1st thematic workshop of the EU project NORMAN

CHEMICAL ANALYSIS OF EMERGING POLLUTANTS

November 27-28. 2006
Maó, Menorca (Balearic island) Spain

PREFACE

We are glad to introduce the Workshop on: **Chemical analysis of emerging pollutants.** This is the first one from the three thematic workshops of the NORMAN `project (Contract No: 018486): " **Network of reference laboratories and related organisations for monitoring and bio-monitoring of emerging environmental pollutants"** This co-ordination action is funded under the 6th EU Framework programme and it will develop and implement a methodology within a network of reference laboratories and related organisations (including standardisation bodies) to enable and improve EU capabilities for monitoring emerging pollutants, thereby ensuring the production of data that are valid, comparable and fit for purpose across EU25. The project will align the activities of the network with the requirements of organisations / stakeholders in charge of risk assessment and management. It will organise, via workshops, the EU-wide exchange of information between monitoring experts, environmental agencies and standardisation and regulatory bodies.

The Workshop is structured in six sessions: EU research on emerging contaminants, polar pesticides, pharmaceuticals, halogenated emerging contaminants, other emerging contaminants and degradation and bioavailablity of emerging contaminants with a total number of 20 key note lectures and 22 poster presentations. Regular scientific papers of this workshop are expected to be published in the Journal of Chromatography A as a special issue. Guidelines for authors can be found on http://ees.elsevier.com/chroma being the deadline for submission is 31 December 2006.

The main objective of the workshop are to evaluate practical aspects of the usefulness of chemical analytical methods techniques for determining a variety of organic and inorganic contaminants in a variety of environmental samples like water, soil/sediment and air. The following practical aspects and state of the art applications will be discussed in the course of the workshop:

- Automated multidimensional GC and on-line solid phase extraction methods for pesticides.
- Liquid chromatography- tandem mass spectrometry for pharmaceuticals and polar pollutants
- Advanced mass spectrometric methods for halogenated contaminants.
- Analytical methods for a broad range of contaminants: fuel oxygenates, organophsophorus flame retardants, bacteria, organometallic compounds, inorganic, PAHs, VOCs, among others

Finally we would like to thank all the participants in the workshop, including lecturers, poster presenters and attendees for their useful contributions in this field and for the stimulating discussions that we are sure will take place in the course of the workshop. We are also specially thankful to Institut Menorqui d Estudis (IME) for the excellent facilities of the Conference room.

Wishing you a very successful and enjoyable stay in Maó

D Barceló and M. Petrovic

Barcelona, November, 10, 2006

MONDAY, 27. November 2006

9.00 – 9.30 Registration

9.30 – 9.45 Welcome and Introductions to the workshops objectives
Damia Barcelo, IIQAB-CSIC, Barcelona, Spain

9.45 – 10.00 Elena Domínguez
European Commission DG Research, Brussels, Belgium
The EU's new Research Framework Programme 2007-2013: FP7

Session 1: EU research on emerging contaminants

Chair: D. Barceló

10.00 – 10.30 Valeria Dulio
INERIS, Verneuil-en-Halatte, France
NORMAN - Network of reference laboratories and related organisations for monitoring and bio-monitoring of emerging environmental pollutants

10.30 – 11.00 Johannes Barth
Eberhard Karls Universität Tübingen, Germany
Consideration of persistent and emerging pollutants in the AQUATERRA project

11.00 – 11.30 Poster session/Coffee break

Session 2: Polar Pesticides

Chair: H. Richnow

11.30 – 12.00 Miren Lopez de Alda
CSIC, Barcelona, Spain
LC-MS/MS methods for analysis of medium to polar pesticides in water and sediment samples and their application in different monitoring programs

12.00 – 12.30 Monica Saez
CSIC, Madrid, Spain
New gas-chromatography-based approaches for the monitoring of pesticides

12.30 – 13.00 Marinella Farré
CSIC, Barcelona, Spain
Surface Plasmon Resonance Immunosensor for Environmental Water Samples Análisis

13.00 – 15.00 Lunch

Session 3: Pharmaceuticals

Chair: J. Barth

15.00 – 15.30 Toine Bovee
RIKILT-Institute of Food Safety, Wageningen, The Netherlands
Monitoring and biomonitoring of hormones and drug residues in the food chain: Advanced screening assays and coupling with accurate mass spectrometry.

15.30 – 16.00 Helene Budzinski
University of Bordeaux; CNRS; Talence, France
Pharmaceutical Substances: Emergent Contaminants of the Aquatic Systems

16.00 – 16.30 Meritxell Gros
IIQAB-CSIC, Barcelona, Spain
Liquid Chromatography-Tandem Mass Spectrometry as a Powerful Tool for The Determination of Pharmaceuticals in Environmental Samples

16.30 – 17.00 Coffee break

17.00 – 17.30 John L. Zhou'
Department of Biology and Environmental Science, School of Life Sciences, University of Sussex, UK
Determination of pharmaceutical compounds in water by solid-phase extraction-liquid chromatography-tandem mass spectrometry

17.30 – 18.00 Tina Kosjek
Institut Jozef Stefan, Department of Environmental Sciences, Ljubljana, Slovenia
Study of pharmaceutical residues removal in a pilot wastewater treatment plant.

18.00 – 18.30 Discussion of sessions 2 and 3

TUESDAY, 28. November 2006

Session 4: Halogenated emerging contaminants

Chair: J. Parsons

9.30 – 10.00 **Guido Vanermen**
Flemish Institute for Techological Research, Mol, Belgium
Brominated flame retardants in industrial effluents: analytical methods and monitoring results.

10.00 – 10.30 **Michael Oehme**
Organic Analytical Chemistry, University of Basel, Switzerland
The never ending story of polychlorinated paraffins: New proposals to overcome the persistent quantification problem.

10.30 – 11.00 **Stefan van Leeuwen**
Institute for Environmental Studies, Vrije Universiteit, Amsterdam
The advances in analysis of perfluorinated organic surfactants (PFCs).

11.00 – 11.30 **Poster session/Coffee break**

Session 5: Other emerging contaminants

Chair: V. Dulio

11.30 – 12.00 **Oliver Gans**
Department of Hazardous Substances and Metabolites, Austrian Federal Environment Agency, Vienna, Austria
Determination of Selected Organophosphorus Flame Retardants (OPFRs) in Water and Sediment River Samples from Austria

12.00 – 12.30 **Teresa Moreno**
Earth Sciences Institute, CSIC; Barcelona
Emerging inorganic pollutants in the atmospheric particulate matter.

12.30 – 13.00 **Ramadan Abuknesha**
King's College London, UK
A new protease-immunoassay tandem assay method for detecting low levels of microbial activity in water samples

13.00 – 15.00 Lunch

Session 6: Degradation and bioavailability of emerging contaminants

Chair: M.J. Lopez de Alda

15.00 – 15.30 Hans - Herrmann Richnow
UFZ-Leipzig, Germany
Compound specific isotope analysis to characterise degradation pathway and to quantify in situ degradation of fuel oxygenates and other fuel derived contaminants

15.30 – 16.00 John Parsons
University of Amsterdam, The Netherlands
Bioavailability of organic contaminants in sediment and soil - emerging insights

16.00 – 16.30 Laura Martín-Díaz
CSIC, Cadiz, Spain
Design and Application of a Set of Analysis of a Battery of Biomarkers as a New Achievement in Dredged Material Characterization and Management

16.30 – 17.00 Discussion of sessions 4, 5 and 6

17.00 End of the workshop

Posters

1.

HOURLY DETERMINATION OF C_6-C_{12} ATMOSPHERIC VOCs USING AN AUTOMATIC ON-LINE GC-MS SYSTEM

N. Durana, M. de Blas, M. Navazo, L. Alonso, J.L. Ilardia, J. A. García, G. Gangoiti and J. Iza
Chemical and Environmental Engineering Department, School of Engineering, University of the Basque Country, Alameda de Urquijo s/n, E-48013 Bilbao, Spain

2.

MICROWAVE-ASSISTED EXTRACTION AND ULTRASOUND EXTRACTION TO DETERMINE POLYCYCLIC AROMATIC HYDROCARBONS IN NEEDLES AND BARK OF PINUS PINASTER AND PINUS PINEA

Nuno Ratola(1), Sílvia Lacorte(2), Damià Barceló(2), Arminda Alves(1)*
(1) LEPAE, Departamento de Engenharia Química, Faculdade de Engenharia da Universidade do Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal
(2) Department of Environmental Chemistry, IIQAB-CSIC, Barcelona, Catalonia, Spain

3.

AN AUTOMATED TECHNIQUE FOR THE CONTINUOUS MONITORING OF ORGANIC MATERIAL CONCENTRATIONS AND TOTAL TOXICITY IN WATER SAMPLES: REAL WORLD APPLICATIONS OF THE BOD-TOXICITY MICROBIAL BIOSENSOR.

T. Diez-Caballero Arnau, Enric Poch, MªJesús Aracil1, Sergio Montoro.
Biosensores, S. L. Avenida Ausias March 1, Moncofar (Castellón) 12593, Spain

4.

IMPROVEMENTS IN DETERMINATION OF LEAD IN ROUMANIAN WINES BY ELECTROTHERMAL ATOMIC ABSORPTION SPECTROMETRY

Mihaela Artimon, Daniela Bălan,Gabriela Luță, Maria Pele*
(*)University of Agronomic Sciences and Veterinary Medicine, Faculty of Biotechnologies, Marasti 59, Bucharest, Romania

5.

DEVELOPMENT, CALIBRATION AND FIELD VALIDATION OF PASSIVE SAMPLING DEVICES FOR MONITORING EMERGING CONTAMINANTS IN AQUATIC ENVIRONMENTS

Branislav Vrana and Katarína Šilhárová, Water Research Institute, National Water Reference Laboratory for Slovakia, Nabr. arm. gen. L. Svobodu 7, 81249 Bratislava, Slovakia

6.

SIMULTANEOUS DETERMINATION OF THREE NITROIMIDAZOLES AND THEIR METABOLITES IN EGGS BY PRESSURIZED FLUID EXTRACTION (PFE) AND LIQUID CHROMATOGRAPHY- TANDEM MASS SPECTROMETRY

Nuria León[a], Vicent Yusà[a], Olga Pardo[a] and Agustín Pastor[b*]
[a]Public Health Laboratory of Valencia, Conselleria de Sanitat, Camí de la Marjal, s/n 46470 Albal, Valencia, Spain
[b]Analytical Chemistry Department, University of Valencia, Spain

7.

USING DECISION TREE ANALYSIS AND GIS IN MODELLING (SEMI)VOC EMISSIONS AT THE EUROPEAN SCALE

Patrik Fauser[1], Alberto Pistocchi[2]
[1]National Environmental Research Institute, Depertment of Policy Analysis, Roskilde, Denmark,
[2]European Commission, DG Joint Research Centre, Ispra (VA) Italy

8.

STUDIES OF LARGE ORGANO - METALLIC POLLUANTS USING ELECTRON SPECTROSCOPY

Al-Taiar A.H., Bentyeb K.
Department of Chemistry, Faculty of Science, University of Science and Technology, P.O.B. 29031 U.S.T.O. ,Oran 31036.

9.

APPLICATION OF HPLC AND ELISA METHODS FOR MONITORING OF SELECTED EMERGING POLLUTANTS IN SLOVAKIAN SURFACE WATERS

Zoltán Krascsenits and Mikuláš Bartal, Water Research Institute, National Water Reference Laboratory for Slovakia, Nabr. arm. gen. L. Svobodu 7, 81249 Bratislava, Slovakia

10.

RESPONSE SURFACE METHODOLOGY FOR THE MICROWAVE ASSISTED EXTRACTION OF INSECTICIDES FROM SOIL SAMPLES

M.C. Hernández-Soriano, A. Peña, M.D. Mingorance
Estación Experimental del Zaidín (CSIC), c/ Profesor Albareda, 1. E-18008 Granada (Spain)

11.

EVALUATION OF PESTICIDES POLLUTION IN THE IRRIGATION AND DRAINAGE CHANNELS OF THE EBRO RIVER DELTA DURING THE GROWING SEASON OF RICE USING CHEMOMETRIC AND GEOSTATISTICAL METHODS

Marta Terrado[1], Marina Kuster[1], Demetrio Raldúa[2], Miren Lopez de Alda[1], Damià Barceló[1] and Romà Tauler[1]*
[1] Department of Environmental Chemistry, IIQAB-CSIC, Jordi Girona 18-26, Barcelona 08034, Spain
[2] Laboratory of Environmental Toxicology, UPC, CN 150 Km 14.5, Terrassa 08220, Spain

12.

SYNERGISTIC AND ANTAGONISTIC EFFECTS OF C_{ORG} AND NO_3^- IN DENITRIFICATION ACTIVITY FROM A WETLAND SOIL IN THE BASQUE COUNTRY

B. Muñoz[1], Antigüedad[2] I. and E. Ruiz[1]
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(2) Hydrogeology Group. University of the Basque Country. 48940 Leioa, Bizkaia. Basque Country (Spain

13.

EC FP6 COORDINATION ACTION (CA) ON RISK-BASED MANAGEMENT OF THE WATER-SEDIMENT-SOIL SYSTEM AT THE RIVER BASIN SCALE (RISKBASE)

Jos Brils[1], Thomas Track[2], Philippe Negrel[3], Werner Brack[4], Dietmar Müller[5], Damia Barcelo[6], M.Silvia Diaz-Cruz[6] ,Winfried Blum[7], Wim Salomons[8], Joop Vegter[9], Vala Ragnarsdottir[10] & Cathy Eccles[11]
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[3]BRGM, Avenue C. Guillemin, 45060, Orléans, France (contractor)
[4]UFZ, Permoserstr. 15, 04318, Leipzig, Germany (contractor)
[5]Umweltbundesamt GmbH, Spittelauerlände 5, 1090, Vienna, Austria (contractor)
[6]CSIC, Jordi Girona 18, 8034, Barcelona, Spain (contractor)
[7]Universität für Bodenkultur, Peter Jordan Str. 82, 1190, Vienna, Austria (contractor)
[8]IVM, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands (contractor)
[9]Amsteldijk Zuid 167, 1189 VM, Amstelveen The Netherlands (contractor)
[10]University of Bristol, Queens Road, BS8 1RJ, Bristol, UK (contractor)
[11]EC DG Research, CDMA 03/155, B-1049, Brussels, Belgium (scientific officer)

14.

TOXICITY ASSESSMENT OF FLUORINATED ALKYL COMPOUNDS USING *Vibrio fischery*

Elena Martínez[1], Marinella Farré[1], Asunción Navarro[1], Marta Villagrasa[1], Fernando Rubio[2], Damià Barceló[1]
[1]Environmental Chemistry Department, IIQAB-CSIC
[2] ABRAXIS, LLC, PA, 18974

15.

EXAMINATIONS OF PHYSICAL AND CHEMICAL PROPERTIES OF SEDIMENTS TOWARDS ADSORPTION OF SELECTED ANTIPHLOGISTIC DRUGS

Katarzyna Styszko-Grochowiak[1], Andrew Parker[2], Janusz Golas[1*], Artur Strzelecki[1], Agnieszka Iwanicha[1], Jerzy Gorecki[1]
[1] Department of Environmental Sciences, AGH University of Science and Technology, 30-059 Krakow, Poland
[2] Department of Soil Science, The University of Reading, Whiteknights, PO Box 227, Reading RG6 6AB, UK

16.

ENVIRONMENTAL ANALYSIS OF FLUORINATED ALKYL SUBSTANCES BY LIQUID CHROMATOGRAPHY-(TANDEM) MASS SPECTROMETRY. A REVIEW.

Marta Villagrasa, Maria López de Alda, Damià Barceló
Department of Environmental Chemistry, IIQAB-CSIC, Jordi Girona 18-26, 08034 Barcelona, Spain

17.

DESIGN AND PERSPECTIVES OF THE USE OF A BATTERY OF BIOMARKERS FOR THE ADVERSE EFFECT ASSESSMENT OF NEW EMERGING POLLUTANTS: PHARMACEUTICALS AND PERSONAL CARE PRODUCTS (PPCPS)

Martín-Díaz, M.L.[1,2], DelValls, T.A[2] Gagné F.[3], Blaise C.[3]
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[3]St. Lawrence Centre, Environment Canada, 105 McGill St., Montreal, Qc., Canada H2Y 2E7

18.

TENAX EXTRACTION AS A TOOL TO EVALUATE THE AVAILABILITY OF EMERGING CONTAMINANTS IN SEDIMENTS.

De la Cal, A.1; Van den Berg, H.3; Eljarrat, E.1; Grotenhuis, J.T.C.2; Murk, A.J.3; and Barceló, D.1
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[3] Section Toxicology, Department ATV. Wageningen University Tuinlaan 5, P.O. Box 8000, 6700 EA Wageningen, The Netherlands.

19.

SPE STRATEGIES AS APPLIED TO THE EXTRACTION OF SULPHONAMIDE ANTIMICROBIALS FROM DIFFERENT WATER SAMPLES.

M.J. García Galán, M.S. Díaz-Cruz, D. Barceló
Department of Environmental Chemistry, IIQAB-CSIC. Jordi Girona, 18-26. 08034 Barcelona, Spain

20.

