ANALYTICAL

Preparation and Storage of Samples

The independent laboratory validation was carried out on three water specimens; surface water, ground water and drinking water. The drinking water was obtained from a "drinking water" tap at the Test Facility (CEMAS), the ground water was obtained from a well near Henley-on-Thames and the surface water was obtained from the River Meon, Meonstoke, Hampshire.

Specimen	CEMAS Sample Reference (CSR) Number
Surface Water	4626-001
Ground Water	4626-002
Drinking Water	4626-003

On receipt the specimens were stored at approximately 4 °C before and after analysis.

Characterisation of Samples

The water specimens were characterised at CEMAS. Details of the characterisation results are as follows:

CSR Number	pH	Total Hardness mg/L as $CaCO_3$	Total Suspended Solids mg/L	Alkalinity mg/L as $CaCO_3$	Total Organic Carbon	Dissolved Organic Carbon
4626-001	7.6	41.0	6.2	247.0	1.45 mg/L	1.47 mg/L
4626-002	7.5	201.2	<1.0	166.0	722 µg/L	607 µg/L
4626-003	7.4	307.3	<1.0	184.0	3.25 mg/L	2.87 mg/L

pH

The pH of the water samples was determined using CEMAS SOP CEM-3373 - Determination of the pH of Water, Soil and Sediment Samples in water and/or Salt Solutions (0.01 M Calcium Chloride, 0.1 M Potassium Chloride, 1.0 M Potassium Chloride).

The pH value reflects the relative number of hydrogen ions (H+) in solution. The more hydrogen ions present, compared to the hydroxyl ions (OH-), the more acidic the solution will be and the lower the pH value. A decrease in hydrogen ions and increase in hydroxyl ions will result in more alkaline or basic conditions.

The pH was determined, potentiometrically, using a glass combination electrode and a pH meter.

Hardness EDTA titration

Total Hardness by EDTA Titration in water was determined using CEMAS SOP CEM-3060 - Determination of Total Hardness by EDTA Titration in Water.

Water hardness is an expression for the sum of the calcium and magnesium cation concentrations in a water sample. The standard method of expressing water hardness is in mg/L calcium carbonate ($CaCO_3$) which has the formula weight of 100.1 g/mole.

Water hardness was determined using a complexometric titration method using a standard ethylenediaminetetraacetic acid (EDTA) solution. Due to steric hindrances EDTA will then complex with calcium and magnesium in a one-to-one molar ratio. Since EDTA and its hardness complexes are not colored, an additional chelating agent, eriochrome black T, was used to facilitate endpoint detection.

Total Suspended Solids

The total suspended solids in the water samples were determined using CEMAS SOP CEM-3448 - Determination of Total Suspended and Volatile Suspended Solids in Waters, which is a standard gravimetric procedure. Total suspended solids are described as those solids which are retained on a glass fibre filter and dried at 103-105°C. 500 mL of sample was filtered, under vacuum, onto a pre-weighed glass fibre filter (GF/F). The paper plus residue was dried for at least two hours and then reweighed. The weight of residue was expressed as mg/L total suspended solids.

Carbonate, Bicarbonate, Carbonate Hardness and Alkalinity

Alkalinity was determined using CEMAS SOP CEM-3384 - Determination of Alkalinity of Water – Carbonate, Bicarbonate and Carbonate Hardness.

Alkalinity is the measure of a water sample's ability to neutralize hydrogen ions (its acid-neutralizing ability). Alkalinity may be caused by dissolved strong bases such as sodium hydroxide or potassium hydroxide (and other hydroxide-containing compounds), and it may, also, be caused by dissolved carbonates, bicarbonates, borates, and phosphates. The measured alkalinity is the total of all of these species found in a water sample. For the sake of simplicity, it is expressed in terms of mg $CaCO_3$/L although many species other than dissolved calcium carbonate may actually contribute to the alkalinity. Total Alkalinity is referred to as Carbonate Hardness.

