Test Material:	Hexazinone
MRID:	45132803
Title:	Independent Laboratory Validation of a Proposed Environmental Chemistry Analytical Method for the Determination of Hexazinone and Metabolites in Water by GC/NPD Analysis and Confirmation by LC/MS/MS
EPA PC Code:	107201
OCSPP Guideline:	850.6100

For CDM Smith

Primary Reviewer: Lynne Binari **Signature:** 

Date: 8/21/14

Secondary Reviewer: Lisa Muto **Signature:** 

Date: 8/21/14

QC/QA Manager: Joan Gaidos **Signature:** 

Date: 8/21/14

Analytical method for hexazinone and its transformation products IN-T3937, IN-A3928, IN-T3935, IN-G3453, IN-JS472, and IN-G3170 in water

Reports: ECM (GC-NPD): EPA MRID No.: 45132803 (Appendix 2, pp. 110-126). Brookey, F. and K. Clark (Appendix 2, p. 126). 1996. DETERMINATION OF HEXAZINONE AND ITS METABOLITES IN WATER. MORSE SOP# Meth-93, Revision #4 (Appendix 2, p. 110). Report prepared by MORSE LABORATORIES, INC., sponsor not specified, submitted by E. I. du Pont de Nemours and Company, Wilmington, Delaware; 17 pages. Method dated May 20, 1996; final review issued June 27, 1996.

ECM (LC/MS/MS): EPA MRID No.: 45132803 (Appendix 2, pp. 127-156). Brill, F. and T. Gardner. 1999. Enforcement Analytical Method for the Determination of Hexazinone and Metabolites of Interest in Soil and Water Using Electrospray-LC/MS/MS. Dupont Project and Report No.: DuPont-2292. Report prepared, sponsored, and submitted by E. I. du Pont de Nemours and Company, Wilmington, Delaware; 30 pages. Draft 2 report issued April 23, 1999.

ILV: EPA MRID No.: 45132803. Zheng, S. 1999. Independent Laboratory Validation of a Proposed Environmental Chemistry Analytical Method for the Determination of Hexazinone and Metabolites in Water by GC/NPD Analysis and Confirmation by LC/MS/MS. Centre Analytical Laboratories Study No.: 008-035. DuPont Project No.: DuPont-2548. Report prepared by Centre Analytical Laboratories, Inc., State College, Pennsylvania, sponsored and submitted by E. I. DuPont de Nemours and Company, Wilmington, Delaware; 193 pages. Final report issued December 16, 1999.

Document No.: MRID 45132803

Guideline: 850.6100

Statements: ECM (GC-NPD): It was not specified whether or not the study was conducted in compliance with USEPA FIFRA Good Laboratory Practice (GLP) standards (Appendix 2, pp. 110-126). Signed and dated No Data Confidentiality, GLP, Quality Assurance, and Authenticity Certification statements were not provided.

ECM (LC/MS/MS): It was not specified whether or not the study was conducted in compliance with USEPA FIFRA Good Laboratory Practice (GLP) standards (Appendix 2, pp. 127-156). Signed and dated No Data Confidentiality, GLP, Quality Assurance, and Authenticity Certification statements were not provided.

ILV: The study was conducted in accordance with USEPA FIFRA GLP standards (p. 3). Signed and dated No Data Confidentiality, GLP, Quality Assurance, and Authenticity Certification statements were provided (pp. 2-5).

Classification: This analytical method is considered unacceptable, for the following reasons.

- For both methods, complete, finalized ECM reports with implemented ILV major modifications were not provided. No originating ECM performance data were reported for either method. Two sets of performance data should be submitted, one for the initial or other internal validation (ECM), and one for the ILV. These reports should be provided separate from each other.
- For ILV analysis of all analytes in lysimeter water, insufficient performance data were provided to validate either method at the LOQ or 10x LOQ (minimum of 5 samples required).
- For ILV ground water analysis of IN-T3935 and IN-G3170 by GC-NPD and all analytes by LC/MS/MS, no performance data at 10x LOQ were provided.
- ILV performance data for 2.0 µg/L (LOQ) fortified hexazinone in ground water using GC-NPD did not meet OCSPP Guideline 850.6100 criteria.
- The determination of LOQ and LOD were not based on scientifically acceptable procedures (see reference at the end of this DER for guidance).
- ILV reported recoveries were corrected if residues were present in the matrix controls.
- Water matrices used in the ILV were not characterized.
- Linearity (r^2) of the calibration standards was not always ≥0.995.

Portions of the study appeared to meet guideline criteria and may be upgradable with the submission of additional information. For ILV analysis of hexazinone and its products IN-T3937, IN-A3928, IN-G3453 and IN-JS472 in ground water using the GC-NPD method, overall mean method recoveries met OCSPP Guideline 850.6100 criteria for both precision and accuracy at 2.0 µg/L (LOQ) and 20 µg/L (10x LOQ), with the exception of 2.0 µg/L (LOQ) hexazinone (mean 67%). For products IN-T3935 and IN-G3170 in ground water using the GC-NPD method, mean recoveries and RSDs met guideline criteria for analysis at 4.0 µg/L (LOQ) and 20 µg/L (5x LOQ); performance data at 10x LOQ were not reported.

For ILV analysis of hexazinone and all six products in ground water using the LC/MS/MS method, overall mean method recoveries met guideline criteria for both precision and accuracy at 0.1 µg/L (LOQ); no performance data at 10x LOQ were reported; however, mean recoveries and RSDs met guideline criteria for analysis at 2.0 µg/L (20x LOQ) for hexazinone, IN-T3937, IN-A3928, IN-G3453 and IN-JS472, and at 4.0 µg/L (40x LOQ) for IN-T3935 and IN-G3170.

PC Code: 107201

Signature: José Luis Meléndez

Reviewer: José L. Meléndez, USEPA Date: October 31, 2014

All page citations refer to the page numbers located in the bottom right corner of the MRID.

Executive Summary

Analytical method DuPont-2550 is designed for the quantitative determination of hexazinone and its products IN-T3937, IN-A3928, IN-T3935, IN-G3453, IN-JS472, and IN-G3170 in water using GC-NPD, and analytical method DuPont-2292 Draft 2 (April 23, 1999) is designed for the confirmatory determination of hexazinone and its products in water and soil using LC/MS/MS. Validation of the LC/MS/MS method with soil is addressed in a separate submission (MRID 45132802). The GC-NPD ECM did not establish LOQs for hexazinone and its products in water. For GC-NPD analysis of ground water, the ILV found the method is quantitative at LOQs of 2.0 µg/L for IN-T3937, IN-A3928, IN-G3453 and IN-JS472, and 4.0 µg/L for IN-T3935 and IN-G3170; recoveries of hexazinone at the LOQ (2.0 µg/L) did not meet OCSPP Guideline 850.6100 criteria. For LC/MS/MS analysis of ground water, the method is quantitative at the stated LOQ of 0.1 µg/L for all analytes. The LOQ is less than the lowest toxicological level of concern in water [based on the aquatic life benchmarks available at the following site: http://www.epa.gov/oppefed1/ecorisk_ders/aquatic_life_benchmark.htm, accessed 10/30/14; furthermore, a lifetime drinking water Health Advisory has been established by the USEPA at 400 ppb, September 1996; finally, a health-protective drinking water level of 170 ppb was derived by the Office of Environmental Health Hazard Assessment (OEHHA, California), based on the available toxicological information using their established approaches]. ILV recoveries were corrected for any residues in the matrix control samples. Successful validation of both the quantitation and confirmation methods for hexazinone and its products in a single matrix was not achieved. For both methods, complete, finalized ECM reports with implemented ILV major modifications were not provided.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Hexazinone	45132803	45132803		Water	GC-NPD: dated 20/05/1996, final review 27/06/1996	E. I. du Pont de Nemours and Company	GC-NPD	2.0 µg/L
IN-T3937								2.0 µg/L
IN-A3928								2.0 µg/L
IN-T3935								4.0 µg/L
IN-G3453								2.0 µg/L
IN-JS472								2.0 µg/L
IN-G3170								4.0 µg/L
Hexazinone					LC/MS/MS[1]: 23/04/1999		LC/MS/MS	0.1 µg/L
IN-T3937								
IN-A3928								
IN-T3935								
IN-G3453								
IN-JS472								
IN-G3170								

1 The LC/MS/MS ECM covers analysis of both water and soil matrices, whereas, this ILV (MRID 45132803) is for analysis of water matrices only. The ILV for analysis of hexazinone and its products in soil is addressed in a separate submission (MRID 45132802).

