ANALYTICAL

Preparation and Storage of Samples

The independent laboratory validation was carried out on three specimens of water of different types: surface water, ground water and drinking water. The drinking water was obtained from a "drinking water" tap at Vergèze (30310 - France), the ground water was obtained from Evian, France, and the surface water was obtained from the Rhôny, Gard (30), France.

Specimen	EAS Chem SAS Sample Reference Number
Surface Water	313
Ground Water	300
Drinking Water	Vergèze

Upon receipt, the specimens were stored at 4 °C.

Characterisation of Samples

The water specimens were characterized by Eurofins IPL Sud (a non-GLP facility, however, one that is ISO certified), 75 chemin de Sommières, 30310 Vergèze, France, details of the characterization results are as follows:

Specimen	pH (20°C)	Total Hardness (°F)	Total Suspended Solids (mg/L)	Calcium (mg/L)	Magnesium (mg/L)	Dissolved Organic Carbon (mg/L)
Method	NFT90-008 / NF EN ISO 10523	Calculation	NF EN 872	NF EN ISO 14911	NF EN ISO 14911	NF EN 1484
Surface Water	8.1	46	< 2	170	8.2	1.9
Ground Water	7.4	34	< 2	90	29	< 0.5
Drinking Water	7.65	40	< 2	150	5.7	2.2

Preparation of Solutions and Standards

Reagents used were of equivalent specifications as described in the analytical method.

The following analytical test substance/analytical standard were utilized during the

independent laboratory method validation:

Test Substance/ Analytical Standard:	Ethalfluralin
Supplier:	Sponsor
Reference Number:	TSN101281
Batch/Lot no:	597-C049-003
Purity:	99.8%
Expiry date:	18 Jun 2017
Storage:	Target 4°C

The certificate of analysis was provided by Dow AgroSciences LLC. This is located in Appendix B.

Analytical standard stock solutions, calibration standard solutions and fortification solutions were prepared as described in the analytical method presented in appendix C. The reference item will be retained until expiry and then disposed of with the approval of the Study Director and the Study Monitor.

Fortification of Recovery Samples

The control specimens were fortified with ethalfluralin as described below:

Matrix	Untreated Control Specimen Reps	Replicates at Fortification Level (LOD)*	Replicates at Fortification Level (LOQ)**	Replicates at Fortification Level (10xLOQ)**
Drinking Water	2	1 at 1.5 ng/L	5 at 5.0 ng/L	5 at 50 ng/L
Groundwater	2	1 at 1.5 ng/L	5 at 5.0 ng/L	5 at 50 ng/L
Surface Water	2	1 at 1.5 ng/L	5 at 5.0 ng/L	5 at 50 ng/L

*LOD – Limit of Detection
**LOQ – Limit of Quantification

One sample was fortified to achieve a fortification level of 1.5 ng/L (LOD), five samples were fortified to achieve the fortification level of 5.0 ng/L (LOQ) and five samples were fortified to achieve the upper fortification level of 50 ng/L for each water type—drinking water, surface water and ground water. The fortification solution was injected directly into the matrix.

Sample Extraction, Purification and Analysis

Specimens were assayed according to the environmental chemistry method Dow AgroSciences Study Number CEMS-5608, "Validation of an Analytical Method for the Determination of Ethalfluralin in Water" (Reference 1). The method was internally referenced at Eurofins Agroscience Services Chem SAS under the number AGR/MOA/ETHALFLURALIN-1.

Residues of ethalfluralin were extracted from water samples and purified using a C-18 solid phase extraction (SPE) column. After elution from the SPE column with 6 mL of ethyl acetate, the ethyl acetate was evaporated from the final eluate and re-dissolved in 1 mL ethyl acetate. Final samples were analysed by gas chromatography with electron-impact mass spectrometry detection (GC/MS).

Full extraction details:

1. Measure 500-mL portions of each water sample into a 1000-mL polypropylene flask.
2. For preparing fortified samples, add appropriate aliquots of the appropriate spiking solutions to untreated control water to encompass the necessary concentration range:

Concentration of Fortified Sample (ng/L)	Volume of Spiking Solution (µL)	Concentration of Spiking Solution (µg/mL)
1.5	150	0.005
5.0	500	0.005
50.0	500	0.05

3. Add 4 mL of methanol to the sample.
4. Mix the samples manually and allow to stand for 5 minutes.
5. Use the following procedure for the clean-up of samples on the Strata C-18 SPE cartridge:
    a. Place a Strata C-18 SPE cartridge (500-mg, 6 mL) for each sample to be analysed on a vacuum manifold box.
    b. Condition the SPE column with 4 mL of ethyl acetate followed by 4 mL of methanol and 4 mL of ultra-pure water. Do not apply vacuum to the SPE

manifold at this stage. Discard the eluates.

c. Place a 100 mL empty reservoir to the top of each cartridge with a suitable adapter.

d. Transfer aliquots of the entire sample to the reservoir. Draw the sample through the column at 4-5 mL/min flow rate by application of vacuum, discarding the eluates. Apply vacuum at the end of the loading process to draw the sample through the column completely.

e. Place a 15 mL centrifuge tube below each cartridge.

f. Elute the analyte from the SPE column with 6 mL of ethyl acetate; do not apply vacuum initially, and then apply vacuum at the end to draw the sample through the column completely.

g. Evaporate the sample using a stream of nitrogen at a room temperature until all ethyl acetate is removed (some remaining water may be present at this stage).

h. Dissolve the samples in 1 mL ethyl acetate and mix well to dissolve ethalfluralin.