The carbonate concentration was determined by titration with hydrochloric acid using phenolpthalein as an indicator and the bicarbonate hardness level was determined by further titration with the same acid using bromophenol blue as the indicator.

Dissolved and Total Organic Carbon

The dissolved organic carbon and the total organic carbon of the water samples were determined using CEMAS SOP CEM-3396 - Determination of the Total and Dissolved Organic Carbon, Inorganic Carbon and Carbon in Water. The dissolved organic carbon (DOC) is a measure of the organic material, contained in a water sample that is soluble and/or colloidal, that can pass through a 0.45um filter.

A Sievers Model 5310C TOC Analyser (GE Analytical Instruments) was used to measure the concentration of Dissolved Organic Carbon (DOC), Inorganic Carbon (DIC), and Carbon (DTC) in the water samples. The analyser principle is based on the oxidation of organic compounds to form carbon dioxide using UV radiation and a chemical oxidising agent (ammonium persulphate). Carbon dioxide is measured using a sensitive, selective membrane-based conductometric detection technique. For each DOC measurement, the concentration of inorganic carbon species (carbonates, bicarbonates and carbon dioxide) is determined and, after oxidation of the organic compounds, the total carbon (DTC) content of the sample is measured. The concentration of the organic compounds (DOC) was calculated from the difference between the concentrations of the dissolved carbon (DC) and dissolved inorganic carbon (DIC).

The total organic carbon (TOC) is a measure of all the organic material, contained in a water sample. The ground water and drinking water samples did not contain any visible particulate matter. The surface water sample appeared to have some small particles present but not sufficient to be a problem for the analysis. The results for this sample are the same, allowing for analytical variation. The dissolved organic carbon and total organic carbon were 1.47 and 1.45 mg/L respectively. The other samples show slightly lower dissolved organic carbon levels than total organic carbon.

Preparation of Solutions and Standards

Reagents used were of equivalent specifications as described in the method. The following analytical test substances/analytical standards (obtained from the Sponsor) were utilized during the independent laboratory method validation:

Test Substance/Analytical Standard:	Fluroxypyr	Fluroxypyr 1-Methylheptyl Ester
Supplier:	Sponsor	Sponsor
AGR/TSN Number:	AGR222210	TSN106348
Batch no:	433-0685-1	XP9-37136-3C
Purity:	99.6%	99.9%
Expiry date:	12 July 2011	20 July 2011
Storage:	Ambient	Ambient

Test Substance/Analytical Standard:	Fluroxypyr Dichloropyridinol	Fluroxypyr Methoxypyridine
Supplier:	Sponsor	Sponsor
AGR/TSN Number:	TSN101651	AGR250194
Batch no:	E0432-71A	N.A.
Purity:	>99%	99.9%
Expiry date:	14 August 2011	24 June 2013
Storage:	Ambient	Ambient

Standard solutions and calibration standard solutions were prepared as described in the method. Full details of these materials are included in the raw data package for the study along with the preparation of all analytical and fortification standards prepared from the primary reference items. The test/reference items will be retained until expiry and then disposed of. A retained sample of each reference item used in this study is kept at Dow AgroSciences Global Test Substance Shipping Department, Zionsville Road, Indianapolis, Indiana 46268, U.S.A.

Fortification of Recovery Samples

The control specimens were fortified as described below:

Matrix	Reference Items	Untreated Replicates	Replicates at Fortification Level (LOQ)	Replicates at Fortification Level (10 x LOQ)
Surface Water	Fluroxypyr 1-MHE	2	5 at 0.05 µg/L	5 at 0.5 µg/L
	Fluroxypyr	2	5 at 0.05 µg/L	5 at 0.5 µg/L
	Fluroxypyr-DCP	2	5 at 0.05 µg/L	5 at 0.5 µg/L
	Fluroxypyr-MP	2	5 at 0.05 µg/L	5 at 0.5 µg/L
Ground Water	Fluroxypyr 1-MHE	2	5 at 0.05 µg/L	5 at 0.5 µg/L
	Fluroxypyr	2	5 at 0.05 µg/L	5 at 0.5 µg/L
	Fluroxypyr-DCP	2	5 at 0.05 µg/L	5 at 0.5 µg/L
	Fluroxypyr-MP	2	5 at 0.05 µg/L	5 at 0.5 µg/L
Drinking Water	Fluroxypyr 1-MHE	2	5 at 0.05 µg/L	5 at 0.5 µg/L
	Fluroxypyr	2	5 at 0.05 µg/L	5 at 0.5 µg/L
	Fluroxypyr-DCP	2	5 at 0.05 µg/L	5 at 0.5 µg/L
	Fluroxypyr-MP	2	5 at 0.05 µg/L	5 at 0.5 µg/L

Sample Extraction, Purification and Analysis

Specimens were assayed according to the analytical method in the Dow AgroSciences report 081042, "Validation Report for the Determination of Residues of Fluroxypyr 1-Methylheptyl Ester, Fluroxypyr and its Major Metabolites in Surface Water, Ground Water and Drinking Water by High Performance Liquid Chromatography and Tandem Mass Spectrometry".

Residues of fluroxypyr 1-MHE and fluroxypyr were extracted from the water sample matrices by acidifying the sample using concentrated formic acid, saturating the water with sodium chloride, then partitioning it twice against ethyl acetate. The two ethyl acetate layers were drawn off and combined in the same graduated tube. A 1.0 mL aliquot of a methanol/water (50:50) solution containing 0.1% acetic acid solution was added. The combined ethyl acetate extracts were concentrated under a stream of nitrogen until approximately 0.9 mL remained. The final sample was adjusted to 2.0 mL using a methanol/water (50:50) solution containing 0.1% acetic acid. The sample was analyzed by liquid chromatography with negative-ion electrospray ionization (ESI) tandem mass spectrometry (LC-MS/MS).

For the determination of residues of fluroxypyr-DCP and fluroxypyr-MP, the water samples were buffered to pH 7 using a potassium dihydrogen phosphate buffer solution. The water sample was loaded onto a Strata -X polymeric sorbent SPE column and eluted with acetonitrile into a graduated tube containing a 1.0 mL aliquot of a methanol/water (50:50) solution with 0.1% acetic acid. The acetonitrile eluate was concentrated under a stream of nitrogen until approximately 0.9 mL remained. The final sample was adjusted to 1.5 mL using a methanol/water (50:50) solution containing 0.1% acetic acid. The sample was analyzed by liquid chromatography with positive-ion atmospheric pressure chemical ionization (APCI) tandem mass spectrometry (LC-MS/MS).

Analytical Instrumentation and Equipment

The instrumental conditions used during the ILV trial were as described in the method, with minor adaptations. The instrumental conditions used were as follows:

Typical LC-MS/MS Operating Conditions for Fluroxypyr 1-MHE and Fluroxypyr

Instrumentation:	Agilent Model 1100 autosampler
	Agilent Model 1100 binary pump
	Agilent Model 1100 degasser
	MDS/Sciex API 5000 LC-MS/MS System
	MDS/Sciex Analyst 1.4.2 data system
Column:	Zorbax SB-C8 4.6 × 75 mm, 3.5 µm

Column Temperature:	25 °C		
Injection Volume:	50 µL		
Run Time:	18.0 minutes		
Mobile Phase:	A – methanol/acetic acid (99.9:0.1) B – water/acetic acid (99.9:0.1)		
Flow Rate:	900 µL/min		
Gradient:	Time, min	Solvent A, %	Solvent B, %
	0.01	60	40
	2.00	60	40
	10.00	100	0
	12.00	100	0
	14.00	60	40
	18.00	60	40
Flow Diverter Program:	1) 0.0 to 1.0 min: flow to waste 2) 1.0 to 10.0 min: flow to source 3) 12.0 min: flow to waste		