I. Principle of the Method

GC-NPD Method:
Water (volume not specified) was brought to volume (100 mL) with HPLC grade water, acidified with glacial acetic acid (20 µL) and filtered (Appendix 2, pp. 110, 112, 114-115). The sample was then loaded onto a solid-phase extraction (SPE) cartridge (Supelclean Envi-Carb SPE Tube, Custom, 12 mL polypropylene tube with Teflon frit packed with 1.5 g of Envi-Carb), preconditioned with acetone:3mM acetic acid (9:1, v:v) and water (Appendix 2, pp. 111, 115). The loaded sample was rinsed with water, followed by hexane, then analytes were eluted with acetone:3mM acetic acid (9:1, v:v, 10 mL) into a silanized glass tube. The eluate was taken to dryness under nitrogen (N-Evap, 40-50°C) in the presence of acetone (Appendix 2, p. 116). Resulting residues were reconstituted in acetone (200 µL), diluted with water, and all trace of solvent removed under nitrogen (final volume 5 mL). The aqueous sample was loaded onto a reverse-phase SPE cartridge (Varian, C18 Mega Bond Elut, 6 cc/1g) preconditioned with methanol:3mM acetic acid (9:1, v:v) and water. The loaded sample was rinsed with water, followed by hexane, then analytes were eluted with methanol:3mM acetic acid (9:1, v:v, 10 mL) into a silanized glass tube (Appendix 2, p. 117). The eluate was taken to dryness under nitrogen (N-evap, *ca*. 50°C) in the presence of methanol, with resulting residues reconstituted in acetone, ethyl acetate and toluene (final solvent ratio 20:50:30, v:v:v) for GC-NPD analysis.

Samples were analyzed for hexazinone (DPX-A3674) and its products IN-T3937 (Metabolite A), IN-A3928 (Metabolite B), IN-T3935 (Metabolite C), IN-G3453 (Metabolite A1), IN-JS472 (Metabolite 1), and IN-G3170 (Metabolite G3170) by GC (RTX-35, 0.53 mm x 15 m, 0.5 µm df column) with nitrogen-phosphorous detection (NPD): carrier gas helium, column gas flow rate 5

mL/minute, injector temperature 290°C, detector temperature 285°C, initial column temperature 150°C, ramp rate (1) 25°C/minute to 275°C and hold for 2.2 minutes, or ramp rate (2) 10°C/ minute, final temperature 285°C and hold for 1.0 minute (Appendix 2, pp. 112, 118). Injection volume was 2 µL.

LC/MS/MS Method:
This method provides for the quantification of hexazinone and its products in both water and soil matrices; however, only methods regarding water matrices are presented in this DER. Methods regarding quantification of analytes in soil are addressed in a separate submission (MRID 45132802).

Water (20 mL) was brought to volume (100 mL) with Milli-Q water and acidified with water: glacial acetic acid (9:1, v:v, 200 µL; Appendix 2, pp. 132, 134). The acidified sample was then extracted, purified and concentrated using Envi-Carb and C18 Mega Bond Elut SPE as described above (Appendix 2, pp. 130, 135-136). The resulting C18 SPE eluate was taken to dryness under nitrogen (N-evap, temperature not specified) in the presence of methanol, with resulting residues reconstituted in HPLC or Milli-Q water and filtered (0.2 µm) for LC/MS/MS analysis.

Samples were analyzed for hexazinone and its products by HPLC (Zorbax RX-C8, 4.6 mm x 25 cm, 5 µm column, with guard column attached, column temperature 30°C) using a mobile phase of (A) aqueous 0.01M acetic acid and (B) acetonitrile [percent A:B at 0.0 min. 100:0, 3.0 min. 90:10, 10.0 min. 50:50, 15.0 min. 25:75, 15.1-20.0 min. 5:95, 20.1-30.0 min. 0:100] with MS/MS-ESI (electrospray ionization, positive) detection and multiple reaction monitoring (MRM; Appendix 2, pp. 131, 138-139). Injection volume was 50 µL. Analytes were identified using one ion transition. Ion transitions monitored were as follows: *m/z* 253.2→171.0 for hexazinone, *m/z* 269.3→171.0 for IN-T3937, *m/z* 239.2→157.0 for IN-A3928, *m/z* 255.3→157.0 for IN-T3935, *m/z* 269.3→171.0 for IN-G3453, *m/z* 267.2→171.0 for IN-JS472, and *m/z* 171.1→71.0 for IN-G3170 (Appendix 2, pp. 138-139).

ILV:
Water samples (50 or 100 mL) were acidified, purified and concentrated using Envi-Carb and C18 SPE as described above (p. 16). The resulting C18 SPE eluate was evaporated to *ca*. 1 mL, brought to volume (4.0 mL) with methanol, and the sample divided into two 2.0-mL aliquots. One sample was evaporated and the resulting residues reconstituted in acetone, ethyl acetate and toluene (final volume 2.0 mL) as described above for GC-NPD analysis (p. 17). GC-NPD analysis was conducted as described above with the following modification: initial column temperature 140°C and hold for 1 minute, ramp rate 20°C/minute, final temperature 290°C and hold for 1 minute (pp. 17-18). The other sample was evaporated and the resulting residues reconstituted in water and filtered as described above for LC/MS/MS analysis (pp. 16-17). LC/MS/MS analysis was conducted as described above with the following modifications: LC column temperature 35°C and changing the re-equilibration solvent from 100% acetonitrile (mobile phase B) to 100% aqueous 0.01M acetic acid (mobile phase A) [percent A:B at 0.0 min. 100:0, 3.0 min. 90:10, 10.0 min. 50:50, 15.0 min. 25:75, 15.1-20.0 min. 5:95, 20.1-30.0 min. 100:0], plus minor changes to optimize MS/MS instrument conditions (pp. 17-19).

In the ILV, LOQs for GC-NPD analysis were reported at 2.0 µg/L (ppb) for hexazinone, IN-T3937, IN-A3928, IN-G3453 and IN-JS472, and 4.0 µg/L for IN-T3935 and IN-G3170 (p. 22); LOQs for the analytes were not reported in the ECM (Appendix 2, pp. 110-126). For GC-NPD analysis, LODs for the analytes were not specified in either the ECM or ILV. For LC/MS/MS analysis, LOQs for all analytes were the same in the ECM and ILV at 0.1 µg/L (p. 22; Appendix 2, p. 144). For LC/MS/MS analysis, LODs for all analytes were set at 0.03 µg/L in the ILV (Appendix 4, pp. 177-193) and estimated at 0.002-0.005 µg/L in the ECM (Appendix 2, p. 144).

II. Recovery Findings

ECMs (Appendix 2, pp. 109-159 of MRID 45132803): Originating ECM performance data were not reported for either the GC-NPD (quantitation) method or LC/MS/MS (confirmation) method.

ILV (MRID 45132803): For hexazinone and its products in lysimeter water, insufficient performance data were provided to validate either the GC-NPD method or LC/MS/MS method at any fortification level (Table 1, p. 24; Table 3, p. 26).

For hexazinone and its products IN-T3937, IN-A3928, IN-G3453 and IN-JS472 in ground water using the GC-NPD method, mean recoveries and RSDs were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis at 2.0 µg/L (LOQ) and 20 µg/L (10x LOQ), with the exception of 2.0 µg/L (LOQ) hexazinone (mean 67%; Table 2, p. 25; DER Attachment 2). For products IN-T3935 and IN-G3170 in ground water using the GC-NPD method, mean recoveries and RSDs were within guideline criteria for analysis at 4.0 µg/L (LOQ) and 20 µg/L (5x LOQ); performance data at 10x LOQ were not reported.

