I. INTRODUCTION

This study was conducted to validate methodologies for the determination of residues of dinotefuran and its metabolites UF, DN and MNG in water, sediment and soil. Untreated water, sediment and soil samples, representative of rice growing regions, were fortified with dinotefuran, UF, DN and MNG at 0.0100 and 0.100 mg/L for water and 0.0100 and 0.100 mg/Kg for sediment and soil. Analyses were then performed to determine quantitative recoveries. Samples were processed and analyzed for the determination of all analytes using high performance liquid chromatography with mass spectral detection (LC/MS/MS) for final quantitation.

This study was conducted by Wildlife International, Ltd. according to the protocol "Analytical Method Validation for the Determination of Dinotefuran, UF, DN and MNG in Water, Sediment and Soil", presented in Appendix I, and identified as Wildlife International, Ltd. Project Number 236C-173. Water and homogenized samples of sediment and soil were subsampled and analyzed from August 18 to 24, 2010. Raw data and the final report generated by Wildlife International, Ltd. are filed under Project Number 236C-173 in archives located on the Wildlife International, Ltd. site.

II. PURPOSE

This study was performed to validate methodologies for the determination of dinotefuran and its metabolites UF, DN and MNG in water, sediment and soil. The method was validated for subsequent application to analyses of water, sediment and soil collected from field sites established in rice growing regions to assess aquatic dissipation of dinotefuran.

III. EXPERIMENTAL DESIGN

Untreated water, sediment and soil were fortified with dinotefuran, UF, DN and MNG and analyzed according to methodologies to be applied for quantitation of residues in field-collected samples. For each substrate, seven replicate fortified samples were prepared and analyzed at the target limit of quantitation (LOQ) of 0.0100 mg/L for water and 0.0100 mg/Kg for sediment and soil. Additionally, three replicate samples for water, sediment and soil were prepared and analyzed at a concentration ten-fold the lower concentration, i.e., 0.100 mg/L for water and 0.100 mg/Kg for sediment and soil. The higher fortification level for each substrate was selected in anticipation that it would exceed the highest measured field residue. The lower fortification concentration was selected based on the anticipated practical limit of quantitation (LOQ) of the method. Reagent and matrix blanks (controls) were also analyzed to evaluate potential matrix or reagent analytical interferences.

IV. MATERIALS AND METHODS

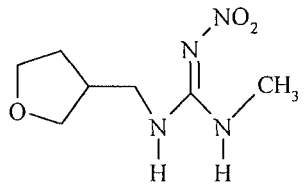
A. Test Substances

Wildlife International, Ltd. received test substances of dinotefuran, DN, UF and MNG from Landis International, Inc. on August 31, 2007, November 12, 2007 and September 30, 2009 (UF and MNG), respectively. Upon receipt, Wildlife International, Ltd. assigned Test Substance numbers 8224, 8293, 9208 and 9209 to

dinotefuran, DN, UF and MNG, respectively. Certificates of Analysis accompanied the test substances and included the information listed below. The structure of MNG was received with earlier correspondence:

Dinotefuran

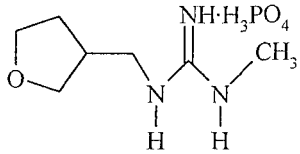
Name: Dinotefuran analytical standrad
Chemical Name: (*RS*)-1-methyl-2-nitro-3-(tetrahydro-3-furylmethyl)guanidine
Structural Formula:



Molecular Weight: 202.21
Appearance: White crystal
Lot Number: TKP-03-149
Purity: 100.00%
Expiration Date: December 21, 2012
Storage Conditions: Dark, ≤-20°C

DN

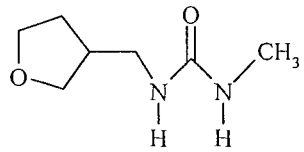
Name: DN analytical standard
Chemical Name: 1-methyl-3-(tetrahydro-3-furylmethyl)guanidinium dihydrogen phosphate
Structural Formula:



Molecular Weight: 255.22
Appearance: White crystal
Lot Number: MU-9428M
Purity: 99.76%
Expiration Date: December 21, 2012
Storage Conditions: Dark, ≤-20°C

UF

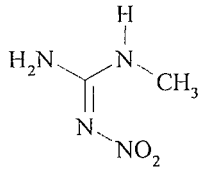
Code Name: UF analytical standard
Chemical Name: 1-methyl-3-(tetrahydro-3-furylmethyl)urea
Structural Formula:



Molecular Weight: 158.20
Appearance: White crystal
Lot Number: TKP-04-096
Purity: 99.74%
Expiration Date: December 21, 2012
Storage Conditions: Dark, ≤-20°C

MNG

Code Name: MNG
Chemical Name: 1-methyl-2-nitroguanidine
Structural Formula:



Lot Number: EBI-3338
Purity: 99.5%
Expiration Date: December 2011
Storage Conditions: Ambient, protected from light

These test/reference substances were used for preparation of standard solutions for instrument calibration and for preparation of fortified samples for method validation. When not in use, solutions prepared from these substances for instrument calibrations and fortifications were stored under refrigerated conditions at approximately 4°C in darkness.

B. Test Systems and Origin of Control Samples

The test system was defined as water, sediment and soil, both fortified and unfortified (untreated) with dinotefuran, UF, DN and MNG for determination of recovery by the methods employed. Untreated water, sediment and soil, typical of rice growing areas was obtained from R & D Research Farm, 7033 Highway 103, Washington, Louisiana 70589. These samples were stored refrigerated when not in use.

C. Reagents and Solvents

All solvents used in this study were Burdick & Jackson®, B & J Brand®, High Purity Solvent or solvents of equivalent purity. Reagents were ACS reagent grade or equivalent quality. Hydrochloric acid, used for preparing 0.1N HCl for reconstitution of samples before injection, was prepared with acid from EMD Sciences Inc. Heptafluorobutyric acid (HFBA), Fluka® Analytical, used in the chromatographic mobile phase was cited on the bottles as adequate for use as an ion-pair reagent.

D. Stock Solutions

A primary stock solution of dinotefuran was prepared in a solution of 15% methanol and 85% HPLC-grade reagent water (v:v) at a concentration of 1.00 mg dinotefuran/mL. Primary stock solutions of UF, DN and MNG were each prepared in HPLC-grade reagent water at a concentration of 1.00 mg active ingredient/mL. For each solution, the percent purity of the reference standard was taken into account such that the final concentrations were 1.00 mg/mL. Therefore, nomenclature of active ingredient (a.i.)/unit volume or mass (e.g., µg a.i./mL, mg a.i. Kg, etc.) is equivalent to the specific analyte per unit volume or mass (e.g., µg/L, mg/Kg, etc.). A combined secondary stock of dinotefuran, DN, UF and MNG was prepared in HPLC-grade reagent water from these individual primary stocks at a concentration for each component of 100 µg/mL. Additional combined stocks at concentrations for each component of 10.0, 1.00 and 0.100 µg/mL in HPLC-grade reagent water were prepared by serial dilution. All stock solutions were quantitatively prepared using gas-tight syringes and volumetric flasks. All stock and calibration standard solutions were stored under refrigerated conditions, approximately 4°C, when not in use.

E. Fortification Solutions and Validation Sample Fortifications

Subsamples of control water, sediment and soil were fortified with dinotefuran, UF, DN and MNG using aliquots of the 1.00 and 10.0 µg/mL combined stock solutions. Control water, sediment and soil were fortified with a combined standard to generate procedural recovery samples containing 0.0100 mg/L (1x the target LOQ) and 0.100 mg/L (10x the target LOQ) of each analyte in water, and 0.0100 mg/Kg and 0.100 mg/Kg in sediment and soil.

