SUMMARY

An analytical method for the determination of dichlobenil (DBN) and its metabolite 2,6-dichlorobenzamide (BAM) in soil was developed and validated at PTRL West (a division of EAG, Inc.).

Dichlobenil and 2,6-dichlorobenzamide were analyzed using internal standardization by gas chromatography with mass spectrometry detection (GC/MS) in SIM mode. Soil samples were individually fortified with DBN at 0.01 mg/kg (ppm) and with BAM at 0.005 mg/kg (ppm) at the LOQ level, and with DBN at 0.1 mg/kg and with BAM at 0.05 mg/kg at the 10X LOQ level. The limit of detection (LOD) was defined as 20% of the LOQ (0.002 mg/kg for DBN and 0.001 mg/kg for BAM).

Validation of DBN and BAM in the soil matrix was conducted with one reagent blank, two untreated controls and ten control samples spiked for two fortification levels: five samples were spiked at the LOQ level and five samples at the 10X LOQ level. The analytes were extracted from soil with acetone/hexane (1:1 v/v) and 0.2% NH_4Cl, followed by liquid-liquid partition with hexane and solid phase extraction (SPE) clean-up for DBN, and by liquid-liquid partition with ethyl acetate and filtration for BAM. For BAM extraction, the aqueous phase was basified (pH > 9) with NH_4OH prior to liquid-liquid partition. All extracts were evaporated with a gentle stream of nitrogen (room temperature for DBN extracts and 40°C for BAM extracts). Same quantity of internal standard, 4-chlorobenzonitrile, was added to all final extracts prior to GC/MS analysis.

Residues of DBN and BAM were quantitated against separate 1/x weighted linear curves of the corresponding reference substances whose concentrations ranged from 4 ng/mL to 400 ng/mL for each analyte. Calibrants containing DBN were dissolved in hexane so as to match the extracts spiked with DBN; similarly, calibrants containing BAM were dissolved in ethyl acetate to match the extracts spiked with BAM. All calibrants and fortified samples contained the internal standard 4-chlorobenzonitrile. The calibration for each analyte yielded acceptable linearity (correlation coefficient $r^2 > 0.997$) over the range examined. The quantitation of each analyte was based on the peak area response (ratio between the corresponding analyte and the internal standard) and concentration of the calibration standards. The amount of DBN was determined with the quantitation ion m/z 171 and two confirmation ions: m/z 173 and m/z 136. The amount of BAM was determined with the quantitation ion m/z 173 and one confirmation ion m/z 175. The amount of internal standard was determined with the quantitation ion m/z 137.

Recoveries from fortified samples were determined by calculating the amount of each individual analyte and dividing by the corresponding amount at each fortification level.

Negligible interferences (< LOD) or no residues (DBN, BAM, and internal standard) were detected in the reagent blank and controls (untreated samples) in all monitored ions.

Matrix effect was assessed so as to determine if signal enhancement or suppression was observed during analysis. Matrix effects were assessed by comparing the GC/MS response of DBN and BAM standard solutions prepared in solvent with standard solutions prepared in matrix (untreated soil samples spiked separately with each analyte solvent-based standard solutions) at the same concentration (20 ng/mL). No significant matrix suppression or enhancement ($\leq$ 110%) was observed in the spiked controls for any of the ions monitored of both compounds; therefore, quantitation and confirmation were conducted relative to solvent-based calibrants.

INTRODUCTION

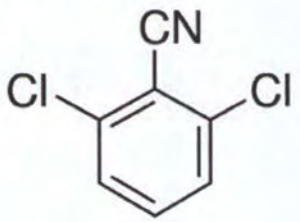
The purpose of this study was to develop and validate the analytical method for the determination of dichlobenil (DBN) and its metabolite 2,6-dichlorobenzamide (BAM) in soil. The analysis of the test substances was performed by gas chromatography with mass spectrometry detection (GC/MS) further developed in this study with the consideration of an analytical method (Reference 1) provided by the Sponsor.

