Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pesticide Name:	Famoxadone
MRID # :	449672-04
Matrix:	Water
Analysis:	GC/ECD

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If you have difficulties in downloading the method, or further questions concerning the methods, you may contact Elizabeth Flynt at 228-688-2410 or via e-mail at flynt.elizabeth@epa.gov.

REPORT

449672-04

Report Title

VALIDATION OF TWO ANALYTICAL METHODS FOR THE DETERMINATION OF FAMOXADONE IN WATER BY GC/ECD

Data Requirement

EEC Directive 91/414/EEC, Annex II 4.2.3

Author of Original Report

Sandra Hausmann

Study Completed on

November 21, 1997

Performing Laboratory

PTRL Europe
Helmholtzstr. 22, Science Park
D-89081 Ulm, Germany

Laboratory Project ID

Study No. PTRL Europe 233 G (Germany)
Report No. B 233
Original 2 of 2

DuPont Report No.

AMR 4720-97

Sponsor

E.I. du Pont de Nemours and Company
Wilmington, Delaware 19898, U.S.A

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA Section 10(d)(1)(A), (B), or (C).

Company: E. I. du Pont de Nemours and Company

Company Agent: Linda G. Carter
(Typed Name)

U. S. Product Registration Manager
(Title)

Linda G Carter
(Signature)

May 12, 1999
(Date)

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

The study forming the subject of this report was performed under the supervision of the Study Director in accordance with the procedures described therein. The report provides an accurate record of the procedures and the results obtained.

The study was conducted in compliance with current German, OECD and U.S. EPA's Good Laboratory Practice Standards 40 CFR part 160.

T. Class

Signature of Study Director:

November 21 1997

Date

Dr. Thomas Class
PTRL Europe
Helmholtzstr. 22, Science Park
D-89081 Ulm, Germany

GLP-CERTIFICATE OF THE TESTING FACILITY

Umweltministerium Baden-Württemberg

GLP-Bescheinigung

Bescbeinigung

Hiermit wird bestatigt, daß die Prüfungseinrichtung

in 89081 Ulm
Helmholtzstr. 22

der PTLR Europe Labor für Umwelt- und Pestizidchemie GmbH

am 11 05 1995

von der für die Überwachung zuständigen Behörde über die Einhaltung der Grundsätze der Guten Laborpraxis inspiziert worden ist

Es wird hiermit bestätigt, daß folgende Prüfungen in dieser Prüfeinrichtung nach den Grundsätzen der Guten Laborpraxis durchgeführt werden

Seite 1/2

Certificate

It is hereby certified that the test facility

in 89081 Ulm
Helmholtzstr. 22

of PTLR Europe Labor für Umwelt- und Pestizidchemie GmbH

on 11.05.1995

was inspected by the competent authority regarding compliance with the Principles of Good Laboratory Practice.

It is hereby certified that studies in this test facility are conducted in compliance with the Principles of Good Laboratory Practice

Stuttgart, den 24.07.1995

page 1/2

GLP-CERTIFICATE OF THE TESTING FACILITY

Translated from German Original:

Page 2/2 of GLP Certificate

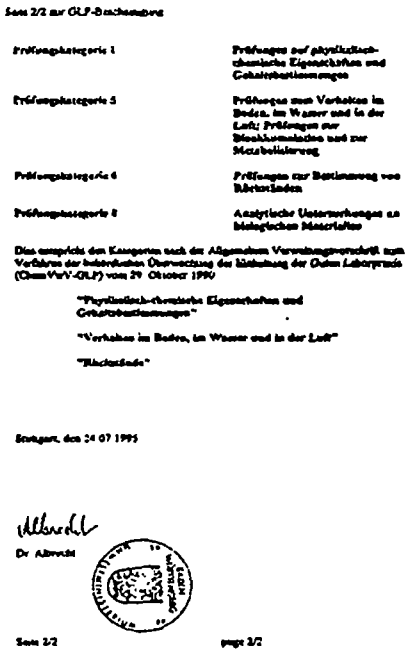
Testing category 1	Physical-chemical testing and content determinations
Testing category 5	Studies on behavior in soil, water and air; bioaccumulation
Testing category 6	Residue studies
Testing category 8	Analytical testing on biological material

This corresponds to the following categories as given in the General Administration Act for Compliance Monitoring Procedures for Good Laboratory Practice (ChemVwV-GLP) from October 29, 1990:

"Physical-chemical properties and content determinations"
"Behavior in soil, in water and in the air"
"Residues"

Stuttgart, 24.07.1995
(Signature)
Dr. Albrecht

Stamp reading: Baden-Württemberg,
Ministery for Environmental Affairs



PTRL QUALITY ASSURANCE UNIT FINAL REPORT STATEMENT

Study No.: PTRL Europe 233 G

Report Title: VALIDATION OF TWO ANALYTICAL METHODS FOR THE DETERMINATION OF FAMOXADONE IN WATER BY GC/ECD.

The Quality Assurance Unit has reviewed this report in accordance with Good Laboratory Practice Standards.

Based upon the documentation provided, the reported data reviewed are determined to be an accurate reflection of the raw data in this study.

The following inspections were performed by the Quality Assurance Unit during the conduct of this study:

Part of Study	Inspection Date	Date of Submission of Inspection Report to Study Director T. Class	Date of Submission of Inspection Report to Management L. Ruzo
Protocol Review	August 01, 1997	August 01, 1997	August 01, 1997
Critical Phase Audit*	August 29, 1997	August 29, 1997	August 29, 1997
Raw Data Review and Draft Report	October 20, 22-23, 1997	October 23, 1997	October 24, 1997
Revised Draft and Final Report	November 21, 1997	November 21, 1997	November 21, 1997

* Spiking of control samples and solid phase extraction.

Prepared by: S. Dmochewitz Date: Nov. 21. 1997

Dr. Susanne Dmochewitz, PTRL Europe
Manager of Quality Assurance

KEY STUDY PERSONNEL

Function	Name
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REPORT COMPLETED BY: T. Class Nov. 27, 1997

Dr. Thomas Class
PTRL Europe,
Study Director

Date

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STUDY IDENTIFICATION

STUDY PROTOCOL TITLE:	Analytical Method for the Determination for Famoxadone (DPX-JE874) in Water: Adaptation / Development and Validation.
REPORT TITLE:	Validation of Two Analytical Methods for the Determination of Famoxadone in Water by GC/ECD.
STUDY NO.:	PTRL Europe 233 G
STUDY SITE:	PTRL Europe Labor für Umwelt- und Pestizidchemie GmbH Helmholtzstr. 22, Science Park D-89081 Ulm, Germany
STUDY DIRECTOR:	Dr. Thomas Class, PTRL Europe Tel. (49)-(0)731-553055, Fax (49)-(0)731-553056
SPONSOR:	E. I. du Pont de Nemours and Company Wilmington, Delaware 19898 U.S.A
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STUDY INITIATION DATE:	August 07, 1997
EXPERIMENTAL	
START DATE:	August 11, 1997
TERMINATION DATE:	October 20, 1997
STUDY COMPLETION DATE:	November 21, 1997

STORAGE LOCATION OF RECORDS, SAMPLE(S), STUDY PROTOCOL AND FINAL REPORT (1ST ORIGINAL): PTRL Europe Archive
Helmholtzstr. 22, Science Park
D-89081 Ulm, Germany

Records can be transferred to the Sponsor upon written request.
No specimens are retained.

1.0 ABSTRACT / SUMMARY

VALIDATION OF TWO ANALYTICAL METHODS FOR THE DETERMINATION OF FAMOXADONE IN WATER BY GC/ECD

Types of Water:
Drinking water, ground water and surface water.

Outline of Solid Phase Extraction (SPE) Method:
Water is sampled through pre-conditioned 1 g C_{18} SPE cartridge (1 L drinking water or ground water, 0.2 L surface water). The cartridge is dried and then the analyte is eluted from the adsorbent with 3 * 2 mL acetonitrile. The eluate is concentrated to dryness and the analyte is redissolved in toluene (e.g. 0.2 to 1.0 mL) for GC/ECD analysis (DB 1: 15 m * 0.32 mm * 0.25 µm).

Outline of Liquid / Liquid Extraction Method:
Water (0.5 L) is extracted three times with 50 mL dichloromethane using an Ultra Turrax homogenizer at ≈ 13500 rpm. The dichloromethane extract is dried over sodium sulfate and then concentrated to dryness. The analyte is redissolved in toluene (e.g. 0.2 to 2.5 mL) for GC/ECD analysis (DB 1: 15 m * 0.32 mm * 0.25 µm).

GC/MS was demonstrated as confirmatory detection method.

Recovery Results and Limits of Quantitation / Determination (LOQ):

	SPE		Liquid / Liquid Extraction	
Matrix	Fortification (µg/L)	Mean Rec.(%)[a] ± RSD[b] (%)	Fortification (µg/L)	Mean Rec. (%)[a] ± RSD[b] (%)
Drinking water:	0.050 (LOQ)	90 ± 5 %	0.050 (LOQ)	88 ± 6 %
	0.25 (5 * LOQ)	96 ± 10 %	0.25 (5 * LOQ)	102 ± 2 %
	Overall average:	93 ± 8 %	Overall average:	95 ± 9 %
Ground water:	0.050 (LOQ)	81 ± 6 %	0.050 (LOQ)	90 ± 14 %
	0.25 (5 * LOQ)	89 ± 3 %	0.25 (5 * LOQ)	98 ± 11 %
	Overall average:	85 ± 6 %	Overall average:	94 ± 12 %
Surface water:	0.25 (LOQ)	72 ± 3 %	0.25 (LOQ)	90 ± 6 %
	1.25 (5 * LOQ)	100 ± 3 %	1.25 (5 * LOQ)	106 ± 9 %
	Overall average:	86 ± 18 %	Overall average:	98 ± 12 %

[a]Rec. = recovery. The mean recovery for each fortification level is calculated from 3 replicates.
[b]RSD = relative standard deviation = (absolute standard deviation / mean recovery) * 100 %.

Estimated Limits of Detection (LOD):
The LODs were not investigated specifically. Estimated LODs: 0.008 µg/L (SPE) or 0.016 µg/L (liquid/liquid extraction) for drinking water and ground water, and 0.040 µg/L (SPE and liquid/liquid extraction) for surface water. LOD may vary with quality of water and performance of GC/ECD system. Detection requires confirmation by GC/MS.

2.0 INTRODUCTION

Background:

Famoxadone (DPX-JE874) is a fungicide used for various crops. The structure of famoxadone and other relevant information on the chemical are given in Figure 1.

Objective:

To provide two analytical methods for the determination of famoxadone (DPX-JE874) in water: One method is based on solid phase extraction (SPE), the other method on liquid / liquid extraction. Both methods are validated for three types of water: drinking water, ground water and surface water. The validation ranges were 0.050 µg/L (LOQ, limit of quantitation) to 0.25 µg/L for drinking water and ground water and 0.25 µg/L (LOQ) to 1.25 µg/L for surface water. Limits of detection (LOD) were not studies but estimated to be 0.008 µg/L (SPE) or 0.016 µg/L (liquid/liquid extraction) for drinking water and ground water, and 0.040 µg/L (SPE and liquid/liquid extraction) for surface water.

Data requirements:

To support requirements by the EEC Directive 91/414/EEC, Annex II 4.2.3.

Method principles for the solid phase extraction (SPE) method:

Water is sampled through pre-conditioned 1 g C_{18} SPE cartridges (1 L drinking water or ground water, 0.2 L surface water). The cartridges are dried and then eluted with 3 * 2 mL acetonitrile. The eluates are concentrated to dryness and the residue is redissolved in toluene (e.g. 0.2 to 1.0 mL) for GC/ECD analysis on a DB 1 capillary column (15 m * 0.32 mm * 0.25 µm).

Method principles for the liquid / liquid extraction method:

Water (0.5 L) is extracted three times with 50 mL dichloromethane using an Ultra Turrax homogenizer at ≈ 13500 rpm. The combined dichloromethane extracts are dried over sodium sulfate and concentrated to dryness. The residue is redissolved in toluene (e.g. 0.2 to 2.5 mL) for GC/ECD analysis on a DB 1 capillary column (15 m * 0.32 mm * 0.25 µm).

For method validation water samples were fortified and then extracted by SPE or liquid / liquid partition. Control water samples were used to demonstrate the absence of famoxadone signals in untreated samples. Furthermore, method validation demonstrated limits of determination / quantitation (LOQ) and the range of applicability.

GC/MS was examined and demonstrated as confirmatory method.

3.0 MATERIALS

Equivalent equipment and materials may be substituted unless otherwise specified; note any specifications in the following description before making substitutions. Substitutions should only be made if equivalency / suitability has been verified with acceptable control and fortification recovery data.

3.1 Standard

Analytical reference standard:

Famoxadone (DPX-JE874) provided by the Sponsor with a Certificate of Analysis: IN # JE874-92, purity: 99.6 %. For detailed information see Figure 1.

3.2 Solvents for Stock, Fortification and Chromatographic Standard Preparation

Solvent used for stock standard preparation:

Methanol (HPLC grade, part no. 3041) (Promochem, Wesel, Germany).

Solvent used for fortification standard preparation:

Acetonitrile (HPLC grade, part no. 2856) (Promochem). DO NOT SUBSTITUTE!

Solvent used for chromatographic standard preparation:

Toluene (residue grade, part no. 8092) (Promochem).

3.3 Equipment and Reagents for Solid Phase Extraction (SPE)

Miscellaneous equipment:

Balance: Sartorius RC 210D (Sartorius, Göttingen, Germany).

SPE processing stations (T.J. Baker, Deventer, Netherlands or Macherey-Nagel, Düren, Germany) equipped with stop cocks for flow control and vacuum gauge for vacuum control

Water suction pumps.

Sample concentration device with water bath and nitrogen supply or rotary evaporators R-114 and RE-111 (Büchi, Flawil, Switzerland).

Ultrasonic bath: Transonic 460 (Elma, Singen, Germany).

Typical glassware and laboratory equipment.

Sampling cartridges:

Mega Bond Elut™, Bonded Phase C_{18}, size 6 mL, part no. 1225-6001 (Varian, Darmstadt, Germany).