F. LC/MS/MS Calibration Standards for Quantitation of Dinotefuran, UF, DN and MNG

Standard solutions containing dinotefuran, UF, DN and MNG were prepared as calibration standards for the LC/MS/MS instrument. Solutions in 0.1N HCl ranging in concentration of dinotefuran, UF, DN and MNG from 0.000500 to 0.0500 µg/mL of each analyte were prepared from the 1.00 and 10.0 µg/mL combined stocks. Combined instrument calibration solutions were prepared at concentrations of 0.000500, 0.00100, 0.00200, 0.00500, 0.0100, 0.0250, and 0.0500 µg/mL of each analyte. All standard solutions were stored refrigerated when not in use.

G. Analytical Methods

1. Water

The analytical method applied to analyses of water for dinotefuran, UF, DN and MNG is described below and presented schematically in Figure 1.

Method validation samples of water were prepared by adding 5-mL aliquots of control water into clean 10-mL class A volumetric flasks. The samples were fortified using aliquots of the appropriate combined stock solutions to achieve the desired concentrations of 0.0100 and 0.100 mg/L for dinotefuran UF, DN and MNG. Seven samples were prepared at the 0.0100-mg/L concentration and three at the 0.0100-mg/L concentration. Control water served as the matrix blanks. Each sample was brought to a final volume of 10.0 mL with high purity water.

The 10.0-mL aqueous samples were transferred to 20-mL glass scintillation vials and the pH adjusted to approximately 8.5 to 9 using Buffer B. Buffer B consisted of a ten-fold aqueous dilution of a 0.5M sodium carbonate – sodium hydrogen carbonate buffer prepared by solubilizing 53 g of sodium carbonate and 42 g of sodium hydrogen carbonate in one liter of water. The water samples were then filtered through a 1.0-µm Acrodisc® filter.

ENVI-Carb (500 mg/6 mL) solid phase extraction (SPE) cartridges were prepared by rinsing with approximately 10 mL of methanol followed by approximately 10 mL of water. The cartridges were not allowed to go dry. A 5.00-mL aliquot of each sample was transferred to a SPE cartridge and allowed to elute at approximately one to two mL/min. Each cartridge was then rinsed with 5 mL of water. The eluates were discarded. The cartridges were subsequently dried under vacuum for approximately 15 to 30 minutes. The columns were then eluted with 10 mL of methanol collecting the eluates in 15-mL culture tubes. The eluates were quantitatively transferred to 125-mL round bottom flasks and rotary evaporated to dryness at a bath temperature of approximately 40 to 50°C. The residues were reconstituted in 20.0 mL of 0.1N HCL. Aliquots of the extracts were transferred to autosampler vials and submitted for LC/MS/MS analysis.

2. Sediment and Soil

The analytical method applied to analyses of sediment and soil of dinotefuran, UF, DN and MNG is described below and presented schematically in Figure 2.

Method validation samples of sediment and soil were prepared by weighing 15.0-g subsamples of control substrates into labeled 250-mL plastic centrifuge bottles. The samples were fortified using aliquots of the appropriate combined stock solutions to achieve the desired concentrations of 0.0100 and 0.100 mg/Kg for dinotefuran, UF, DN and MNG. Control samples served as matrix blanks. Aliquots of 150-mL of acetonitrile:water (CH_3CN:H_2O, 80:20, v:v) and 0.5 mL of concentrated HCL were added to each sample. The samples were placed on a

mechanical reciprocating shaker table at a setting of approximately 250 for approximately 30 minutes.

The samples were centrifuged at approximately 3000 rpm for approximately 5 minutes. The supernatants were decanted and passed through fritted suction funnels each containing a GF/A filter into 1-L round-bottom flasks which was subsequently rinsed with 50 mL of CH_3CN:H_2O (80:20, v:v). To the residual samples in the centrifuge tube, an additional 100 mL of CH_3CN was added and the mixtures extracted again on a mechanical shaker table for approximately 30 minutes at a setting of 250 rpm. The contents were centrifuged and filtered as before and the supernatants combined with the initial extracts in their respective 1-L flasks.

