BASIC STUDY INFORMATION (CONTINUED)

Test Method: Four analytical methods as described in the study report of PTRL Europe ID P 2708 G "Validation of Analytical Methods for the Determination of BAS 455 H (Pendimethalin) and Its Metabolites M455H001, P48 and Reg. No. 4061757 in Surface and Ground Water" [1], with:

- Extraction with pentane (for BAS 455 H, M455H001, and P48) or dichloromethane (for Reg. No. 4061757);
- Concentration, re-dissolution and subsequent detection with liquid chromatography tandem mass spectrometry (LC-MS/MS, for BAS 455 H, M455H001, and P48) or with GC-MS/MS (for Reg. No. 4061757).

Limit of quantification (LOQ): 0.02 µg/L for each analyte and both water matrices

Schedule:		
	Study initiation date:	02-May-2013
	Experimental starting date:	15-May-2013
	Experimental completion date:	19-Jun-2013
	Study completion date:	28-Jun-2013

Guidelines

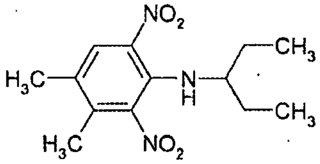
- Regulation (EC) No 1107/2009 of the European Parliament and of the Council (21/10/2009)
- Guidance document SANCO/825/00 rev. 8.1 of 16/11/2010 of the European Commission
- Guidance document SANCO/3029/99 rev. 4 of 11/07/00 of the European Commission
- EPA Guideline OCSPP 850.6100 of January 2012

PROJECT PURPOSE

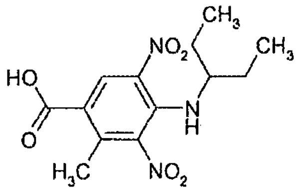
The study objective was to independently validate analytical methods as described in the study report of PTRL Europe ID P 2708 G (BASF DOC ID 2013/1113495) for the determination of residues of BAS 455 H (Pendimethalin) and its metabolites M455H001, Reg. No. 4295966 (P48) and Reg. No. 4061757 in surface and ground water.

TEST / REFERENCE ITEMS

The certified reference items of BAS 455 H, M455H001, P48 and Reg. No. 4061757 were supplied by BASF SE, Limburgerhof, Germany.

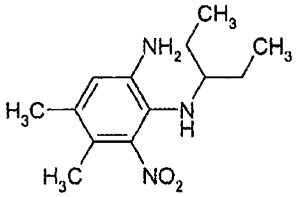
Common Name:	**Pendimethalin (BAS 455 H)**
Chemical Name (IUPAC):	*N*-(1-ethylpropyl)-2,6-dinitro-3,4-xylidine
CAS-Registry-No.:	40487-42-1
BASF-Registry-No.:	900072
Structural Formula:	
Molecular Formula:	$C_{13}H_{19}N_3O_4$
Molecular Mass:	281.3 g/mol
Batch:	AC12251-83
Purity:	99.2 %
Date of Certification:	18 April 2013
Storage Conditions (at test facility):	≤ -18 °C
Date of Expiry:	01 January 2016
Molecular Mass:	248.3

Common Name:	**M455H001**
Chemical Name (IUPAC):	2-methyl-3,5-dinitro-4-(pentan-3-ylamino)benzoic acid
CAS-Registry-No.:	127971-53-3
BASF-Registry-No.:	4108474
Structural Formula:	

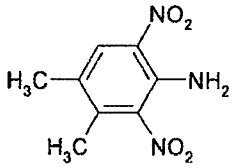


Molecular Formula:	$C_{13}H_{17}N_3O_6$
Molecular Mass:	311.3 g/mol
Batch:	L83-146
Purity:	99.7 %
Date of Certification:	18 April 2013
Storage Conditions (at test facility):	≤ -18 °C
Date of Expiry:	01 Decmber 2014

Common Name:	**P48 (Reg. No. 4295966)**
Chemical Name (IUPAC):	4,5-dimethyl-3-nitro-N^2-(pentan-3-yl)benzene-1,2-diamine
CAS-Registry-No.:	-
BASF-Registry-No.:	4295966
Structural Formula:	