Solvents:

Acetone (residue grade, part no. 0018), acetonitrile (HPLC grade, part no. 2856),

toluene (residue grad, part no. 8092) (Promochem, Wesel, Germany).
Technical acetone (purity < 99 %, part no. 00585) for rinsing of rotary evaporators (Fluka, Neu-Ulm, Germany).
Ampuva water (part no. 1080181, Fresenius, Bad Homburg, Germany).

3.4 Equipment and Reagents for Liquid / Liquid Extraction

Miscellaneous equipment:

Balance: Sartorius RC 210D (Sartorius, Göttingen, Germany).
Ultra Turrax IKA T25 (Janke & Kunkel, Staufen, Germany).
Rotary evaporators R-114 and RE-111 (Büchi, Flawil, Switzerland).
Ultrasonic bath: Transonic 460 (Elma, Singen, Germany).
Typical glassware and laboratory equipment.

Reagents:

Sodium sulfate anhydrous (purity: > 99 %, part no. 0313, T.J. Baker, Deventer, Netherlands).

Solvents:

Dichloromethane (residue grade, part no. 3023), toluene (residue grade, part no. 8092) (Promochem, Wesel, Germany).
Technical acetone for rinsing of rotary evaporators (purity: > 99 %, part no. 2856, Fluka, Neu-Ulm, Germany).
Ampuva water (part no. 1080181, Fresenius, Bad Homburg, Germany).

3.5 GC/ECD System and Evaluation

GC/ECD system:

Varian (Darmstadt, Germany) GC system equipped with:
Varain 8200 Autosampler, Varian 3400 GC with split / splitless injector, Varian ECD, J & W Scientific DB 1 capillary column (15 m, 0.32 mm i.d., 0.25 µm film, J & W Scientific, Folsom, CA, USA).

Evaluation:

PC-based Varian Star chromatographic software, Microsoft Excel.

3.6 Safety and Health

Each analyst must be aquainted with the potential hazards of the reagents, products and solvents used in the method before working in the laboratory. All appropriate material safety data sheets should be read and followed, and proper personal protective equipment should be used

4.0 METHODS

4.1 Principles of the Analytical Methods

4.1.1 Solid Phase Extraction (SPE) Method

Water (1 L drinking water or ground water, 0.2 L surface water) is sampled through 1 g C_{18} SPE cartridges which are preconditioned with acetone, acetonitrile and water. The cartridges are dried and then eluted with 3 * 2 mL acetonitrile. The eluates are concentrated to dryness and the residue is redissolved in toluene (e.g. 0.2 to 1.0 mL) for GC/ECD analysis (DB 1: 15 m * 0.32 mm * 0.25 µm).

A schematic presentation of the method is given in Figure 2.

4.1.2 Liquid / Liquid Extraction Method

Water (0.5 L all types) is extracted three times with 50 mL dichloromethane using an Ultra Turrax homogenizer at ≈ 13500 rpm. The combined dichloromethane extracts are dried over sodium sulfate and then concentrated to dryness. The residue is redissolved in toluene (e.g. 0.2 to 2.5 mL) for GC/ECD analysis (DB 1: 15 m * 0.32 mm * 0.25 µm).

A schematic presentation of the method is given in Figure 3.

4.2 Analytical Procedure

4.2.1 Glassware and Equipment Cleaning Procedure

All reusable glassware should be rinsed with solvent, washed with hot tap water, non-phosphate detergent, rinsed with deionized water (may be performed in laboratory dish washer) and dried fully before use

The Ultra Turrax homogenizers are rinsed between sample extractions in a beaker filled with an appropriate solvent (e.g. dichloromethane) to prevent carry over.

Rotary evaporators are rinsed between sample evaporations with acetone to prevent carry over.

Care should be taken to avoid working with high levels of the analyte being monitored in the same laboratory where samples are being extracted and analyzed.

4.2.2 Stock Standard Solution Preparation (Example) and Stability

A stock standard solution of the reference standard was prepared at 1.00 mg/mL in-methanol: Weigh 10.00 ± 0.10 mg of famoxadone (IN # JE874-92, purity: 99.6 %) into a 10 mL volumetric flask. Dissolve completely in 10.0 mL methanol.

Store refrigerated, stable for at least one month.

4.2.3 Fortification Standard Solution Preparation (Example) and Stability

A 25 µg/mL intermediate solution in acetonitrile was prepared by volumetric dilution:
Dilute 250 µL of stock standard solution in 10.0 mL acetonitrile.
A 250 ng/mL fortification standard solution in acetonitrile was prepared by volumetric dilution:
Dilute 500 µL of intermediate solution in 50.0 mL acetonitrile.

Store refrigerated, stable for at least two weeks.

4.2.4 Chromatographic Standard Solution Preparation (Examples) and Stability

Chromatographic standard solutions were prepared at 25 to 500 pg/µL in toluene by volumetric dilution as exemplified below:
50 ng/µL: 500 µL of stock standard solution (1.00 mg/mL) in 10 mL (intermediate solution).
500 pg/µL: 200 µL of intermediate solution (50 ng/µL) in 20 mL.
300 pg/µL: 150 µL of intermediate solution (50 ng/µL) in 25 mL.
250 pg/µL: 100 µL of intermediate solution (50 ng/µL) in 20 mL.
100 pg/µL: 50 µL of intermediate solution (50 ng/µL) in 25 mL.
50 pg/µL: 25 µL of intermediate solution (50 ng/µL) in 25 mL.
25 pg/µL: 1000 µL of chromatographic solution (500 pg/µL) in 20 mL.
Store refrigerated, stable for at least one month.

4.2.5 Source, Storage and Characterization of Samples

Drinking water: Tap water from the local drinking water supply collected at PTRL Europe, Helmholtzstr. 22, D-89081 Ulm, Germany. See Appendix A1 for representative analysis data provided by the City Water Supplier (column Uni Ulm).
Ground water: Obtained from the well "Fassung 4" (Donauried near D-89129 Langenau, Germany) of the State Water Supplier "Landeswasserversorgung Stuttgart". See Appendix A1 for representative analysis data provided by the supplier.
Surface water: Danube water (sampling point Donau 7 of the State Water Supplier "Landeswasserversorgung Stuttgart" sampled near D-89340 Leipheim, Germany). See Appendix A1 for representative analysis data provided by the supplier.

Drinking water was sampled from the tap as needed. Ground and surface water were sampled once and stored refrigerated in brown glass bottles.

4.2.6 Solid Phase Extraction (SPE) Method

4.2.6.1 Preparation of Samples

Decant ground and surface water for reduction of particles that may clog the frit of the SPE cartridge.

Measure water sample into beaker or bottle: 1 L of drinking water or ground water and 0.2 L of surface water.

4.2.6.2 Sample Fortification Procedure

Fortify untreated water samples with fortification standard (250 ng famoxadone per mL) as exemplified below:

Drinking water and ground water (1 L):	LOQ:	200 µL	=>	0.050 µg/L famoxadone.
	5 * LOQ:	1000 µL	=>	0.25 µg/L famoxadone.
Surface water (0.2 L):	LOQ:	200 µL	=>	0.25 µg/L famoxadone.
	5 * LOQ:	1000 µL	=>	1.25 µg/L famoxadone.

Different volumes may be chosen for modified sample volumes.

4.2.6.3 Analyte Extraction Procedure

1. Place SPE cartridges (1 g Varian Bond Elute C_{18}) on SPE station and pre-condition with 2 cartridge fillings of solvent in the following order: acetone, acetonitrile and bidistilled water.
CAUTION: CARTRIDGE MUST NOT RUN DRY AFTER ADDITION OF FIRST PORTION OF ACETONITRILE!
2. Fill cartridge with bidistilled water.
3. Connect cartridge to water suction pump and immerse the open part of the cartridge in the water sample.
CAUTION: AVOID AIR BUBBLES IN THE CARTRIDGE!
DO NOT ALLOW AIR TO BE SUCKED THROUGH THE CARTRIDGE!
DO NOT IMMERSE TUBING INTO THE WATER SAMPLE!
4. Sample water through the pre-conditioned SPE cartridge (flow rate: ≈ 10 - 15 mL/min).
5. Transfer cartridge onto SPE station and dry the cartridge with air by application of vacuum for ≈ 0.5 hours.

4.2.6.4 Analyte Elution Procedure

1. Place 10 mL vials under the cartridge in the SPE station to collect the eluate.
2. Add 2 mL acetonitrile to the cartridge.
3. Draw ≈ 1 mL solvent into the cartridge to wet the adsorbent.
4. Close stop cock and wait for 1 min.

5. Open stop cock and wait until all solvent has penetrated into the adsorbent.
6. Apply vacuum to remove remaining solvent.
7. Repeat elution twice as above with 2 mL acetonitrile and collect eluats in the 10 mL vial.

4.2.6.5 Concentration of Eluate and Adjustment of Final Volume

1. Concentrate acetonitrile extract to dryness by application of a slight stream of nitrogen (water bath temperature: ≈ 35 °C).
2. Add toluene to adjust final volume as follows:

Blank controls, LOQ samples or samples in which low residues (drinking water and ground water: ≤ 0.1 µg/L, surface water: ≤ 0.5 µg/L) are expected: 0.2 mL.

5 * LOQ samples or samples in which high residues (drinking water and ground water: > 0.1 µg/L, surface water: > 0.5 µg/L) are expected: 1.0 mL.

3. Redissolve analyte by ultra sonication (≈ 1 min).
4. GC/ECD analysis on a DB1 capillary column with split / splitless injection.

It is recommended to analyze final extracts as soon as possible. Final extracts are stable for at least 3 days at room temperature. If storage is necessary store refrigerated (stability > 5 days). Dilution or concentration of final extract may become necessary if the concentration of the sample extract exceeds the established calibration range.

4.2.7 Liquid / Liquid Extraction Method

4.2.7.1 Preparation of Samples

Decant ground and surface water for reduction of particles that may cause problems in phase separation.

Measure 0.5 L of water sample into beaker.

4.2.7.2 Sample Fortification Procedure

Fortify untreated water samples with fortification standard (250 ng famoxadone per mL) as exemplified below:

Drinking water and ground water (0.5 L):	LOQ:	100 µL	=>	0.050 µg/L famoxadone.
	5 * LOQ:	500 µL	=>	0.25 µg/L famoxadone.
Surface water (0.5 L):	LOQ:	500 µL	=>	0.25 µg/L famoxadone.
	5 * LOQ:	2500 µL	=>	1.25 µg/L famoxadone.

Different volumes may be chosen for modified sample volumes.

4.2.7.3 Analyte Extraction Procedure

1. Add 50 mL dichloromethane to water sample.
2. Extract with Ultra Turrax homogenizer at ≈ 13500 rpm for 3 min.

3. Transfer extraction mixture into separatory funnel (e.g. 500 mL).
4. Collect lower dichloromethane phase in a 500 ml erlenmeyer flask with 10 g of sodium sulfate anhydrous (for drying of extract).
5. Transfer upper aqueous phase back into beaker.
6. Repeat extraction twice with 50 mL portions of dichloromethane and combine all dichloromethane phases in the erlenmeyer flask.
7. Wait at least 15 min with occasional shaking for drying of combined dichloromethane extracts.

4.2.7.4 Concentration of Extract and Adjustment of Final Volume

1. Decant dichloromethane extract into 500 mL round bottomed flask.
2. Wash sodium sulfate twice with 10 mL dichloromethane and add rinses to 500 mL round bottomed flask.
3. Concentrate extract to < 5 mL (water bath temperature: ≈ 35 °C).
4. Transfer concentrate into 10 to 15 mL centrifuge vial.
5. Rinse round bottomed flask with 2 * ≈ 2 mL dichloromethane and add rinsates to centrifuge vial.
6. Concentrate extract to dryness (water bath temperature: ≈ 35 °C).
7. Add toluene to adjust final volume as follows:

Drinking water and ground water:

Blank controls, LOQ samples or samples in which unknown residues are expected: 0.2 mL.
5 * LOQ samples or samples in which high residues (> 0.2 µg/L) are expected: 0.5 mL.

Surface water:

Blank controls, LOQ samples or samples in which unknown residues are expected: 0.5 mL.
5 * LOQ samples or samples in which high residues (> 0.5 µg/L) are expected: 2.5 mL.

8. Redissolve analyte by ultra sonication (≈ 1 min).
9. GC/ECD analysis on a DB1 capillary column with split / splitless injection.

It is recommended to analyze final extracts as soon as possible. Final extracts are stable for at least 3 days at room temperature. If storage is necessary store refrigerated (stability > 5 days). Dilution or concentration of final extract may become necessary if the concentration of the sample extract exceeds the established calibration range.

4.3 Instrumentation

4.3.1 Description

See section 3.5 "GC/ECD System and Evaluation" for detailed description of the gas chromatographic system used in this study.
Use a capillary GC instrument with split/splitless injector and electron capture detector (GC/ECD).

The confirmatory method uses a capillary GC/MS ion trap system with temperature programmable SPI injector. For detailed description of GC/MS system see Appendix A2

4.3.2 Operating Conditions

Establish chromatographic conditions for GC/ECD analysis such as (exemplified):

Injection:	1 µL split / splitless injection with autosampler. Splitless time: 0.5 min. Injector temperature: 290 °C.
GC capillary column:	J&W Scientific DB 1 (15 m, 0.32 mm i.d., 0.25 µm film).
Carrier gas:	Helium at 10 psi.
Oven program:	90 °C, 0.5 min; 30 °C/min to 220 °C; 5 °C/min to 240 °C; 30 °C/min to 300 °C; 300 °C, 1.0 min.
Retention time:	Famoxadone (DPX-JE874): ≈ 8.6 - 8.9 min (≈ 239 °C).
Detection:	Electron capture detector (ECD), Argon / CH_4 (90:10) make-up gas. Detector temperature 300 °C.

4.3.3 Calibration Procedure

Prepare at least four chromatographic standards for calibration, intended to bracket the levels of famoxadone (DPX-JE874) in the sample extracts.

REMARK:
It is advised to run chromatographic standards first to demonstrate reproducibility of injection, separation and to establish the calibration curve.

Once the calibration curve is established, chromatographic standards should be interspersed with sample extracts and evaluated as verifications. In the case that verifications indicate increased or decreased response, use verifications to establish new calibration curve.

With the GC/ECD system used in this study linear calibration functions from 25 to 500 pg/µL resulted in acceptable regression coefficients (Figures 4 and 5) and recovery results.

4.3.4 Sample Analysis

Before analyzing blanks or low level extracts, verify contamination or memory effect of syringe and injector by solvent injections. See Figures 6 to 11 for chromatograms of sample extracts.