APPLICATION OF A NEWLY DEVELOPED, HIGHLY SENSITIVE METHOD, BASED ON PRESSURIZED LIQUID EXTRACTION AND ANALYSIS BY LIQUID CHROMATOGRAPHY-ELECTROSPRAY-TANDEM MASS TO THE ANALYSIS OF TWENTY-TWO MEDIUM TO POLAR PESTICIDES IN SEDIMENTS OF THE LLOBREGAT RIVER BASIN

Maria Hosana Conceiçao, Marta Villagrasa, Maria José López de Alda, Damià Barceló
Environmental Chemistry Department, IIQAB-CSIC, Jordi Girona 18-26, 08034 Barcelona, Spain

21.

LC-MS- ION TRAP DETERMINATION OF NATURAL AND SYNTHETIC ESTROGENS IN DRINKING WATER

Claudiu Brumaru[1], Stefano Polesello[2], Sara Valsecchi[2]
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22.

AUTOMATED IN-TUBE SPME COUPLED TO LIQUID CHROMATOGRAPHY FOR THE DETERMINATION OF PHTHALATES IN ENVIRONMENTAL WATER SAMPLES

C. Cháfer-Pericás, P. Campíns-Falcó* and Mari Carmen Prieto-Blanco
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NEWS OF PERSISTENT AND EMERGING POLLUTANTS IN THE AQUATERRA PROJECT AND RELATED WORK

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[3] LAOP Consulting & Research, Germany
[4] Europa Fachhochschule Fresenius, Germany
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[7] Université de Neuchâtel, Neuchâtel, Switzerland

Better understanding of deposition, turnover and movement of persistent and emerging pollutants (POPs) is among the foremost priorities of the EU FP6 project “AquaTerra”. Several innovative analytical methods and field techniques contribute to this goal and lead to first comparable results between basins of the Ebro, the Meuse, the Elbe and the Danube as well as of the 3-km^2 Brévilles catchment.

For instance, in Ebro sediments chlorinated pesticides were detected in much higher concentrations than polar ones. Analytical results, particularly of the latter compound group, were assured in ring experiments within AquaTerra. For less polar compounds, sediment concentration analyses of the sum of the 16 most common polyaromatic hydrocarbons (ΣPAHs) in the Ebro compare to those of Danube sediments while distribution patterns of selected compounds in the two basins remain to be explored. Other specific results of pollutant transport via sediments with new flood sampling techniques revealed highest deposition rates of β-hexachlorocyclohexane ($\tilde{\beta}$HCH) in river sediments at hotspot areas on the Mulde River in the Bitterfeld region (Elbe catchment, Germany).

In selected soils of pollution hotspots, turnover investigations with labelled stable isotope techniques showed decreasing degradation efficiencies from benzene via acenaphtene to naphthalene with increasing soil depth. These varying degradation efficiencies are likely related to molecule size and aerobic versus anaerobic conditions. On the other hand, when considering the retention potential, sorption of persistent organic pollutants is strongly controlled by the water solubility of the compounds and the carbon content of soils. Moreover, the type of carbon is crucial for the retention potential of pollutants and can be determined with detailed modern microscopic organic petrography as well as chemical or thermal oxidation techniques.

When trying to outline sources of for instance PAHs, bulk deposition samplers were employed across Europe. First results show highest deposition rates in the Meuse Basin and largest seasonal variations in the Elbe Basin. Nevertheless, such deposition rates are not capable to reveal differences between for instance wet and dry deposition. Therefore, when POPs are found in plants, their origin (for instance from atmospheric deposition or uptake from soils) and their distribution in the plant tissue can potentially be determined with innovative laser techniques such as two-photon excitation microscopy (TPEM).

LC-MS/MS METHODS FOR ANALYSIS OF MEDIUM TO POLAR PESTICIDES IN WATER AND SEDIMENT SAMPLES AND THEIR APPLICATION IN DIFFERENT MONITORING PROGRAMS

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This work describes the development of various methods based on liquid chromatography-tandem mass spectrometry (LC-MS/MS) for the determination of medium to polar pesticides in environmental water and sediment samples. The list of target analytes includes organophosphates (fenitrothion, malathion, dimethoate, diazinon), triazines (desethylatrazine, deisopropylatrazine, tertbutylazine, simazine, atrazine, cyanazine), phenylureas (chlortoluron, isoproturon, diuron, linuron), anilides (propanil), chloroacetanilides (alachlor, metolachlor), acidic herbicides (bentazone, MCPA, 2,4D, mecoprop), and thiocarbamates (molinate). Previously filtered water samples are concentrated by fully automated on-line solid phase extraction (SPE) using PLRP-s or HySphere Resin GP cartridges (10 × 2 mm; Spark Holand) depending on the target analytes. Sediment samples, once liophylized and sieved (120 µm), are extracted with acetone:methanol (1:1) by pressurized liquid extraction (PLE) and the extract obtained is further purified by SPE using either Oasis HLB (6 cc, 200 mg; Waters) or, preferentially, Carbograph Extract-Clean Column SPE cartridges (1000mg, 15mL; Alltech). Analysis is performed with an electrospray interface operating in negative or positive ion mode depending on the target analytes. Two different types of LC-MS/MS instruments/analysers have been used: a triple quadrupole Quattro LC (Micromass) for analysis of water samples and an hybrid quadrupole-ion trap (4000 Q-Trap (Aplied Biosystems) for analysis of sediment samples. In all cases, two selected reaction monitoring (SRM) transitions, one for quantitation and another one for confirmation, have been monitored per compound. Recoveries achieved with the methods developed have been in most instances higher than 70% and the limits of detection lower than 10 ng/L in water and 1 ng/g in sediment. The methodologies developed have been used in different monitoring studies to evaluate the occurrence and fate of pesticides in, for instance, the Ebro, Llobregat and Anoia rivers, and along the treatment process in the waterworks of Sant Joan Despí, which provides drinking water to the city of Barcelona, and in two artificial recharge plants located in Denmark and Sweden. The results obtained in these studies are also presented.

Acknowledgements

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NEW GAS-CHROMATOGRAPHY-BASED APPROACHES FOR THE MONITORING OF PESTICIDES.

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Introduction

The use of pesticides has an unquestionable benefit for agricultural production. However, after their application, residues of these chemicals remain in the crops and constitute a health risk because of their toxicity. To protect consumers´ health, the use of these deleterious chemicals has been restricted in many countries, which have established legal directives to control their levels in food, through maximum residue levels (MRLs). These restrictions refer to some of the most widely used classes of pesticides, such as triazines and organophophorus pesticides (OPPs), but also to another types of pesticides belonging to the emerging contaminants category, such as pyrethroids.

Most analytical procedures dealing with the determination of pesticides in fruit are protocols involving separate treatments for exhaustive extraction of a relatively large amount of sample (ca. 2−100g) and subsequent purification and concentration of the extracts before conventional chromatographic analysis. This results in very selective but time consuming and expensive protocols not really suitable for routine analysis. Matrix-solid phase dispersion (MSPD) can be regarded as a valuable alternative to these classical multi-step sample preparation methods allowing the extraction and clean-up to be carried out in a single step.

On the other hand, comprehensive two-dimensional gas chromatography (GC×GC) is a powerful separation technique in which two GC columns with different separation mechanism are coupled via an interface called *modulator*. This modulator is used to focus and efficiently transfer (*i.e.* re-inject) the entire effluent from the first column into the second one as consecutive narrow chromatographic bands. Parameters affecting the modulation step such as the cold and hot jet temperatures and the modulation frequency should be carefully optimised to preserve the separation achieved in the first column through the entire separation run. The fast GC separation that takes place in the second column depends on the nature of this stationary phase, its length and the offset of temperature between this column and the main oven. The main features of GC×GC, the influence of the experimental parameters in the final peak capacity and separation power as well as the main advantages of GC×GC as compared to other multidimensional chromatographic separation techniques for different application fields have been discussed in a number of reviews [1-4].

Up to now, the high peak capacity and distinctly superior separation power of GC×GC has been used to unravel classes of compounds in complex samples such as aromas, essential oils, petroleum mixtures, or to identify individual components within families of persistent pollutants with a large number of isomers, including polychlorinated biphenyls[5,6] and polychlorinated dibenzo-*p*-dioxins and furans[7], polybrominated diphenyl ethers and polychlonaftalenes[8]. However, to date, the

feasibility of GC×GC for pesticides analysis has been scarcely investigated[9,10] and, to the best of our knowledge, no research on group separation of close related families of these pollutants has been carried out.

The present study focuses on this latter topic and evaluates the relative merits of several column combinations for the simultaneous screening of selected environmentally relevant classes of pesticides (triazines, OPPs and pyrethroids) in different types of fruit samples. The feasibility of using this approach in combination with a fast miniaturised generic MSPD-based sample preparation method for the fast monitoring of pesticides in real (i.e. non-spiked) samples will be evaluated. The advantages and shortcomings of this type of generic approach as compared to more conventional instrumental analysis procedures based on GC–qMS will be discussed.

Materials and Methods

All solvents were of trace analysis grade and purchased from Merck Co. (Darmstadt, Germany) and Scharlau Chemie (Barcelona, Spain). C8 was purchased form IST (Mid Glamorgan, UK). The working standard solutions were prepared from individual congeners or from technical mixtures in isooctane (**Table 1**) at concentrations between 100 and 1000 pg/μl. Triazines were purchased from Chem Service (West Chester, PA, USA), OPPs from Dr. Ehrenstorfer (Augsburg, Germany) and pyrethroids from Sigma-Aldrich (St. Louise, USA).

Orange, grape, pear and apple samples were purchased from supermarkets in Madrid. After optimisation, the extraction and clean-up was carried out in a single step. Briefly, 100 mg of the untreated peel was dispersed on 100 mg of C8 and the mixture was packed on a solid-phase extraction barrel (3 mL). The extraction was carried out with 700 μL of ethyl acetate after washing of the sample with 15 mL of Milli-Q water (Millipore, Bedford, MA, USA), except for apple for which no clean-up was required. The extract was collected in a vial, concentrated under a gentle nitrogen stream, reconstituted in isooctane and directly subjected to GC–qMS analysis. No extra concentration was required before GC×GC analysis.

An Agilent 6890N (Agilent Technologies, Palo Alto, USA) equipped with the KT2003 loop modulator (Zoex Corporation, Lincoln, Nebraska, USA) was used for analysis. Details of the loop modulator principles have been described elsewhere[1]. Liquid nitrogen was used to create the cold jet, while the temperature of the hot jet heater was kept 80ºC over the main column temperature program. A secondary oven was programmed to track the main oven. Helium was used as carrier gas in the constant flow mode. The μECD was maintained at 300ºC throughout the study and nitrogen was used as make-up gas at a flow rate of 150ml/min. In all instances the modulation period was set at 4s with a 200ms hot jet pulse duration. Injections were performed in the splitless mode (1μl; splitless time, 0.75min) at 250ºC. Data acquisition rate was set at 50 Hz. The following GC columns (typically 30m × 0.25mm, 0.25μm film thickness) were tested as first dimension: ZB-5 (5% phenyl methylpolysiloxane), HT-8 (5% phenyl polysiloxane-carborane) and DB-17 (50% phenyl methylpolysiloxane). As second dimension, HT-8, BPX-50 (50% phenyl polysilphenilene siloxane) and a polyethylene glycol type (Supelcowax-10) columns were assayed (dimensions, 0.8 × 0.10mm, 0.10μm film thickness).

GC–qMS analyses were carried out on an Agilent 6890N equipped with an Agilent 5975 inert XL mass spectrometer detector. The previously described ZB-5 column was used for separation and, because of the intended comparison, the rest of the experimental conditions were kept as similar as possible to those used for GC×GC analysis. Data were acquired in the scan mode for triazines and OPPs (m/z range, 55-550), and in the selected ion monitoring (SIM) mode for pyrethroids (**Table 1**).

Table 1. Analytes investigated, retention time on a ZB-5 under the experimental conditions used and *m/z* ions selected for GC–qMS analysis.

Pesticide	Retention time (min)	*m/z* ion	Pesticide	Retention time (min)	*m/z* ion
OPPs			***Triazines***		
Dichlorvos	9.90	109/185	Atraton	18.37	211/196
Mevinphos	12.93	127/109	Simazine	18.43	201/186
Dimethoate	18.26	125/143	Prometone	18.57	225/210
Diazinon	19.52	179/152	Atrazine	18.62	215/200
Disuloforon	19.65	125/153	Propazine	18.77	229/214
Parathion-Me	21.05	263/125	Terbuthylazine	19.11	229/214
Paraoxon-Et	21.47	109/149	Symetryn	21.16	213/198
Malathion	22.29	125/173	Amtryne	21.33	227/212
Chlorpyrifos-Et	22.56	278/125	Prometryne	21.45	241/226
Fenthion	22.63	314/197	Terbutryn	21.86	241/226
Parathion-Et	22.65	291/139			
Bromophos-Me	23.19	331/125			
Chlorfenvinphos	23.94	267/323			
Bromophos-Et	24.55	331/303			
Ethion	27.01	231/153			
Pyrethroids					
Fenpropathrin	29.75	181/265	Cypermethrin (I)	33.33	265/163
Permethrin (I)	32.34	183/163	Cypermethrin (II)	33.86	265/163
Permethrin (II)	32.55	183/163	Cypermethrin (III)	34.03	265/163
Cyfluthrin (I)	33.31	265/163	Cypermethrin (IV)	34.24	181/163
Cyfluthrin (II)	33.48	265/163	Fenvalatate (I)	35.75	253/125
Cyfluthrin (III)	33.62	265/163	Fenvalatate (II)	36.21	253/125
Cyfluthrin (IV)	33.69	265/163	Deltamethrin (I)	37.56	265/181
			Deltamethrin (I)	38.60	265/181

Results

After optimisation, the proposed sample preparation method allowed fast extraction and (when required) clean-up of the studied pesticides from the tested fruits in a single step with minimum reagent and time consumption (see Materials and Methods section). In principle, these features could make the procedure to be considered as suitable for routine analyses. However, when using GC–qMS for final instrumental analysis, a severe matrix effect was observed for most of the analytes in all sample

types, which resulted in both overestimation and underestimation of the analyte concentration and prevented from direct quantification using the external calibration mode. Addition standard allowed to circumvent this shortcoming but with the obvious drawbacks of longer determination times, the need of using blank sample calibrations, and the requirement of two separate runs per sample, one for triazines and OPPs in the scan mode and another one for pyrethroids in the SIM mode. Under these conditions, satisfactory recoveries in the 80–105% and relative standard deviations (RSD) below 20% were obtained for most of the analytes (spiking level, 0.05 mg/kg, corresponding to the EU MRL set for most of the test analytes). Somehow lower recoveries and higher RSD were obtained for some pesticides in pear.

As regards the GC×GC experiments, the assayed column combinations were selected on the base of data previously reported in the literature[9,10], our experience, and the required orthogonality of the GC×GC separations. In all cases, thermally stable commercial phases with a relatively high upper temperature limit were used to avoid an unnecessary lengthening of the GC run. Although no offset of temperature relative to the primary oven was applied to the second dimension column, wrap-around was not observed within the 4s modulation period used throughout the study.

Although the compounds distribution into the GC×GC retention plane differed depending on the column combination used, all analytes were satisfactory separate both between and within groups under the experimental conditions finally proposed. However, the results obtained with the different test column combinations markedly differed when analysing real-life samples. A satisfactory resolution of the target compounds from other coextracted matrix components, preventing from false positive identification, was achieved with ZB-5×BPX-50. Column combinations involving DB-17 and HT-8 as first dimension did not provide such a separation and consequently were not further considered in this study. The use of polar stationary phases as second dimension column, such as Supelcowax-10, resulted in degradation of some of the coextracted matrix components and hampered proper identification of the analytes in real-life extracts.

As an example of the results obtained, **Figure 1** shows the typical reconstructed GC–qMS fragmentogram and the zoom of the corresponding GC×GC contour plot area obtained for a grape sample prepared using the miniaturised sample preparation method proposed, i.e. 100 mg of sample extracted with 700 μL of ethyl acetate and concentrated to a final volume of 50 μL in the former case, and directly analysed in the later.

Following the implications of these results, we conclude that the analytical approach proposed in the present study based on fast miniaturised MSPD of a small amount of sample (i.e. 100 mg) combined with direct GC×GC analysed of the collected extracts could be considered a valuable alternative to more conventional large scale multistep procedures in use for routine analysis of pesticides in fruits. The satisfactory resolution achieved among the target compounds and the coextracted matrix components could make GC×GC to be considered as a particularly interesting chromatographic approach in monitoring studies in which a larger number of samples of different nature are usually involved.