6. Transfer the samples into suitable autosampler vial.

7. Analyse the sample by gas chromatography with electron-impact mass spectrometry detection (GC/MS).

Analytical Instrumentation and Equipment

- CG-MS: HP6890GC (HP)
- HP 5973 MSD mass spectrometer with Chemstation software
- GC column DB-5MS 30 m x 0.25 mm i.d. 0.25-µm film thickness (Agilent, ref.122-5532)
- Cartridge SPE Strata C-18 (500 mg/6 mL) (Phenomenex, ref. 8B-S002-HCH)
- Heating block under nitrogen flow (Techne)
- Laboratory centrifuge (Thermo Scientific)
- Polypropylene centrifugation tubes (VWR)
- Precision balance (Mettler, Sartorius)
- Standard laboratory glassware (volumetric flasks, measuring cylinders)
- Various pipettes (Thermo Scientific)
- Vortex (VWR)

The instrumental conditions used during the ILV trial were as described in the analytical method, and are given below.

Typical GC/MS Operating Conditions

GC	:	HP 6890 GC
Detector	:	HP 5973 MSD mass spectrometer with Chemstation software
Column	:	DB-5MS (30.0 m × 0.25 mm i.d., df = 0.25 µm)
Injection Port	:	Splitless
Carrier gas, flow rate	:	Helium at 1.4 mL/min constant flow
Injection mode	:	Pulsed splitless
Injection volume	:	4 µL
Injector temperature	:	250°C
Transfer line temperature	:	280°C
Ionisation mode	:	EI
Scan type	:	SIM
Resolution	:	Low
Ion source temperature	:	230°C
Quadrupole temperature	:	150°C

Temperature Programme

Step	Rate (°C/min)	Temperature (°C)	Time (min.)
1	-	100	1.5
2	5	140	-
3	1	150	3.0
4	50	280	5.0

Under these conditions the retention time of ethalfluralin is approximately 18.1 min.

Mass spectrometer conditions

Analyte			Dwell (ms)
Ethalfluralin	Target ion	276 m/z	300
	Qualifier 1	292 m/z	300
	Qualifier 2	316 m/z	300

Calculation of Results

For each analytical batch, 7 calibration standards was injected over the range 0.75 ng/mL to 100 ng/mL. A calibration curve was prepared for the analyte by plotting the quantification peak area obtained versus the analyte concentration.

Example: Ethalfluralin recovery at 5.0 ng/L

A linear calibration curve was calculated using the method of least squares (1/x weighting):

$$Y = A \times C + B$$

Y = detector response (as peak area) for ethalfluralin = 15428

A = slope of the linear least squares fit of the calibration curve = 7441.467

C = Analyte concentration

B = Y-intercept of the linear least squares fit of the calibration curve = -603.015

The concentration determined from standard curve is $C = \frac{(Y-B)}{A} = 2.154$ ng/L

The residue of ethalfluralin in each test specimen is calculated as follows:

$$\text{Residue (ng/L)} = \frac{V_f}{V_1} \times f \times \text{extract concentration (ng/L)}$$

Where:

V_1 (mL) = total extraction volume (500 mL)

V_f (mL) = final volume (1 mL)

f = conversion factor between ng/L and ng/mL (1000)

$$\text{Residue (ng/L)} = \frac{1}{500} \times 1000 \times 2.154$$

Residue (ng/L) = 4.31 ng/L

Procedural recovery data from fortified samples are calculated via the following equation:

$$\text{Recovery (\%)} = \frac{A}{S} \times 100$$

$$\text{Recovery (\%)} = \frac{4.31}{5} \times 100$$

Where:

A = concentration of ethalfluralin found in spiked sample = 4.31 ng/L.

S = concentration of ethalfluralin added in spiked sample = 5 ng/L.

Recovery = 86% (calculation performed on unrounded values)

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 each fortification level for each 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 (n-1), 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.

Confirmation of Residue Identity

The GC/MS method is highly selective for the determination of residues of ethalfluralin in drinking water, surface water and ground water by virtue of the chromatographic separation and selective detection system used. To demonstrate further confirmation, three structurally characteristic fragment ions were monitored for ethalfluralin. Calculations of %Recovery and %RSD were carried out on the confirmatory ions data in addition to the quantitative ions (Tables 1 to 18).