4.4 Calculations

4.4.1 Methods

The famoxadone (DPX-JE874) signals are integrated to give peak areas which are evaluated with the correct calibration function (e.g. linear) to yield a final extract concentration C_{end} reported in pg/µL (calculated by chromatographic software).

Concentration C_{end} (pg/µL = ng/mL; calculate mean value if more than one injection was performed; usually fortified samples were injected twice) is multiplied with the final volume V_{end} (mL) to obtain the total amount of famoxadone (DPX-JE874) in the water sample (µg).

The total amount is divided by the total water sample volume Vol (L) to obtain the concentration of famoxadone in water found (C_{water} in µg/L).

$$C_{water}\ (found) = \frac{(C_{end}) * V_{end}}{1000 * Vol\ (sample)} \quad [\mu g/L]$$

Recoveries for fortified water samples are calculated as follows:

$$Recovery = \frac{C_{water}\ (found)}{C_{water}\ (spiked)} * 100 \quad [\%]$$

Calculations in the Excel evaluation tables were performed with full precision and results were rounded to 2 significant digits.

4.4.2 Example

Drinking water sample ID P233-1159 analyzed with solid phase extraction, fortification level 0.050 µg/mL:

GC run P233a20 and 21 (Figure 6, see Table I for results):

$$C_{water} = \frac{(226\ pg/\mu L + 221\ pg/\mu L) / 2 * 0.2\ mL}{1000 * 1.0\ L} = 0.045\ \mu g/L.$$

The recovery for the water sample fortified with 0.050 µg/L famoxadone (DPX-JE874) and analyzed with solid phase extraction is calculated as follows:

$$\text{Recovery} = \frac{0.045\ \mu g/L}{0.050\ \mu g/L} * 100\ \% = 90\ \%$$

Calculations in the Excel evaluation tables were performed with full precision and resulted in a recovery of 89 % (rounded to 2 significant digits).

5.0 RESULTS AND DISCUSSION

5.1 Method Validation Results

5.1.1 GC/ECD Detector Response

Examples of calibration chromatograms and of a calibration curve are given in Figures 4 and 5 together with the calibration function and correlation coefficient R^2. The linear dynamic range is established from 25 to 500 pg/µL with 1 µL injections.

5.1.2 Controls

Examples for injections of controls, i.e. drinking water, ground water and surface water sample extracts from solid phase extraction and from liquid / liquid extraction are given in Figures 6 to 11. No signals for famoxadone (DPX-JE874) were detected in any of the untreated control samples. (Exception: One blank ground water sample was contaminated with interfering matrix from a different study during the liquid / liquid extraction.)

5.1.3 Recoveries (Accuracy and Precision)

Tables I to IV summarize the recoveries for fortified samples of drinking water, ground water and surface water samples with the two different extraction methods: solid phase extraction and liquid / liquid extraction with dichloromethane. For representative chromatograms for the three types of water and two extraction methods see Figures 6 to 11.

Solid phase extraction (SPE):

Solid phase extraction of fortified drinking water samples (LOQ: 0.050 µg/L, 5 * LOQ: 0.25 µg/L) gave a total average recovery of 93 ± 8 % (n = 6, relative standard deviation).

Solid phase extraction of fortified ground water samples (LOQ: 0.050 µg/L, 5 * LOQ: 0.25 µg/L) gave a total average recovery of 85 ± 6 % (n = 6, relative standard deviation).

Solid phase extraction of fortified surface water samples (LOQ: 0.25 µg/L, 5 * LOQ: 1.25 µg/L) gave a total average recovery of 86 ± 18 % (n = 6, relative standard deviation).

Liquid / liquid extraction:

Liquid / liquid extraction of fortified drinking water samples (LOQ: 0.050 µg/L, 5 * LOQ: 0.25 µg/L) gave a total average recovery of 95 ± 9 % (n = 6, relative standard deviation).

Liquid / liquid extraction of fortified ground water samples (LOQ: 0.050 µg/L, 5 * LOQ: 0.25 µg/L) gave a total average recovery of 94 ± 12 % (n = 6, relative standard deviation).

Liquid / liquid extraction of fortified surface water samples (LOQ: 0.25 µg/L, 5 * LOQ: 1.25 µg/L) gave a total average recovery of 98 ± 12 % (n = 6, relative standard deviation).

The recovery results are considered acceptable according to the following criteria stated in the study protocol:

- All individual recoveries were in the range of 70 and 110 %.
- Average percent recoveries per fortification level fell within the range of 70 to 110 % (with a relative standard deviation of ≤ 20 %).

5.1.4 Limit of Quantitation (LOQ) and Detection (LOD)

The limit of determination / quantitation (LOQ) and the upper fortification level (5*LOQ) validated for famoxadone (DPX-JE874) in drinking water, ground water and surface water are identical for both extraction methods (solid phase extraction and liquid / liquid extraction).

Drinking water and ground water:	LOQ:	0.050 µg/L.	5 * LOQ:	0.25 µg/L.
Surface water:	LOQ:	0.25 µg/L.	5 * LOQ:	1.25 µg/L.

The limits of detection (LOD) were not investigated specifically. However as 25 pg/µL were detectable in a calibration standard solution it is assumed that signals of 40 pg/µL can be detected in water sample extracts (however, they may need confirmation by GC/MS). This results in the following estimated LODs:

Drinking water and ground water:	0.008 µg/L (solid phase extraction).
	0.016 µg/L (liquid / liquid extraction).
Surface water:	0.040 µg/L (solid phase and liquid / liquid extraction).

LOD may vary with quality of water, and performance of GC/ECD system. Doubtful detections need to be confirmed by GC/MS.

5.2 Timing

5.2.1 Solid Phase Extraction Method

The number of water samples analyzed in one set of samples depends on the availability and

size of SPE stations. Recommended number of samples to be analyzed by one technician during one working day: 10 to 20.

The following time schedule is based on the assumption that all samples are treated parallel:
Preparation of 10 - 20 water samples: ≈ 1 h.
Pre-conditioning of SPE cartridges: ≈ 0.5 h.
Sampling of water samples: ≈ 1 - 2 h.
Drying of SPE cartridges: ≈ 0.5 h.
Elution of analyte from the SPE cartridges: ≈ 1 h.
Concentration to dryness and adjusting to final volume: 3 hours.
GC analysis requires approx. 20 min between injections: over night.
Time for re-integration and evaluation: ≈ 3 h.

Provided with sufficient equipment, approx. 30 - 60 samples could be analyzed within 24 h or 3 shifts.

5.2.2 Liquid / Liquid Extraction Method

The amount of water samples analyzed in one set of samples depends on the availability of Ultra Turrax homogenizers and rotary evaporators. Recommended number of samples to be analyzed by one technician during one working day: 10 to 15.

Preparation of 10 water samples: ≈ 0.5 h.
Extraction of 10 water samples: ≈ 5 h.
Drying of dichloromethane extracts: ≈ 1 h.
Concentration to dryness and adjusting of final volume: ≈ 0.5 h per sample
GC analysis requires approx. 20 min between injections: over night.
Time for re-integration and evaluation: ≈ 3 h.

Provided with sufficient equipment, approx. 30 samples could be analyzed within 24 h or 3 shifts.

5.3 Modifications and Special Precautions

Contaminations of glassware, solvent and Ultra Turrax have to be avoided.

Different lots of SPE cartridges have to be checked prior to use for sample analysis.

DO NOT SUBSTITUTE ACETONITRILE FOR ELUTING OF ANALYTE FROM SPE CARTRIDGE. WHEN METHANOL OR ACETONE WERE USED INSTEAD OF ACETONITRILE, SIGNIFICANT LOSSES OF ANALYTE DURING SAMPLE CONCENTRATION WERE OBSERVED.

5.4 Method Ruggedness

5.4.1 Stability

Stability of standard solutions and final extracts are stated in section 4.0 Methods.

5.4.2 Specificity / Potential Interference

Interference from glassware, reagents and matrix:

Interference from glassware and reagents were not observed. However care must be taken to avoid contamination of glassware, reagents and extraction equipment with the analyte or with interfering matrix from different studies.

Selectivity problems in GC/ECD detection may arise from matrix interferences for lower famoxadone concentrations as those validated for the different water types (0.050 µg/L for drinking water and ground water and 0.25 µg/L for surface water).

Interference from other pesticides:

Interferences from other co-eluting pesticides were not investigated.

5.4.3 Confirmatory Method

The two extraction methods validated in this study may be used as confirmatory method for each other.

Additionally a full scan GC/MS method and a GC/MS/MS method were demonstrated as confirmatory methods for the GC/ECD determination. These methods can be used to verify residue results in unknown samples. Representative chromatograms of chromatographic standard solutions, selected samples and a description of the GC/MS and GC/MS/MS methods are given in Appendix A2.

The mass spectrum of famoxadone lacks the M^+ peak for the molecular mass of 374 m/e. However, the distinct mass peak at 330 m/e indicates decarboxylation of the compound (374 m/e - 330 m/e = 44 m/e equivalent to loss of CO_2) which may occur either in the hot injector or in the mass spectrometer.

5.4.4 Second (Independent) Laboratory Tryout

No formal Second Laboratory Tryout was performed.

6.0 CONCLUSIONS

Two methods (solid phase extraction and liquid / liquid extraction) for the determination of famoxadone (DPX-JE874) in three different types of water (drinking water, ground water and surface water) were developed and validated.

Limits of determination / quantitation for both methods (solid phase extraction and liquid / liquid extraction) were determined at 0.050 µg/L for drinking water and ground water and at 0.25 µg/L for surface water.

The recovery rates achieved with the solid phase extraction method were 90 % ± 5 % (n = 3) for LOQ drinking water samples, 81 % ± 6 % (n = 3) for LOQ ground water samples and 72 % ± 3 % (n = 3) for LOQ surface water samples.

The recovery rates achieved with the liquid / liquid extraction method were 88 % ± 6 % (n = 3) for LOQ drinking water samples, 90 % ± 14 % (n = 3) for LOQ ground water samples and 90 % ± 6 % (n = 3) for LOQ surface water samples.

The applicability of both methods at a range of 0.050 µg/L to 0.25 µg/L famoxadone in drinking water and ground water and of 0.25 to 1.25 µg/L famoxadone in surface water was demonstrated.

Blank control samples of the three types of water were analyzed with both methods. No significant signal (above 30 % of LOQ) for famoxadone (DPX-JE874) was detected in any of the control samples.

Both methods described in this report can be used as confirmatory methods for each other. GC/MS or GC/MS/MS is proposed as confirmatory detection method.

Interferences from other active substances were not examined.

7.0 RETENTION OF RECORDS

The raw data of this study, the protocol and the first original of the final report are stored in the GLP archive located at:

PTRL Europe GmbH
Labor für Umwelt- und Pestizidchemie
Helmholtzstrasse 22 Science Park
D-89081 Ulm, Germany

The second original of the final report will be sent to the Sponsor. Records can be transferred to the Sponsor upon written request. No specimens of the water samples are retained.

PTRL Europe will also archive the quality assurance records and a sample of the reference substance according to GPL regulations and SOPs.

8.0 REFERENCES

EEC Directive 91/414/EEC, Annex II 4.2.3.

DIN V 38407-6, April 1995, DEV – 33. Lieferung 1995: German standard methods for the examination of water, waste water and sludge - Jointly determinable substances (group F) - Part 6: Determination of selected organic nitrogen and phosphorous compounds by gas chromatography after solid-liquid-extraction (F6).

DuPont Report No. AMR 4070-96: "A Method for the Analysis of Water for Residues of Flusilazole and DPX-JE874 Resulting From the Use of the Formulated Product DPX-MC444-17".

DuPont Report No. AMR 4212-96 (PTRL Study P 220 G): "Analytical (Multi-Residue Enforcement) Method for the Determination of Famoxadone and Flusilazole in Sugar Beet".

Table I

Recovery results obtained for drinking water: Solid phase extraction method.

P 233 G Famoxadone in Water
Drinking Water (SPE Method)

Sample		Spiked		Final	GC			Famoxadone Found			
ID	Vol.	Amount	C_{water}	V_{end}	Runs	C_{end}	C_{end}	Mean	Amount	C_{water}	Recovery
P233-	L	µg	µg/L	mL	P233a	pg/µL	pg/µL	pg/µL	µg	µg/L	
Blank Control											
1158	1,0	0,000	0,000	0,2	17	nd	na	nd	nd	nd	na
LOQ											
1159	1,0	0,050	0.050	0.2	20/21	226	221	223	0,045	0,045	89%
1160	1,0	0,050	0.050	0,2	22/23	214	219	216	0,043	0,043	86%
1161	1,0	0,050	0.050	0.2	24/25	257	217	237	0,047	0,047	95%
									Average	0,045	90%
									abs. SD	0,002	4%
									rel. SD	5%	5%
5*LOQ											
1162	1,0	0,25	0,25	1,0	27/28	218	208	213	0,213	0,21	85%
1163	1,0	0.25	0,25	1.0	29/30	253	251	252	0,252	0,25	101%
1164	1.0	0.25	0.25	1.0	31/32	249	262	255	0,255	0,26	102%
									Average	0,24	96%
									abs. SD	0,02	9%
									rel. SD	10%	10%
									Total Average		**93%**
									Total abs. SD		**7%**
									Total rel. SD		**8%**

SD. Standard deviation (absolute / relative)

nd: not detected. Signal ≤ 40 pg/µL C_{end} equivalent to ≤ 0.008 µg/L C_{water}.

na: not analysed or applicable

Calculations are performed with full precision, but shown rounded.

Discrepancies may arise when recalculated with a calculator.

Table II

Recovery results obtained for ground water: Solid phase extraction method.