For hexazinone and all six products in ground water using the LC/MS/MS method, mean recoveries and RSDs were within guideline criteria for analysis at 0.1 µg/L (LOQ); no performance data at 10x LOQ were reported (Table 4, p. 27; DER Attachment 2). Mean recoveries and RSDs met guideline criteria for analysis at 2.0 µg/L (20x LOQ) for hexazinone, IN-T3937, IN-A3928, IN-G3453 and IN-JS472, and at 4.0 µg/L (40x LOQ) for IN-T3935 and IN-G3170.

Recovery values were corrected for any residues in the matrix control samples (Appendix 4, pp. 163-193). The independent laboratory reported that two validation sets, one each of lysimeter and ground water, were extracted and analyzed, for each method (p. 10). The lysimeter and ground water matrices used were not characterized (p. 15).

Table 2. Initial Validation Method Recoveries for Analytes in Water

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)[1]	Relative Standard Deviation (%)
Hexazinone	LOQ					
	10x LOQ					
IN-T3937	LOQ					
	10x LOQ					
IN-A3928	LOQ					
	10x LOQ					
IN-T3935	LOQ		No originating ECM performance data were reported for either method (GC-NPD or LC/MS/MS).			
	10x LOQ					
IN-G3453	LOQ					
	10x LOQ					
IN-JS472	LOQ					
	10x LOQ					
IN-G3170	LOQ					
	10x LOQ					

Table 3. Independent Validation Method Recoveries for Analytes in Water

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Lysimeter Water						
Hexazinone (DPX-A367)	GC-NPD (quantitation)					
	2.0 (LOQ)	2	79, 90	*	*	*
	4.0	2	73, 96	*	*	*
	20	2	84, 89	*	*	*
	LC/MS/MS (confirmation)					
	0.1 (LOQ)	2	90, 98	*	*	*
	2.0	2	77, 94	*	*	*
	4.0	2	79, 88	*	*	*
IN-T3937	GC-NPD (quantitation)					
	2.0 (LOQ)	2	93, 112	*	*	*
	4.0	2	77, 105	*	*	*
	20	2	83, 89	*	*	*
	LC/MS/MS (confirmation)					
	0.1 (LOQ)	2	72, 93	*	*	*
	2.0	2	89, 101	*	*	*
	4.0	2	76, 88	*	*	*
IN-A3928	GC-NPD (quantitation)					
	2.0 (LOQ)	2	91, 104	*	*	*
	4.0	2	78, 101	*	*	*
	20	2	88, 92	*	*	*
	LC/MS/MS (confirmation)					
	0.1 (LOQ)	2	107, 108	*	*	*
	2.0	2	78, 89	*	*	*
	4.0	2	80, 96	*	*	*

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
IN-T3935	GC-NPD (quantitation)					
	4.0 (LOQ)	2	85, 110	*	*	*
	8.0	2	74, 94	*	*	*
	20	2	88, 92	*	*	*
	LC/MS/MS (confirmation)					
	0.1 (LOQ)	2	71, 80	*	*	*
	4.0	2	85, 96	*	*	*
	8.0	2	79, 87	*	*	*
IN-G3453	GC-NPD (quantitation)					
	2.0 (LOQ)	2	106, 115	*	*	*
	4.0	2	91, 108	*	*	*
	20	2	93, 98	*	*	*
	LC/MS/MS (confirmation)					
	0.1 (LOQ)	2	82, 97	*	*	*
	2.0	2	80, 96	*	*	*
	4.0	2	76, 89	*	*	*
IN-JS472	GC-NPD (quantitation)					
	2.0 (LOQ)	2	84, 103	*	*	*
	4.0	2	71, 94	*	*	*
	20	2	76, 81	*	*	*
	LC/MS/MS (confirmation)					
	0.1 (LOQ)	2	51, 60	*	*	*
	2.0	2	73, 87	*	*	*
	4.0	2	68, 77	*	*	*
IN-G3170	GC-NPD (quantitation)					
	4.0 (LOQ)	2	89, 93	*	*	*
	8.0	2	81, 116	*	*	*
	20	2	97, 100	*	*	*
	LC/MS/MS (confirmation)					
	0.1 (LOQ)	2	88, 105	*	*	*
	4.0	2	89, 91	*	*	*
	8.0	2	76, 89	*	*	*
Ground Water						
Hexazinone (DPX-A367)	GC-NPD (quantitation)					
	2.0 (LOQ)	5	59-71	**67**	5	7
	20	5	73-100	87	10	11
	LC/MS/MS (confirmation)					
	0.1 (LOQ)	5	110-118	115	3	3
	2.0	5	83-98	90	6	6
IN-T3937	GC-NPD (quantitation)					
	2.0 (LOQ)	5	74-95	85	8	9
	20	5	72-102	87	11	12
	LC/MS/MS (confirmation)					
	0.1 (LOQ)	5	97-102	99	2	2
	2.0	5	76-97	90	8	9
IN-A3928	GC-NPD (quantitation)					

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	2.0 (LOQ)	5	69-89	77	8	10
	20	5	73-103	88	11	13
	LC/MS/MS (confirmation)					
	0.1 (LOQ)	5	109-116	114	3	3
	2.0	5	82-100	91	7	8
IN-T3935	GC-NPD (quantitation)					
	4.0 (LOQ)	5	71-89	82	7	8
	20	5	72-96	85	10	11
	LC/MS/MS (confirmation)					
	0.1 (LOQ)	5	80-87	83	3	4
	4.0	5	74-97	88	9	10
IN-G3453	GC-NPD (quantitation)					
	2.0 (LOQ)	5	73-86	81	5	6
	20	5	76-103	89	10	11
	LC/MS/MS (confirmation)					
	0.1 (LOQ)	5	115-119	117	2	1
	2.0	5	79-98	89	7	8
IN-JS472	GC-NPD (quantitation)					
	2.0 (LOQ)	5	69-81	77	5	6
	20	5	68-94	82	9	11
	LC/MS/MS (confirmation)					
	0.1 (LOQ)	5	90-98	93	3	3
	2.0	5	75-86	82	4	5
IN-G3170	GC-NPD (quantitation)					
	4.0 (LOQ)	5	78-113	100	14	14
	20	5	79-107	98	11	12
	LC/MS/MS (confirmation)					
	0.1 (LOQ)	5	94-114	103	8	7
	4.0	5	71-95	87	9	11

Data were obtained from Tables 1-4, pp. 24-27 and DER Attachment 2 (means, SDs and RSDs per fortification level were not provided by the study author). For LC/MS/MS ground water analysis, reported fortification levels of 2.0 ppb for IN-T3935 and IN-G3170 in Table 4 (p. 27) appear to be typographical errors; the raw data sheets indicate those fortifications were at 4.0 ppb (Appendix 4, pp. 185-186).

* Not calculated, n = 2.

III. Method Characteristics

GC-NPD method: In the ILV, LOQs were reported at 2.0 µg/L (ppb) for hexazinone, IN-T3937, IN-A3928, IN-G3453 and IN-JS472, and 4.0 µg/L for IN-T3935 and IN-G3170 (p. 22); LOQs for the analytes were not reported in the ECM (Appendix 2, pp. 110-126). No justification for the selected LOQs were provided. LODs for the analytes were not specified in either the ECM or ILV; however, the ILV may have set LODs at the lowest calibration standard (0.03 µg/mL for hexazinone, IN-A3928, IN-G3453, IN-T3937, and IN-JS472, and 0.06 µg/mL for IN-3170 and IN-T3935; Appendix 4, pp. 163-176).

LC/MS/MS method: LOQs for all analytes were the same in the ECM and ILV at 0.1 µg/L (p. 22; Appendix 2, p. 144). No justification for the selected LOQ was provided. LODs for all analytes were set at 0.03 µg/L in the ILV (Appendix 4, pp. 177-193) and estimated at 0.002-0.005 µg/L in the ECM (Appendix 2, p. 144). The ECM defined the LOD as the level where the signal to noise ratio is in the range of 3:1 to 5:1 at the expected retention time of the analyte (Appendix 2, p. 144).