Molecular Formula:	$C_{13}H_{21}N_3O_2$
Molecular Mass:	251.3 g/mol
Batch:	L83-102
Purity:	98.0 %
Date of Certification:	18 April 2013
Storage Conditions (at test facility):	≤ -18 °C
Date of Expiry:	01 September 2013

Common Name:	**Reg. No. 4061757**
Chemical Name (IUPAC):	2,6-dinitro-3,4-dimethylaniline
CAS-Registry-No.:	40318-31-8
BASF-Registry-No.:	4061757
Structural Formula:	
Molecular Formula:	$C_8H_9N_3O_4$
Molecular Mass:	211.2 g/mol
Batch:	AC12625-139B
Purity:	96.1 %
Date of Certification:	18 April 2013
Storage Conditions (at test facility):	≤ -18 °C
Date of Expiry:	01 August 2021

TEST SYSTEMS

Specimen origin

Surface water and ground water were provided by the test facility. Characterisation of both water types is given in Appendix 5.

Specimen preparation

The untreated waters were shaken before analysis. Specimens were uniquely identified with internal sample numbers and were stored at 3 - 8 °C in the dark until analysis.

Specimen analysis

Specimens were analysed for BAS 455 H (Pendimethalin) and its metabolites M455H001, P48 (Reg. No. 4295966) and Reg. No. 4061757 using analytical methods described in the study report of PTRL Europe ID P 2708 G "Validation of Analytical Methods for the Determination of BAS 455 H (Pendimethalin) and Its Metabolites M455H001, P48 and Reg. No. 4061757 in Surface and Ground Water" [1] with LC-MS/MS or GC-MS/MS detection. For surface water and ground water, control specimens were analysed in duplicate and fortified specimens were analysed in quintuplicate for each fortification level. For each method applied, one reagent blank was also analysed. Since at least two characteristic mass transitions were used to monitor BAS 455 H (Pendimethalin) and its metabolites M455H001, P48 (Reg. No. 4295966) and Reg. No. 4061757, the methods achieve a high level of specificity.

Representative chromatograms are presented in Appendix 3.

TEST METHOD

Outline of the Method

BAS 455 H (Pendimethalin) and its metabolites M455H001, P48 (Reg. No. 4295966) and Reg. No. 4061757 residues are extracted with pentane (Pendimethalin, M455H001, P48) or dichlormethane (Reg. No. 4061757). After concentration and re-dissolution, detection was performed with liquid chromatography tandem mass spectrometry (LC-MS/MS, for Pendimethalin, M455H001, and P48) or with GC-MS/MS (for Reg. No. 4061757).

The limit of quantification (LOQ) of the method for BAS 455 H (Pendimethalin) and its metabolites M455H001, P48 (Reg. No. 4295966) and Reg. No. 4061757 was 0.02 µg/L each.

The following analytical procedure is in accordance with the procedure described in the original method [1], with minor adaptations due to the use of the available laboratory equipment.

For the extraction of P48, the 50 mL centrifuge tubes were replaced by 25 mL Pyrex glass bottles. For the extraction of 4061757, the final volume could be adjusted to 1.0 mL volumetrically instead of gravimetrically by the use of tapered flasks with a graduation mark at 1.0 mL.

As a deviation, the determination of 4061757 was performed using GC-MS/MS in EI mode instead of NCI mode as in the validation. Due to the different ionisation technique, the mass transitions monitored (211->194, 211->164 and 211->118) differed partly from those used in the validation (211->194 and 211->193).

Nevertheless, these deviations as well as the minor adaptations are considered to have had no impact on the results of the study.