Each filtrate was transfer to a 500-mL separatory funnel. The round bottom flasks were rinsed with 100 mL of hexane and the hexane transferred to its respective separatory funnel. The separatory funnels were shaken for approximately one minute. The lower acetonitrile:water phases were drained into 1-L round bottom flasks. The hexane phases were discarded. Three to four Teflon™ boiling stones/chips were added to each flask. The acetonitrile:water extracts were rotary evaporated to their aqueous remainders using a bath temperature at approximately 50 to 60°C.

The aqueous solutions were quantitatively transferred to 100-mL graduated cylinders with water. The pH of the solutions was adjusted by addition of 5.0 mL of Buffer A. The final volumes were brought to 80.0 mL with water with sonication to mix if necessary. Buffer A consisted of 0.5M sodium carbonate - sodium hydrogen carbonate prepared by addition of 53 g of sodium carbonate and 42 g of sodium hydrogen carbonate per liter of water.

For each sample, 10.0 mL of the aqueous solution were transferred into a 20-mL glass scintillation vial and the pH adjusted to approximately 8.5-9.0 by drop wise addition of 0.5 mL of Buffer B. Buffer B (0.05M sodium carbonate - sodium hydrogen carbonate) consisted of a 10-fold dilution of Buffer A with water. Samples were filtered through a 1.0-µm Acrodisc® filter.

ENVI-Carb (500 mg/6 mL) solid phase extraction (SPE) cartridges were prepared by rinsing with approximately 10 mL of methanol followed by approximately 10 mL of water. The cartridges were not allowed to go dry. A 5.00-mL aliquot of each sample was transferred to a SPE cartridge and allowed to flow though at approximately one to two mL/min. Each cartridge was then rinsed with 5 mL of water. The eluates were discarded. The cartridges were subsequently dried under vacuum for approximately 15 to 30 minutes. The columns were then eluted with 10 mL of methanol collecting the eluates into 15-mL culture tubes. The eluates were quantitatively transferred to 125-mL round bottom flasks and rotary evaporated to dryness at a bath temperature of approximately 40 to 50°C. Residues were reconstituted in 10.0 mL of 0.1N HCL. Aliquots of the extracts were transferred to autosampler vials and submitted for LC/MS/MS analysis.

3. Quantitation of Dinotefuran, UF, DN and MNG by LC/MS/MS

An aliquot of each sample extract was transferred to an autosampler vial for subsequent separation of analytes and quantitation by LC/MS/MS. The liquid chromatograph was connected to the mass spectrometer through a Valco valve that diverted only the eluant from 1 to 10 minutes post-injection to the LC/MS/MS. Dinotefuran, UF, DN and MNG were quantitated in the positive-ion multiple reaction monitoring (MRM) mode. Dinotefuran was quantitated monitoring the 203 to 129 amu transition. A second confirmatory transition of 203 to 157 amu was monitored but not used for quantitation. UF, DN and MNG were quantitated monitoring the 159 to 102 amu transition, the 158 to 102 amu transition and the 119 to 73 amu transition, respectively. No other ions were found for UF, DN and MNG of sufficient intensity for use as confirmatory ions. Attempts to use the secondary ions present for UF, DN and MNG were unsuccessful since they were low molecular weight ions that consistently contributed excessive noise to the responses obtained. Instrument operating conditions for the LC/MS/MS are presented in Table 1.

V. CALCULATIONS

A. Standard Curves by Linear Regression

For dinotefuran, UF, DN and MNG, regression analysis was applied to the chromatographic peak area responses determined from the calibration standard solutions versus the respective nominal concentrations of the analytes. Standard curves were generated by plotting the regression functions consisting of the analyte concentration (µg/mL) on the abscissa and the respective peak area responses on the ordinate. Representative standard curves for dinotefuran, UF, DN and MNG are presented in Figures 3, 4, 5 and 6, respectively. A linear, 1/x weighted, regression analysis was used for quantitation.