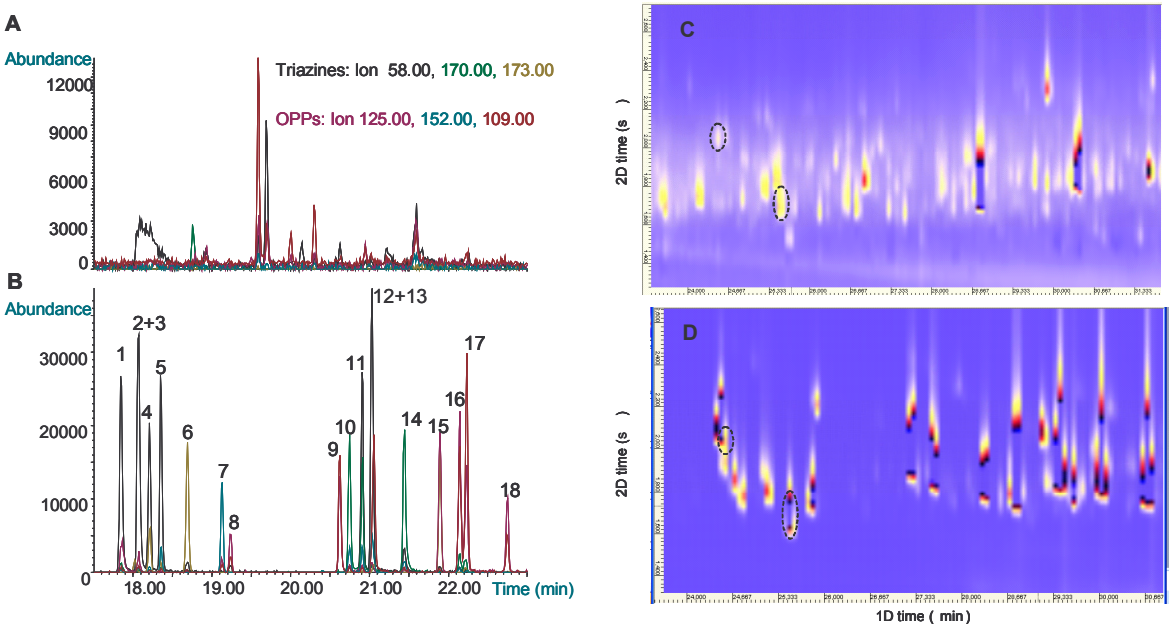

Figure 1. Zoom of a selected area (17.5-23.0 min) of the typical chromatograms obtained for a non-spiked grape by (A) GC–qMS (scan) using a ZB5 columns and (B) a mixture of pesticides (0.05 mg/Kg), and for the same grape extract (C) and mixture of pesticides (D) by GC×GC-microECD using ZB5×BPX50 as column combination. Peak numbering: (1) atratron, (2) simazine, (3) prometron, (4) atrazine, (5) promazine, (6) terbuthylazine, (7) diazinon, (8) disulfoton, (9) parathion-Me, (10) simetryn, (11) ametryn, (12) prometryn, (13) paraoxon-Et, (14) terbutryne, (15) malathion, (16) fenthion, (17) chlorpyrifos, and (18) bromophos-Me.

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SURFACE PLASMON RESONANCE IMMUNOSENSOR FOR ENVIRONMENTAL WATER SAMPLES ANÁLISIS

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The application of the optical sensor to environmental monitoring, as a field analytical method, can reduce the time and cost of environmental pollutants detection.
New immunoassays for continuous monitoring in water have been developed using a portable biosensor platform based on surface plasmon resonance (SPR) technology. A recent developed platform commercialised by the company SENSIA, SL (Spain) will be presented. This is a small size beta-SPR platform would allow its utilization in real contaminated locations.

Different examples of imunoassay applications will be presented, which are based on the binding inhibition between free antibodies with the analyte derivative covalently immobilized on a gold chip.
The use of Self-Assembled Monolayers (SAMs) allowing the regeneration of the same immunosurface throughout 150 cycles.

A validation procedure using a pesticide model example have been carried out for natural water samples analysis. The performance of this assay was evaluated in front of different types of water matrices including (river water, well water, wastewater and ultra-pure water). The validation of this system was accomplished comparing the results obtained by the immunoassay direct on the samples with those from a chromatographyc method based on solid phase extraction (SPE) coupled to gas chromatography- mass spectrometry (GC/MS). The main results of this study will be presented, as well as, the research lines carried out at the present in our group for the analysis of emerging pollutants in environmental samples.

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MONITORING AND BIOMONITORING OF HORMONES AND DRUG RESIDUES: SPECIFIC SCREENING BIOASSAYS AND COUPLING WITH ACCURATE MASS SPECTROMETRY

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Yeast based assays have several advantages. These include robustness, low costs, lack of known endogenous receptors and the use of media that are devoid of steroids. Recombinant yeast cells were constructed that express human estrogen receptor α (hERα), human estrogen receptor β (hERβ) or human androgen receptor (hAR) and yeast enhanced green fluorescent protein (yEGFP) as a reporter protein in response to exposure to estrogens or androgens respectively.

Although transcription activation assays based on human cell lines are more sensitive and may be able to identify compounds that require metabolism for activation into their active state, the latter is not necessarily an advantage. This is demonstrated by the ER-CALUX assay that is based on the T47 D human breast carcinoma cell line. With this ER-CALUX cell line it was demonstrated that estrone (E1) was converted to 17β-estradiol. In addition, estriol showed an unexplainable high potency in this assay and as a result water samples often contained large estrogenic activities.

Especially in the case of androgens, the lack of known endogenous receptors in yeast is a great advantage compared with mammalian cell lines, as androgen responsive elements (AREs) can also be activated by the progesterone and glucocorticoid receptor. As the glucocorticoid receptor is normally expressed in all mammalian cell types, the potential crosstalk with the glucocorticoid receptor is thus always present in these cell types. It is therefore not unexpected that dexamethasone was probably false described as an AR agonist in NIH Publication No: 03-4503, showing androgenic activity in 3 out of 4 mammalian cell reporter gene systems.

The yeast bioassays that were developed in our laboratory are sensitive, robust and very specific. When exposed to 17β-estradiol, the concentration where half-maximal activation is reached (EC_{50}) is in the range of 0.5 to 1.0 nM. Furthermore, this yeast estrogen bioassay showed no response to progesterone, MPA, testosterone and dexamethasone. The yeast androgen bioassay showed an EC_{50} of 40 nM for 17β-testosterone and dexamethasone did not give a response.

The yeast estrogen assay was validated as a qualitative screening method for the determination of estrogenic activity in calf urine and animal feed. This validation was performed, according to EC decision 2002/657, which prescribes the determination of the detection capability (CCβ), the specificity and stability. An ISO 17025 accreditation status was acquired for both matrixes and both methods are in routine use at RIKILT for about 2 years. A study performed on 126 calf urine samples shows that there is a good agreement between the estrogen bioassay result and the GC/MS analyses. In addition, this bioassay was responsible for detecting estrogenic activity

in several feed incidents. Identification of the responsible substances was carried out with an LC/estrogenbioassay/MS approach (see below).

In our latest study the feasibility of the new yeast androgen bioassay in combination with mass spectrometric identification was investigated for trace analysis of designer steroids in urine. Human urine samples were spiked with the designer anabolic steroid tetrahydrogestrinone (THG). Samples were analysed by gradient LC with effluent splitting toward two identical 96-well fraction collectors. One plate was used for androgen bioactivity detection using the yeast androgen bioassay yielding a bioactivity chromatogram. The diagram clearly showed the androgenic activity of THG in one of these fractions. Analysing the corresponding fraction in the duplicate well by LC/QTOFMS resulted in a [M+H]+ ion at m/z 313 and fragments with element compositions that were in full agreement with the recently proposed fragmentation scheme of THG.

Our results show that the yeast hormone bioassays are applicable tools for the detection and identification of hormone active substances in urine, feed, illegal supplements, water and environmental samples.

PHARMACEUTICAL SUBSTANCES: EMERGENT CONTAMINANTS OF THE AQUATIC SYSTEMS

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Beside principal classical chemical contaminants (PAHs, PCBs, pesticides, phtalates, trace metals, dioxins…), we find in the aquatic environment substances such as pharmaceutical substances. Beyond them we can considered various classes according to their therapeutic action: hormones, antidepressant , analgesics, antibiotics, lipid regulators, …. Important quantities of these molecules are consumed in our occidental society (Table 1) and are rejected *in fine* in the aquatic media via sewage treatment plants (incomplete destruction). They are increasingly studied as they could represent a non negligible environmental risk when considering on one hand the potentially important quantities introduced in the aquatic media and on the other hand the fact that they have been designed in order to be biologically active.

These compounds could have important toxic effects towards aquatic organisms but in order to estimate environmental risks there is a need for data documenting the effective contamination of the aquatic environment by these molecules.

The presentation will focuss on different aspects. After a general introduction of the context and the situation, the presentation will deal with development of analytical protocols in order to analyze different classes of pharmaceuticals in aquatic media (dissolved phase, particulate matter, biological tissues). These developments involve both extraction and purification methods such as SPE, SPME and microwave assisted extraction but also analytical developments for identification and quantification by GC/MS or MS/MS.

The work presented will deal with the development of an extraction procedure that makes possible to measure at trace level ($ng.l^{-1}$) (Table 2) many pharmaceuticals belonging to very different chemical classes: anti-inflammatory drugs, antidepressants, hypolipidic drugs…

Reliability and sensitivity have been tested on 18 different compounds (7 neutral compounds and 11 acidic drugs) extracted simultaneously and analyzed with two GC-MS methods. Different applications will demonstrate the multi-residue but also multi-matrix characteristics of the developed method.

The use of semi-permeable membrane devices (POCIS type) in order to get access to integrative sampling procedure (necessary when considering the variability of aquatic contamination) have been be also investigated and results will be presented. The aim of the study was to determine the sampling rates (Rs; expressed as effective volumes of water extracted daily) of POCIS device for 14 pharmaceuticals in several conditions of temperature, salinity and analytes concentration. These values are influenced by significant changes in water temperature, salinity. Overall POCIS Rs values were independent of aqueous concentrations. After laboratory experiments, environmental field has been deployed, showing qualitative application of POCIS

devices on contaminated system: the Seine Estuary (Figure 1). The suitability of the devices for monitoring multiple media under a wide range of environmental conditions will be also discussed.

All the analytical developments have been applied to several environmental case studies. Various French estuaries (Seine, Loire, Gironde, Adour) have been studied as well as marine locations (Arcachon Bay and Marseille coast). In all cases it has been possible to detect quite important concentrations of pharmaceutical substances. Measured concentrations fluctuate between few nanograms per liter and dozens of micrograms per liter depending on compounds, sampling stations and seasons (Table 3). The results have proved that, if dissolved phase is the most contaminated one, particulate phase could have a large part in the pharmaceuticals spread in aquatic systems (Figure 2). When pharmaceuticals occurrence in solid phase is observed, expressed in $ng.g^{-1,}$ some phenomena can be highlighted. High contents have been measured in the upper part of the seine estuary system (dam of Poses), with concentrations up to 1,220 $ng.g^{-1}$ for ketoprofen or 260 $ng.g^{-1}$ for naproxen. The solid phase can participate at a quite important extent to the water column contamination.

The understanding of the transfer of these compounds to aquatic organisms and of their toxicity is under progress as well as their impact on human health in relation to environmental contamination. There is really very few data at this moment on this aspect and investigations are really needed in order to gain a better knowledge. Some examples will be presented showing the possibility of transfer of these compounds.

Table 1: Consumed quantities experessed as tons per year.

Compounds	Therapeutic Class	UK (2000) (a)	Germany (1995-1997) (b)	Australia (c)	France (d)
Paracetamol	Analgesic	2000	-	295	2294
Aspirin	NSAID	770	> 500	20	880
Ibuprofen	NSAID	-	105-180	14	166
Erythromycine	Antibiotic	27	-	11	
Ketoprofen	NSAID	-	0,7	4	
Diclofenac	NSAID	26	75	4	39
Penicilline V	Antibiotic	22	140	9	

(a) (Webb, 2001)
(b) (Hirsch *et al.*, 1999 ; Ternes, 2001 ; Ternes *et al.*, 1998)
(c) (Khan et Ongerth, 2004)
(d) (Janex *et al.*, 2002)

Table 2: Limits of detection obtained for natural waters (expressed in ng.l^{-1}, for 1 l extracted for tap and surface waters, 500 ml for wastewater effluent).

	Tap water	Surface water	Waste water effluent
Amitryptiline	0.7	2.2	3.4
Aspirin	0.2	2.1	7.8
Caffeine	1.5	2.5	14.3
Carbamazepine	0.8	1.4	11.6
Clenbuterol	0.6	0.3	2.0
Diazepam	0.4	1.4	6.9
Nordiazepam	0.4	1.4	6.9
Diclofenac	0.9	0.7	4.5
Doxepine	0.7	2.1	8.3
Gemfibrozil	0.1	0.3	1.6
Ibuprofen	0.1	0.1	2.4
Imipramine	0.7	1.2	6.9
Ketoprofen	0.3	0.7	5.8
Naproxen	0.1	1.0	3.1

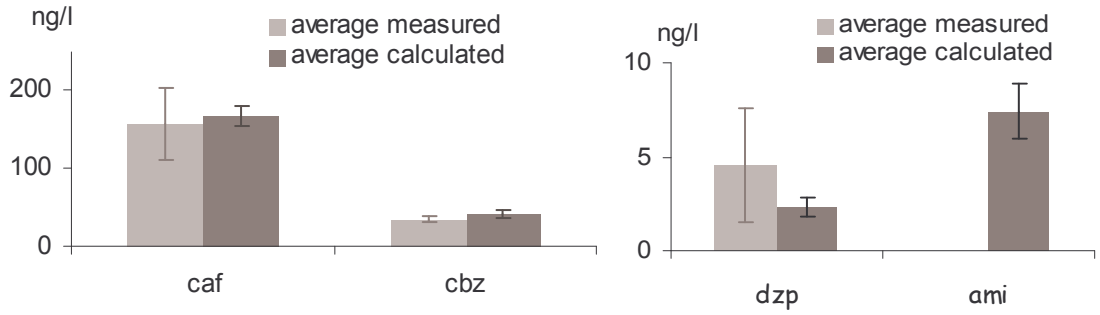

Figure 1 : Comparison of calculated (POCIS) and measured (discrete sampling) concentrations for three days of exposure.

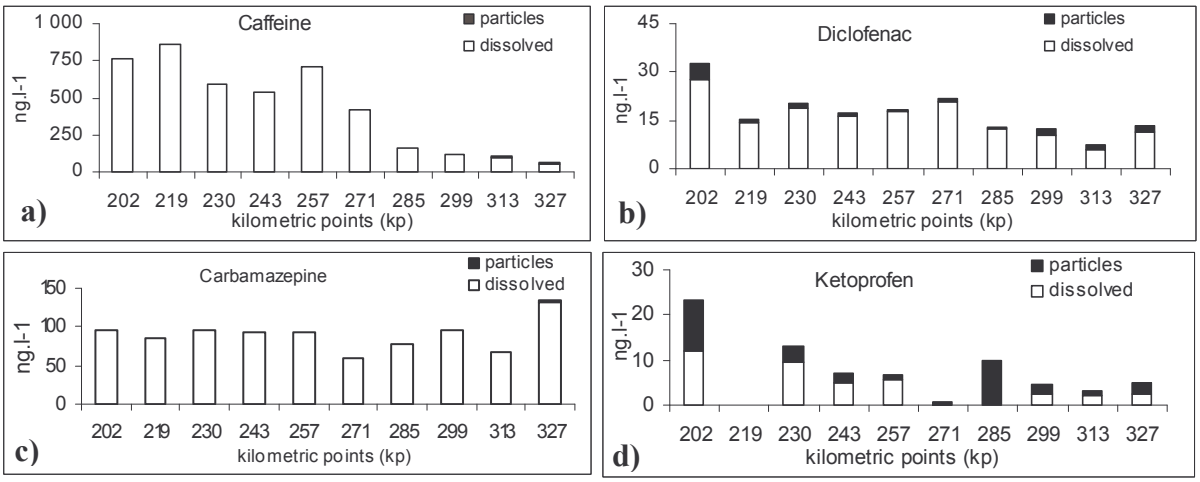

Figure 2: Pharmaceutical partition between dissolved and particulate phases.

a) caffeine; b) diclofenac; c) carbamazepine and d) ketoprofen

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LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY AS A POWERFUL TOOL FOR THE DETERMINATION OF PHARMACEUTICALS IN ENVIRONMENTAL SAMPLES

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Introduction

The occurrence of pharmaceutically active substances in the environment has become an important issue in the last few years. These compounds along with their metabolites, which can be even more harmful than the original compound, are continuously released in the environment, mainly through excreta, disposal of unused or expired drugs or directly from pharmaceutical discharges [1]. Numerous studies have shown that some pharmaceutical compounds are not removed during wastewater treatment processes, being therefore discharged to receiving surface waters, and subsequently found in ground and drinking waters [2,3].

As pharmaceuticals are found in the environment at low concentrations (ng/L and low µg/L), sensitive instrumental techniques are required to assess their occurrence, removal, partition and ultimate fate in the environment. Nowadays, liquid chromatography-tandem mass spectrometry (LC-MS/MS) is the method of choice to assay pharmaceutical residues and their metabolites due to its specificity and selectivity, enabling the detection of target compounds at low environmental levels [4]. Among tandem mass spectrometric techniques coupled to liquid chromatography, the most widely used are triple quadrupole instruments (QqQ). However, hybrid equipments are gaining popularity as they gather the advantages of different mass analyzers, providing great amount of structural information. This is the case of quadrupole-time-of flight (Q-TOF) and quadrupole-linear ion trap (Q-TRAP) instruments. With the former, several applications for the analysis of pharmaceuticals in environmental waters have been developed [5-7], whereas the use of Q-TRAP in the environmental field is still scarce. Since now, it has been widely applied in biomedical science, specifically for pharmacokinetic studies as well as for the determination of pharmaceutical residues in biological fluids [8-10].