Table 4. Method Characteristics

	Hexazinone	**IN-T3937**	**IN-A3928**	**IN-T3935**	**IN-G3453**	**IN-JS472**	**IN-G3170**
Lysimeter Water with GC-NPD (quantitation) and LC/MS/MS (confirmation)							
	GC-NPD						
Limit of Quantitation (LOQ)	2.0 µg/L	2.0 µg/L	2.0 µg/L	4.0 µg/L	2.0 µg/L	2.0 µg/L	4.0 µg/L
Limit of Detection (LOD)	Not reported.						
Linearity (calibration curve r^2 and concentration range)[1]	r^2 = 0.9949 (0.03-0.20 µg/mL)	r^2 = 0.9969 (0.03-0.375 µg/mL)	r^2 = 0.9973 (0.03-0.375 µg/mL)	r^2 = 0.9982 (0.06-0.75 µg/mL)	r^2 = 0.9983 (0.03-0.375 µg/mL)	r^2 = 0.9963 (0.03-0.375 µg/mL)	r^2 = 0.9996 (0.06-0.25 µg/mL)
	LC/MS/MS						
Limit of Quantitation (LOQ)	0.1 µg/L						
Limit of Detection (LOD)	0.03 µg/L						
Linearity (calibration curve r^2 and concentration range)[2]	r^2 = 0.9962 (0.67-10 ng/mL)	r^2 = 0.9961 (0.67-10 ng/mL)	r^2 = 0.9995 (0.67-10 ng/mL)	r^2 = 0.9966 (0.67-10 ng/mL)	r^2 = 0.9963 (0.67-10 ng/mL)	r^2 = 0.9961 (0.67-10 ng/mL)	r^2 = **0.9877** (0.67-10 ng/mL)
Repeatable	No. The ILV failed to analyze a minimum of five matrix control samples fortified at the LOQ for either method. Additionally, there was no justification for the LOQs proposed by the initial validation (ECM).						
Reproducible							
Specific	No. The ILV did not provide sufficient performance data to validate the GC-NPD (quantitative) and LC/MS/MS (confirmation) methods.						
Ground Water with GC-NPD (quantitation) and LC/MS/MS (confirmation)							
	GC-NPD						
Limit of Quantitation (LOQ)	2.0 µg/L	2.0 µg/L	2.0 µg/L	4.0 µg/L	2.0 µg/L	2.0 µg/L	4.0 µg/L
Limit of Detection (LOD)	Not reported.						
Linearity (calibration curve r^2 and concentration range)[1]	r^2 = 0.9971 (0.03-0.20 µg/mL)	r^2 = 0.9982 (0.03-0.375 µg/mL)	r^2 = 0.9949 (0.03-0.375 µg/mL)	r^2 = **0.9933** (0.06-0.75 µg/mL)	r^2 = 0.9990 (0.03-0.375 µg/mL)	r^2 = 0.9978 (0.03-0.375 µg/mL)	r^2 = **0.9943** (0.06-0.25 µg/mL)
Repeatable	No at LOQ (mean 67%) Yes at 10x LOQ	Yes	Yes	Yes[3]	Yes	Yes	Yes[3]
Reproducible[4, 5]	No	Yes	Yes	Yes	Yes	Yes	Yes
Specific[6]	Yes						
	LC/MS/MS						
Limit of Quantitation (LOQ)	0.1 µg/L						
Limit of Detection (LOD)	0.03 µg/L						
Linearity (calibration curve r^2 and concentration range)[2]	r^2 = **0.9928** (0.67-10 ng/mL)	r^2 = 0.9949 (0.67-10 ng/mL)	r^2 = **0.9919** (0.67-10 ng/mL)	r^2 = **0.9878** (0.67-10 ng/mL)	r^2 = **0.9911** (0.67-10 ng/mL)	r^2 = 0.996 (0.67-10 ng/mL)	r^2 = **0.966-0.9907** (0.67-10 ng/mL)
Repeatable[3]	Yes						
Reproducible[5]	Yes						

	Hexazinone	IN-T3937	IN-A3928	IN-T3935	IN-G3453	IN-JS472	IN-G3170
Specific[6]	Yes						

Data were obtained from p. 22; Appendix 4, pp. 163-193.

1 Reviewer-calculated calibration curves verified reported linearity for the ILV (r^2 = 0.9933-0.9996; see DER Attachment 2). Linearity is satisfactory when $r^2 \geq 0.995$.

2 Calibration curve r^2 values determined by reviewer (DER Attachment 2). Calibrations standards were analyzed, but calibration curves were not provided (Appendix 4, pp. 177-193). Linearity is satisfactory when $r^2 \geq 0.995$.

3 Performance data at 10x LOQ were not reported (Table 2, p. 25; Table 4, p. 27)

4 The ILV validated the method at the LOQ, with the exception of 2.0 µg/L (LOQ) hexazinone (mean 67%).

5 Performance data establishing the LOQs for the analytes were not provided with the ECM.

6 Any residues detected in the matrix control samples and reagent blanks were ≤30% of the LOQ (Appendix 4, pp. 170-176, 185-193).

IV. Method Deficiencies and Reviewer's Comments

1. For both the GC-NPD (quantitation) and LC/MS/MS (confirmation) methods, no originating ECM performance data were reported (Appendix 2, pp. 110-159).

2. For ILV analysis of all analytes in lysimeter water, insufficient performance data were provided to validate either method (GC-NPD, LC/MS/MS) at the LOQ or 10x LOQ. For ILV ground water analysis of IN-T3935 and IN-G3170 by GC-NPD and all analytes by LC/MS/MS, no performance data at 10x LOQ were provided.

   A validation sample set should consist of, at a minimum, a reagent blank, two unspiked matrix control samples, five matrix control samples spike at the LOQ, and five matrix control samples spiked at 10x LOQ for each analyte and matrix.

3. For the ILV, the following fortifications did not meet OCSPP Guideline 850.6100 criteria for precision and accuracy (mean recoveries for replicates at each spiking level between 70% and 120%, and relative standard deviations (RSD) ≤20%): GC-NPD analysis of 2.0 µg/L (LOQ) fortified hexazinone in ground water (mean 67%, DER Attachment 2).

4. The determination of the LOQ and LOD were not based on scientifically acceptable procedures. For either method (GC-NPD, LC/MS/MS), no justification for the selected LOQs for the analytes (2.0 or 4.0 µg/L for GC-NPD, 0.1 µg/L for LC/MS/MS) were provided. LODs were not reported for GC-NPD analysis. For LC/MS/MS analysis, LODs for all analytes were set at 0.03 µg/L in the ILV (Appendix 4, pp. 177-193) and estimated at 0.002-0.005 µg/L in the ECM (Appendix 2, p. 144), with the ECM defining the LOD as the level where the signal to noise ratio is in the range of 3:1 to 5:1 at the expected retention time of the analyte. Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples. Additionally, the lowest toxicological level of concern in water was not reported. An LOQ above toxicological levels of concern results in an unacceptable method classification.

5. For the ILV, reported recoveries were corrected for any residues in the matrix control samples (Appendix 4, pp. 163-193). For GC-NPD analysis of lysimeter and ground water matrices, no significant interferences were detected in reagent blank and matrix

control samples at the retention times of the analytes, with the exception of product IN-G3170 in lysimeter water (41.0-47.5% of LOQ; Appendix 4, pp. 163-176). For LC/MS/MS analyses, any residues detected in the reagent blank and matrix control samples were ≤30% of the LOQ, with the exception of product IN-G3170 in lysimeter water (50-60% of LOQ; Appendix 4, pp. 185-193).

The GC-NPD ECM residue calculations specified for the correction of sample recoveries for any residues found in the matrix control samples (Appendix 2, pp. 119-120); however, the LC/MS/MS ECM residue calculations did not specify for correction of sample recoveries (Appendix 2, p. 141).