Apparatus and equipment

Centrifuge tubes with caps, 50 mL (e.g. Sarstedt, Art. No. 62.458.004)

Screw-capped Pyrex glas bottles, 25 mL

Tapered flasks, 25 mL, with graduation mark at 1.0 mL

Graduated cylinder, 250 mL

Separating funnel, 500 mL

Rotary Evaporator Hei-VAP-Advantage HL, HEIDOLPH, Schwabach, Germany, Art. No. 562-01300-00

Volumetric pipettes 'Fortuna', 10 mL

Ultrasonic bath Bandelin Sonorex RK 510 H, BANDELIN, Berlin, Germany., Art. No. 321

Autosampler vials with caps, 1.8 mL, with screw cap, Fa. Supelco or Agilent

Dilutor Model ML530b, Hamilton Deutschland, Martinsried, Germany., Art. No. ML530220

Common laboratory glassware

All glassware was rinsed with water (to remove detergents) and dried before use.

TEST METHOD (CONTINUED)

Reagents

Acetic acid, 100 % (e.g. Merck, Art.No. 1.00063)
Acetonitrile (e.g. Riedel de Haën, Art. No. 34881)
Ammonia solution, 25 % (e.g. Merck, Art.No. 1.05432)
Dichloromethane (e.g. VWR, Art.No. 23373320)
Formic acid, 98 - 100 % (e.g. Riedel-de Haën, Art. No. 33015)
Methanol (e.g. Sigma Aldrich, Art. No. 34860)
n-Pentane (e.g. Sigma Aldrich, Art. No. 34956)
Sodium sulphate, anhydrous (e.g. Merck No..6649)
Toluene (e.g. Honeywell, Art.No. 10313901)
Ultra pure water (e.g. Braun Melsungen, Aqua ad iniectabilia, No. 536108)

Extraction of Pendimethalin

25 mL water sample were measured into a 50 ml Sarstedt centrifuge tube. Fortification was carried out at this step by adding the appropriate fortification solution. 5 mL of n-pentane were added, and the sample was shaken by hand for 2 minutes. Phases were allowed to separate, and the upper phase was transferred into a 25 mL tapered flask. Extraction was repeated twice with each 5 mL of n-pentane, and the pentane phases were combined in the tapered flask. The combined extract was reduced to less than 1 mL using a rotary evaporator with a water bath temperature of max. 40 °C, and then evaporated to dryness under a stream of nitrogen. Subsequently, the residues were immediately dissolved with 1.0 mL of acetonitrile. After addition of 1.0 mL of ultra pure water with 0.2 % formic acid (V_{End} = 2.0 mL), the extract was mixed well and transferred into an autosampler vial for LC-MS/MS analysis.

Extraction of M455H001

25 mL water sample were measured into a 50 ml Sarstedt centrifuge tube. Fortification was carried out at this step by adding the appropriate fortification solution. After the water sample was acidified with 0.5 mL of acetic acid, 5 mL of n-pentane were added, and the sample was shaken by hand for 2 minutes. Phases were allowed to separate, and the upper phase was transferred into a 25 mL tapered flask. Extraction was repeated twice with each 5 mL of n-pentane, and the pentane phases were combined in the tapered flask. The combined extract was reduced to less than 1 mL using a rotary evaporator with a water bath temperature of max. 40 °C, and then evaporated to dryness under a stream of nitrogen. Subsequently, the residues were immediately dissolved with 1.0 mL of acetonitrile. After addition of 1.0 mL of ultra pure water with 0.2 % formic acid (V_{End} = 2.0 mL), the extract was mixed well and transferred into an autosampler vial for LC-MS/MS analysis.

TEST METHOD (CONTINUED)

Extraction of P48

25 mL water sample were measured into a 25 ml screw-capped Pyrex glas bottle. Fortification was carried out at this step by adding the appropriate fortification solution. After the water sample was alkalized with 1 mL of ammonia solution, 5 mL of n-pentane were added, and the sample was shaken by hand for 2 minutes. Phases were allowed to separate, and the upper phase was transferred into a 25 mL tapered flask. Extraction was repeated twice with each 5 mL of n-pentane, and the pentane phases were combined in the tapered flask. The combined extract was reduced to less than 1 mL using a rotary evaporator with a water bath temperature of max. 40 °C, and then evaporated to dryness under a stream of nitrogen. Subsequently, the residues were immediately dissolved with 1 mL of acetonitrile. After addition of 1 mL of ultra pure water (V_{End} = 2.0 mL), the extract was mixed well and transferred into an autosampler vial for LC-MS/MS analysis.