Although QqQ allows a good identification of target compounds by using two MRM transitions, sometimes false positive determinations are committed due to other components present in the matrix having the same transitions as target compounds. When this happens, Q-TOF and Q-TRAP could be the suitable techniques to overcome these drawbacks. Q-TOF is a powerful tool for unequivocal identification and confirmation of target compounds due to their ability for exact mass measurements when recording full scan spectra, but it is not so suitable for quantitation purposes, since [5]. Otherwise, Q-TRAP is a promising and robust technique to be used for target environmental analysis as well as for confirmation purposes, as it can provide large amount of structural information in one single experiment and chromatographic run, due to its ability to combine the properties of quadrupole and ion trap analyzers. This means that it is able to perform quadrupole type scans, such as multiple reaction monitoring (MRM), neutral loss scan, precursor ion scan, among others, as well as the high sensitive ion trap scans. This is an

important advantage against other hybrid and triple quadrupole mass spectrometers, as it provides high accuracy when ensuring the presence of target compounds in complex environmental samples.

In this work, applications of liquid chromatography coupled to QqQ, Q-TOF and Q-TRAP instruments for the determination of pharmaceuticals in surface and wastewaters is described, standing out the advantages and drawbacks of each technique.

HPLC-QqQ TANDEM MS

A method based on off-line solid phase extraction for the simultaneous extraction of an extended list of 29 multiple-class pharmaceuticals followed by LC-tandem MS was developed. Target compounds studied belonged to the group of analgesics and anti-inflammatories, lipid regulators, psychiatric drugs, anti-histaminics, antibiotics and beta-blockers. This method was successfully applied in the analysis of target compounds in both surface and wastewaters from the Ebro river basin (in the North East of Spain). Chromatographic separation was achieved with a Purospher Star RP-18 endcapped column (125x2.0mm, particle size 5µm) and a C_{18} guard column, both supplied by Merck (Darmstadt, Germany). For increased sensitivity and selectivity, data acquisition was performed in multiple reaction monitoring mode (MRM) and for each analyte, two transitions between precursor ions and the two most abundant product ions were monitored, as indicated in table 1. Method detection limits achieved ranged from 1 to 60ng/L.

UPLC-Q-TOF

Q-TOF was coupled to a novel approach of Ultra Performance Liquid Chromatography (UPLC), using Acquity UPLC™ (Waters). In this case, the same 29 multiple-class pharmaceuticals were determined in wastewaters. Chromatographic separation was achieved with a 50x2.1mm ACQUITY C_{18} column. The major benefit from the use of the 1.7µm particles, and one advantage against conventional HPLC, was the increased column efficiency that resulted in narrower peaks and improved separation. Moreover, peak widths ranged from 0.1 to 0.2minutes, against the 1 minutes peak width obtained in HPLC, allowing very good separation of all compounds in a 10 minute run, another advantage against HPLC, where the elution required 30 minutes. Method limits of detection achieved with this technique ranges from 100 to 150ng/L, which were from one to two orders of magnitude higher than the ones achieved with triple quadrupole. However, it was a reliable tool to complement the identification of target pharmaceutical as indicated in Figure 1. First, UPLC-TOF chromatograms were recorded containing full scan spectral data, and the m/z of the target analytes was extracted from the total ion chromatogram (TIC), obtaining the accurate mass spectrum. For further confirmation, a MS-MS scan was performed, comparing the spectra obtained with a standard. XICs were extracted using a narrow 20mDa window, in order to increase selectivity, and errors obtained in the measurements were less than 3ppm.

Table 1. List of MRM transitions for the analysis of target compounds

Group of compounds	Target compounds	RT (min)	Precursor ion	CV-CE	MRM 1*	CV-CE	MRM 2**
Analgesics and antiinflammatories	Ketoprofen	14.71	253 $[M-H]^-$	30-10	253>209	30-15	253>197
	Naproxen	13.89	229 $[M-H]^-$	26-10	229>185	26-20	229>169
	Ibuprofen	18.48	205 $[M-H]^-$	20-10	205>160	-	-
	Indomethacine	20.03	356 $[M-H]^-$	20-20	356>297	20-20	356>312
	Diclofenac	18.87	240 $[M-H]^-$	20-20	294>250	20-15	294>214
	Mefenamic acid	19.42	240 $[M-H]^-$	20-20	240>196	20-30	240>180
	Acetaminophen	4.34	152 $[M-H]^+$	25-15	152>110	30-20	152>93
	Phenylphenazone	19.34	231 $[M-H]^+$	20-20	231>189	20-25	231>201
Lipid regulator and cholesterol lowering statin drugs	Clofibric acid	12.48	213 $[M-H]^-$	20-10	213>127	20-15	213>85
	Gemfibrozil	21.19	249 $[M-H]^-$	20-30	249>121	-	-
	Bezafibrate	16.87	360 $[M-H]^-$	20-20	360>274	20-20	360>154
	Pravastatin	18.14	447 $[M+Na]^+$	30-20	447>327	-	-
	Mevastatin	27.91	391 $[M-H]^+$	20-15	391>185	20-30	391>159
Psychiatric drugs	Carbamazepine	19.58	237 $[M-H]^+$	20-20	237>194	25-20	237>192
	Fluoxetine	21.82	310 $[M-H]^+$	25-10	310>148	20-10	310>44
	Paroxetine	20.44	330 $[M-H]^+$	30-20	330>192	30-30	330>123
Antiulcer agent	Lansoprazole	20.32	370 $[M-H]^+$	20-15	370>252	20-20	370>205
Histamine H_1 and H_2 receptor antagonists	Loratadine	27.94	383 $[M-H]^+$	30-20	383>337	30-30	383>259
	Famotidine	3.58	338 $[M-H]^+$	20-20	338>189	20-10	338>259
	Ranitidine	3.43	315 $[M-H]^+$	20-20	315>176	20-30	315>130
Antibiotics	Erythromycin	19.06	734 $[M-H]^+$	20-20	734>576	20-20	734>558
	Azythromycin	16.14	749 $[M-H]^+$	30-30	749>591	30-30	749>158
	Sulfamethoxazole	15.14	254 $[M-H]^+$	20-25	254>92	20-15	254>156
	Trimethoprim	10.55	291 $[M-H]^+$	30-20	291>230	30-30	291>261
	Ofloxacin	10.11	362 $[M-H]^+$	20-15	362>316	20-20	362>318
ß-blockers	Atenolol	3.30	267 $[M-H]^+$	30-20	267>190	20-30	267>145
	Sotalol	3.82	273 $[M-H]^+$	20-10	273>255	20-20	273>213
	Metoprolol	14.07	268 $[M-H]^+$	25-20	268>159	30-30	268>133
	Propanolol	18.04	260$[M-H]^+$	25-20	260>183	25-20	260>116

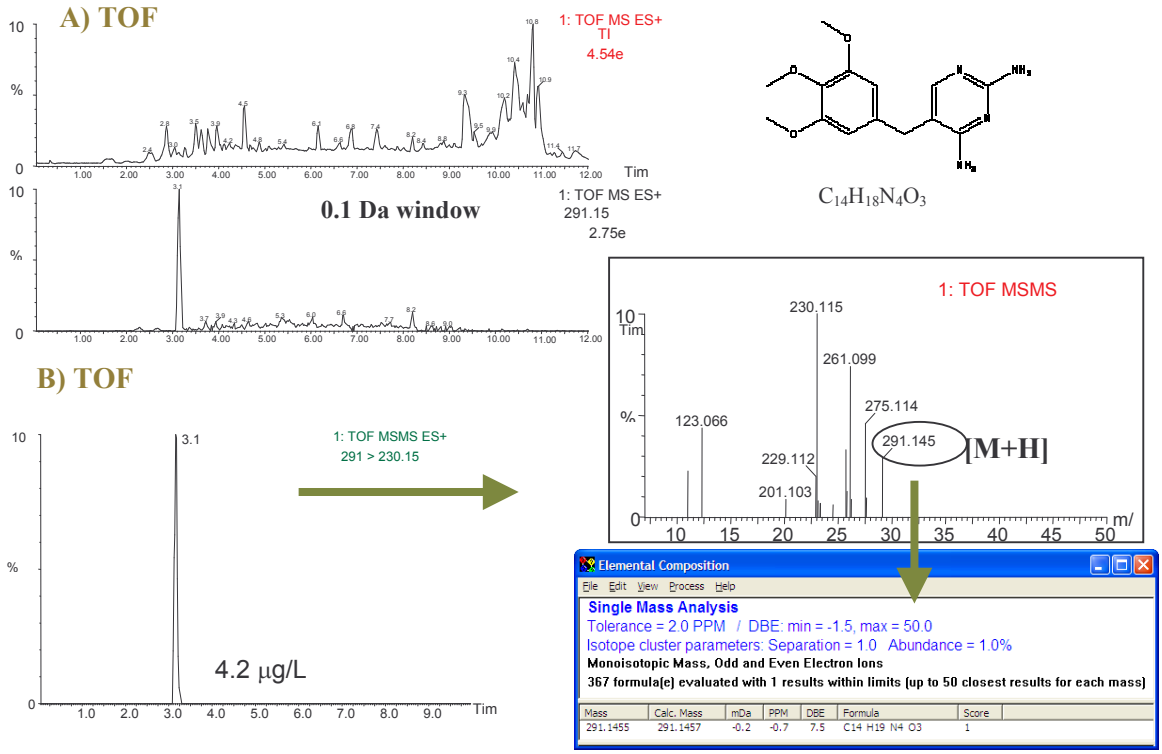

Figure 1. Determination of the antimicrobial trimethoprim in urban wastewater by UPLC-Q-TOF

HPLC-Q-TRAP

A method based on molecularly imprinted polymers (MIP) followed by HPLC-Q-TRAP (4000QTRAP, Applied Biosystems, MSD Sciex) for the determination of eight β-blockers in surface and wastewater was developed. For increased sensitivity and selectivity, target analytes were determined by multiple reaction monitoring mode (MRM), but in order to achieve extra confirmation, an MS/MS scan, with Q3 working in ion trap mode was performed in the same experiment as the MRM. Such combination was performed working in the Information Dependent Acquisition (IDA) mode. In a typical IDA experiment, an MS survey scan is used to generate a peak list of all ions present, which is subjected to a set of user defined criteria to filter out unwanted precursor ions. The remaining ions are then submitted for MS/MS. This cycle is repeated through the duration of the acquisition to generate large amounts of informative data. Chromatographic separation was achieved with the same column as in the HPLC-QqQ method. Method detection limits achieved ranged from 0.2 to 8ng/L, one order of magnitude lower than the ones achieved in QqQ and two orders than QTOF. An example of IDA experiment is illustrated in figure 2. As it can be observed, on the left side of the screen, all target analytes are detected (are indicated by their m/z). By clicking on the desired compound (for atenolol m/z=267), MRM and EPI are illustrated.

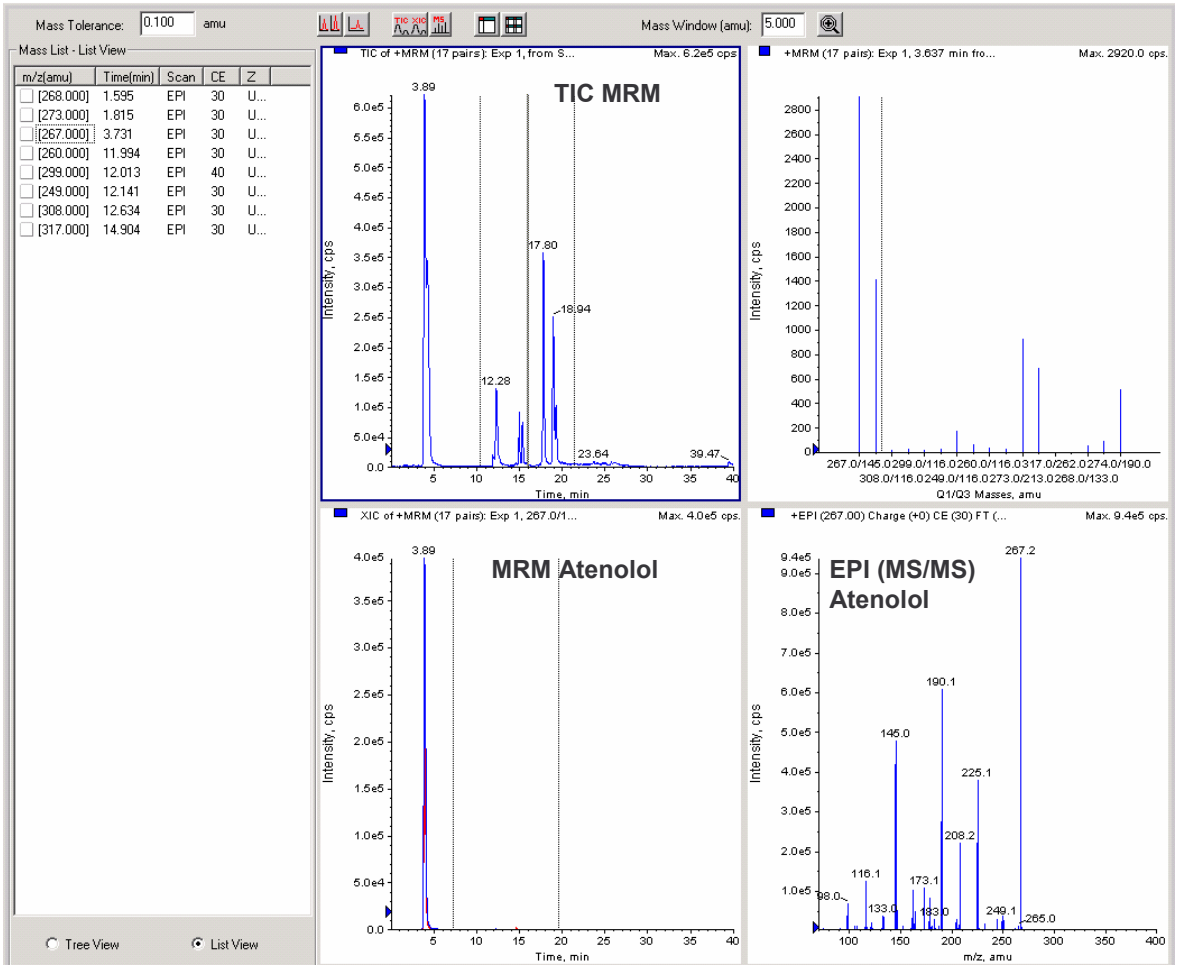

Figure 2. Determination of Atenolol by MRM and extra confirmation with Enhanced Product Ion Scan (EPI) in a wastewater influent sample.

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DETERMINATION OF PHARMACEUTICAL COMPOUNDS IN WATER BY SOLID-PHASE EXTRACTION-LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

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INTRODUCTION

The focus of environmental research has been extended from traditional pollutants such as polychlorinated biphenyls, polycyclic aromatic hydrocarbons and pesticides, to emergent pollutants such as pharmaceuticals and personal care products, some of which may be carcinogenic, mutagenic and reproductively toxic. Pharmaceutical substances are used in human and veterinary medicine and can enter the aquatic environmental following manufacture, use or ingestion/excretion. The rapid rise in the use of pharmaceutical products is a new environmental problem. In 1999, the European Science Foundation organised the “Pharmaceuticals in the Environment” workshop to begin exploring this issue. Considering the large number of registered pharmaceutical ingredients (>3000) and the larger number of corresponding metabolites, analytical methods have only been developed for a small subset of compounds (~150) in environmental matrixes. In the last decade high-performance liquid chromatography (HPLC) coupled to electrospray ionization tandem mass spectrometry (ESI-MS-MS) has become the analytical technique of choice for the determination of polar environmental pollutants. In this article, a method was developed for the determination of various pharmaceuticals by solid-phase extraction (SPE) and LC-ESI-MS-MS, in either positive ionization (PI) or negative ionization (NI) modes. A number of parameters that may affect the recovery of target compounds, such as the type of SPE cartridges, eluents, as well as water properties including pH value, salinity, colloid and surfactant were investigated. The established method was also applied to environmental water samples for the determination of the target pharmaceuticals.

METHOD

All the solvents used including methanol, ethyl acetate, acetone, dichloromethane (DCM), hexane and acetonitrile, purchased from Rathburns, were of distilled-in-glass glade. Formic acid was of HPLC grade. Propranolol, sulfamethoxazole, meberverine, carbamazepine, mecoprop, indomethacine, diclofenac, meclofenamic acid, calmagite and monensin were purchased from Sigma, UK. Internal standard (diuron-d_6 and ^{13}C-phenacetin) were purchased from Cambridge Isotope Laboratories, USA. Separate stock solutions of individual compounds were made up at 1000 mg/l by dissolving an appropriate amount of each substance in methanol. Internal standard solutions (1 mg/l) of diuron-d_6 and ^{13}C-phenacetin were prepared in methanol.