6. For both the GC-NPD and LC/MS/MS methods, complete, finalized ECM reports with implemented ILV major modifications were not provided.

   a) For the GC-NPD analysis method, DuPont-2550, "Analytical Method for the Determination of Hexazinone and Its Metabolites in Water by GC-NPD Analysis" (p. 12), the ECM report was either not adequately identified and/or a complete ECM report was not provided (Appendix 2, pp. 110-126). The ECM is appended to the ILV, and, it appears that in preparation of the ILV report, pages 1-11 of the ECM were omitted. The GC-NPD ECM appended to the ILV report is titled MORSE LABORATORIES SOP# Meth-93, Revision #4, Determination of Hexazinone and Its Metabolites in Water, but does appear to be analytical method DuPont-2550 based on the general description of the method provided by the independent laboratory (pp. 12, 16). The modification to method DuPont-2550 to include a sample splitting procedure at step 4.4.5 of the GC-NPD method to allow for LC/MS/MS confirmation (Appendix 2, p. 159), plus the required LC/MS/MS conditions should be included as part of the finalized GC-NPD ECM. It should also be noted that the modification to method DuPont-2550 (Appendix 2, pp. 158-159) appears to be appended to the LC/MS/MS method (Appendix 2, pp. 127-157), rather than the GC-NPD method to which it pertains (Appendix 2, pp. 110-126).

   b) The LC/MS/MS method appended to the ILV report is labeled as a Draft (Appendix 2, pp. 127-156). The major ILV modification to the method, changing the re-equilibration solvent from 100% acetonitrile (mobile phase B) to 100% aqueous 0.1M acetic acid (mobile phase A), was not implemented in, or even appended to, the ECM report (pp. 17-18; Appendix 2, pp. 138-139). In addition, the ECM covers analysis of both water and soil matrices, whereas, this ILV (MRID 45132803) is for analysis of water matrices only. The ILV for analysis of hexazinone and its products in soil is addressed in a separate submission (MRID 45132802).

7. The lysimeter and ground water matrices used in the ILV were not characterized. For the lysimeter water used in the ILV, selected lysimeter water samples from DuPont Study No. AMR 32-2-94 (CAL 9903356-99003364) were combined to yield a composite sample (CAL 9903427, p. 15). Similarly for the ground water used in the ILV, selected

ground water samples from Dupont Study No. AMR 3202-94 (CAL 9902704-9902712 and 9903307-990335) were combined to form a composite sample (CAL 9903428).

8. Linearity (r^2) of the calibration standards was not always ≥0.995 (see Table 4 above). Calibration curves were not provided for LC/MS/MS analyses. Calibrations standards were analyzed, but calibration curves were not generated as part of the ILV report (Appendix 4, pp. 185-193). Fortification recoveries were determined based on average response factor (RF) of bracketed standards (p. 20). When the RSD value of the average RF was >20%, samples were re-analyzed (Appendix 4, pp. 177, 184-185, 192-193).

9. It was reported for the ILV that a single analyst completed a sample set consisting of eleven lysimeter samples (one reagent blank, two matrix control samples, and eight fortified samples) in *ca*. 3.5 days; 2 days, or 16 person hours, to complete extraction, plus *ca*. 3 hours for GC-NPD analysis and *ca*. 8 hours for LC/MS/MS analysis (p. 21). For ground water, a single analyst completed a sample set consisting of eighteen samples (one reagent blank, two matrix control samples, and fifteen fortified samples) in *ca*. 4.5 days; 3 days, or 24 person hours, to complete extraction, plus *ca*. 4 hours for GC-NPD analysis and *ca*. 9.5 hours for LC/MS/MS analysis.

V. References

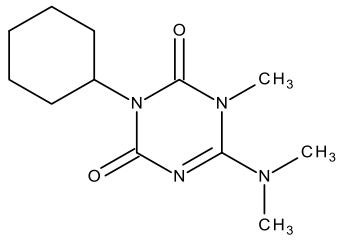
U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.

Attachment 1: Chemical Names and Structures

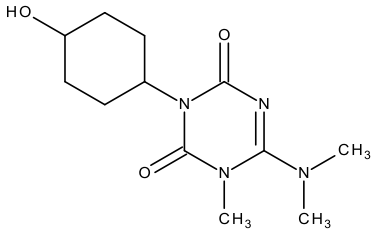
Hexazinone (DPX-A367)

IUPAC Name: 3-Cyclohexyl-6-dimethylamino-1-methyl-1,3,5-triazine-2,4(1H,3H)-dione
CAS Name: 3-Cyclohexyl-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1H,3H)-dione
CAS Number: 51235-04-2
SMILES String: O=C(N=C(N(C1=O)C)N(C)C)N1C(CCCC2)C2



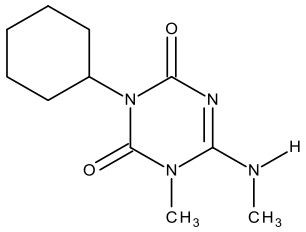
IN-T3937 (Metabolite A)

IUPAC Name: 6-(Dimethylamino)-3-(4-hydroxycyclohexyl)-1-methyl-1,3,5-triazine-2,4-dione
CAS Name: 3-(4-Hydroxycyclohexyl)-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1H,3H)dione
CAS Number: Not reported
SMILES String: Cn1c(nc(=O)n(c1=O)C2CCC(CC2)O)N(C)C



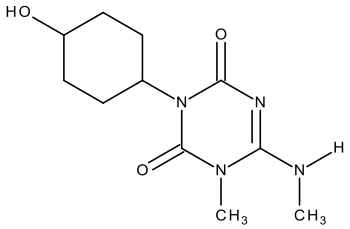
IN-A3928 (Metabolite B)

IUPAC Name: 3-Cyclohexyl-1-methyl-6-(methylamino)-1,3,5-triazine-2,4-dione
CAS Name: 3-Cyclohexyl-6-(methylamino)-1-methyl-1,3,5-triazine-2,4(1H,3H)dione
CAS Number: 56611-54-2
SMILES String: [H]N(C)c1nc(=O)n(c(=O)n1C)C2CCCCC2



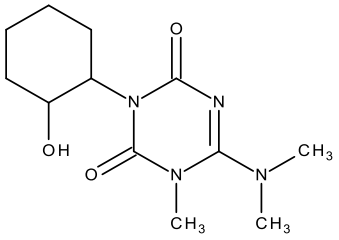
IN-T3935 (Metabolite C)

IUPAC Name: 3-(4-Hydroxycyclohexyl)-1-methyl-6-(methylamino)-1,3,5-triazine-2,4-dione
CAS Name: 3-(4-Hydroxycyclohexyl)-6-(methylamino)-1-methyl-1,3,5-triazine-2,4(1H,3H)dione
CAS Number: 72585-88-7
SMILES String: [H]N(C)c1nc(=O)n(c(=O)n1C)C2CCC(CC2)O



IN-G3453 (Metabolite A1)

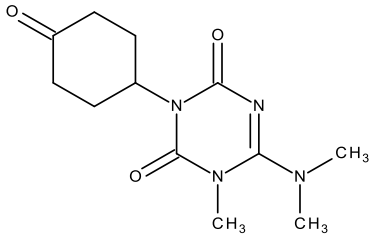
IUPAC Name: 6-(Dimethylamino)-3-(2-hydroxycyclohexyl)-1-methyl-1,3,5-triazine-2,4-dione
CAS Name: 3-(2-Hydroxycyclohexy)-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1H,3H)dione
CAS Number: Not reported
SMILES String: Cn1c(nc(=O)n(c1=O)C2CCCCC2O)N(C)C



IN-JS472 (Metabolite 1)

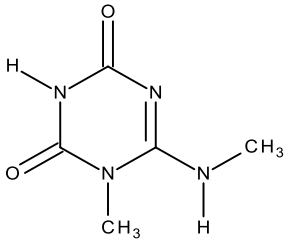
IUPAC Name: 6-(Dimethylamino)-1-methyl-3-(4-oxocyclohexyl)-1,3,5-triazine-2,4-dione

CAS Name: 3-(4-Ketocyclohexyl)-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1H,3H)dione
CAS Number: Not reported
SMILES String: Cn1c(nc(=O)n(c1=O)C2CCC(=O)CC2)N(C)C



IN-G3170 (Metabolite G3170)

IUPAC Name: 1-Methyl-6-(methylamino)-1,3,5-triazine-2,4-dione
CAS Name: 6-(Dimethylamino)-1-methyl-1,3,5-triazine-2,4(1H,3H)dione
CAS Number: Not reported
SMILES String: [H]n1c(=O)nc(n(c1=O)C)N([H])C



