIALS AND METHODS

This study was conducted according to the protocol "Validation of a Method for the Determination of Chlorpropham in Soil for Support of Terrestrial Field Dissipation Studies" (Appendix 1).

Test Substance

The Chlorpropham (PIN-NIP 98%) test substance was received from 1,4Group on September 20, 2013 and was assigned Wildlife International identification number 11281 upon receipt. The test substance, described as a solid, was identified on the label as: PIN-NIP 98% Chlorpropham; Batch Number: 1364. The test substance had a reported purity of 99.47% and an expiration date of October 05, 2015. The test substance was stored under ambient conditions. A Certificate of Analysis for the test substance is presented in Appendix 2.

Analytical Reference Substance

The Chlorpropham reference substance was received from ChemService on September 26, 2013 and was assigned Wildlife International identification number 11294 upon receipt. The reference substance, described as a solid, was identified on the label as: Chlorpropham; Lot/Batch Number: 1538900. The reference substance had a reported purity of 99.6% and an expiration date of September 30, 2015. The reference substance was stored under refrigerated conditions. A Certificate of Analysis for the analytical reference substance is presented in Appendix 3.

Reagents and Solvents

All solvents used in this study were HPLC grade or equivalent. All reagents used were ACS reagent grade or higher quality.

Test System

The soil used to prepare the method validation samples for this study was obtained from Agvise Laboratories and was characterized in compliance with GLP standards. This particular soil was chosen to best represent the soil type for the region of potential terrestrial field dissipation study. It was characterized as a sandy loam according to USDA textural class (hydrometer method). The soil characterization report is presented in Appendix 4.

Test Substance Stocks/Standards Preparation

A primary stock solution of Chlorpropham (PIN NIP 98%) was prepared by weighing 0.1005 grams (weight corrected for purity) of the test substance on an analytical balance. The test substance was transferred to a 100-mL class A volumetric flask, and brought to volume using acetonitrile to achieve a 1.00 mg a.i./mL stock solution. This primary stock solution (1000 µg a.i./mL) was serially diluted in the same solvent solution to prepare 100, 10.0 and 1.00 µg a.i./mL working stock solutions. The following shows the dilution scheme for the working standards of Chlorpropham (PIN NIP 98%):

Stock Concentration (µg a.i./mL)	Aliquot (mL)	Final Volume (mL)	Stock Concentration (µg a.i./mL)
1000	10.0	100	100
100	10.0	100	10.0
10.0	10.0	100	1.00

The 100 and 10.0 µg a.i./mL stock solutions were used to prepare the method validation samples for this study.

Analytical Reference Substance Stocks/Standards Preparation

A primary stock solution of Chlorpropham was prepared by weighing 0.0502 grams (weight corrected for purity) of the test substance on an analytical balance. The test substance was transferred to a 50-mL class A volumetric flask, and brought to volume using acetonitrile to achieve a 1.00 mg a.i./mL stock solution. This primary stock solution (1000 µg a.i./mL) was serially diluted in the same solvent solution to prepare 100, 10.0, 1.00 and 0.100 µg a.i./mL working stock solutions. The following shows the dilution scheme for the working standards of Chlorpropham reference substance:

Stock Concentration (µg a.i./mL)	Aliquot (mL)	Final Volume (mL)	Stock Concentration (µg a.i./mL)
1000	5.00	50.0	100
100	5.00	50.0	10.0
10.0	5.00	50.0	1.00
1.00	5.00	50.0	0.100

Calibration standards of Chlorpropham were prepared in acetonitrile: water (50:50, v/v) using the 1.00 and 0.100 µg a.i./mL stock solutions. The following shows the dilution scheme for a set of calibration standards:

Stock Concentration (µg a.i./mL)	Aliquot (mL)	Final Volume (mL)	Calibration Standard Concentration (µg a.i./L)
0.100	0.200	100	0.200
0.100	0.500	100	0.500
0.100	1.00	100	1.00
1.00	0.250	100	2.50
1.00	0.500	100	5.00
1.00	1.00	100	10.0

Analytical Method

Soil (10.0 gram aliquots) were weighed into individual 50-mL plastic graduated centrifuge tubes, fortified at two different concentrations (0.0500 and 0.500 mg a.i./kg) and analyzed based on methodology developed by Wildlife International. One reagent and two matrix blanks were prepared for analysis to evaluate potential analytical method interferences.

