



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

PC Code: 044312
DP Barcode: 393117
September 15, 2011

MEMORANDUM

Subject: Data Evaluation Record on the Analytical Method Validation for the Determination of Dinotefuran, UF, DN and MNG in Water, Sediment and Soil

To: Debra Rate, PhD
Registration Division
RIMUERB / Section 18 Team
Office of Pesticide Programs

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Office of Pesticide Programs

Ronald Parker 9/15/11
Rochelle F. H. Bohaty 9/15/11
Dana Spatz 9/15/11

The Environmental Fate and Effects Division (EFED) have completed the review of Analytical Method Validation for the Determination of Dinotefuran, UF, DN and MNG in Water, Sediment and Soil. This study was conducted to validate methodologies for the determination of residues of dinotefuran and its metabolites, 1-Methyl-3-(tetrahydro-3-furylmethyl)urea (UF), 1-Methyl-3-(tetrahydro-3-furylmethyl)guanidinium dihydrogen phosphate [DN phosphate] (a salt of DN) and 1-Methyl-2-nitroguanidine [N-Methyl-N'-nitro-guanidine (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.

The methodology used in this method validation study is scientifically valid and can be used for measuring dinotefuran concentrations at environmentally relevant levels.



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PMRA Submission Number {.....}

EPA MRID Number 48548801

Data Requirement: PMRA Data Code:
EPA DP Barcode: 393117
OECD Data Point:
EPA Guideline: Fate, Transport and Transformation Test Guidelines
OPPTS 835.6200 Aquatic (Sediment) Field Dissipation and OPPTS
835.6100 Terrestrial Field Dissipation-guideline

Test material:

Common name: Dinotefuran
Chemical name: (RS)-1-methyl-2-nitro-3-(terahydro-3-furylmethyl) guanidine
IUPAC name:
CAS name:
CAS No.: 165252-70-0
Synonyms
SMILES String: C1(CCOCl)CNC(=NN(=O)=O)NC; (NC)(=NN(=O)=O)NCCl(CCOCl)

Primary Reviewer: Ronald Parker

Signature:

Date: 09/14/2011

Secondary Reviewer: Rochelle F. H. Bohaty

Signature:

Date: 09/14/2011

Company Code:

Active Code:

Use Site Category:

EPA PC Code: 044312

CITATION: MacGregor, J.; Nixon, W. (2011) Analytical Method Validation for the Determination of Dinotefuran, UF, DN and MNG in Water, Sediment and Soil. Project Number: 236C-173. Unpublished study prepared by Wildlife International, Ltd. 89p. SPONSOR: MITSUI CHEMICALS AGRO, INC., Agrochemicals Division 1-5-2, Higashi-Shimbashi Minato-ku, Tokyo 105-7117, Japan. Experimental start date August 18, 2010 and completion date August 24, 2010. Final report issued March 9, 2011.



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EXECUTIVE SUMMARY

This study was conducted to validate methodologies for the determination of residues of dinotefuran and its metabolites, 1-Methyl-3-(tetrahydro-3-furylmethyl)urea (UF), 1-Methyl-3-(tetrahydro-3-furylmethyl)guanidinium dihydrogen phosphate [DN phosphate] (a salt of DN) and 1-Methyl-2-nitroguanidine [N-Methyl-N'-nitro-guanidine (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 according to the protocol "Analytical Method Validation for the Determination of Dinotefuran, UF, DN and MNG in Water, Sediment and Soil". Water and homogenized samples of sediment and soil were subsampled and analyzed from August 18 to 24, 2010.

Recoveries of dinotefuran, UF, DN and MNG were acceptable for the target limit of quantitation of 0.0100 mg/L in water, and 0.0100 mg/Kg in sediment and soil.