P 233 G Famoxadone in Water
Ground Water (SPE Method)

Sample		Spiked		Final	GC			Famoxadone Found			
ID	Vol.	Amount	C_{water}	V_{end}	Runs	C_{end}	C_{end}	Mean	Amount	C_{water}	Recovery
P233-	L	µg	µg/L	mL	P233a	pg/µL	pg/µL	pg/µL	µg	µg/L	
Cartridge Blank Control											
1172	na	0,000	na	0,2	37	nd	na	nd	nd	nd	na
Blank Control											
1165	1.0	0,000	0.000	0.2	38	nd	na	nd	nd	nd	na
LOQ											
1166	1,0	0,050	0.050	0.2	41/42	206	199	203	0,041	0,041	81%
1167	1,0	0,050	0,050	0,2	43/44	207	223	215	0,043	0,043	86%
1168	1,0	0,050	0.050	0,2	45/46	203	180	192	0,038	0,038	77%
									Average	0,041	81%
									abs. SD	0,002	5%
									rel. SD	6%	6%
5*LOQ											
1169	1,0	0,25	0,25	1,0	48/49	232	222	227	0,227	0,23	91%
1170	1,0	0.25	0,25	1,0	50/51	217	214	215	0,215	0,22	86%
1171	1,0	0,25	0,25	1.0	52/53	229	219	224	0,224	0,22	90%
									Average	0,22	89%
									abs. SD	0,01	2%
									rel. SD	3%	3%
									Total Average		**85%**
									Total abs. SD		**5%**
									Total rel. SD		**6%**

SD: Standard deviation (absolute / relative)

nd: not detected. Signal ≤ 40 pg/µL C_{end} equivalent to ≤ 0.008 µg/L C_{water}

na: not analysed or applicable

Calculations are performed with full precision. but shown rounded.

Discrepancies may arise when recalculated with a calculator.

Table III

Recovery results obtained for surface water: Solid phase extraction method.

P 233 G Famoxadone in Water
Surface Water (SPE Method)

Sample		Spiked		Final	GC			Famoxadone Found			
ID	Vol.	Amount	C_{water}	V_{end}	Runs	C_{end}	C_{end}	Mean	Amount	C_{water}	Recovery
P233-	L	µg	µg/L	mL	P233a	pg/µL	pg/µL	pg/µL	µg	µg/L	
Blank Control											
1174	0,2	0,000	0,000	0,2	62	nd	na	nd	nd	nd	na
LOQ											
1175	0,2	0,050	0,25	0,2	65/66	171	180	176	0,035	0,18	70%
1176	0,2	0,050	0,25	0,2	67/68	178	191	185	0,037	0,18	74%
1177	0,2	0,050	0,25	0,2	69/70	177	190	184	0,037	0,18	73%
									Average	0,18	72%
									abs. SD	0,005	2%
									rel. SD	3%	3%
5*LOQ											
1178	0,2	0,25	1,25	1,0	72/73	246	250	248	0,248	1,24	99%
1179	0,2	0,25	1,25	1,0	74/75	262	256	259	0,259	1,29	103%
1180	0,2	0,25	1,25	1,0	76/77	248	238	243	0,243	1,21	97%
									Average	1,25	100%
									abs. SD	0,04	3%
									rel. SD	3%	3%
									Total Average		**86%**
									Total abs. SD		**15%**
									Total rel. SD		**18%**

SD: Standard deviation (absolute / relative)

nd: not detected. Signal ≤ 40 pg/µL C_{end} equivalent to ≤ 0.040 µg/L C_{water}.

na: not analysed or applicable

Calculations are performed with full precision, but shown rounded.

Discrepancies may arise when recalculated with a calculator

Table IV

Recovery results obtained for drinking water: Liquid / liquid extraction method.

P 233 G Famoxadone in Water
Drinking Water (Liquid / Liquid Extraction with Dichloromethane)

Sample		Spiked		Final	GC			Famoxadone Found			
ID	Vol.	Amount	C_{water}	V_{end}	Runs	C_{end}	C_{end}	Mean	Amount	C_{water}	Recovery
P233-	L	µg	µg/L	mL	P233a	pg/µL	pg/µL	pg/µL	µg	µg/L	
Reagent Blank											
1188	0,5	0,000	0,000	0,2	102	nd	na	nd	nd	nd	na
Blank Control											
1181	0,5	0,000	0.000	0,2	103	nd	na	nd	nd	nd	na
LOQ											
1182	0,5	0,025	0,050	0,2	95/96	119	117	118	0,024	0,047	94%
1183	0,5	0,025	0,050	0,2	90/91	113	103	108	0,022	0,043	87%
1184	0,5	0,025	0,050	0,2	92/93	102	109	105	0,021	0,042	84%
									Average	0,044	88%
									abs. SD	0,003	5%
									rel. SD	6%	6%
5*LOQ											
1185	0.5	0,125	0,25	0,5	107/108	252	249	250	0,125	0,25	100%
1186	0,5	0,125	0,25	0.5	109/110	259	250	254	0,127	0,25	102%
1187	0.5	0.125	0,25	0.5	111/112	266	252	259	0,129	0,26	104%
									Average	0,25	102%
									abs. SD	0,004	2%
									rel. SD	2%	2%
									Total Average		**95%**
									Total abs. SD		**8%**
									Total rel. SD		**9%**

SD. Standard deviation (absolute / relative)

nd: not detected. Signal ≤ 40 pg/µL C_{end} equivalent to ≤ 0.016 µg/L C_{water}.

na: not analysed or applicable

Calculations are performed with full precision, but shown rounded
Discrepancies may arise when recalculated with a calculator.

Table V

Recovery results obtained for ground water: Liquid / liquid extraction method.

P 233 G Famoxadone in Water
Ground Water (Liquid / Liquid Extraction with Dichloromethane)

Sample ID P233-	Vol. L	Spiked Amount µg	Spiked C_{water} µg/L	Final V_{end} mL	GC Runs P233a	Famoxadone Found C_{end} pg/µL	C_{end} pg/µL	Mean pg/µL	Amount µg	C_{water} µg/L	Recovery
Blank Control											
1189[a]	0,5	0,000	0,000	0,2	122	na	na	na	na	na	na
1203	0,5	0,000	0,000	0,2	195/196	nd	nd	nd	nd	nd	na
LOQ											
1190[a]	0.5	0,025	0,050	0,2	126/127	na	na	na	na	na	na
1191	0,5	0,025	0.050	0,2	128/129	128	134	131	0,026	0,052	104%
1192	0,5	0,025	0,050	0,2	130/131	96	106	101	0,020	0,040	81%
1204	0,5	0,025	0,050	0,2	199/200	114	100	107	0,021	0,043	85%
									Average	0,045	90%
									abs. SD	0,006	13%
									rel. SD	14%	14%
5*LOQ											
1193	0,5	0,125	0,25	0,5	134/135	256	243	249	0,125	0,25	100%
1194	0,5	0,125	0.25	0,5	136/137	270	267	269	0,134	0,27	107%
1195	0.5	0,125	0,25	0.5	138/139	223	208	215	0,108	0,22	86%
									Average	0,24	98%
									abs. SD	0,027	11%
									rel. SD	11%	11%
										Total Average	**94%**
										Total abs. SD	**11%**
										Total rel. SD	**12%**

SD: Standard deviation (absolute / relative)

nd: not detected. Signal ≤ 40 pg/µL C_{end} equivalent to ≤ 0.016 µg/L C_{water}.

na not analysed or applicable

[a] Contaminated with interfering matrix from a different study.

Calculations are performed with full precision, but shown rounded.

Discrepancies may arise when recalculated with a calculator.

Table VI

Recovery results obtained for surface water: Liquid / liquid extraction method.

P 233 G Famoxadone in Water
Surface Water (Liquid / Liquid Extraction with Dichloromethane)

Sample ID P233-	Vol. L	Spiked Amount µg	Spiked C_{water} µg/L	Final V_{end} mL	GC Runs P233a	C_{end} pg/µL	C_{end} pg/µL	Famoxadone Found Mean pg/µL	Amount µg	C_{water} µg/L	Recovery
Blank Control											
1196	0,5	0,000	0,000	0,5	166	nd	na	nd	nd	nd	na
LOQ											
1197	0.5	0.125	0,25	0,5	172/173	223	220	222	0,111	0,22	89%
1198	0,5	0.125	0.25	0.5	174/175	268	209	238	0,119	0,24	95%
1199	0,5	0,125	0.25	0.5	176/177	239	184	212	0,106	0,21	85%
									Average	0,22	90%
									abs. SD	0,01	5%
									rel. SD	6%	6%
5*LOQ											
1200	0.5	0,625	1.25	2,5	179/180	240	241	241	0,602	1,20	96%
1201	0.5	0,625	1,25	2,5	181/182	240	294	267	0,667	1,33	107%
1202	0,5	0,625	1.25	2,5	183/184	283	299	291	0,727	1,45	116%
									Average	1,33	106%
									abs. SD	0,125	10%
									rel. SD	9%	9%
									Total Average		**98%**
									Total abs. SD		**12%**
									Total rel. SD		**12%**

SD: Standard deviation (absolute / relative)

nd. not detected. Signal ≤ 40 pg/µL C_{end} equivalent to ≤ 0.040 µg/L C_{water}.

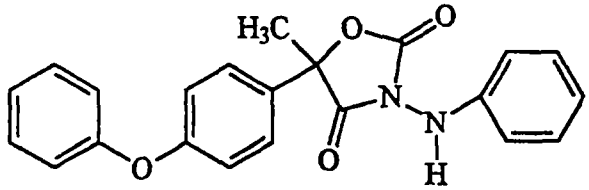
na. not analysed or applicable

Calculations are performed with full precision, but shown rounded.

Discrepancies may arise when recalculated with a calculator.

Figure 1

Chemical structure of famoxadone (DPX-JE874) and related information.



IUPAC name: 3-anilino-5-methyl-5-(4-phenoxyphenyl)-2,4-oxazolidinedione.

CA name: 5-methyl-5-(4-phenoxyphenyl)-3-(phenylamino)-2,4-oxazolidinedione.

CAS RN.: [131807-57-3].

Standard obtained from the Sponsor with the following information:

IN # JE874-92, Ref. # E72194-122.

Purity: 99.6 %.

Expiration date: January 21, 1998.

Figure 2

Schematic presentation of the solid phase extraction (SPE) method.

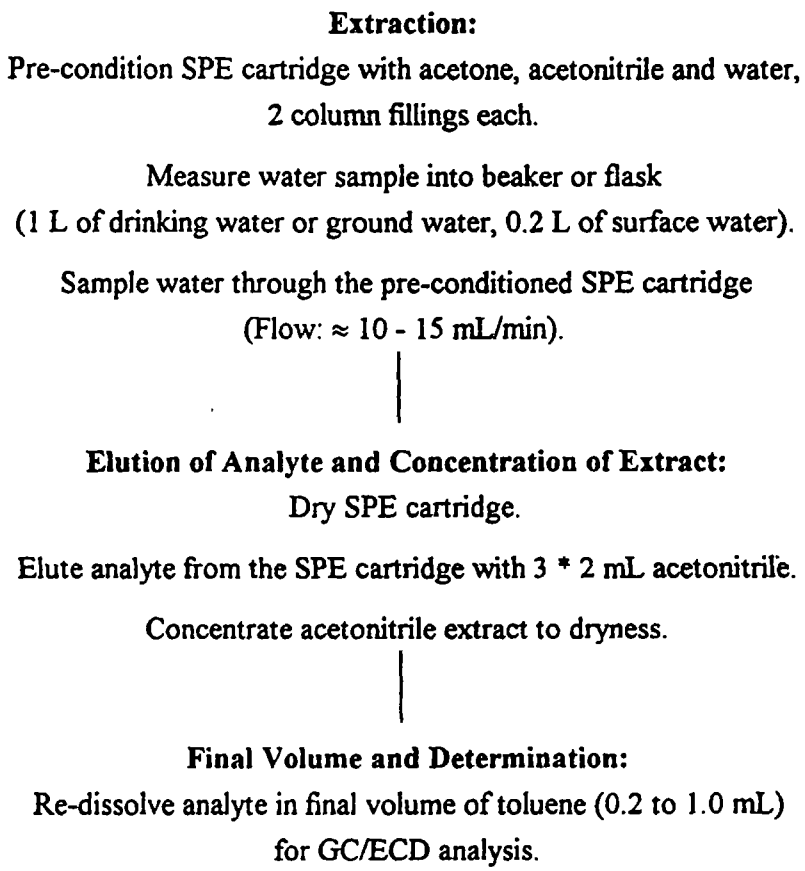


Figure 3

Schematic presentation of the liquid / liquid extraction method.

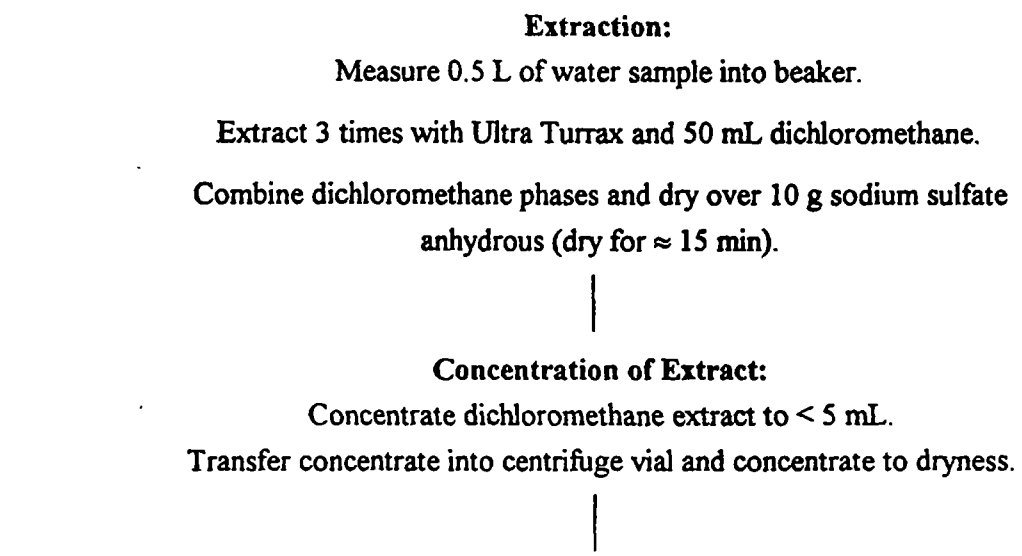


Figure 4

GC/ECD calibration chromatograms (examples).

Top:	Solution ID K233-1151	conc. 25 pg/µL	(GC Run P233a013).
Bottom:	Solution ID K233-1146	conc. 500 pg/µL	(GC Run P233a004).