Typical Mass Spectrometry Operating Conditions for Fluroxypyr 1-MHE and Fluroxypyr

Interface:	ESI
Polarity:	Negative
Scan Type:	MRM
Resolution:	Q1 - unit, Q3 - unit
Curtain Gas (CUR):	20
Collision Gas (CAD):	5
Temperature (TEM):	550
Ion Source Gas 1 (GS1):	30
Ion Source Gas 1 (GS1):	60
Period 1	
Acquisition Time:	18 min
IonSpray Voltage (IS):	-4500
Entrance Potential (EP):	-10

Analytes:	Precursor Ion Q1	Product Ion Q3	Dwell Time (ms)	Collision Energy (CE)	Declustering Potential (DP)	Cell Exit Potential (CXP)
Fluroxypyr 1-MHE (quantification)	365.2	194.1	150	-36	-140	-20
Fluroxypyr 1-MHE (confirmation)	367.2	196.3	150	-36	-140	-20
Fluroxypyr (acid) (quantification)	253.2	232.9	150	-10	-50	-15
Fluroxypyr (acid) (confirmation)	255.1	197.0	150	-20	-50	-10

Typical LC-MS/MS Operating Conditions for Fluroxypyr-DCP and Fluroxypyr-MP

Instrumentation:	Agilent Model 1100 autosampler		
	Agilent Model 1100 binary pump		
	Agilent Model 1100 degasser		
	MDS/Sciex API 4000 LC-MS/MS System		
	MDS/Sciex Analyst 1.4.2 data system		
Column:	Zorbax SB-C8 4.6 × 75 mm, 3.5 µm		
Column Temperature:	Ambient		
Injection Volume:	20-50 µL		
Run Time:	16.0 minutes		
Mobile Phase:	A – methanol/acetic acid (99.9:0.1) B – water/acetic acid (99.9:0.1)		
Flow Rate:	900 µL/min		
Gradient:	Time, min	Solvent A, %	Solvent B, %
	0.01	40	60
	2.00	40	60
	10.00	100	0
	12.00	40	60
	16.00	40	60
Flow Diverter Program:	1) 0.0 to 3.0 min: flow to waste 2) 3.0 to 9.0 min: flow to source 3) 9.0 min: flow to waste		

Typical Mass Spectrometry Operating Conditions for Fluroxypyr-DCP and Fluroxypyr-MP

Interface:	APCI
Polarity:	Positive
Scan Type:	MRM
Resolution:	Q1 - unit, Q3 - unit
Curtain Gas (CUR):	20
Collision Gas (CAD):	7
Temperature (TEM):	600
Ion Source Gas 1 (GS1):	60
Nebulizer Current (NC):	5
Period 1	
Acquisition Time:	16 min
Declustering Potential (DP)	66

Analytes:	Precursor Ion Q1	Product Ion Q3	Dwell Time (ms)	Collision Energy (CE)	Entrance Potential (EP)	Cell Exit Potential (CXP)
Fluroxypyr-DCP (quantification)	199.14	181.00	150	31	13	4
Fluroxypyr-DCP (confirmation)	199.14	154.00	150	37	13	14
Fluroxypyr-MP (quantification)	211.17	113.00	150	49	10	8
Fluroxypyr-MP (confirmation)	211.17	195.90	150	29	10	12

Statistical Treatment of Data

The mean recoveries for the fortified samples were calculated using the "AVERAGE" function of the Microsoft Excel spreadsheet computer program, which divides the sum of the selected cells by the number of determinations. The standard deviation of the recoveries for a fortification level of one matrix type was calculated using the "STDEV" function of the same spreadsheet program, which sums the squares of the individual deviations from the mean, divides by the number of degrees of freedom, and extracts the square root of the quotient. Percent relative standard deviation, % RSD, was calculated by dividing the standard deviation by the mean, and then multiplying by 100.