Results Synopsis:

Recoveries of Dinotefuran, UF, DN and MNG from Water, Sediment and Soil

Recoveries of dinotefuran, UF, DN and MNG from fortified water, sediment and soil were overall acceptable. Recoveries for individual samples, mean recoveries for concentrations of 0.0100 and 0.100 mg/L from water, mean recoveries for concentrations of 0.0100 and 0.100 in g/Kg from sediment and soil, standard deviations and relative standard deviations are summarized below.

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Substrate	Analyte	Fortification Concentrations	
		0.0100 mg/L	0.100 mg/L
Water	Dinotefuran	93.8 ± 2.8 (3.0%)	93.9 ± 1.7 (1.9%)
	UF	98.0 ± 4.4 (4.5%)	95.3 ± 3.2 (3.4%)
	DN	86.7 ± 3.1 (3.5%)	92.0 ± 1.1 (1.2%)
	MNG	91.6 ± 2.1 (2.3%)	94.0 ± 1.8 (2.0%)

Substrate	Analyte	Fortification Concentrations	
		0.0100 mg/Kg	0.100 mg/Kg
Sediment	Dinotefuran	91.4 ± 6.7 (7.3%)	92.3 ± 4.3 (4.6%)
	UF	94.5 ± 1.7 (1.8%)	91.2 ± 3.9 (4.2%)
	DN	82.0 ± 9.1 (11%)	80.9 ± 4.0 (5.0%)
	MNG	98.1 ± 4.8 (4.9%)	95.9 ± 3.8 (4.0%)
Soil	Dinotefuran	94.6 ± 5.1 (5.4%)	92.6 ± 0.7 (0.8%)
	UF	85.9 ± 1.9 (2.2%)	82.6 ± 1.3 (1.6%)
	DN	76.3 ± 5.3 (6.9%)	77.0 ± 1.4 (1.8%)
	MNG	92.9 ± 3.0 (3.3%)	96.7 ± 4.1 (4.3%)

Study Acceptability: This method validation is classified as acceptable and meets the criteria of the Fate, Transport and Transformation Test Guidelines OPPTS 835.6200 Aquatic (Sediment) Field Dissipation and OPPTS 835.6100 Terrestrial Field Dissipation.

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: Fate, Transport and Transformation Test Guidelines OPPTS 835.6200 Aquatic (Sediment) Field Dissipation and OPPTS 835.6100 Terrestrial Field Dissipation

COMPLIANCE: This study was conducted by Wildlife International, Ltd. in compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency, 40 CFR Part 160, 17 August 1989 with the following exceptions:
Reference substance characterization and stability were not determined in accordance with Good Laboratory Practice

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Standards for storage conditions at the test site performing this analytical phase.

A. MATERIALS:

1. Test Material

(RS)-1-methyl-2-nitro-3-(terahydro-3-furylmethyl) guanidine (p. 12).

Chemical Structure:

See DER Attachment 1.

Description:

Technical grade; white crystal

Purity:

Unlabeled

Lot No.: TKP-03-149

Analytical purity: 100.00%.

Storage conditions of test chemicals:

>20°C in the dark.

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Summary of Dinotefuran Physiochemical Parameters ¹		
Parameter	Value	Reference/ Comments
<i>Selected Physical Chemical Parameters</i>		
Chemical Classification	Nitroguanidine	
Pesticide Classification	Systemic Insecticide	
Molecular Weight	202.2 g/mol	MRID # 45640101
Water Solubility (20°C, pH 6.98)	Parent: 39,830 mg/L MNG: 11,480 mg/L DN-2-OH: 1,000,000 mg/L DN: 619,400 mg/L UF: 4,171 mg/L	MRID# 45640112, 45639702, 45639706, 45639707 (USEPA Pesticide Fact Sheet, 2004)
Vapor pressure (30°C)	<1.275x10 ⁻⁸ mm Hg; <1.7 x 10 ⁻⁶ Pa	MRID# 45640105, 45639702
Henry's Law Constant	8.63 x 10 ⁻¹⁴ Atm m ³ /mol	Calculated
Octanol/Water Partition, K _{ow}	0.283 @25°C	MRID # 45639702
log K _{ow}	-0.549	MRID # 45639702
Dissociation constant (pKa)	12.6 @20°C (estimated)	MRID # 45639702
Melting point	107.5°C	MRID # 45639702
pH	5.6 @25°C	MRID # 45654201
Density	1.40 g/cm ³ @20°C	MRID # 45639702

¹Data were obtained from OPP/EFED Problem Formulation (2011)

2. Test Commodities:

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.