Chemical: Hexazinone
PC: 107201
MRIDs: 45132803
Guideline: 850.6100
ILV for Determination of Hexazinone and its Products in Ground Water

	Hexazinone (DPX-A3674)							IN-T3937							IN-A3928						
Fortified (µg a.i./L)	Recovery (%)	Mean (%)	SD[1] (%)	RSD[2] (%)	Max	Min	n =	Recovery (%)	Mean (%)	SD[1] (%)	RSD[2] (%)	Max	Min	n =	Recovery (%)	Mean (%)	SD[1] (%)	RSD[2] (%)	Max	Min	n =
	GC-NPD (quantitation)							GC-NPD (quantitation)							GC-NPD (quantitation)						
2.0	66							95							74						
(LOQ)	70							86							80						
	71							88							75						
	70							82							89						
	59	67	5	7	71	59	5	74	85	8	9	95	74	5	69	77	8	10	89	69	5
20	73							72							73						
	85							87							91						
	100							102							103						
	85							90							83						
	91	87	10	11	100	73	5	85	87	11	12	102	72	5	91	88	11	13	103	73	5
	LC/MS/MS (confirmation)							LC/MS/MS (confirmation)							LC/MS/MS (confirmation)						
0.1	116							98							109						
(LOQ)	110							102							113						
	116							97							114						
	116							98							116						
	118	115	3	3	118	110	5	99	99	2	2	102	97	5	116	114	3	3	116	109	5
2.0	83							76							82						
	98							97							100						
	91							92							94						
	92							92							95						
	88	90	6	6	98	83	5	92	90	8	9	97	76	5	86	91	7	8	100	82	5

Results from Table 2, p. 25; Table 4, p. 27 of MRID 45132803.
Means and standard deviations calculated using Microsoft program functions =AVERAGE(A1:A2) and =STDEV(A1:A2).
Any discrepancies between reviewer calculated values and reported results most likely due to rounding.
1 SD = Standard Deviation; determined using the “unbiased” or “n-1” method.
2 RSD = Relative Standard Deviation; calculated as (SD/mean) x 100.

Chemical: Hexazinone
PC: 107201
MRIDs: 45132803
Guideline: 850.6100
ILV for Determination of Hexazinone and its Products in Ground Water

	IN-T3935							IN-G3170						
Fortified (µg a.i./L)	Recovery (%)	Mean (%)	SD[1] (%)	RSD[2] (%)	Max	Min	n =	Recovery (%)	Mean (%)	SD[1] (%)	RSD[2] (%)	Max	Min	n =
	GC-NPD (quantitation)							GC-NPD (quantitation)						
4.0	81							113						
(LOQ)	84							103						
	89							110						
	83							97						
	71	82	7	8	89	71	5	78	100	14	14	113	78	5
20	72							79						
	83							98						
	96							107						
	93							101						
	83	85	10	11	96	72	5	106	98	11	12	107	79	5
LC/MS/MS (confirmation)								LC/MS/MS (confirmation)						
0.1	85							106						
(LOQ)	82							100						
	87							94						
	81							114						
	80	83	3	4	87	80	5	100	103	8	7	114	94	5
4.0	74							71						
	97							95						
	90							89						
	91							89						
	86	88	9	10	97	74	5	89	87	9	11	95	71	5

Results from Table 2, p. 25; Table 4, p. 27 of MRID 45132803; except the 2.0 ppb fortifications for IN-T3937 and IN-G3170 appear to typographical errors and should be 4.0 ppb (Appendix 4, pp. 185-186).
Means and standard deviations calculated using Microsoft program functions =AVERAGE(A1:A2) and =STDEV(A1:A2).
Any discrepancies between reviewer calculated values and reported results most likely due to rounding.
1 SD = Standard Deviation; determined using the “unbiased” or “n-1” method.
2 RSD = Relative Standard Deviation; calculated as (SD/mean) x 100.

Chemical: Hexazinone
PC: 107201
MRIDs: 45132803
Guideline: 850.6100
ILV for Determination of Hexazinone and its Products in Ground Water

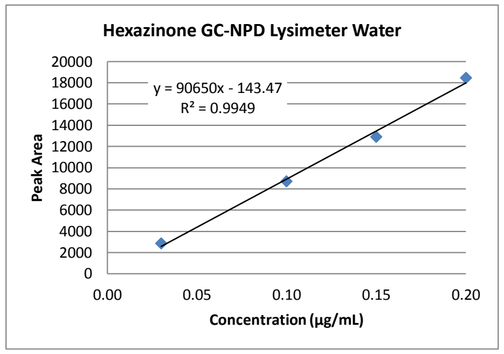
	IN-G3453							IN-JS472						
Fortified (µg a.i./L)	Recovery (%)	Mean (%)	SD[1] (%)	RSD[2] (%)	Max	Min	n =	Recovery (%)	Mean (%)	SD[1] (%)	RSD[2] (%)	Max	Min	n =
	GC-NPD (quantitation)							GC-NPD (quantitation)						
2.0 (LOQ)	80							80						
	82							81						
	85							77						
	86							76						
	73	81	5	6	86	73	5	69	77	5	6	81	69	5
20	76							68						
	87							82						
	103							94						
	89							85						
	91	89	10	11	103	76	5	81	82	9	11	94	68	5
	LC/MS/MS (confirmation)							LC/MS/MS (confirmation)						
0.1 (LOQ)	118							98						
	119							90						
	115							93						
	118							91						
	117	117	2	1	119	115	5	94	93	3	3	98	90	5
2.0	79							75						
	98							84						
	91							86						
	92							82						
	86	89	7	8	98	79	5	82	82	4	5	86	75	5

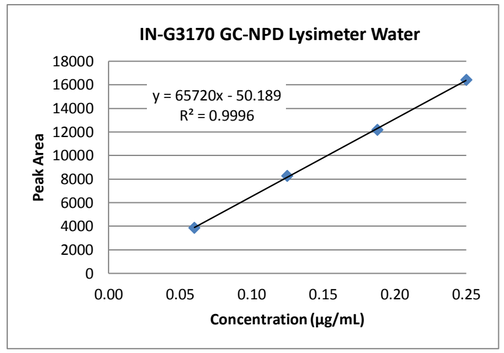
Results from Table 2, p. 25; Table 4, p. 27 of MRID 45132803.
Means and standard deviations calculated using Microsoft program functions =AVERAGE(A1:A2) and =STDEV(A1:A2).
Any discrepancies between reviewer calculated values and reported results most likely due to rounding.
1 SD = Standard Deviation; determined using the “unbiased” or “n-1” method.
2 RSD = Relative Standard Deviation; calculated as (SD/mean) x 100.

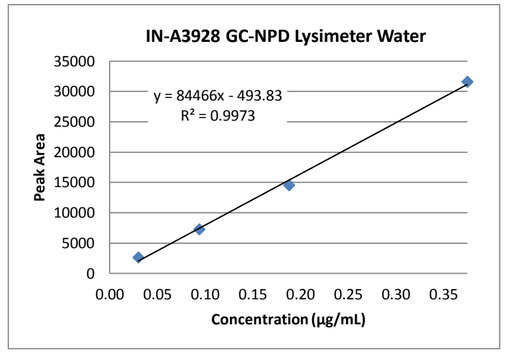
Chemical: Hexazinone
PC: 107201
MRIDs: 45132803
Guideline: 850.6100
ILV Calibration Curves: GC-NPD Method (quantitation)/Lysimeter Water