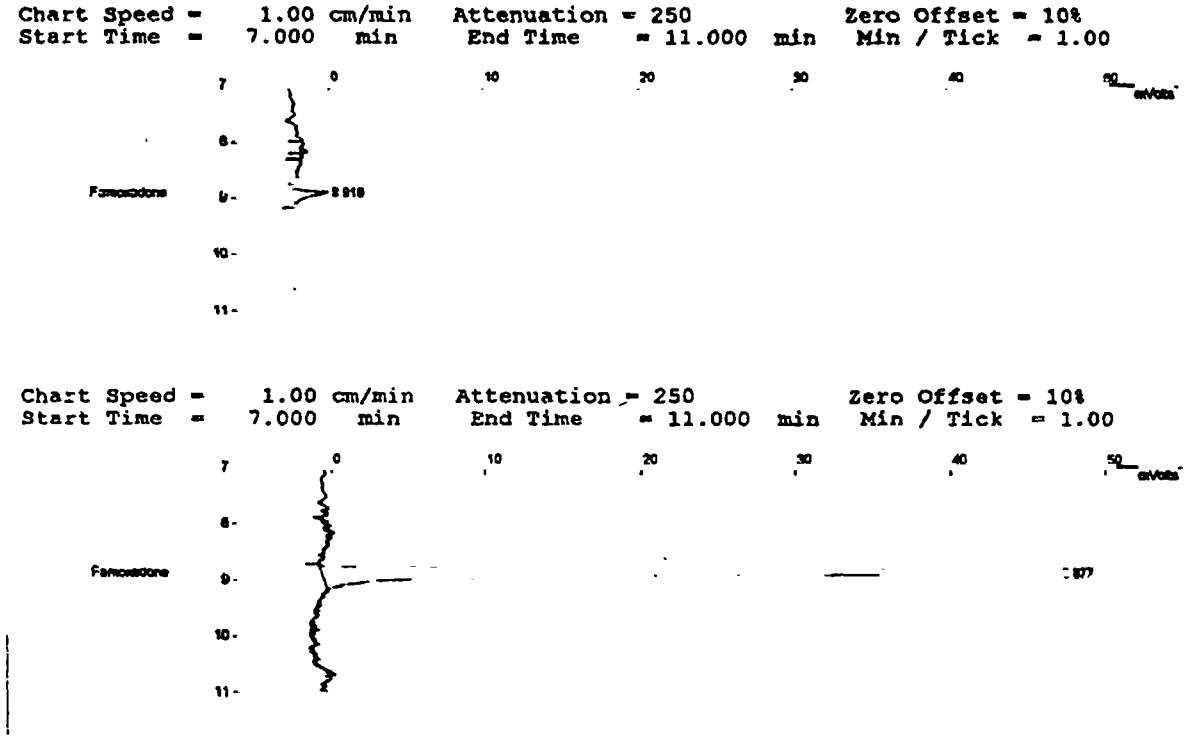


Figure 5

Representative GC/ECD calibration curve.

Peak sizes are given as peak areas.

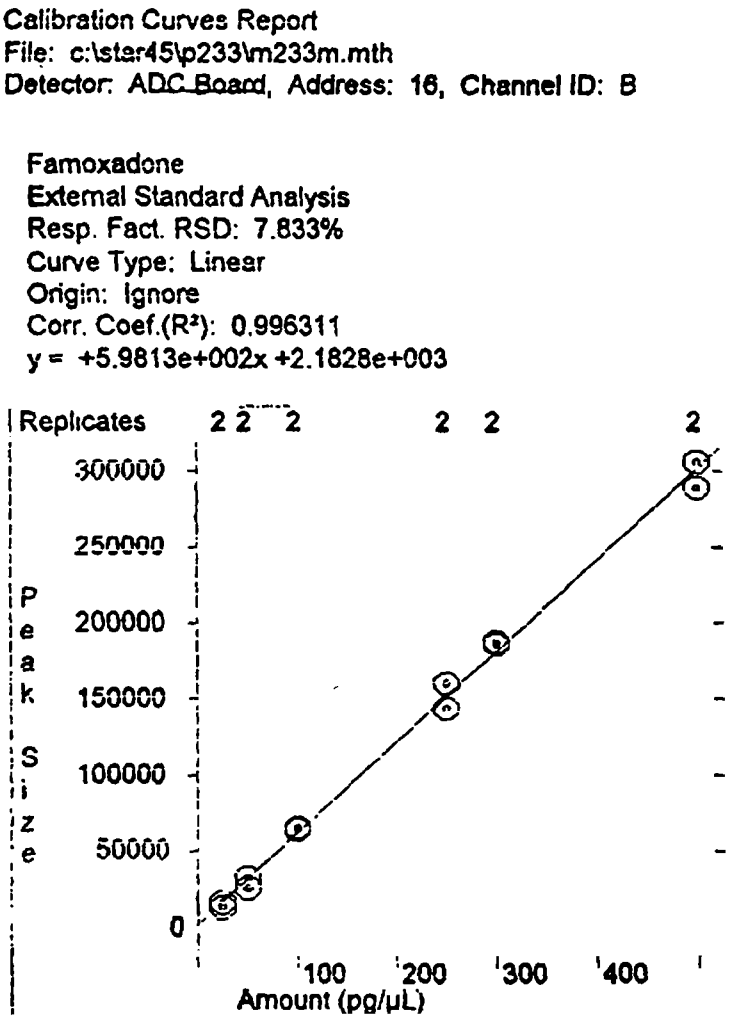


Figure 6

GC/ECD chromatograms of drinking water samples (1 L) analyzed by solid phase extraction.

Top: Blank sample (ID P233-1158, V_{end} = 0.2 mL) (GC Run P233a017)

Middle: LOQ sample fortified at 0.050 µg/L (ID P233-1159, V_{end} = 0.2 mL) (GC Run P233a021).

Bottom: 5 * LOQ sample fortified at 0.25 µg/L (ID P233-1162, V_{end} = 1.0 mL) (GC Run P233a027).

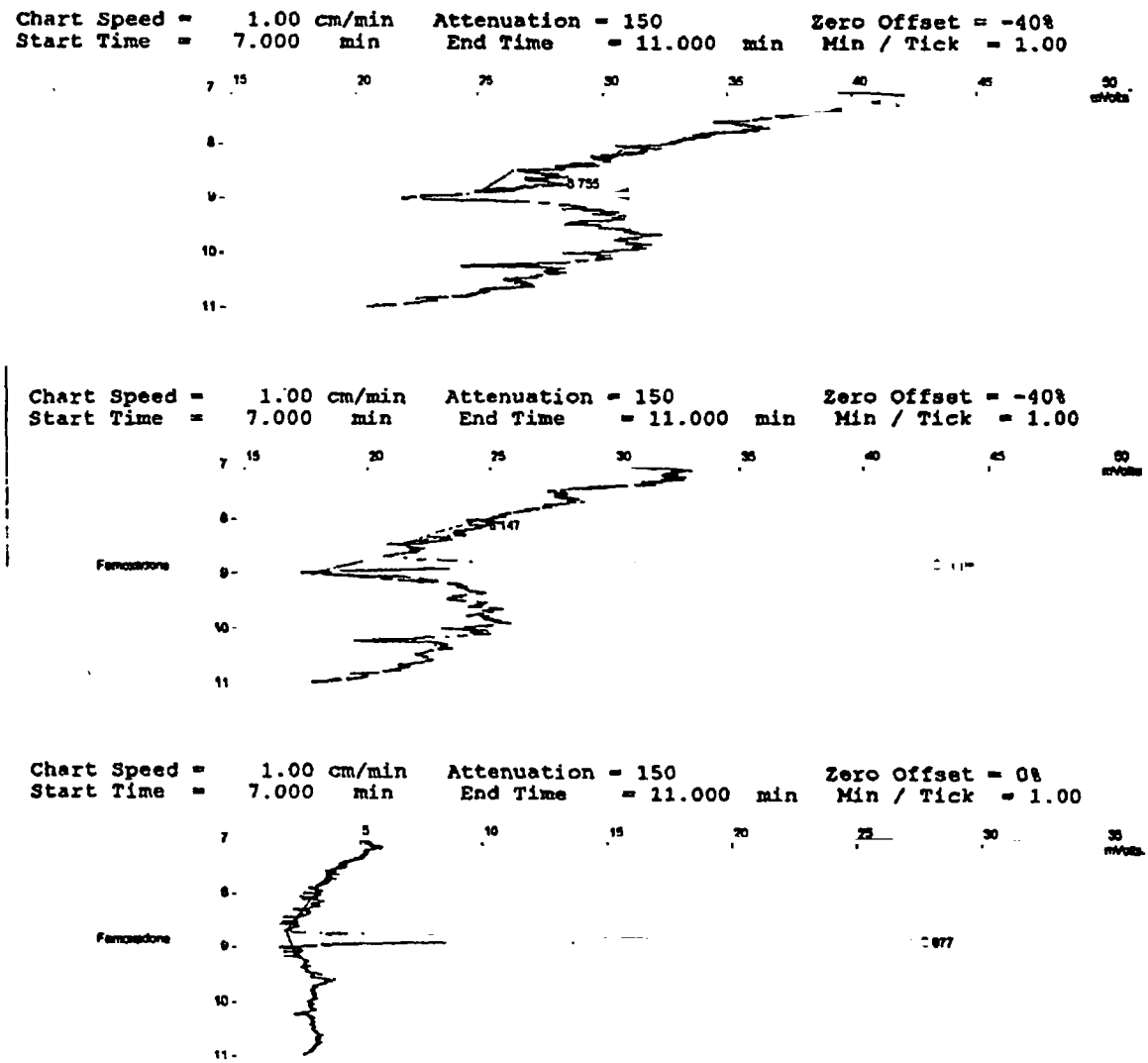


Figure 7

GC/ECD chromatograms of ground water samples (1 L) analyzed by solid phase extraction.

Top: Blank sample (ID P233-1165, V_{end} = 0.2 mL) (GC Run P233a038).

Middle: LOQ sample fortified at 0.050 µg/L (ID P233-1166, V_{end} = 0.2 mL) (GC Run P233a041).

Bottom: 5 * LOQ sample fortified at 0.25 µg/L (ID P233-1169, V_{end} = 1.0 mL) (GC Run P233a049).

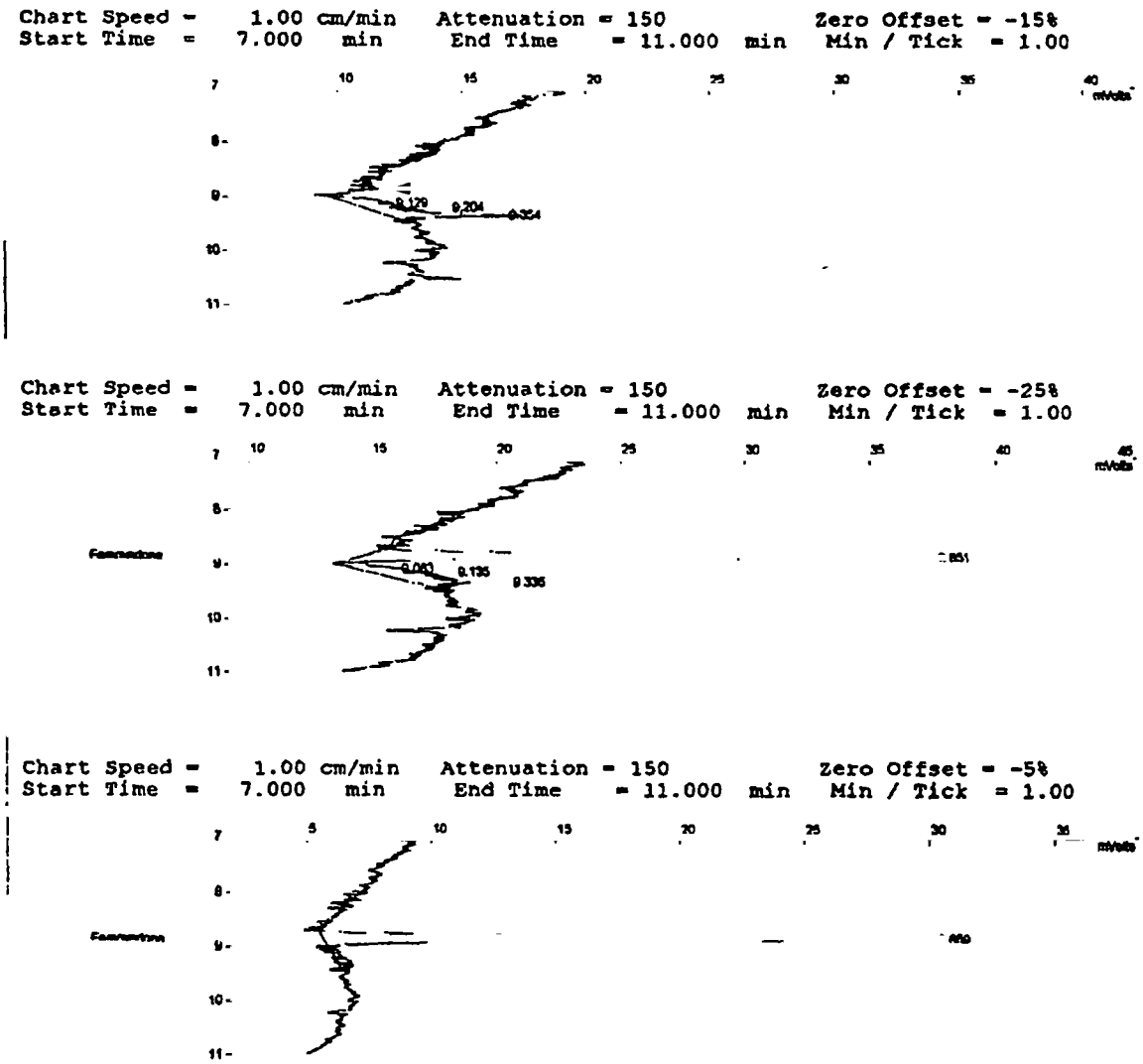


Figure 8

GC/ECD chromatograms of surface water samples (0.2 L) analyzed by solid phase extraction.

Top: Blank sample (ID P233-1174, V_{end} = 0.2 mL) (GC Run P233a062).

Middle: LOQ sample fortified at 0.25 µg/L (ID P233-1175, V_{end} = 0.2 mL) (GC Run P233a065).

Bottom: 5 * LOQ sample fortified at 1.25 µg/L (ID P233-1178, V_{end} = 1.0 mL) (GC Run P233a073).