The target compounds were extracted from water samples by different SPE cartridges (Table 1). One hundred nanogram of each target compounds were spiked

in 1 L of ultrapure water for the recovery test. All the cartridges were first conditioned with 10 ml of methanol, ultrapure water (3×5ml) was passed through the cartridges at a rate of 1-2 ml/min. Then, water samples were extracted at a flow rate of 5-10 ml/min. After the extraction, the cartridges were dried under vacuum for 30 min, with the analytes being eluted to 20 ml vials from the sorbents with 10 ml of solvents (e.g. methanol) at a flow rate of 1 ml/min. The solvents were blown down to 0.5 ml under a gentle nitrogen stream and then subjected to LC-MS-MS analysis.

Table 1. A summary of the different types of SPE cartridges being studied

Cartridges	Descriptions	Manufacturer
DSC-C18 (0.5g, 3	Polymerically bonded. Octadecyl	Supelco
DSC-Si (0.5g, 3ml)	Unbonded acid washed silica sorbent	Supelco
DSC-SCX (0.5g, 3	Aliphatic sulfonic acid, Na^+ counterion	Supelco
DSC-SAX (0.5g,	Quaternary amine, Cl^- counterion	Supelco
Strata X-CW (0.5g,	Polymeric weak cation	Phenomenex
Strata SDB-L (0.2g,	styrene-divinylbenzene polymeric	Phenomenex
Chrom.-Easy (0.2g,	Bifunctionally modified polystyrene-	Macherey-Nagel
Chrom.-C18	Octadecyl-modified silica	Macherey-Nagel
Chrom.-Drug (0.2g,	Modified silica	Macherey-Nagel
Isolute C18 (1g,	Octadecyl	Int. sorbent
Isolute C18/ENV$^+$	C18 Hydroxylated polystyrene-	Int. sorbent
Oasis HLB (0.2g,	Poly(divinylbenzene-co-N-	Waters

The LC separation was performed using a Waters 2695 HPLC separations module (Mild ford, MA, USA) equipped with a Waters Symmetry C_{18} column (4.6×75 mm, particle size 3.5 μm). Eluent A was 0.1% formic acid in Mili-Q water, eluent B was acetonitrile and eluent C was methanol at a flow rate of 0.2 mL/min. The elution started with 10% of eluent B, followed by a 25-min gradient to 80% of eluent B and a 3-min gradient to 100% of eluent B, and then changed to 100% of eluent C within 8-min and held for 10 min, and back to the initial conditions within 4 min. The re-equilibration time was 10 min. The injection volume was 5 or 10 μl. The tandem MS analyses were carried out on a Micromass Quattro triple-quadrupole mass spectrometer equipped with a Z-spray electrospray interface.

RESULTS AND DISCUSSION

After preliminary experiments, a good chromatographic separation of the compounds was achieved using the gradient described earlier, both in PI and NI mode (Fig. 1), with retention times in the range 9-29 min for the analysis in the PI mode and in the range 19-28 min in NI mode. The ESI interface parameters were optimised for all individual compounds in the PI and NI mode in order to obtain the best instrumental conditions for the identification of target compounds. Most of the compounds showed better sensitivity operating in the PI mode with the exception of mecoprop and calmagite. For the internal standard, ^{13}C-phenacetin was analysed in the PI mode, diuron-d_6 could be detected in both PI and NI mode. The protonated ($[M+H]^+$) or deprotonated molecules ($[M-H]^-$) were the base peak for all compounds and selected as precursor ions, except for diclofenac with 250 as the precursor ions. Precursor ions and their daughter ions used for confirmation and quantitation are listed in Table

2. Limits of detection (LOD) for the method were 34-1245 pg/L and limit of quantification (LOQ) 0.11-4.16 ng/L.

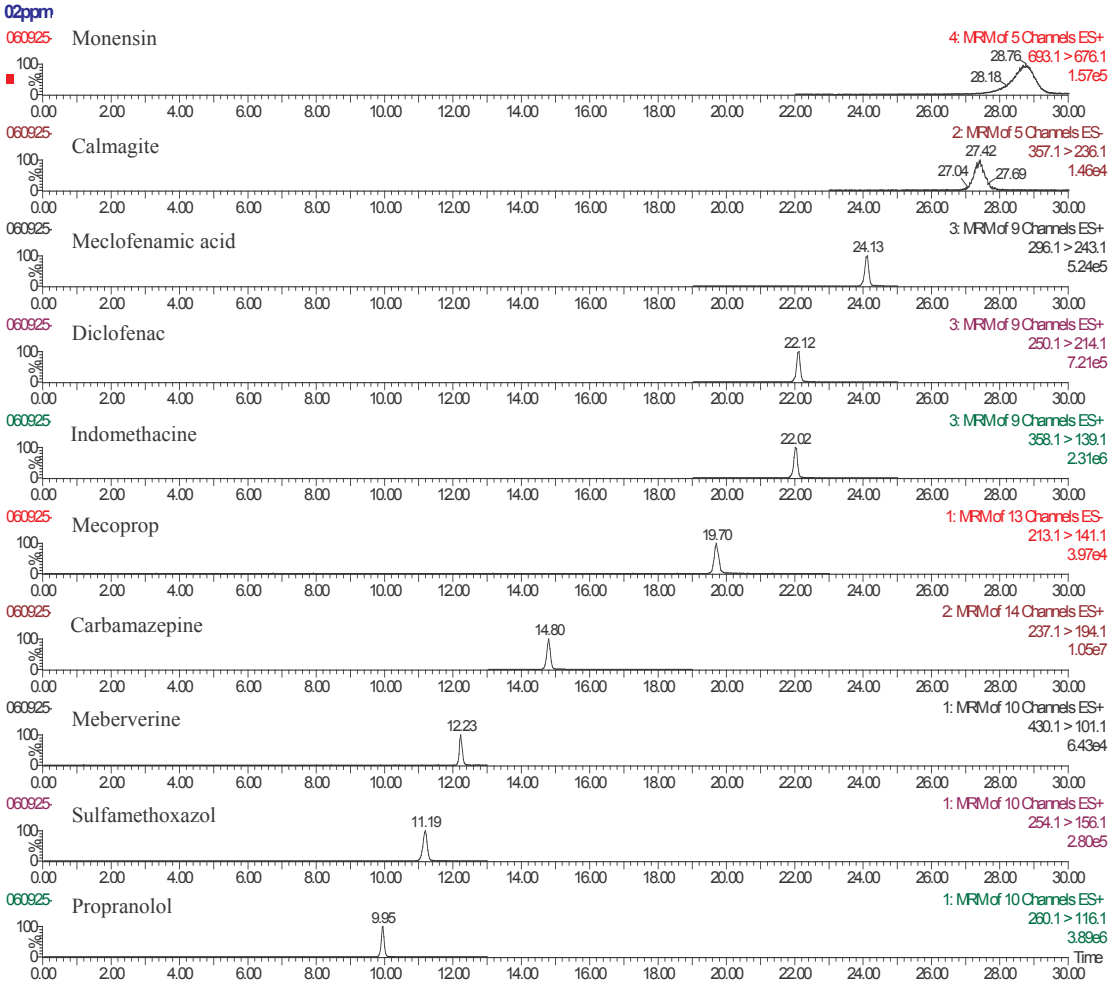

Fig.1. Chromatograms for multi reaction monitoring (MRM) of target pharmaceuticals

The optimisation of an appropriate SPE cartridge with different sorbent materials plays a key role in the achievement of high and reproducible recovery for contaminants. In this study, several types of cartridges (Table 1) from different manufacturers were selected for the evaluation of extraction efficiency of pharmaceuticals. When methanol was used as elution solvent, poor recoveries (< 40%) for all of the compounds were observed on DSC-Si (1g, 6 ml), DSC-SCX (0.5g 3 ml) and DSC-SAX (0.5g 3 ml). DSC-C18 with polymerically bonded octadecyl, Chromabond-Easy (0.2g, 6 ml), Isolute C18 (1g, 6 ml) and C18/ENV+ (0.4g, 6 ml) showed slightly better but still unsatisfactory recovery (<70%). Much improved recoveries (from 70% to 98%) for carbamazepine, indomethacine, diclofenac and meclofenamic acid were observed on Strata X-CW (0.5g, 6 ml), Strata SDB-L (0.2g, 3 ml), Chromabond-C18 Hydra (0.5g, 6 ml) and Chromabond-Drug (0.2g, 3 ml). Of all the cartridges, Waters Oasis HLB (0.2g, 6 ml) copolymer cartridges showed the best recoveries overall (70-95%) and were therefore used for further testing.

Table 2 LC-ESI-MS-MS condition and LOD (LOQ) for the analysis of pharmaceuticals by MRM in PI and NI mode

Compounds	Ionization mode	RT (min)	Molecular Weight	Collision Energy (ev)	Prescursor ion(m/z)	Product ion (m/z)	LOD (pg/L)	LOQ (ng/L)
Propranolol	PI	9.95	259	20	260	116,183	64	0.21
Sulfamethoxazole	PI	11.19	253	25	254	156,92,108,155	555	1.84
Meberverine	PI	12.23	429	25	430	101,135	755	2.51
Carbamazepine	PI	14.80	236	20	237	194,193	34	0.11
Mecoprop	NI	19.70	214	20	213	141,140	1245	4.16
Indomethacine	PI	22.02	357	25	358	139,141,174	138	0.46
Diclofenac	PI	22.12	295	25	250	214,213,215	156	0.52
Meclofenamic acid	PI	24.13	295	25	296	243,242	810	2.71
Calmagite	NI	27.42	358	20	357	236,235	820	2.73
Monensin	PI	28.76	692	25	693	676,677,678	188	0.63

The recovery of target compounds by SPE is highly dependent on the polarity of the eluents. Acetone, DCM, ethyl acetate, hexane and methanol as eluents were tested for the elution recovery of pharmaceuticals (spiked at 100 ng/l) from Oasis HLB cartridges. The results show that hexane produced poor recovery for most of the compounds at less than 30%, which may be due to the relatively polar nature of these compounds. Better recoveries were obtained with DCM, ethyl acetate and acetone as the elution solvents, with most varying between 50% and 105%. The best recoveries (80-100%) were achieved with elution by methanol. Accordingly, methanol was chosen as the solvent for the simultaneous extraction of all pharmaceuticals.

Natural waters can have different properties such as varying salinity, pH value, colloid content and surfactant concentration, which may affect the extraction efficiency on the SPE. It was decided to investigate the effects of different salinity (0-35), pH value (4-10), colloids (0-10 mg/L) and surfactant concentration (0-10 μg/L) on recovery. The extraction efficiency for propranolol, sulfamethoxazole and carbamazepine was enhanced with increasing salinity in water. Some of these parameters did not have any effect on the recovery, for example, the effect of pH is minimal. The method developed was applied to the determination of target pharmaceuticals in river water samples from East Sussex, UK. The results show levels of pharmaceuticals comparable to river waters elsewhere.

PHARMACEUTICAL RESIDUES REMOVAL IN A PILOT WASTEWATER TREATMENT PLANT

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It is known that conventional wastewater treatment plants (WWTP) effectively remove easily biodegradable matter, while persistent organic pollutants can penetrate through the natural filtration steps or man-made treatments to contaminate the aquatic environment (Öllers et.al., 2001). This is especially true for trace amounts of polar persistent pollutants: pharmaceutical residues being a case in point. Pharmaceutical compounds need to be relatively hydrophilic as they reach their target site via bodily fluids; further, they need to be chemically stable so as to avoid first-pass degradation by metabolic enzymes.

How pharmaceuticals enter into the environment is a function of several factors: the quantity manufactured and consumed; dosage (amount, frequency, duration); the excretion efficiency of the parent compound and its metabolites; adsorption/desorption onto soil; and metabolic decomposition during sewage treatment. The actual amount of pharmaceuticals and their bioactive contaminants entering into the environment maybe low but their continuous input may lead to a high, long-term concentration resulting in adverse effects on aquatic and terrestrial organisms (Díaz-Cruz et. al., 2003). Municipal WWTPs have been recognised as a point source of pharmaceutical discharge, originating from urinary or faecal excretions or by the improper disposal of unused or expired drugs down the toilet. Other important sources of these compounds are discharges from hospitals and the pharmaceutical industry. By recognising the possibilities for their removal in WWTPs it is possible to limit or prevent their occurrence in the environment.

The aim of our study was to develop new or improve existing technology for the removal of pharmaceutical product residues during wastewater treatment. To achieve this we designed a pilot wastewater treatment plant (PWWTP) using an actual activated sludge from a Slovenian municipal waterworks. To determine the rate of removal and degradation of pharmaceuticals we applied our own previously developed analytical procedure (Kosjek et.al., 2005) and selected a range of pharmaceuticals according to their high rate of consumption, known presence in the aquatic environment (Kümmerer, 2001; Kosjek et.al, 2005), reported toxicity (Oaks et.al, 2003) and their environmental persistence (Daughton, 2001; Díaz-Cruz et.al., 2003). The compounds we chose to study were acidic pharmaceuticals including four members of the group of nonsteroidal anti-inflammatory drugs referred to as NSAIDs: ibuprofen, ketoprofen, diclofenac and naproxen, and clofibric acid (CLA), which is an active and stable metabolite of clofibrate used as a blood lipid regulator. The common structural characteristics of these pharmaceuticals i.e., the presence of a

carboxylic group, an aromatic ring, an alkyl chain, enabled us to determine their presence in aqueous samples using a single analytical procedure (Kosjek et.al, 2005). The procedure involves solid phase extraction (Strata^{TM} X, Phenomenex), followed by derivatisation with MSTFA (N-methyl-N-(trimethylsilyl) trifluoroacetamide) and separation and identification using gas chromatography with mass spectrometric detection (HP 6890 instrument). Separation of the trimethylsilyl derivatives was achieved chromatographically on a 30m x 0.25mm x 0.25µm Hewlett-Packard HP-5 MS capillary column. Compounds were quantified using Mecoprop as an internal standard.

Our pilot wastewater treatment plant consists of three parallel reactors (R0, R1 and R2), two of which are operated under continuous input of high (R1 = 50 µg L^{-1}) and low (R2 = 5 µg L^{-1}) concentrations of NSAIDs and CLA, while the third (R0) was the control with no pharmaceuticals added to the nutrient-mineral medium. The efficiency of elimination in R1 and R2 was calculated as the difference between the influent and effluent concentration. The removal rate of a single compound was determined using Equation 1, where % el.eff. is the percent elimination efficiency, $c_{X\text{-}INF}$ the concentration of the compound in the influent and $c_{X\text{-}EFF}$ the concentration of the same compound present in the effluent.

$$\%\ el.eff. = \frac{\left(c_{X-INF} - c_{X-EFF}\right)}{c_{X-INF}} \times 100 \qquad \text{Equation 1}$$

The results (Table 1) show a high and constant removal of ibuprofen, ketoprofen and naproxen (≥ 89 %) after 2 years of continuous operation. In contrast, the elimination efficiency of diclofenac elimination was much lower (54 – 56 %) with a greater standard deviation in the results. According to the literature, diclofenac is present in wastewater effluents in several countries in concentrations from 0,25 to 49 µg/L, resulting from its incomplete elimination (50 -75%) in WWTP (Buser et.al., 1998; Ternes, 1998).

PHARMACEUTICAL	REACTOR	% el.eff	STANDARD DEVIATION (%)
IBUPROFEN	R1	95,6	4
	R2	96,4	6
NAPROXEN	R1	96,1	3
	R2	88,8	8
KETOPROFEN	R1	94,9	5
	R2	90,3	6
DICLOFENAC	R1	54,4	23
	R2	56,4	36
CLOFIBRIC ACID	R1	24,2	22
	R2	29,5	20

Table 1: % elimination efficiency and standard deviation of acidic pharmaceuticals in the PWWTP

Clofibric acid (CLA) was included in our study only recently (may 2006) and as yet no adaptation of the biomass has taken place despite us operating the model reactors at a high sludge age (estimated to be > 100 days). Clofibric acid is known to be highly resistant to biodegradation and, according to the literature (Heberer, 2002; Tauxe-

Wuersch et.al., 2005; Zwiener and Frimmel, 2003), we do not expect a substantially higher removal rate than that we give in Table 1.

To our knowledge, hydraulic retention time (HRT) can influence significantly biodegradation. Therefore, a HRT of 48 hours was selected, which means a daily feed rate of 2.0 L in the 4 L model reactor. Similarly, although temperature can be an important factor influencing the rate of (bio) degradation including abiotic thermal degradation, we did not consider it as such since no significant fluctuations of the temperature within the PWWTP were recorded during the two years of operation [T = 22 ºC ± 5 %],.

In addition to biodegradation, we are also interested in other possible removal mechanisms, such as abiotic degradation or adsorption on to the activated sludge and onto the reactor walls. At the end of the experiment, we intend to extract the reactor walls, tubes and other surfaces in contact with the test compounds, including the activated sludge, to determine the amount of adsorbed compounds. Because the compounds under study are polar we expect them to be highly mobile in water and therefore do not expect the actual losses due to adsorption to be significant (Urase et.al., 2005). Since photodegradation (Andreozzi et.al., 2003; Buser et.al., 1998; Pérez-Estrada et.al., 2005) and oxidation by ozone and H_2O_2 (Zwiener and Frimmel, 2000) are also successful mechanisms for the degradation of diclofenac, we will also investigate technology based on such phenomena as a potential complementary treatment methods.