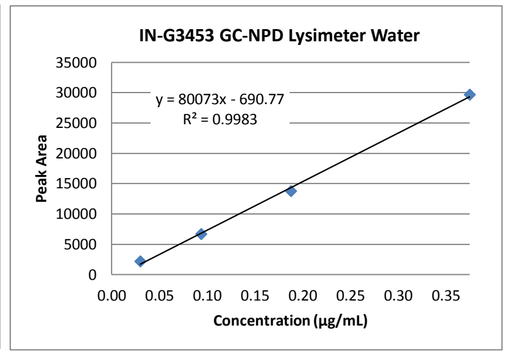
Calibration Curve Data	Hexazinone (DPX-A3674)		IN-G3170		IN-A3928		IN-G3453		IN-T3937		IN-JS472		IN-T3935	
	Conc. (µg/mL)	Peak Area counts	Conc. (µg/mL)	Peak Area counts	Conc. (µg/mL)	Peak Area counts	Conc. (µg/mL)	Peak Area counts	Conc. (µg/mL)	Peak Area counts	Conc. (µg/mL)	Peak Area counts	Conc. (µg/mL)	Peak Area counts
	0.030	2860.971	0.060	3861.518	0.030	2653.002	0.030	2176.697	0.030	2176.458	0.030	2622.132	0.060	4015.834
	0.100	8718.644	0.125	8279.670	0.094	7264.214	0.094	6682.637	0.094	6308.144	0.094	7458.516	0.188	11263.817
	0.150	12890.985	0.188	12170.257	0.188	14528.104	0.188	13735.000	0.188	13702.604	0.188	15159.703	0.375	23408.359
	0.200	18467.506	0.250	16431.504	0.375	31607.260	0.375	29652.477	0.375	30267.068	0.375	33798.848	0.750	50213.262

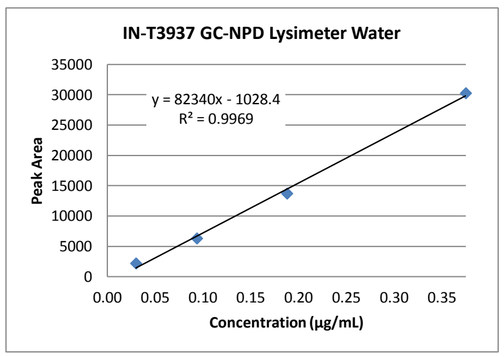
Results (Peak Area) from Appendix 4, pp. 163-169.

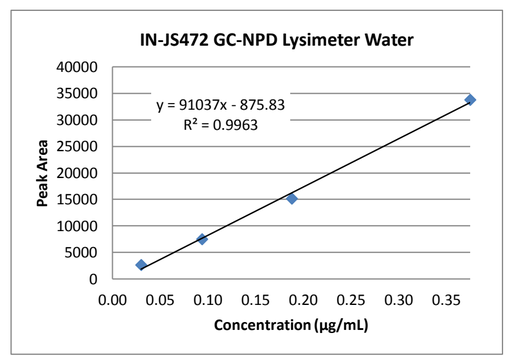


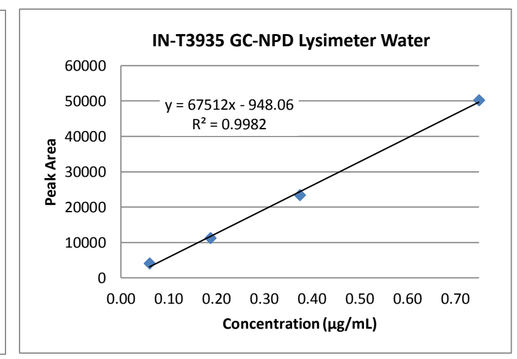








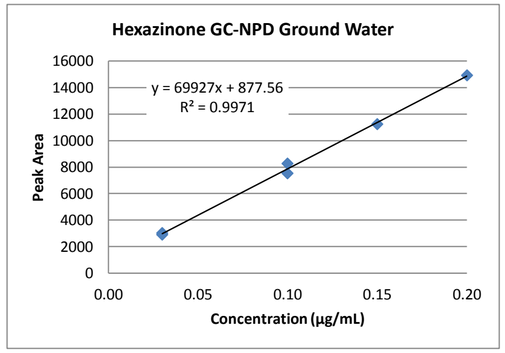


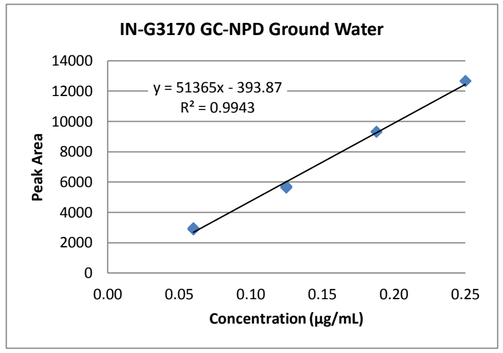


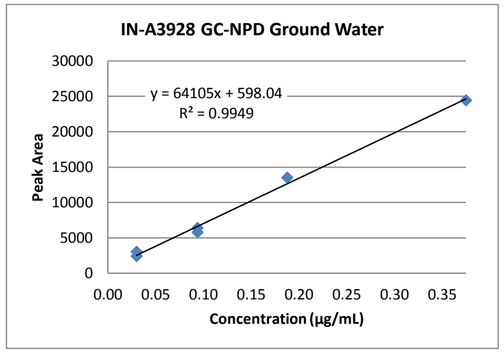
Chemical: Hexazinone
PC: 107201
MRIDs: 45132803
Guideline: 850.6100
ILV Calibration Curves: GC-NPD Method (quantitation)/Ground Water

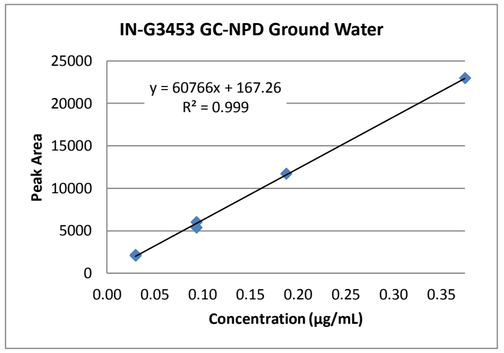
Calibration Curve Data	Hexazinone (DPX-A3674)		IN-G3170		IN-A3928		IN-G3453		IN-T3937		IN-JS472		IN-T3935	
	Conc. (µg/mL)	Peak Area counts	Conc. (µg/mL)	Peak Area counts	Conc. (µg/mL)	Peak Area counts	Conc. (µg/mL)	Peak Area counts	Conc. (µg/mL)	Peak Area counts	Conc. (µg/mL)	Peak Area counts	Conc. (µg/mL)	Peak Area counts
	0.030	3036.780	0.060	2879.075	0.030	3033.127	0.030	2142.665	0.030	2265.241	0.030	2633.348	0.060	3654.146
	0.030	2916.027	0.060	2951.607	0.030	2392.868	0.030	2081.365	0.030	2341.865	0.030	2702.277	0.060	3773.007
	0.100	8280.390	0.125	5629.439	0.094	6396.730	0.094	6001.780	0.094	5993.913	0.094	6966.089	0.188	9644.192
	0.100	7519.083	0.125	5706.031	0.094	5798.450	0.094	5379.724	0.094	5435.412	0.094	6230.454	0.188	8865.586
	0.150	11241.388	0.188	9317.021	0.188	13523.676	0.188	11708.634	0.188	12230.240	0.188	14184.629	0.375	21959.865
	0.200	14926.941	0.250	12656.379	0.375	24432.359	0.375	22970.844	0.375	23761.291	0.375	26963.941	0.750	39075.768

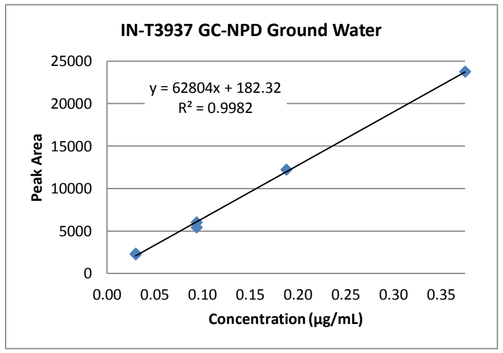
Results (Peak Area) from Appendix 4, pp. 170-176.