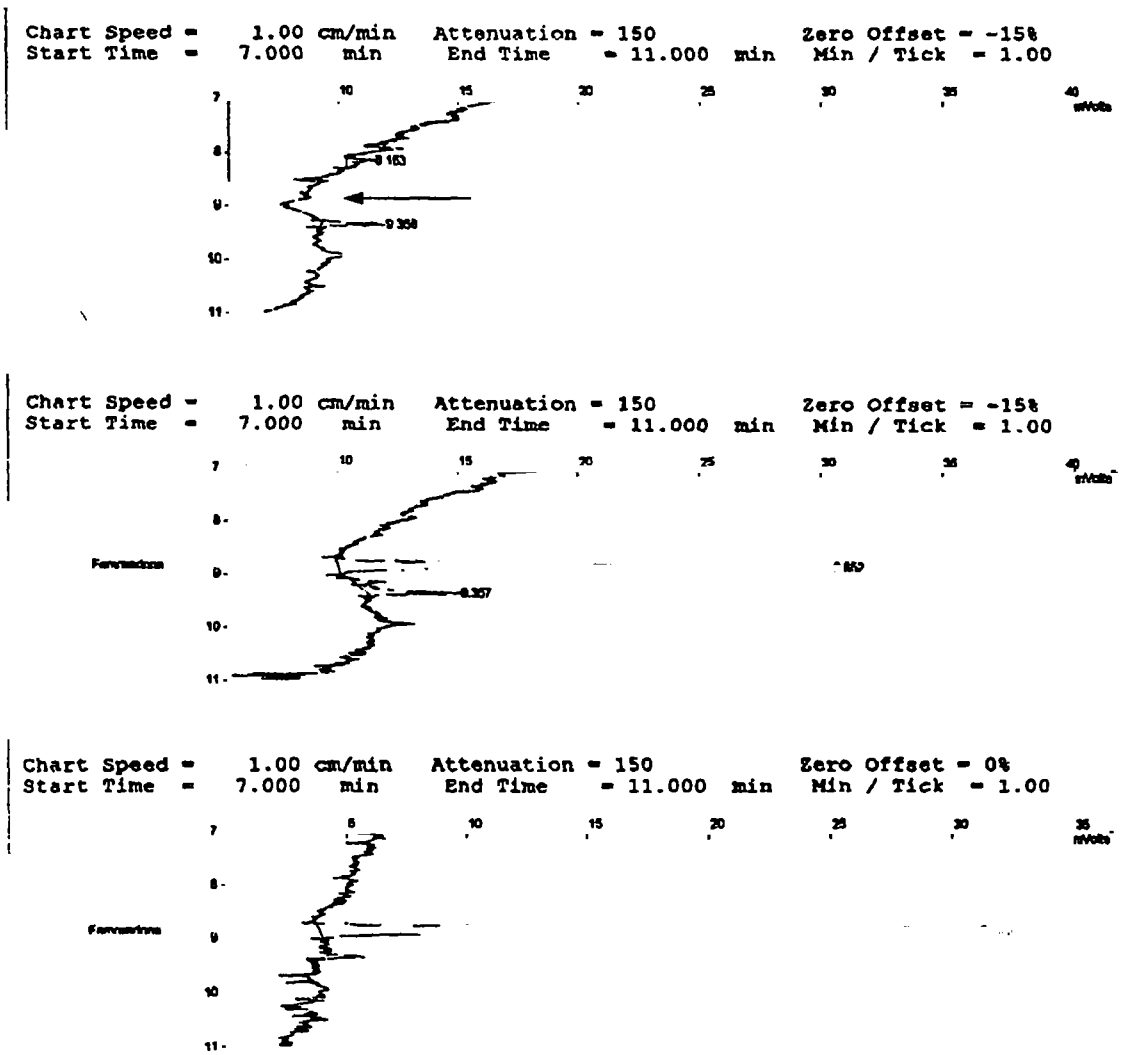


Figure 9

GC/ECD chromatograms of drinking water samples (0.5 L) analyzed by liquid / liquid extraction

Top: Blank sample (ID P233-1181, V_{end} = 0.2 mL) (GC Run P233a103).

Middle: LOQ sample fortified at 0.050 µg/L (ID P233-1182, V_{end} = 0.2 mL) (GC Run P233a095).
Signals smaller than LOQ may require confirmation by GC/MS.

Bottom: 5 * LOQ sample fortified at 0.25 µg/L (ID P233-1185, V_{end} = 0.5 mL) (GC Run P233a107).

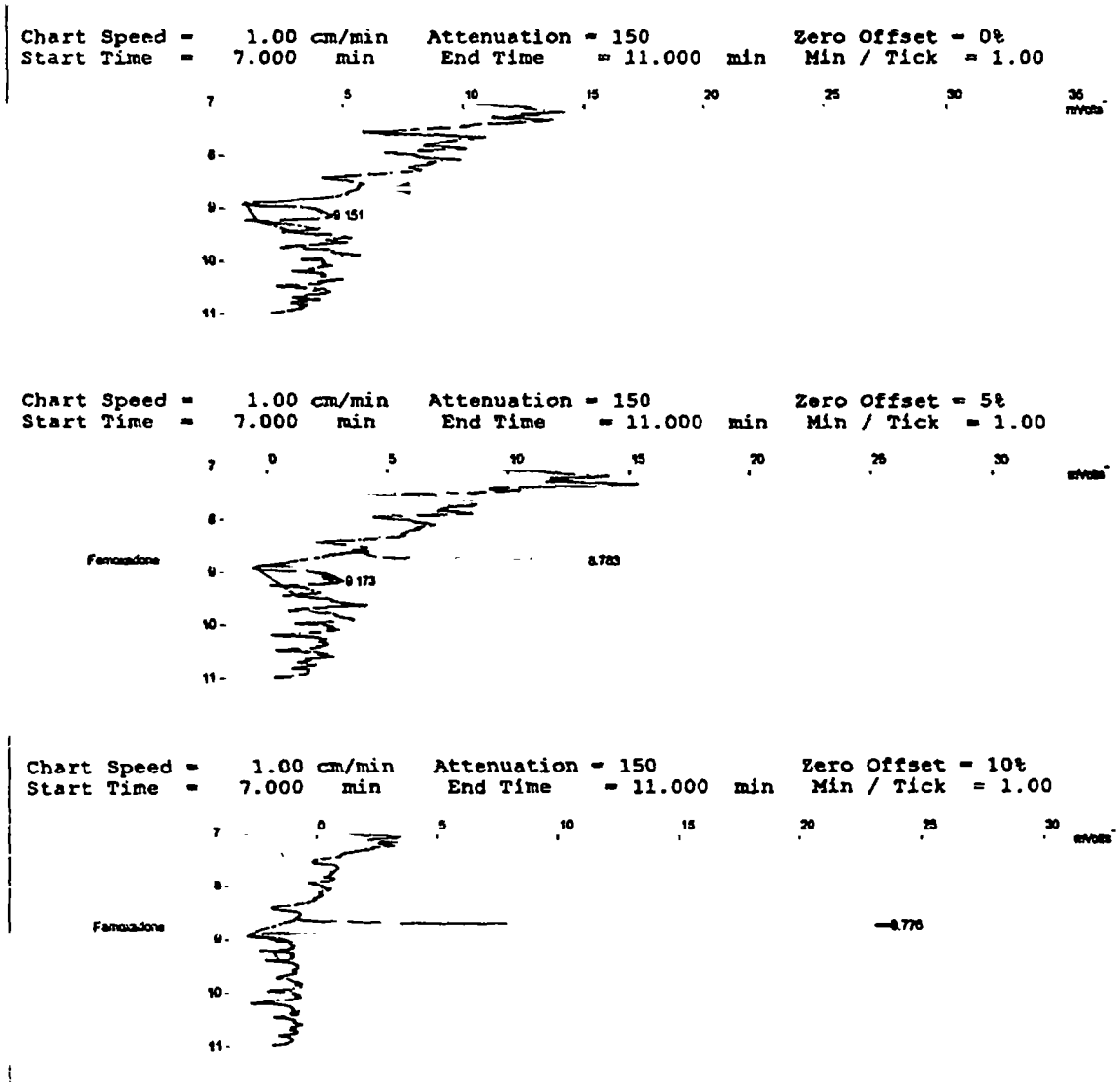


Figure 10

GC/ECD chromatograms of ground water samples (0.5 L) analyzed by liquid / liquid extraction.

Top: Blank sample (ID P233-1203, V_{end} = 0.2 mL) (GC Run P233a196).

Middle: LOQ sample fortified at 0.050 µg/L (ID P233-1204, V_{end} = 0.2 mL) (GC Run P233a199).

Bottom: 5 * LOQ sample fortified at 0.25 µg/L (ID P233-1193, V_{end} = 0.5 mL) (GC Run P233a134).

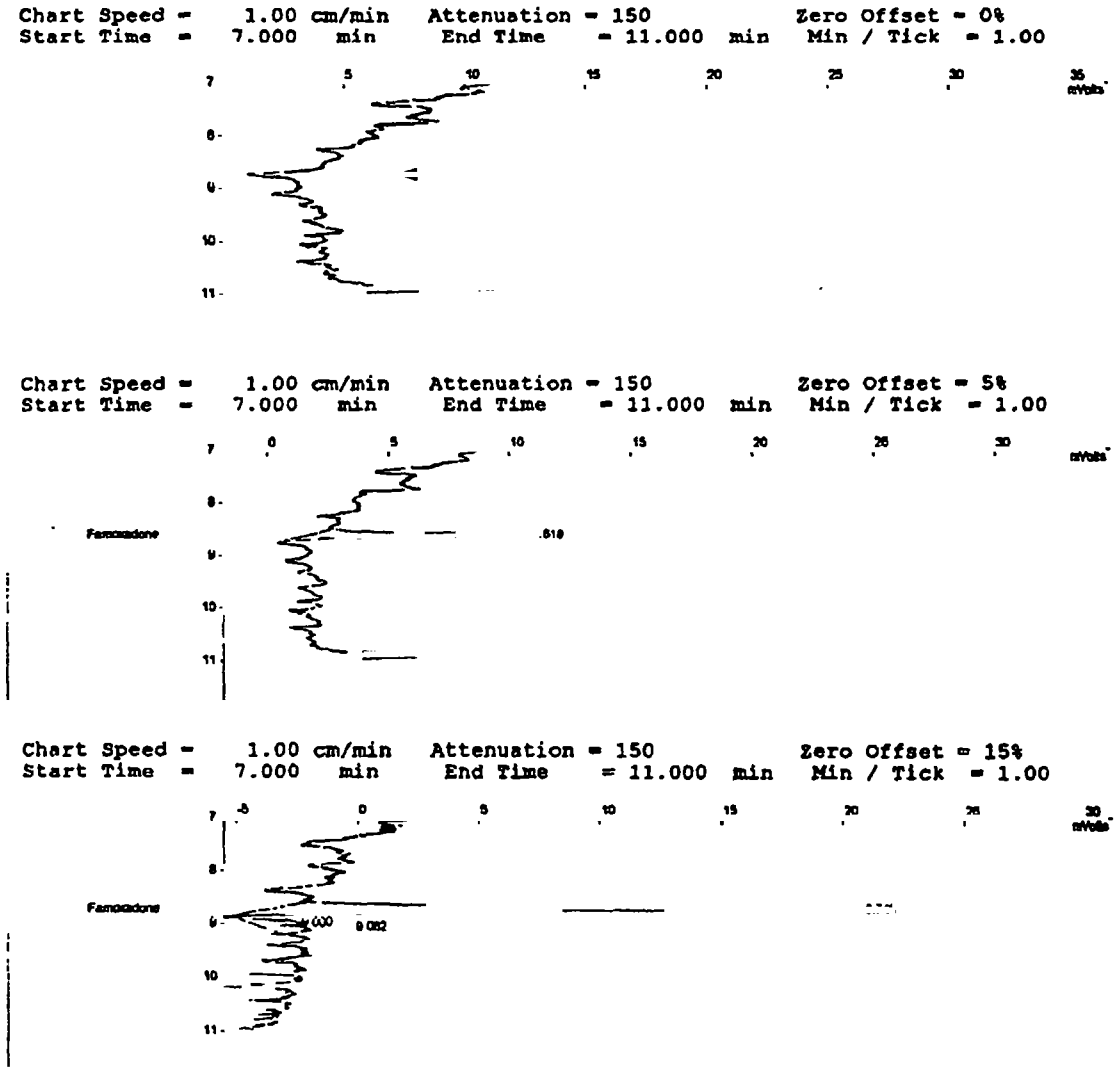


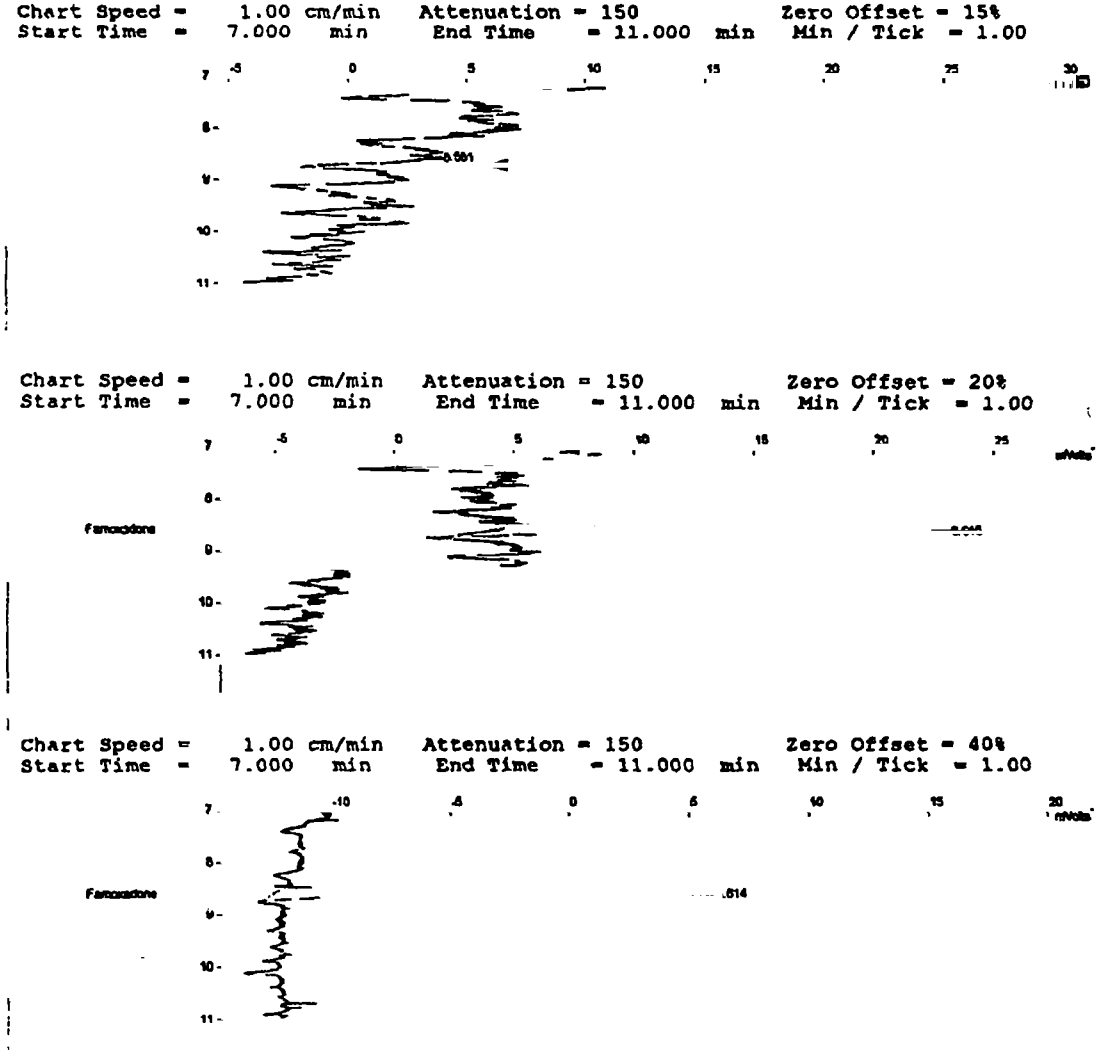
Figure 11

GC/ECD chromatograms of surface water samples (0.5 L) analyzed by liquid /liquid extraction.