This study was designed to satisfy US EPA Guideline requirements described in OCSPP 850.6100. The study was initiated on September 17, 2015. The experimental work was conducted from September 17 to November 18, 2015, at PTRL West, 625-B Alfred Nobel Drive, Hercules, CA 94547 under an approved protocol (Appendix A) and according to the US EPA FIFRA Good Laboratory Practice Standards, 40 CFR §160.

MATERIAL AND METHODS

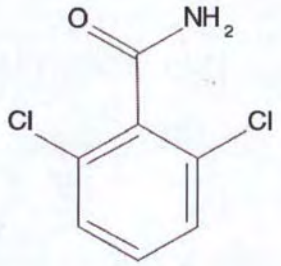
Test/ Reference Substances

Dichlobenil (DBN) and 2-6-Dichlorobenzamide (BAM) test/reference substances were provided by the Sponsor on August 25, 2015. Upon receipt at PTRL West, the test/reference substances were assigned the PTRL inventory numbers 2792W-001 and 2792W-002, respectively. The reference/test substance were maintained frozen (typically < -4°C) when not in use. The certificates of analysis are provided in Appendix B.

Name:	Dichlobenil (DBN)
CAS Name:	2,6-Dichlorobenzonitrile
IUPAC Name:	2,6-Dichlorobenzonitrile
CAS no.:	1194-65-6
Purity:	99.8%
Lot no.:	2757-105-RRG
Molecular formula:	$C_7H_3Cl_2N$
Molecular weight:	172.01 g/mole
Structure:	

Name:	2-6-Dichlorobenzamide (BAM)
CAS Name:	2-6-Dichlorobenzamide
IUPAC Name:	2,6-Dichlorobenzamide
CAS no.:	2008-58-4
Purity:	99.7%
Lot no.:	2757-103-RRG
Molecular formula:	$C_7H_5Cl_2NO$
Molecular weight:	190.03 g/mole

Structure:



Other Chemicals

HPLC grade water, acetone, hexanes, and ethyl acetate were obtained from Burdick & Jackson; ammonium chloride (NH_4Cl) and ammonium hydroxide (NH_3 28-30%) were obtained from Sigma-Aldrich; sodium chloride (NaCl) was obtained from EMD; sodium sulfate (Na_2SO_4) was obtained from Fisher Scientific. The internal standard, 4-Chlorobenzonitrile (100W-0052), was purchased from Sigma-Aldrich with commercial certificate of analysis (Appendix B) and stored at room temperature when not in use.

Equipment/Materials List

Laboratory Balances
Plastic disposable centrifuge bottles (50 mL capacity)
4 mm SS grinding balls
SPEX GenoGrinder
Whatman glass fiber GF8 filters
Buchner funnels
IKA® rotary evaporators with water bath
Thermometers
J.T. Baker Bakerbond Alumina neutral 1g, 6 mL SPE cartridges
SPE chamber
Turbovap® LV nitrogen evaporator
Nitrogen evaporator N_2 Evap
Whatman pH indicator paper
0.2 µm nylon filters
Pipetmen with plastic disposable tips (adjustable volume pipetors)
Sonicator
Vortex mixer
Glassware:

Flasks (125 mL capacity)
Separatory funnels (250 mL capacity)
Fluted filters
Round bottom flasks (125 mL capacity)
Graduated centrifuge tubes (15 mL capacity)
Pasteur pipettes
Graduated cylinders
Beakers
Hamilton glass precision syringes
Volumetric flasks (10 mL capacity)
Amber bottles with Teflon® lined caps

Agilent 6890 Gas Chromatograph (GC) equipped with Agilent 5973 Mass Selective Detector (MSD) and 7673 Autosampler (GC/MSD # 2)
Mass Hunter software

Test System

Source of the Test System

A sandy loam soil was collected at Hickman, CA and received on May 28, 2015. Upon arrival at PTRL West, the test system was assigned the inventory no. 2705W-015 and stored refrigerated (typically < 4°C) in the dark when not in use.