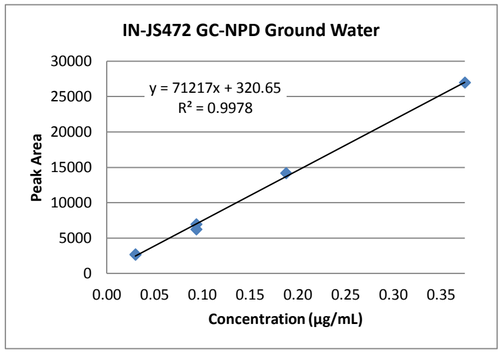


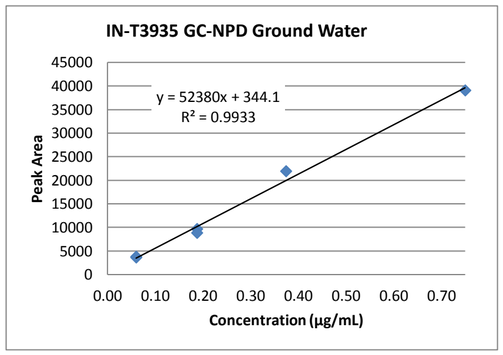








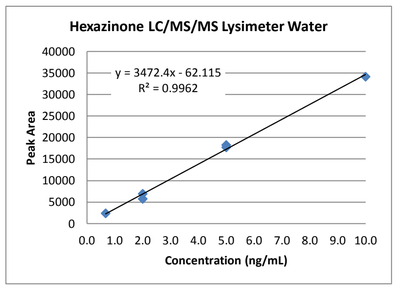


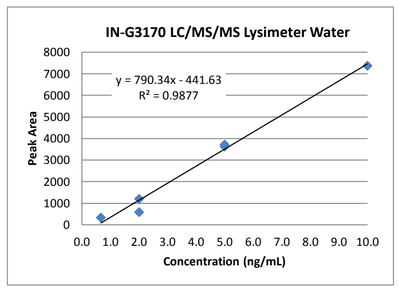


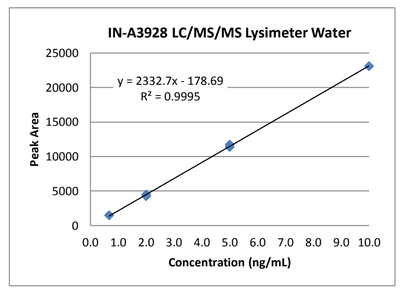
Chemical: Hexazinone
PC: 107201
MRIDs: 45132803
Guideline: 850.6100
ILV Calibration Curves: LC/MS/MS Method (confirmation)/Lysimeter Water

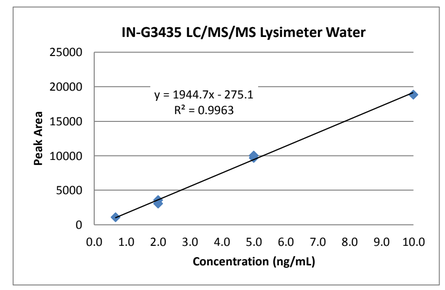
Calibration Curve Data	Hexazinone (DPX-A3674)		IN-G3170		IN-A3928		IN-G3453		IN-T3937		IN-JS472		IN-T3935		IN-G3170 (re-analysis)	
	Conc. (ng/mL)	Peak Area counts	Conc. (ng/mL)	Peak Area counts	Conc. (ng/mL)	Peak Area counts	Conc. (ng/mL)	Peak Area counts	Conc. (ng/mL)	Peak Area counts	Conc. (ng/mL)	Peak Area counts	Conc. (ng/mL)	Peak Area counts	Conc. (ng/mL)	Peak Area counts
	0.67	2396	0.67	336	0.67	1487	0.67	1085	0.67	1084	0.67	1077	0.67	709	0.67	491
	2.0	6941	2.0	1201	2.0	4514	2.0	3580	2.0	3361	2.0	3288	2.0	2417	2.0	1561
	2.0	5781	2.0	581	2.0	4252	2.0	3058	2.0	2837	2.0	3052	2.0	1792	2.0	1363
	5.0	18272	5.0	3627	5.0	11738	5.0	9711	5.0	8881	5.0	9411	5.0	6248	2.0	1391
	5.0	17752	5.0	3724	5.0	11372	5.0	10024	5.0	8833	5.0	9629	5.0	5925	2.0	1330
	10.0	34150	10.0	7379	10.0	23113	10.0	18867	10.0	16698	10.0	18046	10.0	12643	5.0	3830
															10.0	7572

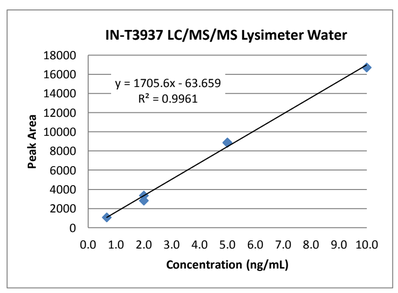
Results (Peak Area) from Appendix 4, pp. 177-184.

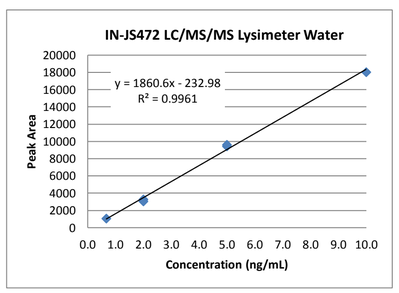


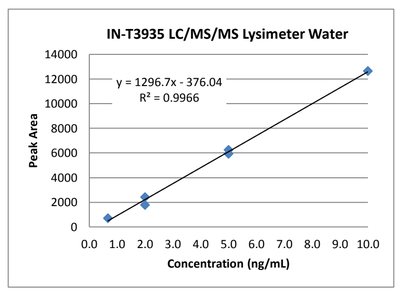


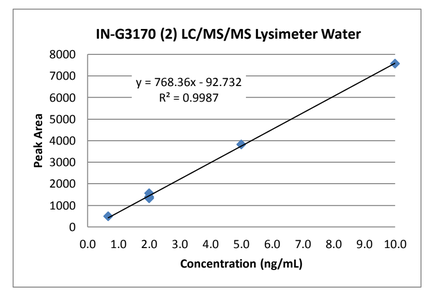












Chemical: Hexazinone
PC: 107201
MRIDs: 45132803
Guideline: 850.6100
ILV Calibration Curves: LC/MS/MS Method (confirmation)/Ground Water

Calibration Curve Data	Hexazinone (DPX-A3674)		IN-G3170		IN-A3928		IN-G3453		IN-T3937		IN-JS472		IN-T3935		IN-G3170 (2)		IN-G3170 (3)	
	Conc. (ng/mL)	Peak Area counts	Conc. (ng/mL)	Peak Area counts	Conc. (ng/mL)	Peak Area counts	Conc. (ng/mL)	Peak Area counts	Conc. (ng/mL)	Peak Area counts	Conc. (ng/mL)	Peak Area counts	Conc. (ng/mL)	Peak Area counts	Conc. (ng/mL)	Peak Area counts	Conc. (ng/mL)	Peak Area counts
	0.67	2990	0.67	524	0.67	2342	0.67	1630	0.67	1339	0.67	1413	0.67	802	0.67	54	0.67	589
	0.67	2976	0.67	670	0.67	2359	0.67	1487	0.67	1332	0.67	1626	0.67	813	2.0	114	2.0	1065
	2.0	8508	2.0	1722	2.0	6634	2.0	4546	2.0	3816	2.0	4237	2.0	2357	5.0	341	5.0	2271
	2.0	8903	2.0	2183	2.0	6725	2.0	4689	2.0	3893	2.0	4210	2.0	2728	5.0	307	10.0	5364
	5.0	21708	5.0	4670	5.0	17145	5.0	11827	5.0	10168	5.0	10929	5.0	6347	5.0	299		
	5.0	20822	5.0	4367	5.0	16323	5.0	11284	5.0	9814	5.0	10514	5.0	6002	5.0	343		
	10.0	39822	10.0	8441	10.0	31147	10.0	22453	10.0	18066	10.0	19718	10.0	11602	10.0	674		
	10.0	36602	10.0	6848	10.0	28532	10.0	20033	10.0	17520	10.0	19019	10.0	10344				

Results (Peak Area) from Appendix 4, pp. 185-193.