The linear regression equation, derived from regression of peak areas and known nominal concentrations of calibration standard solutions, was expressed as follows:

$$\text{Peak Area} = \text{Slope} \times \text{Concentration} + \text{y-Intercept}$$

The concentrations of dinotefuran, UF, DN and MNG in the final solutions of samples were calculated using a rearrangement of the above equation:

$$\text{Concentration} = \frac{\text{Peak Area - y-Intercept}}{\text{Slope}}$$

Calculations of concentrations for injected calibration standards (µg/mL), water (mg/L), sediment and soil (mg/Kg) were performed using Analyst Version 1.5.1, Applied Biosystems/MDS Sciex software. Entry of dilution factors for water, conversion factors for sediment and soil (relating mass extracted and equivalent final volumes) and sample identifiers were entered into the software.

Excel 2000, in full-precision mode, was used for calculation of means, standard deviations, etc., using un-rounded values generated by Analyst Version 1.5.1. Values calculated using rounded numbers as presented in the text and tables might differ slightly. The following calculation sections for water and sediment/soil are illustrative of the procedure used by Analyst Version 1.5.1.

B. Dinotefuran, UF, DN and MNG Concentrations in Water, Sediment and Soil

Water

A representative calculation is presented below consisting of quantitation of dinotefuran in water fortified at 0.0100 mg/L, the target LOQ. UF, DN and MNG in water were quantitated analogously. Using the results from the linear regression analysis as follows:

Slope = 5.22714×10^7
y-Intercept = 326.235
r = 0.9998 equivalent to r^2, the coefficient of determination = 0.9997

$$\text{Concentration} = \frac{\text{Peak Area - (326.235)}}{5.22714 \times 10^7}$$

the concentration of dinotefuran was determined by substituting the resulting analyte peak area into the above equation. Using the peak area for a 0.0100-mg/L fortification, Sample Number 236C-173-WVMAS-1 (122,400 counts), the concentration in the final sample solution was calculated as:

$$\text{Concentration in } \mu g/mL \text{ or } mg/L = \frac{122400 - 326.235}{5.22714 \times 10^7}$$

The residue concentration (mg/L) of dinotefuran in fortified water was determined as the product of the solution concentration determined above, the equivalent final volume and initial extracted volume of the sample as follows:

$$\text{Sample Concentration (mg/L)} = \text{Concentration} \times \frac{\text{(Equivalent Final Volume)}}{\text{(Initial Sample Volume)}}$$

where: Equivalent Final Volume = 40 mL

Note: Only 5 mL of the initial 10-mL aliquot of water was processed through the entire method. This aliquot was reconstituted in 20 mL of 0.1N HCl after rotary evaporation to dryness. Thus, the equivalent final volume for a 10-mL initial volume was 40 mL (i.e., a factor of 4 fold).

Initial Sample Volume = 10.0 mL

Using the nominal concentration for Sample Number 236-173-WVMAS-1, 0.0100-mg/L, the concentration of dinotefuran was calculated as follows:

$$\text{Sample Concentration (mg/L)} = 0.002335\ \mu g/mL \times \frac{40.0\ mL}{10.0\ mL}$$

Sample Concentration in µg/mL or mg/L = 0.00934 mg/L

Note: Calculations of concentrations, µg/mL for the injected calibration standards and mg/L for water, were performed using Analyst Version 1.5.1 Applied Biosystems/MDS Sciex software algorithms following entry of dilution factors and sample identifiers. Excel 2000, in full-precision mode, was used for calculation of means, standard deviations, etc. Values calculated using rounded numbers, either above or in the tables, might differ slightly.