Characterization of the Test System

The soil used in the study was characterized by Agvise Laboratories, Inc. (604 Highway 15 West, Northwood, North Dakota) under the PTRL West Soil Repository study P2705W. Soil characterization included USDA classification for textural class (% sand, % silt, % clay), bulk density, Cation Exchange Capacity (CEC in meq/100 g), moisture capacity at 0.33 bar and at 0.1 bar), % organic matter, and pH. The soil was sieved through a 6-mm sieve. The soil characterization report and collection documentation are presented in Appendix C.

Analytical Method

The analytical method for the analysis of dichlobenil (DBN) and its metabolite 2,6-dichlorobenzamide (BAM) was developed and validated at PTRL West by gas chromatography with mass spectrometry detection (GC/MS).

Soil samples were spiked separately with known concentrations of each of the analytes. The analytes were extracted from soil samples with acetone/hexane (1:1 v/v) and 0.2% NH_4Cl followed by liquid-liquid partition with hexane and solid phase extraction (SPE) clean-up for DBN and then by liquid-liquid partition with ethyl acetate and filtration for BAM. For BAM extraction, the aqueous phase was basified (pH > 9) with NH_4OH prior to liquid-liquid partition. All extracts were evaporated with a gentle stream of nitrogen (room temperature for DBN extracts and 40°C for BAM extracts). Same quantity of internal standard, 4-chlorobenzonitrile, was added to all final extracts prior to GC/MS analysis.

The percent recovery was determined using internal standardization where separate linear curves for each analyte and each monitored ion were analyzed along with the samples.

Preparation of Internal Standard Solutions

Preparation of Internal Standard Stock Solution

An internal standard (IS) stock solution was prepared by weighing an aliquot (50.17 mg) of 4-chlorobenzonitrile into a 50 mL volumetric flask. The solution was diluted to the mark with ethyl acetate. The final solution was sonicated to ensure all solids have completely dissolved. Stock solution yielded a nominal assay concentration of 1.0 mg/mL. The final solution was transferred into a bottle and stored frozen (typically < -4°C) when not in use.

Preparation of Internal Standard Working Solution

An internal standard working solution was prepared by measuring an aliquot (0.1 mL) of the IS stock solution (1.0 mg/mL), transferring into a 200 mL volumetric flask, and diluting to the mark with ethyl acetate. Final solution yielded a nominal assay concentration of 0.5 µg/mL. The IS working solution was transferred into an amber bottle and stored frozen (typically < -4°C) when not in use.

Preparation of Reference Substance Solutions

Preparation of Stock Solutions

Separate stock solutions of DBN and BAM were prepared by weighing aliquots of the reference substances in weighing boats, transferring to volumetric flasks, and diluting to the mark with ethyl acetate. Stock solutions were sonicated to ensure all solids have completely dissolved. Additional ethyl acetate was added as necessary to achieve a nominal concentration of 1.0 mg/mL after adjusting for the appropriate reference substance purity. Actual weights and volumes used for the preparation of stock solutions are shown below:

Analyte	Weight (mg)	Final volume (mL)	Purity (%)	Theoretical conc.[1] (µg/mL)	solvent	Std ID
DBN	50.53	50.40	99.8	1,001	ethyl acetate	stock A
BAM	25.07	25.0	99.7	1,000	ethyl acetate	stock B
IS	50.17	50.0		1,003	ethyl acetate	stock C

[1]Theoretical conc. (µg/mL) = [weight (mg) x 1,000 µg/mg ÷ final volume (mL)] x [purity (%) ÷ 100]

The stock solutions were transferred into amber bottles and stored in frozen (typically < -4°C) when not in use.