B. EXPERIMENTAL DESIGN:

1. Sample Preparation

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.

2. Description of Analytical Methods:

1. Water

The analytical method applied to analyses of water for dinotefuran, UF, DN and MNG is described below.

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

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0.100 mg/L for dinotefuran UF, DN and MNG. Seven samples were prepared at the 0.0100mg/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-um 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 inL/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.

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 (CH₃CN:H₂O, 80:20, v:v) and 0.5 mL of concentrated HCL were added to each sample. The samples were placed on mechanical reciprocating shaker table at a setting of approximately 250 for approximately 30 minutes.

The samples were centrifuged at 3000 rpm for 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 CH₃CN:H₂O (80:20, v:v). To the residual samples in the centrifuge tube, an additional 100 mL of CH₃CN was added and the mixtures extracted again on a mechanical shaker table for 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.

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Each filtrate was transferred 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 aqueous phase ($\text{CH}_3\text{CN}:\text{H}_2\text{O}$) were drained into 1 L round bottom flasks. The hexane phase was discarded. This was followed by a second extraction. Three to four Teflon™ boiling stones/chips were added to each flask. The aqueous extracts were rotary evaporated to remove CH_3CN using a 50 to 60°C water bath.

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 0.5 M 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 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 um Acrodisc® filter.

ENVI-Carb (500 mg/6 mL) solid phase extraction (SPE) cartridges were prepared by rinsing using approximately 10 mL of methanol followed by approximately 10 mL of water. The cartridges were not allowed to dry. A 5.00 mL aliquot of each sample was transferred to a SPE cartridge and allowed to flow through 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 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 using a 40 to 50°C water bath. 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

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for UF, DN and MNG were unsuccessful since they were low molecular weight ions that consistently contributed excessive noise to the responses obtained.

4. Calculations: 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 ($\mu\text{g/mL}$) on the abscissa and the respective peak area responses on the ordinate. 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:

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

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

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

Calculations of concentrations for injected calibration standards ($\mu\text{g/mL}$), 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 for the calculation.

5. Detection limits (LOD, LOQ) for dinotefuran:

Although the target LOQ values were 0.0100 and 0.100 mg/L for water, and 0.0100 and 0.100 mg/Kg for sediment and soil, replication of the low-fortification level was adequate to calculate LODs and LOQs using the method cited in Assigning Values to Non-Detected/Non-Quantified Pesticide Residues in Human Health Food Exposure Assessments, Office of Pesticide Programs, U.S. Environmental Protection Agency, March 23, 2000. A summary of the calculated LOQ values is presented below. Calculated LOD values are numerically one-third of the calculated LOQ values presented.

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	Water	Sediment	Soil
Dinotefuran	0.0026 mg/L	0.0063 mg/Kg	0.0048 mg/Kg
UF	0.0041 mg/L	0.0016 mg/Kg	0.0018 mg/Kg
DN	0.0029 mg/L	0.0086 mg/Kg	0.0050 mg/Kg
MNG	0.0020 mg/L	0.0045 mg/Kg	0.0029 mg/Kg

In all instances, the adopted target LOQ is greater than the calculated LOQ. It is noted that the calculated LOQ for DN in sediment, 0.0086 mg/Kg, indicates the adopted method LOQ of 0.0100 mg/Kg is near the intrinsic LOQ of the method.

II. RESULTS AND DISCUSSION

RECOVERY FROM CONTROL SAMPLES:

There was no integrated noise or extraneous peaks in the reagent blank regions of dinotefuran, UF, DN or MNG elution.