For polar compounds like acidic pharmaceuticals microbial degradation is the most important removal pathway in the activated sludge wastewater treatment process. While transformation of pharmaceuticals in the human body and other mammals has been studied extensively, the microbial degradation of such compounds including possible degradation pathways and products is yet to be fully investigated and remains largely unknown (Quintana et. al., 2005). Therefore, we focus our attention on detecting the degradation products of the tested compounds during the treatment in our PWWTP. By comparing total ion chromatograms (TIC) (Figure 1) we observed additional peaks in the chromatograms of the effluent extracts, possibly originating from NSAID degradation. On the basis of the similarity between the mass spectra (mass fragments and their abundance) we were able to identify and explain the origin of these peaks. We were also able to make a preliminary identification of these compounds by matching their spectra to the spectra of known compounds held in the NIST library (National Institute of Standards and Technology). In this manner we identified three possible degradation products of diclofenac: (2,6-dichlorophenyl)-1,3-dihydro-2H-indol-2-one; 2-((2,6-dichlorophenyl)-amino)-benzyl alcohol; 2-((2,6-dichlorophenyl)-amino)-benzyl alcohol methyl ether] and one possible degradation product of naproxen [(6-methoxy-2-naphtyl) ethanol. Additionally, we were able to identify other potential degradation products the structure of which we intend to determine more precisely as part of our future work. We will apply high resolution mass spectrometry to characterise the chemical structures of all the identified compounds.

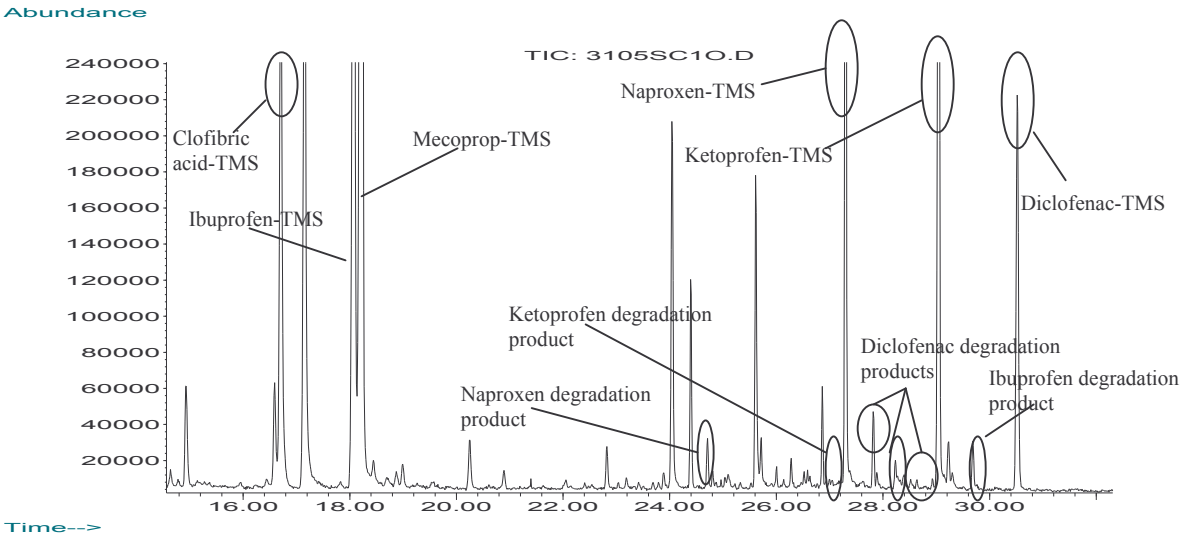

Figure 1: Total ion chromatogram of PWWTP effluent: trimethylsilyl esters (TMS) of the parent compounds, internal standard mecoprop and degradation products are signposted

Clearly, when assessing the risk that pharmaceutical residues pose to the environment it is also important that we do the same for their degradation products, that is by examining their toxicity since in some instances degradation products can be more toxic than the parent compound and looking at possible combination effects with other compounds. We intend to make ecotoxicity and genotoxicity tests of parent compounds vs. their degradation products as part of our future work. In addition, to obtain a better risk assessment regarding WWTP effluents and pharmaceutical residues in the environment, our toxicity testing will also include the PWWTP influent and effluent.

Finally, we used the polymerase chain reaction (PCR) method to analyse samples of biomass taken from each of our model reactors to reveal changes resulting from bacterial acclimation after two years of continuous operation. Our preliminary results reveal that the biomass composition in R1 (model reactor operating under high concentration of pharmaceuticals) differed from R2 and R0. In the future the biomass will be sampled repeatedly over a three month period in all three reactors and examined in detail using PCR and DGGE (denaturating gradient gel electrophoresis).

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BROMINATED FLAME RETARDANTS IN INDUSTRIAL EFFLUENTS: ANALYTICAL METHODS AND MONITORING RESULTS

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Introduction

Brominated flame retardants (BFRs) are a diverse group of chemicals that are used to improve fireproof properties of materials. They are incorporated into a wide range of products such as computers, TV sets, car parts, electric cables, textiles, paints and upholstery. Some of them, such as polybrominated diphenyl ethers (PBDEs), hexabromocyclododecane (HBCD) and to a lesser extent tetrabromobisphenol-A (TBBPA) have led to both scientific and public concern since they have been found to be persistent and accumulate in man and wildlife[1]. BFRs, in particular the lower brominated PBDEs, show tendency to disrupt thyroid hormones, cause neurobehavioral deficits and possibly cause cancer[2].

BFRs are classified into two groups, additive and reactive, depending on the mode of addition during the manufacturing process. Reactive BFRs (e.g. TBBPA) are incorporated covalently into the polymeric materials, additive BFRs (e.g. PBDEs and HBCD) are not, therefore it is thought that additive BFRs are more easily released into the environment.

BFRs are widespread in the environment. Most of the monitoring focuses on the occurrence of BFRs in sediments and biota[3]. Also in the Flemish Region such monitoring studies have been conducted [4,5]. In addition the Flemish Authority has built out a pollutant monitoring network for public water bodies using eel (*Anguilla anguilla*) as a biomonitor[6]. The monitoring results show that there exists a clear spatial variation in contamination which can be linked to differences in industrial activity and processing of waste streams. The Environmental Inspectorate Division (EID) therefore decided to start an investigation on the discharge of BFRs in industrial waste water. At first the EID selected different industrial sites which probably use BFRs in their production process (mainly textile processors and plastic manufacturers) and water treatment installations which treat the waste water from these production processes. Different samples of these waste waters were analysed. In order to get better insight in the amounts and types of BFRs used, a survey was conducted in the Flemish region. The results of the survey allowed to improve the selection of potentially BFR discharging companies and to extent the list of BFR compounds which might be relevant to monitor. In this study the results of the survey, the analytical methods used and the results of the monitoring campaigns are shown.

Survey of the use of BFRs in the Flemish Region

A priority list of potentially BFR discharging companies and BFRs could be established on the basis of trade data and questionnaires sent to over 500 companies. The companies had to provide information on the production or the use of BFRs and had to give an indication on the used amounts of the different

substances. An overview of the most widely used substances is given in table 1. For the lower molecular weight compounds the analytical feasibility was evaluated. Pentabromotoluene, pentabromoethylbenzene and 1,2-bis(2,4,6-tribromofenoxy) ethane were added to the list, on the basis of environmental levels reported in the States[7].

Table 1: overview of most widely used BFRs in Flanders (sorted from high to low)

BFR	**CAS**
Brominated polystyrene	88497-56-7
Decabromodiphenyl ether (DBDE)	1163-19-5
Hexabromocyclododecane (HBCD)	25637-99-4; 3194-55-6
Ammonium bromide	12124-97-9
Decabromodiphenylethane (DBDPE)	84852-53-9
Tetradecabromodiphenoxybenzene	58965-66-5
(Poly)pentabromobenzylacrylate	59447-57-3; 59447-55-1
TBBPA bis(2,3-dibromopropyl ether)	21850-44-2
Tetrabromobisphenol A (TBBPA)	79-94-7; 30496-13-0
Tris(2,3-dibromopropyl)isocyanurate	52434-90-9
N,N´-Ethylene bis(tetrabromophtalimide)	32588-76-4
TBBPA carbonate oligomer; 2,4,6-tribromo-phenol terminated	71342-77-3
Polydibromostyreen	148993-99-1
TBBPA diglycidyl ether	3072-84-2
TBBPA carbonate oligomer, phenoxy end capped	94334-64-2
Tris(tribromoneopentyl)phosphate	19186-97-1

Analytical methodologies for the determination of BFRs

For an overview of analytical methodologies see e.g. Covaci et al [8]. Due to the complex composition of some industrial effluents extracts are subject to appropriate clean up and measured with selective detection techniques. Multilayer acid/base silica clean up can be used for PBDEs, HBCD and other GC-amenable BFRs; sometimes an additional GPC clean up is necessary. Much attention has to be paid to the gas chromatographic conditions: on-column injection, short columns with an appropriate phase and a maximum oven temperature not exceeding 300°C are obvious[9]. The compounds can be analysed with conventional GC-EI-MS, but the technique is not very sensitive and not very selective. Most laboratories prefer NCI-MS detection which is very sensitive but the method still lacks some specificity (for most congeners only Br^- can be monitored and interference with other brominated compounds is likely to occur) and does not allow the use of isotopically labeled internal standards. Very selective detection can be realised with tandem MS or high resolution MS and both techniques were used in the monitoring campaigns.
HBCD can alternatively be analysed with LC-MS, using electrospay ionisation in negative mode. This allows the separation of the α-, β- and γ-isomers[10], but this is of minor importance in case of waste water analysis. TBBPA can selectively be analysed with LC-ES(-)MS/MS; alternatively in-vial derivatisation of the BFR extract allows the rapid and sensitive determination of TBBPA with GC-MS.

Monitoring

Experimental

Different monitoring campaigns were organized by the EID. Samples were collected at selected discharge points and analysed for PBDE, HBCD and other BFRs.

PBDE, HBCD and other GC-amenable BFRs: The samples were extracted with methylene chloride (DCM). If a "latex" emulsion was present the latex was separated from the water phase by coagulation with acid and centrifugation. The latex was subject to a soxhlet extraction with DCM. The combined water and latex extract was evaporated to 1 ml and a clean up was carried out using acid silica. The final extract was injected into a GC-ion trap-MS/MS (Thermo PolarisQ) or a GC-HRMS (Micromass Autospec Ultima) using a cold injection technique, a short column and electron impact ionisation. In case of GC-IT-MS/MS PBDEs were analysed in MS/MS mode, HBCD in single MS mode. Identification and quantification (by the internal standard method) were done according to ISO/DIS 22032. HBCD isomers were quantified assuming the same response factor for all isomers. To validate the method a waste water sample, resulting from the mixing of different BFR free textile effluents, was spiked with BDE and HBCD congeners. For all congeners recoveries were >77% and repeatabilities <13%. The limit of detection for BDE-209 was <0.3 µg/l, for other BDE congeners <0.02 µg/l and for HBCD <1 µg/l (500 ml intake, GC-IT-MS/MS). In case of GC-HRMS fifty to hundredfold lower detection limits were obtained.
TBBPA: Samples were extracted with SPE on C18 and eluted with methanol. Analysis was performed with LC-ES(-)MS/MS (Micromass Quattro II, upgraded with Z-spray)
Antimony: Samples were treated with $HCl/HNO_3/H_2O_2$ and measured with ICP-AES (PE Optima 3000 DV)
Total organic bromine: DCM extracts were solvent exchanged to tetrahydrofuran, diluted with water and injected into an ICP-HRMS (Finnigan Element 2)

Results and discussion

*PBDE and HBCD***:** The EID executed 2 monitoring campaigns in 2005 and collected 59 samples (29 in April and 30 in November) at 43 selected discharge points of industrial waste water. All samples were analysed on PBDEs and HBCD. Among the PBDEs, only the presence of BDE-209 was confirmed (20 'positive' samples), other PBDEs were not detected. HBCD was detected in 8 samples. An overview of the significant results is given in tables 1 and 2. The highest level of BDE-209 was 360 µg/l and the highest level of HBCD 480 µg/l. In 2006 a similar monitoring was conducted at 41 different discharge points, showing the same results: absence of the non-deca BDE congeners at concentrations >0.02 µg/l and about 50% and 15% positive samples for BDE-209 and HBCD resp. Lower levels of PBDE or HBCD were measured in effluents from companies that make master batches for the plastics industry and in effluents from installations where waste waters and liquid wastes from different companies were treated. The higher levels of these substances were found in effluents from textile finishing waste water treatment plants.

Table 1: overview of BDE-209 results (#59)

Concentration (µg/l)	number of results
< 1	39
1 – 10	6
10 – 100	10
> 100	4

Table 2: overview of HBCD results (#59)

Concentration (µg/l)	number of results
< 1	51
1 – 10	3
10 – 100	2
> 100	3

Other BFRs: in the most recent campaign pentabromoethylbenzene, 1,2-bis(2,4,6-tribromophenoxy)ethane, pentabromotolueen, decabromodiphenylethane, pentabromobenzylalcohol, tris(2,3-dibromopropyl)isocyanurate and TBBPA were analyzed. No significant results could be reported for TBBPA, pentabromobenzylalcohol and tris(2,3-dibromopropyl)isocyanurate. In only 2 samples pentabromotoluene and pentabromoethylbenzene were detected with values ranging from 0.1 to 2 µg/l. 11 samples were positive for decabromodiphenylethane with values ranging from 0.1 to 7 µg/l.

Antimony: Sb_2O_3 is known as a substitute for some BFRs and is often used in conjunction with BFRs. Antimony is also mentioned in the annex of the European Directive as a “dangerous substance” for the discharge in water. For that reason the samples were analyzed on Sb. The results show a correlation between the presence of Sb and the presence of BFRs in the waste waters. An overview of the results of the 2005 campaign is given in table 3. The highest level of Sb found was 21.500 µg/l!

Table 3: overview of Sb-results (41 samples)

Concentration (µg/l)	number of results
< 20	16
20 – 100	8
100 – 1000	11
> 1000	6

Total organic bromine:
BFR positive samples were selected for the determination of the total organic bromine content. The organic bromine concentration ranged from 4 to 500 µg/l. In most samples the ratio of measured BFRs to organic bromine was >50% indicating that at this moment BDE-209 and HBCD are still the most relevant BFRs when dealing with effluents of the textile industry.

Conclusion

Depending on the type of industrial activity significant levels of PBDE, HBCD and Sb have been found in the discharges, which explains the spatial variation of PBDE and HBCD content in sediment and biota. Decabromodiphenylethane has been detected in 25% of the samples and has to become part of the BFR priority list. Some other BFRs have only been detected in a few effluents and at lower concentrations. The observed correlation between antimony and BFR content makes the monitoring of antimony advisable.

It is clear that the monitoring results are an important instrument for the EID by which the release of this persistent compounds in the environment can be reduced. The results are used to initiate an administrative and judicial procedure against the BFR discharging companies. Companies are enforced to diminish or even stop the

discharge of these substances by taking appropriate waste water treatment measures and/or by using less harmful flame retardant alternatives.

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THE NEVER ENDING STORY OF POLYCHLORINATED PARAFFINS: NEW PROPOSALS TO OVERCOME THE PERSISTENT QUANTIFICATION PROBLEM AND NEW ENVIRONMENTAL SURVEYS

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Polychlorinated paraffins or alkanes (PCAs) are very complex mixtures containing carbon chains of variable length and degree of chlorination. They are e.g. available as short chain (sPCAs, C_{10}-C_{13}) or medium chain (mPCAs, C_{14}-C_{17}) products and were produced as bulk chemicals in large quantities (>300'000 t/a). PCA are applied as additives, flame retardants and plasticizers. The knowledge about toxic effects is incomplete, but information is available about high aquatic toxicity and carcinogenic properties in mice. PCAs have physical properties which make them suitable for global long range transport as well as a high potential of bioaccumulation. The analysis of CP is very demanding, since the technical products contain ten thousands of congeners. Therefore, only few data are available indicating a global ubiquity and concentrations comparable to polychlorinated biphenyls.

Until 2001, only one major method was available for CP quantification based on negative ion chemical ionisation combined with preferably high resolution mass spectrometry. Since then, our research group has developed alternative analysis methods both suitable for screening using low cost instrumentation as well as congener specific techniques. They are based on electron ionisation and the MS/MS detection of fragments common to all CP or negative ion mass spectrometry using methylene chloride as reagent gas. The currently available methodologies will be presented and compared, and the problems of CP analysis discussed.

Fish livers (cod, dab, flounder) were collected during two monitoring expeditions at five to six sites in the North and Baltic Sea in August/September 2002/2004. Livers were pooled to obtain minimum 5 g of sample. Moreover, six cod liver samples were obtained from the north and south coast of Iceland and the Lofot Islands. s+mPCA levels in fish liver from the North and Baltic Sea showed no species-specific concentration dependence. Concentration ranges were comparable for the North Sea (54-3880 ng/g lipid weight (lw), mean 985 ng/g lw) and the Baltic Sea (90-3170 ng/g lw, mean 615 ng/g lw). The highest s+mPCA levels were far above 1 ppm, which is remarkably high.