Preparation of Fortification Solutions

Fortification solutions were prepared by measuring aliquots of the DBN and BAM stock solutions (1.0 mg/mL) and/or of the previously prepared diluted solutions and transferring into separate volumetric flasks. Final solutions were diluted to the mark with ethyl acetate. Actual volumes and concentrations are shown below:

Solution used	Aliquot (mL)	Final volume (mL)	Theoretical conc.[2] (µg/mL)	Std ID
stock A	0.100	10	10.0	Fort A
stock B	0.125	25	5.0	Fort B
Fort A	1.000	10	1.0	Fort C
Fort B	1.000	10	0.5	Fort D

[2]Theoretical conc. (µg/mL) = [theoretical conc. soln used x aliquot (mL)] ÷ final volume (mL)

Preparation of Intermediate Solution for DBN Analysis

An intermediate solution for DBN analysis was prepared by measuring an aliquot (1 mL) of the fortification solution A (10.0 µg/mL) and transferring into a 10 mL volumetric flask. Final solution was diluted to the mark with hexane to yield a nominal assay concentration of 1.0 µg/mL. The intermediate solution was transferred into an amber bottle and stored in the freezer (typically < -4°C) when not in use.

Preparation of Intermediate Solutions for BAM Analysis

Intermediate solutions for BAM analysis were prepared by measuring aliquots of the fortification solution B (5.0 µg/mL) and transferring into volumetric flasks. Final solutions were diluted to the mark with ethyl acetate. Actual volumes and concentrations are shown below:

Solution used	Aliquot (mL)	Final volume (mL)	Theoretical conc.[3] (µg/mL)	Std ID
Fort B	1.0	10	0.5	Intermediate I
Fort B	2.0	10	1.0	Intermediate II

[3]Theoretical conc. (µg/mL) = [theoretical conc. soln used x aliquot (mL)] ÷ final volume (mL)

The intermediate solutions were transferred into amber bottles and stored in the freezer (typically < -4°C) when not in use.

Preparation of Solvent-Based Calibrants for DBN Analysis

Seven solvent-based calibrants in hexane containing DBN were prepared by transferring an appropriate volume of the intermediate solution (1.0 µg/mL) into separate 10 mL volumetric flasks. An aliquot (2 mL) of the IS working solution (0.5 µg/mL) was volumetrically added to each calibrant and diluted to the mark with hexane. The final concentration for the IS in all calibrants was 0.1 µg/mL. The concentration of DBN ranged from 4 ng/mL to 400 ng/mL as shown below:

Solution used	Aliquot (mL)	Final volume (mL)	Theoretical conc.[4] (ng/mL) DBN
Intermediate	0.040	10	4.0
Intermediate	0.100	10	10
Intermediate	0.200	10	20
Intermediate	0.500	10	50
Intermediate	1.000	10	100
Intermediate	2.000	10	200
Intermediate	4.000	10	400

[4]Theoretical conc. (ng/mL) = {[theoretical conc. soln used x aliquot (mL)] ÷ final volume (mL)} x 1000 ng/µg

Calibrants were stored frozen (typically <- 4°C) when not in use.

Preparation of Solvent-Based Calibrants for BAM Analysis

Seven solvent-based calibrants in ethyl acetate containing BAM were prepared by transferring appropriate volumes of the intermediate solutions I and II (0.5 µg/mL and 1.0 µg/mL) into separate 10 mL volumetric flasks. An aliquot (2 mL) of the IS working solution (0.5 µg/mL) was volumetrically added to each calibrant and diluted to the mark with ethyl acetate. The final concentration for the IS in all calibrants was 0.1 µg/mL. The concentration of BAM ranged from 4 ng/mL to 400 ng/mL as shown below:

Solution used	Aliquot (mL)	Final volume (mL)	Theoretical conc.[5] (ng/mL) BAM
Intermediate II	0.040	10	4.0
Intermediate	0.200	10	10
Intermediate	0.500	10	25
Intermediate	1.000	10	50
Intermediate	2.000	10	100
Intermediate	5.000	10	250
Intermediate II	4.000	10	400

[5]Theoretical conc. (ng/mL) = {[theoretical conc. soln used x aliquot (mL)] ÷ final volume (mL)} x 1000 ng/µg

Calibrants were stored frozen (typically <- 4°C) when not in use.

Preparation of Spiked Solutions for Matrix Effects Assessment

Preparation of Matrix-Based and Solvent-Based Calibrants for DBN Analysis

20 ng/mL matrix-based calibrant was prepared by combining 1.95 mL of a mixture of two control extracts, 0.05 mL of fortification solution C (1.0 µg/mL) and 0.5 mL of IS working solution (0.5 µg/mL) into a 4 mL amber vial. Similar procedure was conducted for a solvent-based calibrant except that 1.95 mL hexane was used instead of the control. Spiked solutions were stored frozen (typically < - 4°C) when not in use.