Extraction of Reg. No. 4061757

250 mL water were measured into a 500 ml separatory funnel. Fortification was carried out at this step by adding the appropriate fortification solution. 25 mL of dichloromethane were added, and the sample was shaken for 2 minutes. Phases were allowed to separate, and the lower phase was drained into a 150 mL beaker. Extraction was repeated twice with each 25 mL of dichloromethane, and the dichloromethane phases were combined in the beaker. The combined extract was filtered into a 100 mL tapered flask through a funnel equipped with anhydrous sodium sulphate (wetted with dichloromethane). Beaker and sodium sulphate were washed two times with each 10 mL of dichloromethane. The extract was concentrated to less than 5 ml using a rotary evaporator with a water bath temperature of max. 40°C, and then transferred into a 25 mL tapered flask with 3 x 5 mL of dichloromethane. 1 mL of toluene was added as keeper, and the extract was concentrated to less than 1 mL using a rotary evaporator with a water bath temperature of 40°C. The extracts were adjusted with toluene to a final volume of 1.0 mL, then sonicated and transferred into an autosampler vials for GC/MS/MS analysis at BASF SE.

Final Determination of Pendimethalin and M455H001 by LC-MS/MS

Extracts were analysed for Pendimethalin and M455H001 using an HPLC (Agilent 1200 Binary Pump SL) coupled to a PE Sciex API 4000 tandem mass spectrometer with electrospray nebuliser. Typical HPLC and mass spectral operating conditions are summarized in the following tables.

TEST METHOD (CONTINUED)

HPLC Conditions

System:	Hewlett-Packard Series 1200 HPLC (Agilent Technologies)		
Column:	Thermo Betasil C18, 100 × 2.1 mm, 5 µm		
Column Temperature:	35 °C		
Injection Volume:	50 µL		
Mobile Phase Conditions:	A: Methanol + 0.1 % formic acid; B: Water + 0.1 % formic acid		
Flow Rate:	0.60 mL/min		
Gradient Steps:	Time (min)	Phase A (%)	Phase B (%)
	0.0	34	66
	2.0	74	26
	4.0	90	10
	6.50	90	10
	6.60	100	0
	10.0	100	0
	10.1	34	66
	12.0	34	66
Retention Times:	Pendimethalin: approx. 5.0 min / M455H001: approx. 4.6 min		
Valco Valve:	0 - 3 min to waste; 3 - 7 min to MS		

Mass Spectrometer Conditions

MS System:	PE-Sciex API 4000 Tandem mass spectrometer			
Analyte Monitored	Ions Monitored	Declustering Potential	Collision Energy	Dwell Time (Seconds)
Pendimethalin	282 → 194	21	27	0.20
	282 → 212	21	17	0.20
M445H001	310 → 266	- 50	-14	0.20
	310 → 236	- 50	-18	0.20
Ion Mode:	Positive Multiple Reaction Monitoring (MRM) for Pendimethalin; Negative Multiple Reaction Monitoring (MRM) for M455H001			
Curtain Gas Flow:	25			
Gas Flow 1:	40			
Gas Flow 2:	70			
Ion Source Temperature:	450 °C (TurboIon Spray = Electrospray ionisation, ESI)			
Capillary voltage:	5200 V for Pendimethalin / - 4500 V for M455H001			

ESI-MS/MS spectra of Pendimethalin and M455H01 are given in Appendix 2, Figures A and B.

TEST METHOD (CONTINUED)

Final Determination of P48 by LC-MS/MS

Extracts were analysed for P48 using an HPLC (Agilent 1200 Binary Pump SL) coupled to a PE Sciex API 4000 tandem mass spectrometer with electrospray nebuliser. Typical HPLC and mass spectral operating conditions are summarized in the following tables.