Sediment and Soil

Residues in sediment and soil were calculated analogously to those in water. Linear regression was applied to measured peak areas of analytes in solutions of known concentrations to derive equations relating peak areas and concentrations. The concentrations of dinotefuran, UF, DN and MNG in the injected sediment and soil extract solutions were calculated using a rearrangement of these equations as follows:

$$\text{Extract Concentration in µg/mL or mg/L} = \frac{\text{Peak Area - y-Intercept}}{\text{Slope}}$$

The residue concentration (mg/Kg) of dinotefuran, UF, DN and MNG in fortified sediment and soil were determined as the product of the solution concentration determined above, the equivalent final volume and initial extracted mass of the sample as follows:

$$\text{Sample Concentration in µg/g} = \text{Extract Concentration} \times \frac{\text{(Equivalent Final Volume)}}{\text{(Initial Sample Weight)}}$$

where the Initial Sample Weight was 15.0 g and the Equivalent Final Volume was 168 mL. The initial 15.0-g sample was extracted, rotary evaporated and brought to 80 mL total volume with buffered aqueous solution. Ten mL of this extract was processed further by addition of 0.5 mL of buffer. Five mL of this intermediate solution was processed ending in a final extract volume of 10.0 mL. Thus, the Equivalent Final Volume was calculated as follows:

$$\text{Equivalent Final Volume (mL)} = 80mL \times \frac{10.5\ mL}{10.0\ mL} \times \frac{10.0\ mL}{5.00\ mL}\ mL = 168\ mL$$

and

$$\text{Sample Concentration in µg/g (or mg/Kg)} = \text{Extract Concentration} \times \frac{168\ mL}{15\ g}$$

Sample Concentration in µg/g (or mg/Kg) = Extract Concentration x 11.2 mL/g

Note: Calculations of concentrations, µg/mL for the injected calibration standards and mg/Kg for sediment or soil, were performed using Analyst Version 1.5.1 Applied Biosystems/MDS Sciex software algorithms following entry of dilution (or conversion) factors and sample identifiers. Excel 2000, in full-precision mode, was used for calculation of means, standard deviations, etc. Values calculated using rounded numbers, either above or in the tables, might differ slightly.

C. Procedural Recoveries

The percent recoveries were determined by dividing the concentrations of the analyte recovered in the fortified samples by the nominal concentration added as shown below:

$$\text{Percent Recovery} = \frac{\text{mg/L (water) or mg/Kg (sediment or soil) Found}}{\text{mg/L (water) or mg/Kg (sediment or soil) Added}} \times 100$$

For the above example calculation with water fortified at 0.0100-mg/L, Sample Number 236-173-WVMAS-1, the percent recovery of dinotefuran was calculated as:

$$\text{Percent Recovery} = \frac{0.00934 \text{ mg/Kg Found}}{0.0100 \text{ mg/Kg Added}} \times 100$$

$$\text{Percent Recovery} = 93.4\%$$

Mean recoveries for dinotefuran, UF, DN and MNG for each fortification level were calculated as well as the standard deviation (correlation coefficient) and relative standard deviation (coefficient of variation) for the recoveries.

D. Limits of Detection and Quantitation

Limits of detection (LOD) and quantitation (LOQ) were determined in accordance with procedures presented by the Office of Pesticide Programs in the document titled "Assigning Values to Non-Detected/Non-Quantified Pesticide Residues in Human Health Food Exposure Assessments", March 23, 2000. The method requires the analysis of seven or more control (untreated) samples fortified at the defined target LOQ. The standard deviation of the area responses of these samples was determined and the LOD and LOQ were calculated using the following equations:

$$LOD = (t_{0.99})(S)$$

$$LOQ = 3 \text{ X } LOD$$

where t = the one-tailed t-statistic at the 99% confidence level for n-1 replicates

S = standard deviation of n samples fortified at the defined/target LOQ.

As an example, the standard deviation (S), 0.0002801, for the seven replicate dinotefuran fortified samples of water at the target LOQ of 0.0100 mg/L was calculated from the recovery values presented in Table 2. For seven replicates, the $t_{0.99}$ value is 3.143 (n - 1 = 6 degrees of freedom). The LOD and LOQ were then calculated as follows:

LOD = (3.143)(0.0002801) = 0.000880 mg/L

LOQ = (3)(0.000880) = 0.00264 mg/L

Table 1. Representative High Performance Liquid Chromatography/Mass Spectrometry Operating Conditions.