Preparation of Matrix-Based and Solvent-Based Calibrants for BAM Analysis

20 ng/mL matrix-based calibrant was prepared by combining 1.90 mL of a mixture of two control extracts, 0.05 mL of fortification solution D (0.5 µg/mL) and 0.5 mL of IS working solution (0.5 µg/mL) into a 4 mL amber vial. Similar procedure was conducted for a solvent-based calibrant except that 1.90 mL ethyl acetate was used instead of the control. Spiked solutions were stored frozen (typically < - 4°C) when not in use.

Fortification Procedure

Fortification procedure of DBN and BAM was conducted individually in separate sample sets. Fortification of untreated soil samples was conducted at two fortification levels as shown below:

Fortification Level (mg/kg)	DBN
0.01	0.1 mL of 1.0 µg/mL in 10 grams of soil
0.1	0.1 mL of 10.0 µg/mL in 10 grams of soil

Fortification Level (mg/kg)	BAM
0.005	0.1 mL of 0.5 µg/mL in 10 grams of soil
0.05	0.1 mL of 5.0 µg/mL in 10 grams of soil

Fortification was conducted to determine the percent recovery within the method validation. This procedure was performed in quintuplicate during method validation at each fortification level for each analyte.

Extraction Method for DBN and BAM in Soil

1. Weigh 10.0 g of soil into a 50mL plastic disposable centrifuge bottle.
2. Fortify the sample as necessary (DBN and BAM spiked in separate samples).
3. Add 30 mL of acetone/hexanes (1:1 v/v), 3.4 mL 0.2 % freshly prepared NH_4Cl solution, and (4) 4mm SS grinding balls to each sample. Place on SPEX GenoGrinder at 1,500 rpm for 3 minutes.
4. Filter through a Whatman glass fiber GF8 filter in a Buchner funnel into a 125 mL filtering flask. Rinse the filter cake with approximately 6 mL of acetone/hexanes (1:1 v/v).
5. Transfer the extract into a 125 mL separatory funnel, add 60 mL of water and 2 mL of saturated NaCl solution and shake for 1 minute. Allow the phases to separate and collect the hexanes layer through a fluted filter, containing 4 g of anhydrous Na_2SO_4, into a 125 mL round bottom flask.
6. Add 20 mL of hexanes to the aqueous layer and shake for 1 minute. Allow the layers to separate and collect the hexanes through the same fluted filter containing anhydrous Na_2SO_4.
7. Repeat extraction twice with 20 mL portions of hexanes and combine hexanes extracts. Keep the aqueous phase in a 100 mL beaker.
8. Evaporate the extract at ambient temperature using a rotary evaporator until a volume of ~ 5 mL remains.
9. Transfer the extract to a 10 mL volumetric flask. Rinse and sonicate the round bottom flask with additional 5 mL hexanes and transfer to the volumetric flask. Bring up to 10 mL total volume with hexanes. This is the DBN extract A.
10. Condition an alumina SPE cartridge with 5 mL x 2 of hexanes.
11. Take 5.0 mL of DBN extract A onto the cartridge into 15 mL graduated glass centrifuge tube.
12. Elute with 4 mL of 2% acetone/hexanes into the same tube twice.
13. Evaporate the eluate in a gentle stream of nitrogen to approximately 2 mL at room temperature. Add 0.5 mL IS working solution (0.5 µg/mL) with glass pipet and sonicate well. This is the final extract A for GC/MSD analysis of DBN.
14. Add 1 drop of NH4OH in the aqueous phase of step 7, swirl contents to mix, and measure pH with a pH indicator paper (pH has to be > 9).