HPLC Conditions

System:	Hewlett-Packard Series 1200 HPLC (Agilent Technologies)		
Column:	Luna C18, 50 × 2.0 mm, 5 µm		
Column Temperature:	35 °C		
Injection Volume:	50 µL		
Mobile Phase Conditions:	A: Methanol + 0.1% formic acid B: Water + 0.1 % formic acid		
Flow Rate:	0.60 mL/min		
Gradient Steps:	Time (min)	Phase A	Phase B
	0.0	40	60
	2.0	40	60
	2.1	100	0
	6.0	100	0
	6.1	40	60
	8.0	40	60
Retention Time:	P48: approx. 3.3 min		
Valco Valve:	0 - 2 min to waste; 2 - 6 min to MS		

Mass Spectrometer Conditions

MS System:	PE-Sciex API 4000 Tandem mass spectrometer			
Analyte Monitored	Ions Monitored	Declustering Potential	Collision Energy	Dwell Time (Seconds)
P48	252 → 119	41	35	0.50
	252 → 146	41	27	0.50
Ion Mode:		Positive Multiple Reaction Monitoring (MRM)		
Curtain Gas Flow:		25		
Gas Flow 1:		40		
Gas Flow 2:		70		
Ion Source Temperature:		450 °C (TurboIon Spray) (= Electrospray ionisation, ESI)		
Capillary voltage:		4500 V		

An ESI-MS/MS spectrum of P48 is given in Appendix 2, Figure C.

TEST METHOD (CONTINUED)

Final Determination of 4061757 by GC-MS/MS

GC-MS/MS-System:	Agilent Technologies GC 7890A with CombiPal Autosampler, coupled to an Agilent Technologies 7000 GC/MS Triple Quad
Column:	30 m fused silica capillary column DB-5 MS (J&W) Internal diameter 0.25 mm, film thickness 0.25 µm
Gas:	Carrier: helium, 1.5 mL/min
Temperatures:	Oven: initial 90 °C (hold for 2 min) heat rate 50 °C/min to 150 °C, heat rate 10 °C/min to 200 °C, heat rate 100 °C/min to 310 °C (hold for 4 min) Injector: 250 °C
Injection volume:	2 µL, splitless
Ionisation method:	Electron ionisation (EI)
Source temperature:	230 °C
Collision energy:	10 eV (211->194), 20eV (211->164 and 211->118)
Selected mass transitions:	211->194 (quantification); 211->164 and 211->118 (confirmation)
Dwell time:	70 ms
External standards:	ranging from 1.0 ng/mL to 100 ng/mL of 4061757 (matrix-matched standards for both ground and surface water)
Retention time:	approx. 8.5 min
Integration:	MassHunter GC/MS Acquistition B.05.02.1032 MassHunter Workstation Software: Quantitative Analysis V. B.05.00 for QQQ

CALCULATION OF RESULTS

Principle

Calculation of results is based on peak area measurements of the peak representing Pendimethalin, M455H001, P48, or 4061757. The residues were calculated from the calibration curve using matrix-matched standard solutions, except for P48, where solvent standards were used.

Calculation

The individual concentrations in µg/L are calculated as shown in the following equation:

$$R = \frac{A_A \times AvF \times V_{End} \times DF}{V}$$

where:

CALCULATION OF RESULTS (CONTINUED)

R: Residue of the analyte in µg/L

A_A: Peak area of the analyte in final solution in counts

AvF: Average response factor: average of standard conc. (ng/mL) / sum of the peak areas calculated from the calibration standards in each sequence

The average response factor was calculated as follows:

$$AvF = \frac{(C_{St1} / A_{St1} + C_{St2} / A_{St2} + ... C_{StN} / A_{StN})}{N}$$

C_{St}: Concentration of analyte in external standard solution, in ng/mL

A_{St}: Sum of the peak areas of analyte in external standard solution, in counts

N: Number of external standard solutions

V_{End}: Final volume: 2.0 mL (for Pendimethalin, M455H001; P48) or 1.0 mL (for 4061757)

DF: Dilution factor (1 = no dilution)

V: Sample volume: 25 mL (for Pendimethalin, M455H001, P48) or 250 mL (for 4061757)