Instrumentation:	Agilent Model 1100 High Performance Liquid Chromatograph with a Applied Biosystems/MDS Sciex API 3000 Triple Quadrupole Mass Spectrometric Detector (LC/MS/MS) with Heated Nebulizer Ion Source
Analytical Column:	Phenomenex Luna C18 (150 mm x 3 mm, 5-µm)
Guard Column	Phenomenex Security Guard C18 (4 mm x 3 mm)
Mobile Phases:	A: Methanol:Water:HFBA (5:95:0.1; v:v:v)* B: Methanol:HFBA (100:0.1, v:v)* Gradient Elution Program: Time / %A / %B / Flow Rate (µL/min) 0.00 / 100 / 0 / 500 2.00 / 100 / 0 / 500 7.00 / 20 / 80 / 500 10.0 / 20 / 80 / 500 10.1 / 100 / 0 / 800 15.0 / 100 / 0 / 500 16.0 / 100 / 0 / 500 * HFBA – heptafluorobutyric acid
Injection Volume:	50 µL
Divert Valve:	Only the eluant from 1.0 to 10.0 minutes was loaded to the column.
Total Run Time:	16 minutes, 1 second
Mass Spectrometer Conditions:	Ionization Mode: Positive Scan Type: MRM Number of Scans: 754 scans Dwell Time: 250 msec/mass transition Pause Time: 5 msec Mass Transitions Dinotefuran / UF / DN / MNG Q1 Mass (amu): 203 / 159 / 158 / 119 Q3 Mass (amu): 129 / 102 / 102 / 73 Confirmatory Q1 Mass(amu): 203 Confirmatory Q1 Mass(amu): 157
MS Voltage, Temperature and Gas Settings:	NEB: 12; CUR: 8; NC: 5.0; Temp: 450°C; CAD: 8; DP: 21; FP: 80; EP: 10; CE: 17; CXP: 8.00
Dinotefuran Retention Time:	Approximately 7.5 to 7.7 minutes
DN Retention Time:	Approximately 8.0 to 8.2 minutes
UF Retention Time:	Approximately 6.3 to 6.6 minutes
MNG Retention Time:	Approximately 2.5 to 2.7 minutes

Method Outline for Analysis of Dinotefuran, UF, DN and MNG in Water

1. **Sample Preparation**

Prepare method validation samples by adding 5 mL aliquots of aqueous control matrix into clean 10-mL class A volumetric flasks. Fortify the samples using the appropriate combined stock solutions to achieve the desired concentration. Control samples serve as the matrix blanks. Bring to a final volume of 10.0 mL.

2. **Sample Clean Up**

Transfer the 10.0-mL aqueous samples into 20-mL glass scintillation vials and adjust the pH to approximately 8.5 to 9 using Buffer B. [Buffer solution B consist of ten fold dilution of 0.5 M sodium carbonate – sodium hydrogen carbonate prepared by solubilizing 53 g of sodium carbonate and 42 g of sodium hydrogen carbonate per liter of water.]

OPTIONAL: Depending on sample appearance, filter the aqueous samples through a 1.0-µm Acrodisc® filter (or equivalent).

Prepare ENVI-Carb (500 mg/6 mL) solid phase extraction (SPE) cartridges by rinsing with approximately 10 mL of methanol followed by approximately 10 mL of water (do not allow the columns to go dry).

Transfer 5.00 mL aliquots to SPE cartridges and allow elution at approximately one to two mL/min. Rinse each cartridge with 5 mL of water. Discard the eluates. Dry the columns under vacuum for approximately 15 to 30 minutes.

Elute the columns with 10 mL of methanol collecting the eluates in 15-mL culture tubes. Quantitatively transfer the eluates to 125-mL round bottom flasks.

Rotary evaporate the extracts to dryness at a bath temperature of approximately 40 to 50° C.

Reconstitute the residues in 20.0 mL of 0.1N HCL. Mix well.

OPTIONAL: If particulates are visually present, filter sufficient extract through a 1.0-µm Acrodisc® filter (or equivalent).