Twenty-five mL volumes of acetonitrile solvent were added to each soil sample and mixed well by a combination of hand shaking and vortexing. The mixture was extracted for approximately one minute using a BRANSONIC ultrasonic disruption sample processor at an amplitude setting of approximately 45%. The resulting extracts were then centrifuged at approximately 4500 rpm for approximately five minutes and the supernatant decanted into a glass 100-mL graduated cylinder. Following, a second 25 mL volume of acetonitrile was added to each soil pellet. The samples were capped and placed securely on a gyratory shaker table apparatus and shaken at a setting of approximately 250 excursions/ minute for approximately 15 minutes. The extracts were centrifuged again at ~4500 rpm for ~5 minutes and the supernatant combined in the same 100-mL graduated cylinder from above. Lastly, a 25 mL volume of acetonitrile: water (75:25, v/v) was added to each soil pellet and the shaking, centrifugation, and combining of extracts into the same 100-mL graduated cylinder procedure from above was repeated. The final volume in the graduated cylinder was then adjusted to 75.0 mL using acetonitrile. The final extracts were transferred to 150-mL beakers and swirled gently to ensure mixing. An aliquot (~3-5 mL) of each was filtered using an assembly of a 5-mL BD disposable plastic syringe connected to a 0.2 µm WHATMAN PURADISK 25 TF syringe filter into a 20-mL glass scintillation vial. A portion (~20 mL) of each unfiltered final intermediate extract was transferred to an appropriate storage container and saved. The remaining extract was then discarded. Next, a 1.00 mL aliquot of each final filtered extract was volumetrically transferred to 15-mL plastic graduated centrifuge tube and adjusted to 10.0 mL final volume using a solution of acetonitrile: water (45:55, v/v) to achieve an approximate final solvent composition of acetonitrile: water (50:50,v/v). The dilutions were mixed well. If necessary, dilute the final sample extract dilution further using a solution of acetonitrile: water (50:50, v/v). Aliquots of the final diluted sample extracts were transferred to auto-sampler vials and submitted for analysis.

Concentrations of Chlorpropham in soil samples were determined using an Agilent Technologies Infinity Series High Performance Liquid Chromatograph (HPLC) coupled with an Applied Biosystems/MDS Sciex API 3000 Mass Spectrometer (MS/MS) using a Turbo-Ion Spray source operated in the positive, multiple reaction monitoring (MRM) scan mode. Chromatographic separations were achieved using a THERMO EC Betasil C-18 column (50 mm x 2.1 mm, 5 µm particle size), preceded by a THERMO EC Betasil C-18 guard column (20 mm x 2.1 mm) utilizing a gradient elution profile. The High Performance Liquid Chromatography/Mass Spectrometer (HPLC/MS/MS) operating parameters are summarized in Table 1. A detailed analytical method outline is provided in Figure 1.

Calibration curves were generated from analyses of Chlorpropham reference standard solutions analyzed concurrently with the series of method validation samples.

Method Limit of Quantitation (LOQ)

The method LOQ for the soil method validation was set at 0.0500 mg a.i./kg, the lowest level fortified and analyzed during the validation set. Reagent blank and matrix blank samples were further screened to confirm any potential interference to be < 30% of the fortified LOQ level. The theoretical LOD was 0.0150 mg a.i./kg, calculated as the product of the lowest calibration standard (0.200 µg a.i./L) and the dilution factor of the matrix blank samples (75.0).

Example Calculations

The Chlorpropham analytical result and percent recovery for soil method validation sample number 535C-148-S-VMAS-1, nominal concentration of 0.0500 mg a.i./kg, were calculated using the following equation:

Chlorpropham
(mg a.i./kg) in sample:

$$= \frac{\text{Peak area - (Y-intercept)}}{\text{Slope}} \text{ X } \frac{\text{Final Volume (mL)}}{\text{Initial Mass (g)}} \text{ X SDF X CF}$$

Peak area = 7585.1
Y-intercept = 270.77
Slope = 11159
Initial Mass (g) = 10.0
Final Volume (mL) = 75.0
Secondary Dilution Factor (SDF) = 10.0
Conversion Factor (CF) = 1 mg/1000 µg

$$\text{Concentration (mg a.i./kg) in sample} = \frac{7585.1 - 270.77}{11159} \text{ X} \frac{75.0}{10.0} \text{ X } 10.0 \text{ X } \frac{1 \text{ mg}}{1000 \text{ µg}}$$

Concentration in sample (mg a.i./kg) = 0.0492

$$\text{Percent of nominal concentration} = \frac{0.0492 \text{ (mg a.i./kg)}}{0.0500 \text{ (mg a.i./kg)}} \text{ X } 100$$

Percent of nominal concentration = 98.3%*

*Results were generated using Analyst Software Version 1.5.2 in the full precision mode. Manual calculations may differ slightly.