Percent recovery from fortified specimen was calculated using the following expressions:

$$\text{Recovery} = \frac{R_F \times 100\,\%}{F}$$

R_F = Analyte found in fortified specimen in µg/L

F = Fortification level in µg/L

Calculation of Recoveries

Example of a calculation for a Pendimethalin specimen (internal sample no. 103, MRM 282→212; please compare with Raw Data Sheet in Appendix 1, Table 2):

Residues found in µg/L were calculated as follows:

$$R = \frac{61900 \times 3.6316 \times 10^{-6} \times 2 \times 1}{25} = \underline{0.0180\ \mu g/L\ (rounded)}$$

$$\text{Recovery} = \frac{0.0180 \times 100\,\%}{0.0200} = 90\,\%$$

Recoveries were not corrected for blank values.

STOCK, FORTIFICATION, WORKING AND STANDARD SOLUTIONS

Stock solution no. 8268: 400 µg/mL Pendimethalin

10.08 mg Pendimethalin (99.2 %; page 14) = 10.0 mg (100 %) were dissolved in acetone and diluted to 25 mL in a volumetric flask.

Stock solution no. 8270: 400 µg/mL M455H001

10.04 mg M455H001 (99.7 %; page 15) = 10.0 mg (100 %) were dissolved in acetone and diluted to 25 mL in a volumetric flask.

Stock solution no. 8271: 400 µg/mL P48

10.20 mg P48 (98.0 %; page 15) = 10.0 mg (100 %) were dissolved in methanol and diluted to 25 mL in a volumetric flask.

Stock solution no. 8269: 400 µg/mL Reg. No. 4061757

10.41 mg 4061757 (96.1 %; page 16) = 10.0 mg (100 %) were dissolved in acetone and diluted to 25 mL in a volumetric flask.

Fortification Solutions

The following fortification solutions of Pendimethalin, M455H001 and P48 were prepared in methanol using a dilutor and volumetric flasks.

Pendimethalin:

Solution used	Volume used [mL]	Dilution volume [mL]	Concentration obtained [µg/mL]	Working / Fortification Solution
Stock solution no. 8268	0.060	2.40	10	DZ1
DZ1	0.050	5.0	0.10	Z1
Z1	0.20	2.0	0.010	Z2

M455H001:

Solution used	Volume used [mL]	Dilution volume [mL]	Concentration obtained [µg/mL]	Working / Fortification Solution
Stock solution no. 8270	0.060	2.4	10	DZ2
DZ2	0.050	5.0	0.10	Z3
Z3	0.20	2.0	0.010	Z4

STOCK, FORTIFICATION, WORKING AND STANDARD SOLUTIONS (CONTINUED)

P48:

Solution used	Volume used [mL]	Dilution volume [mL]	Concentration obtained [µg/mL]	Working / Fortification Solution
Stock solution no. 8271	0.060	2.4	10	DZ7
DZ7	0.050	5.0	0.10	Z13
Z13	0.50	5.0	0.010	Z14

The following fortification solutions of 4061757 were prepared in acetone using a dilutor.

Solution used	Volume used [mL]	Dilution volume [mL]	Concentration obtained [µg/mL]	Working / Fortification Solution
Stock solution no. 8269	0.12	2.4	20	DZ4
DZ4	0.12	2.4	1.0	Z7
Z7	0.20	2.0	0.10	Z8
Stock solution no. 8269	0.12	2.4	20	DZ5
DZ5	0.12	2.4	1.0	Z9
Z9	0.10	1.0	0.10	Z10

Working Solutions

Working solutions of Pendimethalin and M455H001 were prepared in acetonitrile/water (1/1, v/v) + 0.1% formic acid using a dilutor and volumetric flasks.

Working Solution	Volume used [mL]	Dilution volume [mL]	Solution used	Concentration of Pendimethalin / M455H001 [µg/mL]
DL1	0.060	2.4	Stock solution no. 8268	10
DL2	0.050	5.0	DL1	0.10
DL3	0.060	2.4	Stock solution no. 8270	10
DL4	0.050	5.0	DL3	0.10

STOCK, FORTIFICATION, WORKING AND STANDARD SOLUTIONS (CONTINUED)

Working solutions of P48 were prepared in acetonitrile/water (1/1, v/v) using a dilutor and a volumetric flask.