Sediments were collected during monitoring expeditions in August/September 2001, August/September 2002, May/June 2003 and spring 2004 (13 sediments from 7 sites in the Baltic sea and 20 sediments from 16 sites in the North Sea). Moreover, 8 river and sea sediments and suspended particulate matter (11 samples) were analysed for comparison. PCAs were present in all sediments (s+mPCA: 5-377 ng/g dry weight (dw)). s+mPCA levels in sediments from the Baltic Sea (45-377 ng/g dw) were generally higher than in those from the North Sea (5-355 ng/g dw, ten of sixteen samples below 50 ng/g dw). However, they were quite equal when expressed on TOC basis (North Sea 2.3-33.1 ng/g TOC, Baltic Sea 2.1-9.4 ng/g TOC). The TOC content was a good marker for PCA concentrations. Concentrations of mPCA (42-303 ng/g dw) were always higher than for sPCA (18-128 ng/g dw). The ratio mPCA/sPCA varied between 1.7 and 3.2. Higher TOC levels indicated usually also a

higher PCA burden. The highest PCA concentrations were found at sites in the Elbe estuary, where chemical waste and sewage sludge had been dumped several years ago.

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ADVANCES IN THE ANALYSIS OF PER AND POLYFLUORINATED COMPOUNDS

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Introduction

Poly- and perflourinated compounds (PFASs) have been and are still being used widely for their surface tension lowering properties in a variety of domestic and industrial applications such as polymerization aid for production of fluorinated polymers, for metal plating, in photographic industry, in the semi-conductor industry, in the aviation industry (hydraulic fluids), in fire fighting foams and as fat, and water repellents for textiles, paper and leather [1, 2]. After initial reports on the presence of PFASs in the environment [3-5] in the past five years many researchers have started to investigate this class of compounds. Initial studies focussed on perfluoroctanesulfonate (PFOS) and perfluorooctanoic acid (PFOA) and those have received most attention. However, a range of other PFASs receive increasing attention because they are produced as alternatives for PFOS and PFOA, as intermediates in PFAS production, as by-products or as (bio)degradation products. These PFASs include perfluorocarboxylates (PFCAs) and perfluorosulfonates (PFSAs) with different chain lengths (typically between C4-C14), fluorotelomer carboxylic acids (FTCAs, both saturated and unsaturated) and non-ionic (volatile) compounds such as fluorotelomer alcohols (FTOHs) and FFOSA and N-substituted sulfonamides. Initially, laboratories analysed ionic PFASs in biota samples employing an extraction method according to Hansen et al. [6], which is based on ion pairing of the ionic PFASs with tetra-n-butylammonium hydrogensulfate (TBA), followed by a liquid-solid extraction (LSE) with methyl-tert-butylether (MTBE), filtration of the extract and final determination by liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS/MS). With the rapid expansion of the PFAS research area, more dedicated methods are developed for efficient and accurate analysis of a variety of PFASs in wide range of matrices (e.g. sewage treatment samples, air, sediment, soil, blood and milk).

Table 1. Full names, abbreviations and chemical formulas of PFASs

Full name	Abbreviation	Chemical formula
Perfluorinated carboxylic acids	PFCAs	$CF_3(CF_2)_nCOOH$, with n=2-13
6:2, 8:2 or 10:2 fluorotelomer carboxylic acids	6:2, 8:2 or 10:2 FTCA	$CF_3(CF_2)_nCH_2COOH$, with n=5,7,9
6:2, 8:2 or 10:2 fluorotelomer unsaturated carboxylic acid	6:2, 8:2 or 10:2 FTUCA	$CF_3(CF_2)_nCF=CHCOOH$, with n=4,6,8
Perfluorinated sulfonates	PFSA	$CF_3(CF_2)_nSO_3^-$, with n=3,5,7,9
6:2 fluorotelomersulfonate	6:2 FTS	$CF_3(CF_2)_5CH_2CH_2SO_3^-$
4:2, 6:2, 8:2 or 10:2 fluorotelomer alcohol	4:2, 6:2, 8:2 or 10:2 FTOH	$CF_3(CF_2)_nCH_2CH_2OH$, with n=3,5,7,9
Perfluorosulfonamide	PFOSA	$CF_3(CF_2)_7SO_2NH_2$
N-ethyl perfluorooctane sulfonamidoethanol	NEtFOSE	$CF_3(CF_2)_7SO_2N(CH_2CH_3)CH_2CH_2OH$
N-methyl perfluorooctane sulfonamidoethanol	NMeFOSE	$CF_3(CF_2)_7SO_2N(CH_3)CH_2CH_2OH$
N-ethyl perfluorooctane sulfonamide	NEtFOSA	$CF_3(CF_2)_7SO_2N(CH_2CH_3)CH_2CH_2OH$
N-methyl perfluorooctane sulfonamide	NMeFOSA	$CF_3(CF_2)_7SO_2N(CH_3)CH_2CH_2OH$

Extraction

Due to their different polarities, the PFASs mentioned in Table 1 require different extraction strategies. The ionic PFCAs and PFSAs require moderately polar media (Oasis WAX SPE or methanol and acetonitrile) for efficiently trapping of polar short-chain (C4-C6) compounds. For longer chains, less polar or apolar SPE phases (C18 and Oasis HLB) may be applied. When an ion-pairing agent is used that decreases the polarity of the ion pair complex, an apolar solvent (MTBE) may be used. Non-ionic PFASs may be extracted from the matrix by apolar media (C18 SPE or hexane). Moderate polar media (Oasis HLB and Oasis WAX SPE, a hexane-acetone mixture or acetonitrile) have also been applied for extraction of non-ionic PFASs.

Water - PFASs concentrations reported in water typically are in the ng-pg/L range [7], requiring enrichment of the sample. SPE is a suitable technique for this purpose, applying purely hydrophobic (C18) [8-10], mixed hydrophobic/polar (e.g. Oasis HLB) [11, 12] and wax-type phases [11]. Relative polar short chain PFASs (C4-6) are trapped more efficiently on a Oasis-Wax column [11], whereas longer chains are (also) efficiently trapped on C18 and Oasis HLB phases. Care should be taken to avoid laboratory consumables such as blank contributions from filters, chemicals, sample vials and septa [12]. Eliminating those improves detection limits to PPQ levels [12]. On-line SPE may reduce manual sample handling time [13].

Human blood and milk - Typical PFASs concentrations found in human blood matrices are in the range of 0.01-100 ng/mL, with PFOS in the highest concentrations observed [14-17]. Although some authors have applied the IPE method or LLE, the most frequently applied extraction technique is SPE (off-line and on-line). Karrman et al. developed a SPE/LC-MS method for PFASs in whole blood

[18]. They tested ten SPE sorbent materials ranging from apolar (C18) to medium polar (phenyl). Best extraction efficiencies (for PFOS) were obtained by large particle size (120 µm) C18 and on the phenyl sorbent [18]. Most of the until now reported SPE methods require sample pre-treatment to prevent clogging of columns or for removal of e.g. proteins. Proteins are removed by denaturising and subsequent centrifugation.

Sewage sludge, sediment, soil and suspended matter - Typical PFASs concentrations in sediments range from approx. 10 pg/g to the mid-ng/g range [19-22], whereas concentrations in sewage sludge may be much higher ranging from low ng/g to low µg/g range [20, 23]. A recently presented extraction techniques is LSE of ionic and non-ionic PFASs [24] with methanol or acetonitrile. Despite it's potential, PLE was only used in limited number of PFASs studies [21, 25, 26]. A wider application of PLE is hindered by the considerable amounts of PTFE tubing in the instrument, resulting in unacceptable blank contributions for several PFCAs.

Biota - Methods for extraction of biota are based on LSE of the sample with LC-mobile phase [27], methanol [28] or by Soxhlet extraction (of non-ionic compounds) with a hexane-acetone solvent mixture [29]. 8:2 FTOH can be extracted from biological tissues with hexane and perchloric acid, followed by silica column clean-up [30].

Air - Typical PFAS concentrations in air are in the pg/m^3 range. Sampling is often based on flow through large volume samplers. The sorbents retaining the volatile PFASs are XAD resin sandwiched between polyurethane (PUF) plugs [31, 32] or just PUF plugs [33]. Jahnke et al. [34] applied simple SPE cartridges in a flow-through set-up. Air particles can be analysed in the same way as solid samples (e.g. methanol extraction [35]). Extraction of the XAD resins and/or PUF plugs is done by (a combination of) medium polar organic solvents like methanol, petroleumether and ethylacetate. An Env+ SPE column was eluted with ethylacetate. Prior to the sampling, the XAD resins and PUF plugs require thorough precleaning.

Clean-up strategies

For various sample types (e.g. fish liver, lipid rich samples, sediments, sewage sludge samples) extracts require further clean-up to remove co-extracted lipids and other matrix constituents, that may lead to enhancement or suppression of the electrospray ionization, resulting in inaccuracies [27]. The co-extraction of lipids from biological matrices can be reduced by the use of medium polar extraction solvents such as methanol and acetonitrile [27, 28]. Crude extracts may be pufified with graphitized carbon (Envi-carb) [28], silica column chromatography [29, 36]. Apolar extracts can be purified by sulfuric acid washing [29]. Clean-up of water samples is generally performed by a washing step after sample enrichment on the SPE cartridge or by additional clean-up with a fluorous silica column [37]. Extracts of abiotic matrices (soil, sediment, sewage sludge) can be cleaned-up by addition of Envi-carb or by C18-SPE [23]. As a final clean-up step, extracts may be filtrated over e.g. nylon filters to remove solids from the final extract, but care should be taken to avoid PFAS losses or contamination of the sample extract. During sample manipulation care should be taken to avoid losses of sulfonamides [16] and 6:2 FTS [38], FTOHs [30] and short chain PFCAs [38] when extracts are concentrated by evaporation (to dryness). Losses may be avoided by using a keeper and by not blowing the extract down to dryness.

Final determination by LC-MS(/MS) or GC-MS(/MS)

Most studies focus on the analysis of PFCAs, PFSAs and PFOSA and employed LC-MS/MS for final determination. LC-MS(/MS) combined with a selective extraction and clean-up provides a sensitive and selective method for detection of PFCAs and PFSAs. Furthermore, LC-MS(/MS) can also be employed for detection of PFOSA, N-EtFOSA, N-MeFOSA, N-EtFOSE, N-MeFOSE and the telomer alcohols. Therefore, broad multi-PFAS detection methods can be developed using LC-ESI-MS(/MS) detection. However, some disadvantages of LC-ESI-MS(/MS) are (i) electrospray ionization enhancement or suppression (matrix effects) [39] and (ii) poor ionization yields for non-ionic FOSA/FOSE type PFASs, reducing the sensitivity when detected by ESI-MS [39].

GC-MS in combination with positive or negative chemical ionization (PCI, NCI) may overcome these problems. Several researchers applied GC-MS on the detection of PFCAs after derivatisating to methylesters, butylesters or 2,4-difluoroanilides (recently reviewed by De Voogt and Saez [40]). Derivatisation techniques improve the selectivity of the analytical method, thereby reducing disturbing matrix effects. Selectivity is further improved with the application of CI-MS detection. Furthermore, GC may provide a better resolution than LC, enabling identification of branched PFCA isomers [41]. Unfortunately, it is difficult to create sufficiently stable PFSA derivates, making them unsuitable for GC analysis. Finally, an advantage of GC-MS is the suitability for sensitive analysis of the non-ionic (volatile) PFASs like PFOSA and N-ethyl-FOSA, N-methyl-FOSA, N-ethyl-FOSE, N-methyl-FOSE and the 6:2, 8:2 and 10:2 FTOHs [29-31].

QA/QC

The results of the first international interlaboratory study on PFASs in human samples showed a good comparability of the different methods applied by the participants as 61-73% of the participants had satisfactory z-scores for PFOS and PFOA [42]. This shows that the methods for human matrices generally include accurate extraction and clean-up of samples. On the other hand, the environmental matrices results showed that matrix effects may cause large inaccuracies [42]. It is expected that this has improved recently, with the development of dedicated extraction and clean-up techniques. Furthermore, a wide range of native standards and 13C and 18O labeled internal standards became commercially available for accurate determination. A new round of PFC interlaboratory studies on human and environmental matrices will be organized in 2006/2007 and will show if indeed progress is made over the last few years.

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DETERMINATION OF SELECTED ORGANOPHOSPHORUS FLAME RETARDANTS (OPFRs) IN WATER AND SEDIMENT RIVER SAMPLES FROM AUSTRIA

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Organophosphate triesters are used in multitude of applications such as flame retardants, plasticizers and lubricants.

According to the European Flame Retardant Association (EFRA), flame retardants are additives that can be added or applied as a treatment to organic materials such as plastics, textiles and timber. Alternatively they can be used during the production process as a chemical modifier of some plastic materials [1]. In essence, they are compounds which, when integrated into or coated over the surface of a fibre or material will provide a flame retardant barrier and so protect against fire. However, many of them are toxic and have been directly linked to health problems.

Concentrations of organophosphorus flame retardants (OPFRs) in surface water were reported in the literature only in a few studies from Germany [2, 3, 4] and USA [5, 6] during the last 6 years. The last one within a reconnaissance program to determine the occurrence and persistence of a chemically diverse suite of emerging contaminants in US streams. Up to now, scarce studies have been published about their presence in sediment samples [7]. Comparable data for Austria are not yet supplied until the present study.

The purposes of the present work were:

1. To develop reliable analytical methods to evaluate 9 priority OPFRs in river water and sediments, by liquid-liquid extraction (LLE) and ultrasonic solvent extraction (USE), respectively. It comprises three chlorinated alkyl phosphates: tris (2-chloroethyl) phosphate (TCEP), tris (1,3-dichloro-2-propyl) phosphate (TDCP), tris (2-chloropropyl) phosphate (TCPP); four non chlorinated alkyl phosphates: tris (2-butoxyethyl) phosphate (TBEP), tributyl phosphate (TBP), triethyl phosphate (TEP), tris (2-ethylhexyl) phosphate (TEHP); and two aryl phosphates: triphenyl phosphate (TPhP), tritolyl phosphate (TCP). Liquid chromatography coupled to tandem electrospray mass spectrometry (LC-ESI-MS/MS) was used for the identification of the analytes.

2. To report on the first assessment of the occurrence of the selected target compounds in rivers of Austria with the aim of evaluating their distribution, transport and fate.

An exhaustive validation procedure of both methods was developed. It comprised the determination of linearity, sensitivity, recovery, precision, procedural blanks,

surrogate recoveries and the study of matrix effects. Recoveries of the organophosphate triesters ranged from 63 % to 94 % (SD ≤ 12) in water and from 74 to 104 % (RSD ≤ 18) in sediment samples with estimated method quantification limits between 2.8 and 9.2 ng/L and between 0.48 and 11 ng/g, respectively.

The development methods were successfully applied to the determination of the organophosphate triesters in several Austrian rivers.

Seven of the nine compounds were detected in most surface waters. The highest concentrations were measured for TBEP, TCPP, TCEP and TBP. The values, for example, of TBEP varied from 11 up to 1470 ng/L with a median of 190 ng/L. The concentrations of TCPP ranged from 11 up to 720 ng/L with a median of 150 ng/L. Lower concentrations for TCEP and TBP were detected in river water samples with a median of 55 and 96 ng/L, respectively.

The concentrations levels of the analyzed sediments ranged from the low µg/kg to low mg/kg range. Especially, TBP, TCPP and TBEP were found in higher concentration levels, a maximum of 95 µg/kg and 50 µg/kg dry weight (dw) were determined for TCPP and for TBP, respectively. TBEP was quantified in all samples but with mean concentrations lower than 10 µg/kg, detecting only in one sediment sample 130 µg/kg. TEHP, which was not detected in water samples, was found in this instance frequently.

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EMERGING INORGANIC POLLUTANTS IN ATMOSPHERIC PARTICULATE MATTER

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One of the major early 21st century scientific advances in environmental health has been the emergence of the epidemiological confirmation that enhanced chronic levels of inhalable airborne particulate matter (PM) significantly increase the risk of premature death (e.g. Krewski et al. 2000; Hoek et al. 2002; Pope et al. 2002 & 2004; Jarett et al. 2005). Concurrent with the gestation and arrival of these conclusions, many European countries have installed sophisticated national air pollution monitoring networks, and a huge and rapidly burgeoning amount of data is now being generated. Many of these data are concerned with the measurement of particle mass, a criterion given emphasis by the legal demands for limits on annual averages and numbers of exceedence days. Thus 2005 limits within the European Union (EU Directive 1999/30/EC) demand maximum daily values of 50μg/m^3 (not to be exceeded more than 35 times a year) and mean annual values of 40μg/m^3.

There is, however, no known limit on PM concentrations below which there are no health effects at all, and the objective must therefore be to reduce levels to as low as is socially, economically, and therefore politically possible. With this reality in mind, the latest World Health Organisation (WHO) guidelines on PM_{10} and $PM_{2.5}$ (particles <10μm and < 2.5μm in size) identify four pollution thresholds. These thresholds cascade down through decreasing concentrations via three "Interim Targets" (IT1-3), aiming towards a recommended Air Quality Guideline (AQG) value of 20μg/m^3 (annual mean) and 50μg/m^3 (24-hour mean) for PM_{10}, and half these levels for $PM_{2.5}$. To add weight to the urgency of achieving these objectives, WHO emphasises the increasing health cost as air quality declines, with long-term mortality at IT1 being around 15% higher than at AQG. More specifically there will be a predictable increase in asthma, chronic bronchitis, and other respiratory diseases, cardiac and respiratory hospital admissions, and days lost from work, with every increase in 10μg/m^3 PM_{10} (WHO 2006), with an estimated 288,000 premature deaths and some 80,000 hospital admissions attributed to PM inhalation in the 25 EU countries during 2000.