15. Add 20 mL of ethyl acetate to the basified aqueous phase and shake for 1 minute. Allow the phases to separate and collect the ethyl acetate through a fluted filter containing 4 g of anhydrous Na_2SO_4, into a 125 mL round bottom flask.
16. Repeat extraction twice with 20 mL portions of ethyl acetate and combine ethyl acetate extracts.
17. Evaporate the combined extract to ~ 2 mL using a rotary evaporator at room temperature. Sonicate and filter the extract through a 0.2 µm nylon filter into a 15 mL graduated glass centrifuge tube. Add 2 mL ethyl acetate to the flask, sonicate, and filter through another 0.2 µm nylon filter into the same centrifuge tube. This is the BAM extract B.
18. Evaporate the filtered extract at 40°C to 2 mL. Add 0.5 mL IS working solution (0.5 µg/mL) with glass pipet and sonicate well. This is the final extract B for GC/MSD analysis of BAM.
19. Aliquot extracts A and B into autosampler vials for analysis by GC/MS.

A schematic diagram of the soil extraction method is presented in Figure 1.

Gas Chromatography with Mass Spectral Detection Method (GC/MSD) for DBN and BAM Analysis

GC Conditions

Instrumentation:	Agilent 6890 Gas Chromatograph (GC) equipped with Agilent 5973 Mass Selective Detector (MSD) and 7673 Autosampler
Column:	Agilent J & W DB-17 Capillary Column 30m x 0.25mm i.d. x 0.5 µm film thickness
Inlet:	Mode: splitless with constant Helium flow of 1 mL/min Injector Temperature: 250 °C Injection Volume: 1 µL Liner: Sky ™ double goose neck 4 mm x 6.5 x 78.5 for Agilent GCs (catalog # 23308.5)
Oven:	Initial temperature: 60 °C (2 minutes hold) Ramp rate: 15 °C per minute Final temperature: 270 °C (5 minutes hold) Run time: 21 minutes

Detector: Transfer line Temperature: 280 °C
MS quadrupole Temperature: 150°C
MS ion source Temperature: 230°C

Retention Times:

DBN = ~ 11.8 minutes
BAM = ~ 15.3 minutes
IS = ~ 9.6 minutes

MS Conditions

Solvent delay: 5 minutes

Scan mode: SIM

Segments:

Analyte	m/z Monitored	Dwell Time
IS	137	400
DBN	171 (quantitation ion)	100
DBN	173 (confirmation ion)	100
DBN	136 (confirmation ion)	100
BAM	173 (quantitation ion)	100
BAM	175 (confirmation ion)	100

GC/MS Analysis

Samples were analyzed interspersed between the solvent-based calibrants so as to assess the response of the calibrants if they had been affected by matrix samples (signal suppression or enhancement). Since separate linearity curves were prepared for each analyte, samples for DBN quantitation were interspersed between DBN calibrants and samples for BAM quantitation were interspersed between BAM calibrants. Hexane was analyzed as the solvent blank for the DBN quantitation and ethyl acetate was analyzed as the solvent blank for the BAM quantitation. Calibrants and samples were analyzed in single injection.

At least one standard solution (100 ng/mL) was reanalyzed as check standard (quality control standard) at the end of the sequence of each analyte validation set to ensure good chromatography and consistent instrument performance. The stability of the signal was

monitored by comparing the response (ratio between the peak area of the analyte and peak area of internal standard) of a quality control standard injection with that of a comparable standard from the linearity curve within the sequence.

For each analyte validation set, a matrix-based calibrant (20 ng/mL) was analyzed after a solvent-based calibrant at the same concentration followed by a QC standard at the end of the sequence.

Methods of Calculation

Quantitation

Each analyte (DBN or BAM) was quantitated separately by the external standard method with addition of the internal standard, 4-chlorobenzonitrile, using a seven-point linear curve regression.