Working Solution	Volume used [mL]	Dilution volume [mL]	Solution used	Concentration of P48 [µg/mL]
DL13	0.060	2.4	Stock solution no. 8271	10
DL14	0.050	5.0	DL13	0.10

Working solutions of 4061757 were prepared in toluene using a dilutor.

Working Solution	Volume used [mL]	Dilution volume [mL]	Solution used	Concentration of 4061757 [µg/mL]
DL7	0.12	2.4	Stock solution no. 8269	20
DL8	0.12	2.4	DL7	1.0
DL9	0.12	2.4	Stock solution no. 8269	20
DL10	0.12	2.4	DL9	1.0

Standard Solutions

External solvent standard solutions of Pendimethalin were prepared by diluting a working solution with acetonitrile/water (1/1, v/v) + 0.1% formic acid using a dilutor.

Calibration Solution	Volume used [mL]	Dilution volume [mL]	Working / Calibration solution used	Concentration of Pendimethalin [ng/mL]
L11	0.10	1.00	DL2	10
L12	0.060	1.20	DL2	5.0
L13	0.060	2.40	DL2	2.5
L14	0.10	1.00	L11	1.0
L15	0.060	1.20	L11	0.50
L16	0.060	2.40	L11	0.25
L17	0.125	1.00	L14	0.125
L18	0.060	1.20	L14	0.050

STOCK, FORTIFICATION, WORKING AND STANDARD SOLUTIONS (CONTINUED)

External solvent standard solutions of M455H001 were prepared by diluting a working solution with acetonitrile/water (1/1, v/v) + 0.1% formic acid using a dilutor.

Calibration Solution	Volume used [mL]	Dilution volume [mL]	Working / Calibration solution used	Concentration of M455H001 [ng/mL]
L21	0.10	1.00	DL4	10
L22	0.060	1.20	DL4	5.0
L23	0.060	2.40	DL4	2.5
L24	0.10	1.00	L21	1.0
L25	0.060	1.20	L21	0.50
L26	0.060	2.40	L21	0.25
L27	0.125	1.00	L24	0.125
L28	0.060	1.20	L24	0.050

External solvent standard solutions of P48 were prepared by diluting a working solution with acetonitrile/water (1/1, v/v) using a dilutor.

Calibration Solution	Volume used [mL]	Dilution volume [mL]	Working / Calibration solution used	Concentration of P48 [ng/mL]
L71	0.10	1.00	DL14	10
L72	0.060	1.20	DL14	5.0
L73	0.060	2.40	DL14	2.5
L74	0.10	1.00	L71	1.0
L75	0.060	1.20	L71	0.50
L76	0.060	2.40	L71	0.25
L77	0.125	1.00	L74	0.125
L78	0.060	1.20	L74	0.050

STOCK, FORTIFICATION, WORKING AND STANDARD SOLUTIONS (CONTINUED)

The following matrix-matched calibration solutions of Pendimethalin were prepared by diluting working or calibration solutions with control matrix extract using volumetric pipettes:

Calibration Solution	Volume used [µL]	Dilution volume [µL]	Working / Calibration solution used	Concentration of Pendimethalin [ng/mL]
Ground water				
M111	50	500	DL2	10
M112	20	400	DL2	5.0
M113	20	800	DL2	2.5
M114	40	400	DL2	1.0
M115	20	400	M111	0.50
M116	20	400	M111	0.25
M117	20	400	M112	0.125
M118	40	400	M113	0.050
Surface water				
M121	50	500	DL2	10
M122	20	400	DL2	5.0
M123	20	800	DL2	2.5
M124	40	400	M121	1.0
M125	20	400	M121	0.50
M126	20	400	M122	0.25
M127	20	400	M123	0.125
M128	40	400	M125	0.050