Table 1

Typical High Performance Liquid Chromatography/Mass Spectrometer (LC/MS/MS)
Operational Parameters for the Analysis of Chlorpropham

Instrument:	Agilent Technologies 1200 Infinity Series High Performance Liquid Chromatograph (HPLC) coupled with an Applied Biosystems MDS SCIEX API 3000 Tandem Mass Spectrometer (MS/MS) with a Turbo-Ion Spray Source operated in the positive ion multiple reaction monitoring (MRM) mode.
Analytical Column:	THERMO EC Betasil C-18 (50 x 2.1 mm, 5 µm particle size)
Guard Column:	THERMO EC Betasil C-18 (20 x 2.1 mm)
Column Oven Temperature:	40°C
Mobile Phases:	A - 0.1% formic acid in water B – 0.1% formic acid in acetonitrile

Gradient Elution Profile :

Time (min.)	Flow Rate (uL/min.)	Percent A	Percent B
0.00	350	80	20
1.00	350	80	20
4.00	350	5	95
6.00	350	5	95
6.10	350	80	20
10.0	350	80	20

Injection Volume:	50.0 µL
Ion Source:	Turbo-Ion Spray, positive mode

Parameter Table:

CUR:	8.00	IS:	5500.00
NEB:	12.00	DP:	15.00
FP :	150.00	EP:	10.00
CAD:	4.00	CE:	13.00, 25.00, 35.00
TEM:	500.00	CXP:	11.00, 18.00, 20.00

Monitored Transition(s):	214 → 172 amu – Quantitation (dwell time 500 msec) 214 → 154 amu – Confirmation (dwell time 250 msec)
Approximate Retention Time:	~6.24 minutes

FLOWCHART FOR THE ANALYSIS OF CHLORPROPHAM IN SOIL

Prepare calibration standards in acetonitrile: water (50:50, v/v) using volumetric flasks and gas-tight syringes. **STORE REFRIGERATED.**

↓

Weigh 10.0 gram of soil matrix into 50-mL plastic graduated centrifuge tubes or equivalent.

↓

Fortify samples as needed, using the appropriate stock solution(s).

↓

Add 25 mL of acetonitrile and mix well by hand shaking/vortexing.

↓

Extract samples for ~ one minute using a BRANSONIC ultrasonic disruption sample processor at 45% amplitude setting.

↓

Centrifuge the samples at ~ 4500 rpm for ~ 5 minutes. Decant the supernatant into a 100-mL glass graduated cylinder.

↓

Add a second 25 mL volume of acetonitrile to each soil pellet. Cap and place securely on a gyratory shaker table apparatus. Shake samples at a setting of ~ 250 for ~ 15 minutes. Centrifuge the samples at ~4500 rpm for ~ 5 minutes and combine the extract supernatant in the 100-mL graduated cylinder from above.

↓

Add a 25 mL volume of acetonitrile: water (75:25, v/v) to each soil pellet and repeat the shaking procedure in the previous step.

↓

Centrifuge the samples at ~4500 rpm for ~ 5 minutes and combine the extract supernatant in the 100-mL graduated cylinder from above.

↓

Adjust to final volume of 75.0 mL with acetonitrile. Transfer to a 150-mL beaker or equivalent and gently swirl to mix. Filter an appropriate volume (~3-5 mL) of each extract using a BD disposable 5.0-mL plastic syringe and a syringe filter into a 20-mL glass scintillation vial. Transfer a portion (~20 mL) of each remaining unfiltered final intermediate extract to appropriate storage container. Discard the remainder of each extract.

↓

Volumetrically transfer a 1.00 mL aliquot of each filtered extract to a 15-mL plastic centrifuge tube and adjust to 10.0 mL final volume graduation using a solution of acetonitrile: HPLC grade bottled water (45:55, v/v) to achieve an approximate final extract solvent composition of acetonitrile: water (50:50, v/v). Mix well.

↓

Dilute sample extracts further, if necessary, using acetonitrile: water (50:50, v/v). Transfer an aliquot of the final extract to an auto-sampler vial and submit for LC/MS/MS analysis.

Figure 1. Analytical method outline for the analysis of Chlorpropham in Soil