Transfer aliquots of the extracts to autosampler vials and submit for LC/MS/MS analysis. Dilute samples further with mixing, if necessary, using 0.1 N HCL.

Figure 1. Method outline for the analysis of dinotefuran, UF, DN and MNG in water.

Method Outline for Analysis of Dinotefuran, UF, DN and MNG in Sediment and Soil

1. Sample Extraction

Prepare method validation samples by weighing 15.0 g of control matrix into labeled 250-mL plastic centrifuge bottles. Fortify the samples using the appropriate combined stock solutions to achieve the desired concentration. Control samples serve as the matrix blanks.

Add 150 mL of acetonitrile:water (CH_3CN:H_2O, 80:20, v:v) followed by 0.5 mL of concentrated HCL to each sample. Place samples on mechanical reciprocating shaker table at a setting of approximately 250 rpm for approximately 30 minutes.

Centrifuge the samples at approximately 3000 rpm for approximately 5 minutes. Decant the supernatants through fritted suction funnels each containing a GF/A filter into 1-L round-bottom flasks. Rinse each with 50 mL of CH_3CN:H_2O (80:20, v:v). Add an additional 100 mL of CH_3CN to each sample and place on a mechanical shaker table for approximately 30 minutes at a setting of 250 rpm. Centrifuge and filter the supernatants as before combining them in their respective 1-L flasks.

Transfer each filtrate into a 500-mL separatory funnel. Rinse the round bottom flasks with 100 mL of hexane and combine it in its respective separatory funnel. Shake the separatory funnels for approximately one minute. Drain the lower acetonitrile:water phases into 1-L round bottom flasks and discard the hexane phases. Add 3-4 Teflon™ boiling stones/chips to each flask.

Rotary evaporate each sample to its aqueous remainder at approximately 50 to 60°C.

Quantitatively transfer the aqueous solutions into 100-mL graduated cylinders with water. Add 5.0 mL of Buffer A and adjust the final volume to 80.0 mL with water. Sonicate to aid in rinsing, if necessary. [Buffer A consist of 0.5M sodium carbonate - sodium hydrogen carbonate prepared by addition of 53 g of sodium carbonate and 42 g of sodium hydrogen carbonate per liter of water.]

2. Sample Clean-Up

For each sample, transfer 10.0 mL of the aqueous solution from above into a 20-mL glass scintillation vial and add 0.5 mL of Buffer B drop wise until the pH is approximately 8.5-9.0. [Buffer B, 0.05M sodium carbonate - sodium hydrogen carbonate, consist of a 10-fold dilution of Buffer A with water.]

OPTIONAL: Depending on sample appearance, filter through a 1.0-µm Acrodisc® filter (or equivalent).

- continued -

Figure 2. Method outline for the analysis of dinotefuran, UF, DN and MNG in sediment and soil.

- continued -

Prepare ENVI-Carb (500 mg/6 mL) solid phase extraction cartridges (SPE) by rinsing with approximately 10 mL of methanol followed by approximately 10 mL of water (do not let the columns go dry).

Transfer the 5.00 mL aliquots to separate SPE cartridges and elute at approximately one to two mL/min. Rinse the cartridge with 5 mL of water. Discard the eluate. Dry the columns under vacuum for approximately 15 to 30 minutes.

Elute the columns with 10 mL of methanol collecting the eluates in culture tubes. Quantitatively transfer the eluates to 125-mL round bottom flasks.

Rotary evaporate the extracts to dryness at a bath temperature of approximately 40 to 50° C.

Reconstitute the residues in 10.0 mL of 0.1N HCL. Mix well.

OPTIONAL: If particulates are present, filter sufficient extract through a 1.0-µm Acrodisc® filter (or equivalent).

Transfer aliquots of the extracts to autosampler vials and submit for LC/MS/MS analysis. Dilute samples further with mixing, if necessary, using 0.1 N HCL.

Figure 2 (continuation). Method outline for the analysis of dinotefuran, UF, DN and MNG in sediment and soil.