STOCK, FORTIFICATION, WORKING AND STANDARD SOLUTIONS (CONTINUED)

The following matrix-matched calibration solutions of M455H001 were prepared by diluting working or calibration solutions with control matrix extract using volumetric pipettes:

Calibration Solution	Volume used [µL]	Dilution volume [µL]	Working / Calibration solution used	Concentration of M455H001 [ng/mL]
Ground water				
M211	50	500	DL4	10
M212	20	400	DL4	5.0
M213	20	800	DL4	2.5
M214	40	400	M211	1.0
M215	20	400	M211	0.50
M216	20	400	M212	0.25
M217	20	400	M213	0.125
M218	40	400	M215	0.050
Surface water				
M221	50	500	DL4	10
M222	20	400	DL4	5.0
M223	20	800	DL4	2.5
M224	40	400	M221	1.0
M225	20	400	M221	0.50
M226	20	400	M222	0.25
M227	20	400	M223	0.125
M228	40	400	M225	0.050

STOCK, FORTIFICATION, WORKING AND STANDARD SOLUTIONS (CONTINUED)

The following matrix-matched calibration solutions of 4061757 were prepared by diluting working or calibration solutions with control matrix extract using volumetric syringes:

Calibration Solution	Volume used [mL]	Dilution volume [mL]	Working / Calibration solution used	Concentration of 4061757 [ng/mL]
Ground water				
M411	25	250	DL8	100
M412	15	200	DL8	75
M413	10	200	DL8	50
M414	50	200	M411	25
M415	20	200	M411	10
M416	20	200	M413	5.0
M417	20	200	M414	2.5
M418	20	200	M415	1.0
Surface water				
M431	25	250	DL10	100
M432	15	200	DL10	75
M433	10	200	DL10	50
M434	50	200	M431	25
M435	20	200	M431	10
M436	20	200	M433	5.0
M437	20	200	M434	2.5
M438	20	200	M435	1.0

All solutions were stored at 3 – 8 °C in the dark.

FORTIFICATIONS

Control (untreated) specimens of both water types were fortified with Pendimethalin prior to extraction with 50 µL of fortification solution Z1 or Z2 as follows:

Matrix	Specimen volume	Fortification solution	Fortification level
Ground water	25 mL	Z2	0.020 µg/L
	25 mL	Z1	0.20 µg/L
Surface water	25 mL	Z2	0.020 µg/L
	25 mL	Z1	0.20 µg/L

Control (untreated) specimens of both water types were fortified with M455H001 prior to extraction with 50 µL of fortification solution Z3 or Z4 as follows:

Matrix	Specimen volume	Fortification solution	Fortification level
Ground water	25 mL	Z4	0.020 µg/L
	25 mL	Z3	0.20 µg/L
Surface water	25 mL	Z4	0.020 µg/L
	25 mL	Z3	0.20 µg/L

Control (untreated) specimens of both water types were fortified with P48 prior to extraction with 50 µL of fortification solution Z13 or Z14 as follows:

Matrix	Specimen volume	Fortification solution	Fortification level
Ground water	25 mL	Z14	0.020 µg/L
	25 mL	Z13	0.20 µg/L
Surface water	25 mL	Z14	0.020 µg/L
	25 mL	Z13	0.20 µg/L

Control (untreated) specimens were fortified with 4061757 prior to extraction with 50 µL of fortification solution Z7 or Z8 (for ground water), or Z9 or Z10 (for surface water) as follows:

Matrix	Specimen volume	Fortification solution	Fortification level
Ground water	250 mL	Z8	0.020 µg/L
	250 mL	Z7	0.20 µg/L
Surface water	250 mL	Z10	0.020 µg/L
	250 mL	Z9	0.20 µg/L

LITERATURE

[1] Report to study PTRL Europe ID P 2708 G “Validation of Analytical Methods for the Determination of BAS 455 H (Pendimethalin) and Its Metabolites M455H001, P48 and Reg. No. 4061757 in Surface and Ground Water”, N. Heinz, 22 April 2013; BASF DOC ID 2013/1113495.