Separation of DBN and BAM was achieved by GC/MS with detection in the SIM mode. The analytes were identified by the coincidence of their retention times with their respective reference substances and MS characteristics. The quantitation of DBN and BAM was conducted by peak area response (PAR or response ratio) of each compound relative to the theoretical concentration of the calibrants. The peak area of each analyte divided by the peak area of the internal standard yields a PAR for samples and calibrants. The content of each compound in samples was quantitated against separate 1/x weighted linear curves ($y = mx + b$) of DBN and BAM calibrants where:

y = PAR (ratio between peak area analyte and peak area internal standard)
x = ng/mL compound injected
m = slope
b = intercept

Weighting of the calibration curve of each compound was applied so as to provide better curve fit at the lower concentration levels of each compound. The calculation of weighted curve equations (linear regression) and concentration (ng/mL) present in samples and calibrants was conducted using Mass Hunter software.

The percent recovery of each analyte from fortified samples was determined by averaging the recovered amount of each compound and dividing by the corresponding amount at each fortification level.

Residue in soil

ng recovered = calculated conc (ng/mL) x final volume (mL) x dilution factor

Where:

Calculated conc (ng/mL) as determined by Mass Hunter software (1/x weighted curve)
Final volume (mL) = 2 mL extract + 0.5 mL IS = 2.5
Dilution factor = 10 mL ÷ 5 mL = 2 (DBN extract A) or
5 mL ÷ 5 mL = 1 (BAM extract B)

Example:
Analyte = DBN
Sample ID = F1A LOQ (m/z 171)

ng recovered = 14.7328 x 2.5 x 2 = 73.664

Percent recovery of DBN and BAM in soil

$$\% \text{ Recovery} = \frac{\text{ng recovered - ng control}}{\text{ng fortified}} \times 100$$

Where:

ng fortified = sample weight (g) x fortification level (mg/kg) x 1000
Sample weight (g) = 10
Fortification level (mg/kg) = 0.01 and 0.1 (DBN) or
0.005 and 0.5 (BAM)

Example:
Analyte = DBN
Sample ID = F1A LOQ (m/z 171)

ng fortified = 10 g x 0.01 mg/kg x 1000 = 100

$$\% \text{ DBN} = \frac{73.664 \text{ ng} - 0.000 \text{ ng}}{100 \text{ ng}} \times 100 = 74\%$$

DBN and BAM residues were detected at < LOD in the controls.

Transcriptions (spreadsheets) of the method validation raw data for DBN and BAM to support calculations are presented in Appendix D and Appendix E respectively.

Limit of Quantitation

The limit of quantitation (LOQ) was defined as the lowest fortification level of each analyte validated by the analytical method. The LOQ was established at 0.01 mg/kg for DBN and 0.005 mg/kg for BAM in soil which represented 20 ng/mL of each analyte in calibration standard solution using the current methodology.

Limit of Detection

The limit of detection (LOD) was defined as approximately 20% of the LOQ (0.002 mg/kg for DBN and 0.001 mg/kg for BAM) which represented 4 ng/mL of each analyte in calibration standard solution using the current methodology.

Time Required for Completion of a Sample Set

A sample set consisted of a reagent blank, two controls (untreated soil samples) and five fortified soil samples at each level (LOQ and 10X LOQ). Time required for one sample set from initiation of extraction until the completion of instrumental analysis and data evaluation is as follows:

- DBN sample preparation (extraction and clean-up) takes approximately 10 hours
- BAM sample preparation(extraction and clean-up) takes approximately 9 hours
- GC/MS analysis and data processing (three transition ions for DBN and two transition ions for BAM) take approximately 5 hours

TOTAL = approximately 24 hours for one analyst to complete a sample set. This does not include preparation of calibrants and fortification solutions as well as instrument initial setup.

Statistical Methods

Means, standard deviation, relative standard deviation, and 1/x linear regression were the only statistical methods employed in this study.

Method validation of dichlobenil (DBN) and its metabolite 2,6-dichlorobenzamide (BAM) in soil, was conducted separately. Each set comprised of one reagent blank, two untreated soil controls and five soil samples spiked either with DBN or BAM at two fortification levels: 0.01 mg/kg (LOQ) and 0.10 mg/kg (10X LOQ) for DBN and at 0.005 mg/kg (LOQ) and 0.05 mg/kg for BAM. The analytical procedure to determine both analytes in soil was evaluated for linearity, precision, accuracy, limits of detection (LOD), and limits of quantitation (LOQ). The precision of the method as well as LOQ were evaluated by examining results from replicate (five) fortified samples. The accuracy of the method was demonstrated by the percent recovery of each analyte from the fortified samples.