Top: Blank sample (ID P233-1196, V_{end} = 0.5 mL) (GC Run P233a166).

Middle: LOQ sample fortified at 0.25 µg/L (ID P233-1197, V_{end} = 0.5 mL) (GC Run P233a172).

Bottom: 5 * LOQ sample fortified at 1.25 µg/L (ID P233-1200, V_{end} = 2.5 mL) (GC Run P233a179)



Appendix A 1

Representative analysis data for the three water types used in this study.

1. Drinking Water

Analysis data (copy of original in German) provided by the local drinking water supplier (best available copy). Tap water is described in the column "Uni Ulm".

Analytical evaluation of Ulm/New Ulm drinking water		Water sample	Acceptable Level	Ulm	New Ulm	Ensingen	University of Ulm	Lehr
[redacted]								
Coloring (SAK 436nm)	m^{-1}	1	0.5	0.02	0.02	0.02	<0.02	0.02
Turbidity	TE/F	2	1.5	0.12	0.15	0.15	0.04	0.14
Odor threshold	-	3	3	1	1	1	1	1
Temperature	°C	4	25	10.4	10.0	15.4	10.2	12.2
pH	-	5	6.5-9.5	7.57	7.45	7.39	7.49	7.23
Conductive capability (25C)	µS/cm	6	2000	498	528	637	512	676
Oxidizability of Mn	mg/l	7	5	0.7	0.7	0.4	0.5	0.5
Aluminum	mg/l	8	0.2	<0.03	<0.03	<0.05	<0.05	No data
Ammonium	mg/l	9	0.5	<0.05	<0.05	<0.05	<0.05	<0.05
Barium	mg/l	10	1.0	0.05	0.05	0.02	0.03	No data
Boron	mg/l	11	1.0	<0.05	0.05	<0.05	<0.05	<0.05
Calcium	mg/l	12	400	72	75	100	80	121
Chloride	mg/l	13	250	18	19	16	31	44
Iron	mg/l	14	0.2	<0.01	<0.01	<0.01	0.01	<0.01
Potassium	mg/l	15	12	1.8	2.5	2.6	1.6	1.1
Kjeldahl nitrogen	mg/l	16	1.0	0.2	0.2	<0.5	<0.1	No data
Magnesium	mg/l	17	50	13	17	22	11	5
Manganese	mg/l	18	0.05	<0.01	<0.01	<0.01	<0.01	<0.01
Sodium	mg/l	19	150	7.9	9.3	5.0	7.2	12.1
Phosphate	mg/l	21	6.7	0.11	<0.05	<0.05	0.62	0.07
Silver	mg/l	22	0.01	<0.0005	<0.0005	<0.0001	<0.0005	No data
Sulfate	mg/l	23	240	17	21	35	50	17
Mineral Oil	mg/l	24	0.01	<0.01	<0.01	<0.02	<0.01	No data
Surface active material	mg/l	26	0.2	<0.01	<0.01	<0.02	<0.01	No data
[redacted]								
Arsenic	mg/l	1	0.04	<0.001	<0.001	<0.001	<0.001	<0.001
Lead	mg/l	2	0.04	<0.001	<0.001	<0.002	<0.001	<0.003
Cadmium	mg/l	3	0.005	<0.0001	<0.0001	<0.0005	<0.0001	<0.0001
Chromium	mg/l	4	0.05	<0.001	<0.001	<0.005	<0.001	<0.001
Cyanide	mg/l	5	0.05	<0.01	<0.01	<0.01	<0.01	<0.01
Fluoride	mg/l	6	1.5	0.07	0.08	0.11	<0.05	<0.05
Nickel	mg/l	7	0.05	<0.001	<0.001	<0.005	<0.001	<0.003
Nitrate	mg/l	8	50	15	13	12	24	39
Nitrite	mg/l	9	0.1	<0.01	<0.01	<0.01	<0.01	<0.01
Mercury	mg/l	10	0.001	<0.00001	<0.00005	<0.00005	<0.00005	<0.00001
Polycyclic Aromatic Hydrocarbons	mg/l	11	0.0002	<0.00005	<0.00005	<0.00005	<0.00005	<0.00005
1,1-Trichloroethane, Trichloroethylene, Tetrachloroethylene, Dichloromethane, Carbon Tetrachloride	mg/l	12	0.01	<0.001	<0.001	<0.001	<0.001	<0.001
Pesticides and polyhalogenated Bi- and terphenyl substances	mg/l	13	0.0001	<0.0001	<0.0001	<0.0002	<0.0001	<0.0002
Each or the sum of the individuals	mg/l	13	0.0003	<0.0001	<0.0001	<0.0002	<0.0001	<0.0003
Antimony	mg/l	14	0.01	<0.001	<0.001	<0.001	<0.001	No data
Selenium	mg/l	15	0.01	<0.001	<0.001	<0.001	<0.001	No data
[redacted]								
Chlorine	mg/l	1	0.3	<0.01	<0.01	-	-	<0.01
Trihalomethane	mg/l	1	0.01	<0.001	<0.001	-	<0.001	<0.001
Chloride dioxide	mg/l	2	0.2	0.04	0.05	-	0.05	-
Chlorite	mg/l	2	0.2	0.07	0.06	-	0.10	-
[redacted]								
Copper	mg/l	1	3	<0.01	<0.01	No data	No data	No data
Zinc	mg/l	2	5	0.04	0.07	No data	No data	No data
[redacted]								
Oxygen	mg/l			5.8	6.4	7.3	11.0	8.6
Total alkaline earth	mol/m³			2.36	2.56	3.40	2.40	3.23
Total hardness	dH			13.2	14.3	19.1	13.4	18.1
Acid capacity to pH 4.3	mol/m³			4.09	4.54	5.57	3.53	4.88
Carbonate hardness	dH			11.5	12.7	16.0	9.9	13.7
Silicate	mg/l			2.3	2.2	3.2	3.0	2.9
Dissolved organic carbon	mg/l			1.5	1.0	1.0	0.4	No data
UV ext. coeffic (SAK 254 nm)	m^{-1}			1.8	0.9	0.5	0.8	1.0
								Stand 1997

Appendix A 1 continued

Representative analysis data for the three water types used in this study.

2. Ground Water

Analysis data (copy of original in German, page 1 of 2) for well water "Fassung 4" provided by the State Water Supplier.

Zweckverband Landeswasserversorgung
Betriebs-und Forschungslabor

Analysis of Groundwater for Drinking Water Law

Sampling Plan Section 4

Timing of Analysis Half Year

Parameter Measured	Units	Water sample	Acceptable Level	10.09.97
			Drinking Water Law, Section 4	
Coloring (SAK 436nm)	1/m	1	0.5	<0.02
Turbidity	TE/F	2	1.5	9 6
Odor threshold	-	3	2	n.b
Temperature	°C	4	25	11.7
pH (measured)	-	5	6.5-9.5	6.89
pH (after $CaCo_3$ neutralization)	-	5	-	
Conductive capability (25C)	µS/cm	6	2000	807
Oxidizability of Mn	mg/l	7	5	1.6
Aluminum	mg/l	8	0.2	0.005
Ammonium	mg/l	9	0.5	0.17
Berium	mg/l	10	1.0	0.033
Boron	mg/l	11	1.0	
Calcium	mg/l	12	400	152
Chloride	mg/l	13	250	29.3
Iron	mg/l	14	0.2	1 6
Potassium	mg/l	15	12	1 3
Kjeldahl nitrogen	mg/l	16	1	
Magnesium	mg/l	17	50	18.4
Manganese	mg/l	18	0.05	0.3358
Sodium	mg/l	19	150	3.8
Phosphate	mg/l	21	6.7	0.04
Silver	mg/l	22	0.01	<0.0001
Sulfate	mg/l	23	240	98
Mineral Oil	mg/l	24	0 01	<0.01
Anionic surface active material	mg/l	26	0.2	
Nonionic surface active material	mg/l	26		

Appendix A 1 continued

Representative analysis data for the three water types used in this study.

2. Ground Water (continued)

Analysis data (copy of original in German, page 2 of 2) for well water "Fassung 4" provided by the State Water Supplier.

Zweckverband Landeswasserversorgung
Betriebs-und Forschungslabor

Analysis of Groundwater for Drinking Water Law

Sampling Plan — Section 4

Timing of Analysis — Half Year

Parameter Measured	Units	Water sample	Acceptable Level	10.09.97
			Drinking Water Law, Section 2	
Arsenic	mg/l	1	0.01	0.0020
Lead	mg/l	2	0.04	0.0006
Cadmium	mg/l	3	0.005	<0.00005
Chromium	mg/l	4	0.05	0.0002
Cyanide	mg/l	5	0 05	<0 005
Fluoride	mg/l	6	1.5	
Nickel	mg/l	7	0.05	0.0009
Nitrate	mg/l	8	50	0.8
Nitrite	mg/l	9	0.1	<0 01
Mercury	mg/l	10	0.001	<0.00001
Polycyclic Aromatic Hydrocarbons	mg/l	11	0.0002	
1,1-Trichloroethane, Tridchloroethylene, Tetrachloro-ethylene,				
Dichloromethane,	mg/l	12	0 01	<0.00001
Carbon tetrachloride	mg/l	12	0.003	<0 00001
Pesticide and polyhalogenated bi- and terphenyl substances				
each or	mg/l	13	0.0001	<0.000005
the sum of the individual	mg/l	13	0.0005	<0.000005
Antimony	mg/l	14	0.01	<0.0005
Selenium	mg/l	15	0.01	<0.0005
Acid capacity to pH 4.3	mol/m3			7 03
Carbonate hardness	dH			19.70
Total alkaline earth	mol/m3			4 51
Total hardness	dH			25.25
Dissolved organic carbon	mg/l			2.8
UV ext. coeffic. (SAK 254 nm)	1/m			6.5
Oxygen	mg/l			<0.1
Chloride dioxide	mg/l			
Choate	mg/l			
Total haloform	mg/l			<0.00001

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Appendix A 1 continued

Representative analysis data for the three water types used in this study.

3. Surface Water

Analysis data (copy of original in German, page 1 of 2) for surface water "Danube 7" provided by the State Water Supplier.

Zweckverband Landerswasserversorgung
Betriebs-und Forschungslabor

Analysis of Groundwater for Drinking Water Law

Sampling Plan		Donau, Leipheim			
Tuning of Analysis		August		07.08.97	
Parameter Measured	Units	Water sample	Acceptable Level		
		Drinking Water Law, Section 4			
Coloring (SAK 436nm)	1/m	1	0.5	0.34	
Turbidity	TE/F	2	1.5	2.0	
Odor threshold	-	3	2		
Temperature	°C	4	25	18.0	
pH (measured)	-	5	6.5-9.5	8.21	
pH (after $CaCO_3$ neutralization)	-	5	-		
Conductive capability (25C)	µS/cm	6	2000	499	
Oxidizability of Mn	mg/l	7	5		
Aluminum	mg/l	8	0.2	0.008	
Ammonium	mg/l	9	0.5	0.04	
Barium	mg/l	10	1.0	0.052	
Boron	mg/l	11	1.0	0.06	
Calcium	mg/l	12	400	73.5	
Chloride	mg/l	13	250	19.7	
Iron	mg/l	14	0.2	0.017	
Potassium	mg/l	15	12	2.0	
Kjedahl nitrogen	mg/l	16	1	0.52	
Magnesium	mg/l	17	50	11.1	
Manganese	mg/l	18	0.05		
Sodium	mg/l	19	150	9.7	
Phosphate	mg/l	21	6.7	0.12	
Silver	mg/l	22	0.01	<0.0001	
Sulfate	mg/l	23	240	19.1	
Mineral Oil	mg/l	24	0.01	<0.01	
Anionic surface active material	mg/l	26	0.2	<0.01	
Nonionic surface active material	mg/l	26		<0.02	

Appendix A 1 continued

Representative analysis data for the three water types used in this study.

3. Surface Water (continued)

Analysis data (copy of original in German, page 2 of 2) for surface water "Danube 7" provided by the State Water Supplier.