III. STUDY DEFICIENCIES

1. Sample refrigeration temperature was given as 4⁰C in most but not all cases. No other deficiencies were noted.

IV. REVIEWER'S COMMENTS

1. The methodology used in this method validation study is scientifically valid and can be used for measuring dinotefuran concentrations at environmentally relevant levels.
2. The recoveries for UF and DN are <90% (76% being the lowest recovery). The method is not representative of aged residues.
3. The most sensitive species is bees (representing terrestrial invertebrates). Based on the interim level of concern for listed terrestrial invertebrates (LOC = 0.05), the calculated concentration of concern from a bee contact study (LD₅₀ = 0.024 ug ai/bee) is 9 ppb (see calculation below). For parent, the assumed LOQ is 10 ppb (calculated 2-9 ppb) with an assumed LOD of 1/3 that value (calculated 0.6-3 ppb) depending on the matrix. Dinotefuran can be detected at concentrations of concerns. Calculation:

$$0.024 \text{ ug ai/bee (LD}_{50}) \times 1 \text{ bee}/0.128^2 \text{ g} = 0.1875 \text{ ug ai/g bee (187 ppb)}$$
$$187 \text{ ppb} \times 0.05 \text{ (LOC)} = 9.375 \text{ ppb}$$

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(²Based upon an average fresh weight per honey bee of 128 milligrams, the LD₅₀ of honey bees (g/bee) can be multiplied by 7.8 to determine the ppm toxicity. (Mayer and Johansen. 1990)).

4. The cumulative level of concern (parent plus degradates of concern) for drinking water is above the 91.3 ppb in the most recent EFED assessment. This is effective for modeling concentrations in drinking water and ecological exposure assessments.
-

V. REFERENCES

1. (Mayer, D. & C. Johansen. 1990. *Pollinator Protection: A Bee & Pesticide Handbook*. Wicwas Press. Cheshire, Conn. p. 161)
2. U.S. Environmental Protection Agency. 2008. Fate, Transport and Transformation Test Guidelines, OPPTS 835.6100, Terrestrial Field Dissipation. Office of Pesticide and Toxic Substances, Washington, DC. EPA 712-C-08-020.
3. U.S. Environmental Protection Agency. 2008. Fate, Transport and Transformation Test Guidelines, OPPTS 835.6200, Aquatic (Sediment) Field Dissipation. Office of Pesticide and Toxic Substances, Washington, DC. EPA 712-C-08-020.

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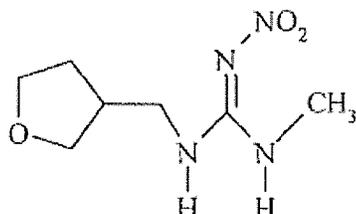
Attachment 1: Structure of Test Materials

Dinotefuran

Name: Dinotefuran analytical standard

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

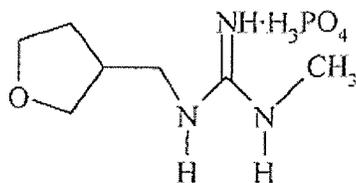
Storage Conditions: Dark, $\leq -20^{\circ}\text{C}$

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, $\leq -20^{\circ}\text{C}$

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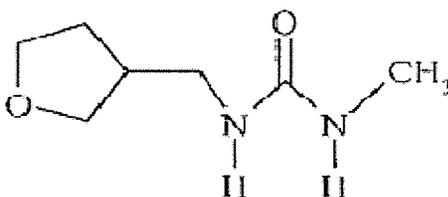
EPA MRID Number 48548801

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

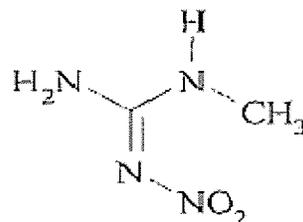
Storage Conditions: Dark, $\leq -20^{\circ}\text{C}$

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

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