All samples were extracted and analyzed by GC/MS in SIM mode with the internal standard method. Three fragment ions: m/z 171 (quantitation ion), m/z 173 and 136 were monitored for DBN analysis; two fragment ions: m/z 173 (quantitation ion) and m/z 175 were monitored for BAM analysis. An attempt has been made to select a third fragment ion (m/z 189) for BAM, but the signal to noise ratio of this ion was too weak to achieve meaningful confirmation/quantitation. The fragment ion for the internal standard, 4-chlorobenzonitrile was m/z 137. The GC/MS full scans of DBN, BAM, and the internal standard are provided in Figure 2, Figure 3, and Figure 4 respectively.

Transcriptions (spreadsheets) of the method validation raw data for DBN and BAM to support calculations are presented in Appendix D and Appendix E respectively.

Linearity

Separate linear regression analyses of DBN and its metabolite, BAM, were determined. DBN and BAM contents were quantitated against separate 1/x weighted linear curves of the reference substances DBN and BAM whose concentrations ranged from 4 ng/mL to 400 ng/mL for each analyte. The quantitation of each analyte was based on the peak area

Matrix Effects

Matrix effect was assessed so as to determine if signal enhancement or suppression was observed during analysis. Matrix effect was evaluated by comparing the response ratio (ratio between peak area of the corresponding compound and peak area of the internal standard) of a matrix-based calibrant (control spiked with known amounts of fortification standard solution) with a solvent-based calibrant (hexane or ethyl acetate spiked with known amounts of fortification standard solution) at the same concentration level for each compound (20 ng/mL).

The matrix effect for the control and solvent spiked with DBN was ≤ 110% for the three ions monitored (m/z 171, 173, and 136); similarly, the matrix effect for the control and solvent spiked with BAM was ≤ 107% for both monitored ions (m/z 173 and 175). Therefore, solvent-based calibrants were used for the quantitation of DBN and BAM since the percent recovery of matrix-based calibrants was within acceptable limits (80% - 120%). Table V shows the summary of results. Representative chromatograms of solvent-based and matrix-based calibrants for DBN analysis are presented in Figure 19. Representative chromatograms of solvent-based and matrix-based calibrants for BAM analysis are presented in Figure 20.

CONCLUSIONS

An analytical method for the determination of dichlobenil (DBN) and its metabolite 2,6-dichlorobenzamide (BAM) has been successfully validated at both LOQ and 10X LOQ levels in a sandy loam soil by GC/MS in SIM mode. The limit of quantitation (LOQ) was established at 0.01 mg/kg for DBN and at 0.005 mg/kg for BAM. Likewise, the limit of detection (LOD) was estimated to be 0.002 mg/kg for DBN and 0.001 mg/kg for BAM in soil.

The average recoveries of each analyte in soil at the LOQ and 10X LOQ levels were within the acceptable range (70 – 120%) and acceptable criteria of RSD < 20%. Therefore, the analytical method validated in this study yielded acceptable precision and accuracy, and is suitable for the determination of DBN and BAM in the soil matrix as the method was designed for.

Negligible interferences (< LOD) or no residues were detected in the control matrices or reagent blanks at the fragment ions monitored for each analyte. No significant matrix suppression or enhancement was observed in controls (untreated soil samples) spiked with individual analyte standard solutions.

This study meets the requirements outlined in EPA guideline OCSPP 850.6100. The study was also in compliance with Good Laboratory Practices (GLP) as stated in 40 CFR Part 160.

REFERENCE

1. Bacher, Reiner. "Validation of an Analytical Method for the Determination of Dichlobenil and its Metabolite 2,6-Dichlorobenzamide (BAM) in Soil by GC/ECD, Demonstrating GC/MS as Confirmatory Method", PTRL Europe, study no. P 556 G, March 27, 2002.

Figure 1. Schematic Diagram of the Analytical Method.