Zweckverband [illegible] ,pang
Betriebs-und Forschungslabor

Analysis of Groundwater for Drinking Water Law

Sampling Plan: Donau, Leipheim
Timing of Analysis: August

Parameter Measured	Units	Water sample	Acceptable Level	07.08.97
		Drinking Water Law, Section 2		
Arsenic	mg/l	1	0.01	0.0005
Lead	mg/l	2	0.04	0.0009
Cadmium	mg/l	3	0.005	<0.00005
Chromium	mg/l	4	0.05	0 0002
Cyanide	mg/l	5	0.05	<0.005
Fluoride	mg/l	6	1.5	
Nickel	mg/l	7	0.05	<0.0001
Nitrate	mg/l	8	50	13.1
Nitrite	mg/l	9	0.1	0 05
Mercury	mg/l	10	0.001	<0.00001
Polycyclic Aromatic Hydrocarboos	mg/l	11	0.0002	0.00003
1,1-Trichloroethane, Tridchloroethylene, Tetrachloro-ethylene,				
Dichloromethane,	mg/l	12	0.01	0.00013
Carbon tetrachloride	mg/l	12	0.003	<0.00001
Pesticide and polyhalogenated bi- and terphenyl substances				
each or	mg/l	13	0.0001	0.000006
the sum of the individual	mg/l	13	0.0005	0.000006
Antimony	mg/l	14	0.01	<0.0005
Selenium	mg/l	15	0.01	<0.0005
Acid capacity to pH 4.3	mol/m3			4.23
Carbonate hardness	dH			11.85
Total alkaline earth	mol/m3			2.43
Total hardness	dH			13.60
Dissolved organic carbon	mg/l			2.8
UV ext. coeffic. (SAK 254 nm)	l/m			7.1
Oxygen	mg/l			9 6
Chloride dioxide	mg/l			
Choate	mg/l			
Total haloform	mg/l			0.00002

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Appendix A 2

Description of confirmatory GC/MS and MS/MS methods with representative mass spectra and chromatograms.

GC/MS system:

A Varian GC/MS system equipped with a Varian 8100 autosampler, a Varian 3400 GC with a temperature programmed SPE injector, a Varian Saturn 3 Iontrap MS (EI ionization, MS/MS option) and Compaq Data System was used.

GC/MS and GC/MS/MS methods:

Injection: 1 µL splitless injection using autosampler and temperature programmed SPI injector (120 °C, 0.10 min, 180 °C/min to 260 °C, 1 min).

Column: BPX-5 (25 m, 0.32 mm i.d., 0.25 µm film, SGE, Weiterstadt, Germany).

Oven program: 90 °C for 1 min; 30 °C/min to 240 °C; 10 °C/min to 300 °C; 300 °C for 2 min.

Full scan MS detection method:

Mass range: 70 to 400 m/e. The sum of the major fragment ions 330/224/196 m/e is used for detection and quantitative evaluation . Representative spectra and chromatograms of chromatographic standards and water sample extracts are given on the following pages.

MS/MS detection method:

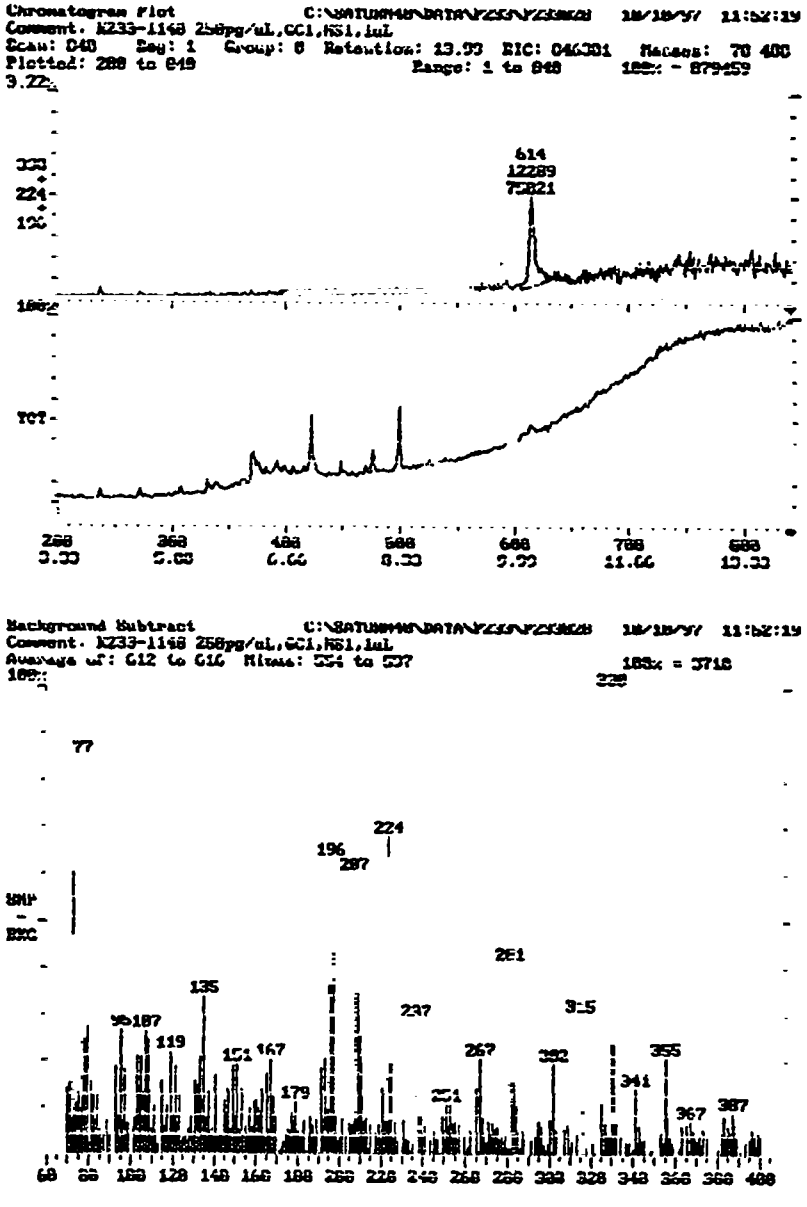
EI-MS/MS with resonant collision-induced dissociation (CID) of the 330 m/e parent ion. The 193 m/e daughter ion is used for evaluation.
Mass range: 180 to 200 m/e.
Resonant excitation: 20 msec with an amplitude of 1.7 V.
Parent fragment ion: 330 m/e, isolation window 3 m/e.
Excitation storage level: 130 m/e.
Representative chromatograms of a chromatographic standard and water sample extracts are given on the following pages.

With the detection method 2 (EI-MS/MS) the 330 m/e fragment ion is isolated in the ion trap and then exposed to an additional resonant excitation voltage. The 330 m/e fragment ion dissociates to the specific 193 m/e daughter ion. This procedure results in improved selectivity and better sensitivity for the monitored 193 m/e daughter ions

Appendix A 2 continued

Description of confirmatory GC/MS methods, representative mass spectra and chromatograms.

GC/MS chromatogram (top) and full scan mass spectrum (bottom) of a chromatographic standard solution: 250 pg/µL ID K233-1148 GC/MS Run P233 028.
(Best available copy)

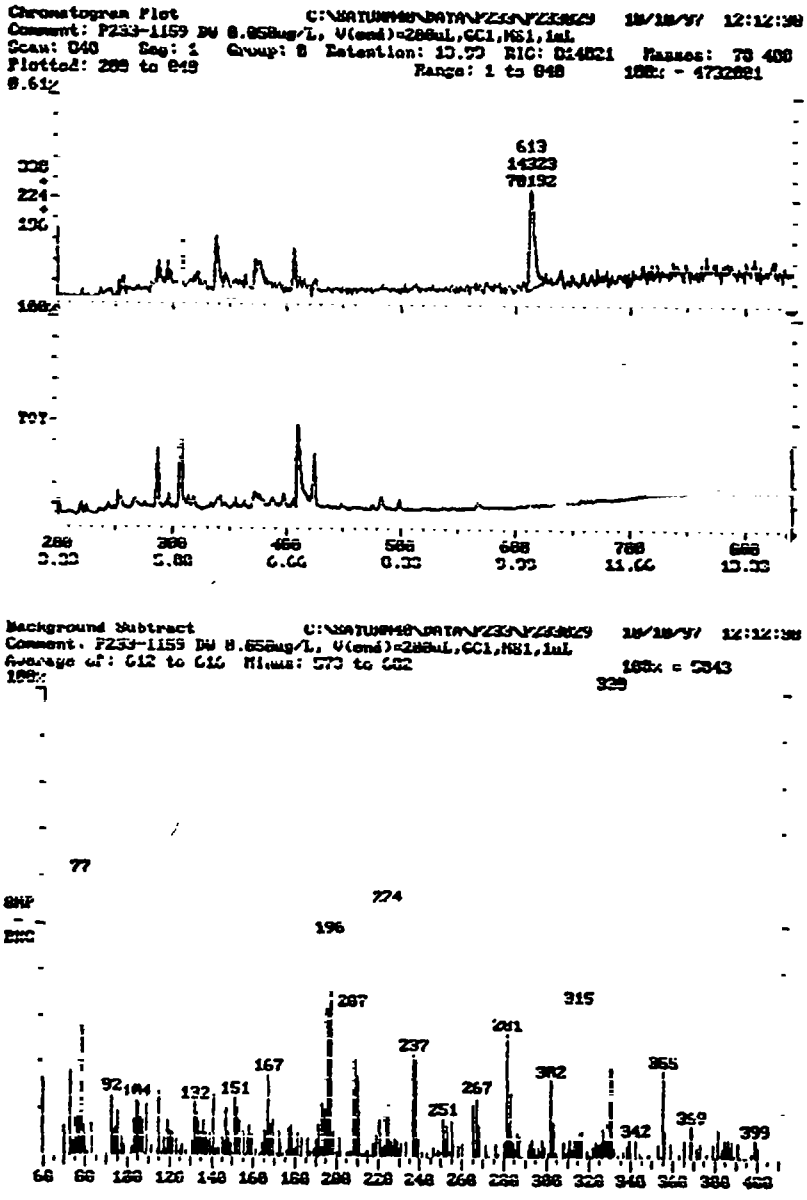


Appendix A 2 continued

Description of confirmatory GC/MS methods, representative mass spectra and chromatograms.

GC/MS chromatogram (top) and full scan mass spectrum (bottom) of a drinking water sample (1 L) from solid phase extraction (best available copy):

LOQ sample fortified at 0.050 µg/L, V_{end} = 0.2 mL ID P233-1159 GC/MS Run P233 029.

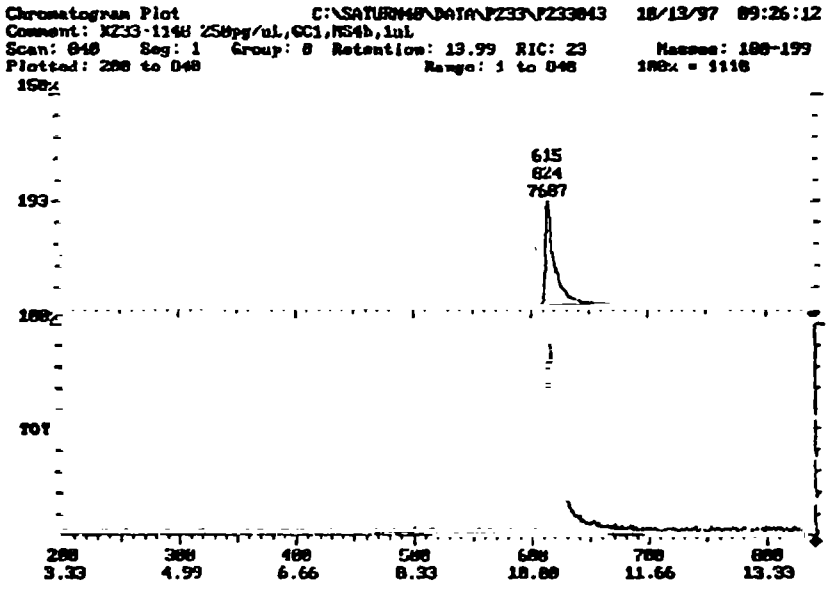


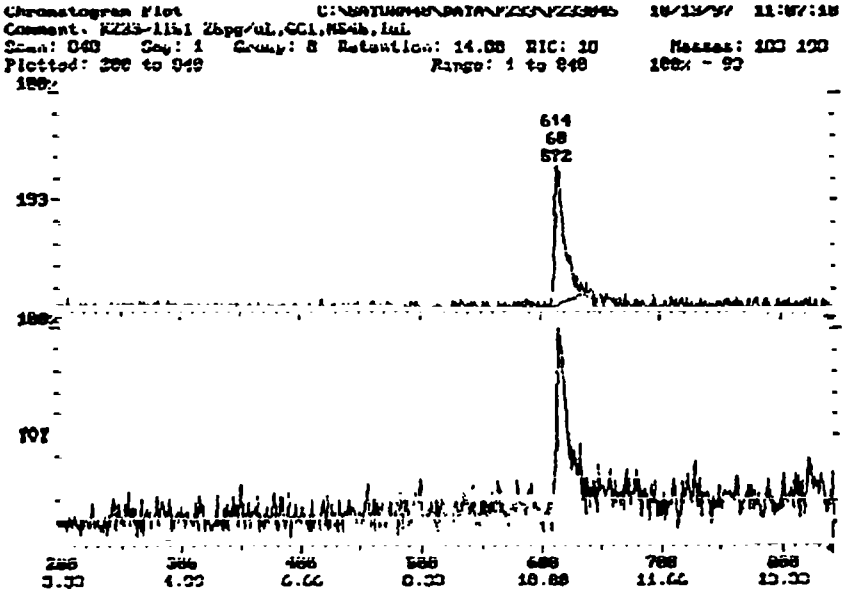
Appendix A 2 continued

Description of confirmatory GC/MS methods, representative mass spectra and chromatograms.

GC/MS/MS chromatograms (upper trace daughter ion, lower trace total ion current) of chromatographic standard solutions (best available copy):

Top:	250 pg/µL	ID K233-1148	GC/MS Run P233 043.
Bottom:	25 pg/µL	ID K233-1151	GC/MS Run P233 045.





Appendix A 2 continued

Description of confirmatory GC/MS methods, representative mass spectra and chromatograms.

GC/MS/MS chromatograms (upper trace daughter ion, lower trace total ion current) of a ground water sample (1L) from solid phase extraction (best available copy).

Top: LOQ sample fortified at 0.050 µg/L, V_{end} = 0.2 mL ID P233-1166
GC/MS Run P233 046.

Bottom: LOQ sample fortified at 0.050 µg/L, V_{end} = 2.0 mL ID P233-1166V1
GC/MS Run P233 047.