Within Europe, Spain proved itself one of the first to push ahead with a national monitoring and source apportionment programme, supplying data daily to AIRBASE since 1997, currently from 240 recording stations (Querol et al. 2004). Furthermore Spain (along with Germany and Italy) has not been shy to place many of its monitoring stations in pollution hot spots, notably urban traffic, in recognition that we need realistic worst-case data, as well as "urban background", on what our urban European populations are really breathing as they live, work and travel in the city. A

study in Amsterdam, for example, estimated that around 10% of the population lives adjacent to roads carrying >10,000 vehicles/day (Roemer & van Wijnen 2001).

We are therefore now in a position, based on a huge database, to recognise that in Spain the typical urban citizen breathes air containing inhalable ambient airborne particles at average concentrations of 30-46μg/m^3 (PM_{10}) and 20-30μg/m^3 ($PM_{2.5}$). This places them above WHO Interim Target limit IT2, and therefore presumably on average around 10% more at risk than if the recommended AQC were to be reached in Spanish cities. These levels of urban PM10 contamination are significantly higher than in some more northerly European countries, such as Germany, and are due to a combination of several factors. Much of Spain has a semi-arid and often windy climate, making aeolian resuspension of dry particles commonplace, especially in the south and east. Regional atmospheric stagnation events, sea breeze effects, wintertime temperature inversions, intrusions of African dust, industrial and traffic (especially diesel) emissions all contribute further to urban pollution.

Epidemiological correlations between particle mass and health effects, such as that championed by the WHO, provide the necessary scientific basis for (and highlight the urgency of) effective air pollution legislation. The next step to refine such legislation will probably emerge from an increasing recognition that epidemiology focussed only on PM mass ignores the fact that the air we breathe as individuals contains a complex and highly variable mixture of different chemical elements and compounds from different sources. This becomes important if health problems can be shown to be related to specific components in the particulate matter rather than just simple increases in mass (e.g. Adamson et al. 2000). Differences in aerosol toxicity are likely to be linked not only to increasing particle mass, but also to variations in particle size (Schwartz & Meas 2000), shape and chemistry (Richards 1997; Adamson et al. 2000), and the presence of small amounts of highly toxic elements such as cadmium (Nawrot et al. 2006), especially if these are soluble (Fernández-Espinosa et al. 2002; Birmili et al. 2006). In this context trace metals, although usually very low in mass, are in particular emerging as important pollutants because in both their inorganic and organometallic forms they are commonly highly bioreactive. Furthermore many trace metals in airborne particles occur in the very fine (<1μm) and ultrafine (<0.1μm) size fraction (Utsunomiya et al. 2004; Birmili et al. 2006), and therefore are able to reach alveolar regions in the lungs (Schaumann et al. 2004).

While some trace metal emissions are due to natural processes (e.g. volcanic eruptions, dust storms, rock weathering, forest fires), many are of anthropogenic origin. For example V, Co, Mo, Ni, Sb, Cr, Fe, Mn and Sn are emitted during combustion of fossil hydrocarbons (e.g. Pacyna 1986; Lin et al. 2005), As, Cr, Cu, Mn and Zn are released into the atmosphere by metallurgical industries (Pacyna 1986; Querol et al. 2002; Alastuey et al. 2006), and traffic pollution involves a wide range of trace element emissions which include Fe, Ba, Pb, Cu, Zn and Cd (Pacyna 1986; Birmili et al. 2006). Even firework displays during fiestas add a transient but considerable burden of metals such as K, Al, Ti, Mg, Pb, Ba, Sr, Co, and Sb (Moreno et al 2006a). Because of the possible health hazards linked to the more toxic trace elements (especially when carcinogenic effects are suspected or proven e.g. Blot & Fraumeni 1975; Sorahan & Waterhouse 1985; Nawrot et al. 2006), the WHO has again established air quality guidelines (WHO 2000). Similarly, the European Union has set annual limits on selected trace elements such as 500ng/m^3 for Pb (1999/30/CE), and has established target values for As (6ng/m^3), Ni (20ng/m^3) and Cd (5ng/m^3) (2004/107/CE). Thus we see the emergence of an awareness that the

epidemiological evidence on air pollutants has to be combined with detailed physical and chemical characterisation of PM samples, with an emphasis on spatial variations in components of especial concern from toxicological studies. In this context we can demonstrate abundant evidence from Spain that illustrates how the chemical cocktail of inhalable metalliferous particles varies substantially from place to place.

Spain has a history of metal mining stretching back over 2000 years. These mines are almost all now abandoned and commonly surrounded by contaminated land. Perhaps the most spectacular example is Almaden in central Spain, once by far the most important mercury mine in the world. Recent study of metalliferous PM_{10} around the mines, processing plants, and in urban road dust, has revealed pervasive high levels of contamination, with the mercury being strongly fractionated into the finer, more deeply inhalable dust fraction (Moreno et al. 2005). A similarly study around a former gold mine, this time at Rodalquilar in southern Spain, revealed unvegetated waste dumps being actively eroded by aeolian resuspension to produce PM_{10} containing levels of >1500ppm As and >40ppm Sb, both clastogenic metalloids with proven negative health effects (Moreno et al. 2006b).

Modern industry in Spain further adds to regional variations in levels and composition of metalliferous PM_{10}. Thus the town of Puertollano in central Spain regularly shows increases of V and Ni when particles are sourcing from the local refinery (Moreno et al. 2006c). Similarly, the important ceramics industry based in the Castellon area of eastern Spain can be linked to local increases in Pb, Zn, As, Cs, Zr (Querol et al. 2006). A comparison of trace metal concentrations and source origins in airborne particles at a range of urban sites in Spain, each registering “normal” levels of urban air pollution in terms of mass, showed that chemical mixture of trace elements breathed at these sites varies enormously, and further confirmed that the most toxic metals are usually concentrated in the finer particle fraction (Moreno et al. 2006d). Whereas in general the most abundant trace element metals in absolute terms at our sites are Zn, Cu, Ti, Mn, Pb and Ba, one industrially-influenced site (at Llodio in northern Spain) showed levels of Sn, Zn, Mn, Ni, Cr, Pb and Mo up to 20 times higher than at the other sites. In contrast, the industrial town of Huelva in southern Spain showed a completely different metal mixture (with strongly elevated levels of Cu and As). Figure 1 plots PM_{10} and $PM_{2.5}$ compositional variations in mixtures of metals from four Spanish urban sites to illustrate how the chemical complexity and variability of the inorganic urban aerosol cocktail is such that the air pollution signature for each town needs to be individually characterised, especially if there are nearby sources of industrial metalliferous emissions.

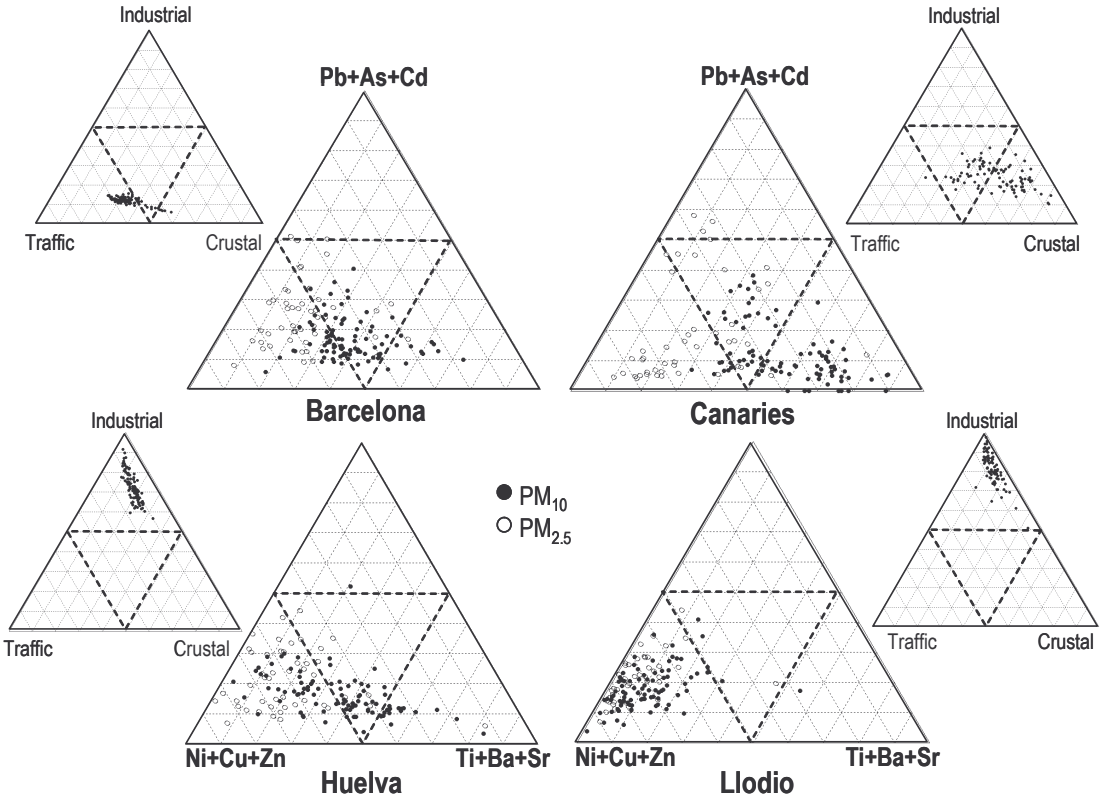

Fig. 1. Comparison between concentrations of selected trace metals at four sites in Spain grouped according to their potential health concern, varying from most (Pb+As+Cd) to least (Ti+Ba+Sr) toxic. Traffic-Industrial-Crustal (TIC) source apportionment triangles for trace elements in the PM_{10} fraction (modified from Moreno et al. 2006d).

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A NEW PROTEASE-IMMUNOASSAY TANDEM ASSAY METHOD FOR DETECTING LOW LEVELS OF MICROBIAL ACTIVITY IN WATER SAMPLES.

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The detection of microbial activity in water is required for the protection of consumers from exposure to pathogenic microbes. Acceptable microbial content of surface water bodies, water bodies for recreational purposes, water from treatment plants and consumable water vary, but all demand relatively frequent testing and ability to detect increasingly lower numbers of bacteria. Useful microbial analysis techniques ideally should be both sensitive and rapid in addition to being cost-effective in order to encourage higher testing frequencies and should be employed by a wider range of users. The technical challenges posed by the regulations governing the safety of drinking water, water supplies for food manufactures and clinical usage are difficult to meet by any of the currently available techniques. The majority of tests that meet the required sensitivity range take far too long to perform and in reality the water body under testing, at any one time, would reach the consumer before the test result is known. It is therefore obvious that in order to provide full protection to consumers, alternative improved testing technologies that meet the principal requirements of effective surveillance methods are required. The techniques for detecting microbial activity may be classified into five main categories according to principle of the testing procedure [1]: culture, catalytic or enzymatic, immunochemical, nucleic acid methods and biosensing techniques. The methods vary in terms of accuracy and sensitivity and to a much greater extent in convenience and speed [2, 3]. The catalytic methods have the distinct advantages of amplification and high sensitivity by virtue of the cellular enzyme action on sensitive synthetic substrates [4]. The use of microbial marker glycosidases (β-D-galactosidase and β-D-glucuronidase) with fluorescent substrates provided a panel of useful tests that have served the analysis field for a long time and have changed little since their introduction [5]. The tests provide a very high sensitivity and a good measure of convenience particularly for rapid front line screening of water and food samples.

However, the glycosidase-based tests require the use of relatively high concentrations of substrates which need to be presented and measured as solution reagents. This requirement may become a technical limitation if the tests are to be improved in terms of convenience or reconfigured to enable the construction of practical biosensor devices.

Principle of the method

This presentation describes the preliminary results of a novel enzymatic method for detecting low numbers of microbial activity in water samples. The method was developed using the cellular protease marker enzymes in conjunction with natural protein substrates. In order to allow convenient detection of enzyme-substrate action, natural protein substrates were modified by the covalent attachment of

antigenic species, hapten molecules. Digestion of the protein-hapten conjugates by the marker proteases provides product species with different properties (small fragments) that distinguish these products from the starting substrate (Fig. 1).

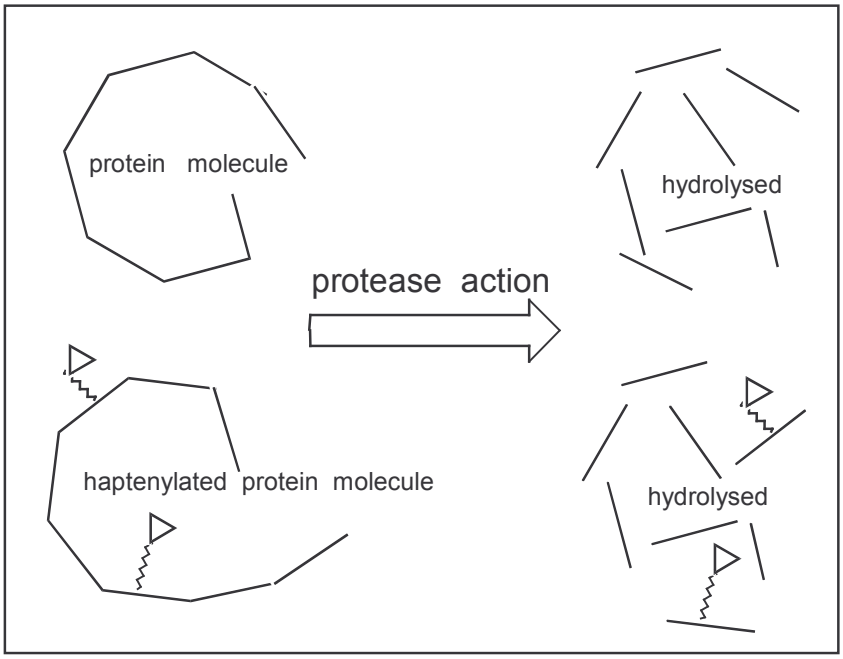

Figure 1: A schematic showing the result of hydrolysis of protein and protein-hapten substrates.

This allows specific detection using anti-hapten antibodies labelled with chosen enzymes. In addition to the introduction of a protease-based microbial detection system, the substrate was presented as a solid phase reagent by adsorbing the protein-hapten conjugates to polystyrene test tubes or microtitre plates. The digestion of the adsorbed substrate was found to result in quantitative desorption of the conjugates. This enabled the enzymatic hydrolysis and the solid phase ELISA steps to be used in tandem. The immunoassay step made possible the measurement of the unhydrolysed substrate, or the material left associated with the solid phase surface (Fig 2).

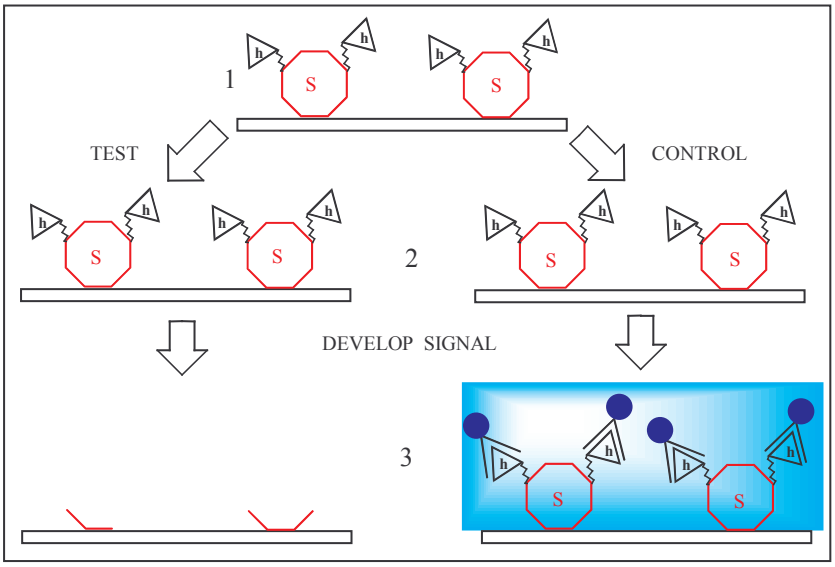

Figure 2: A schematic showing the enzymatic and the immunoassay steps.

Initial Results

In the initial phases of the study, gelatine, a natural substrate for bacteria, was modified by covalent coupling of hapten molecules (cholic acid or fluorescein). The conjugates were adsorbed, as monolayers, to polystyrene test tubes or 96-well microtitre plates. Hapten antibodies were purified by affinity chromatography and labelled with horse radish peroxidase [6]. The adsorbed gelatine-hapten conjugate was shown to be hydrolysable by protease preparations and by contaminated river water samples. This lead to desorption of the attached conjugates and a proportional decrease in the detectable levels of hapten binding activity in the ELISA steps (Fig 3).

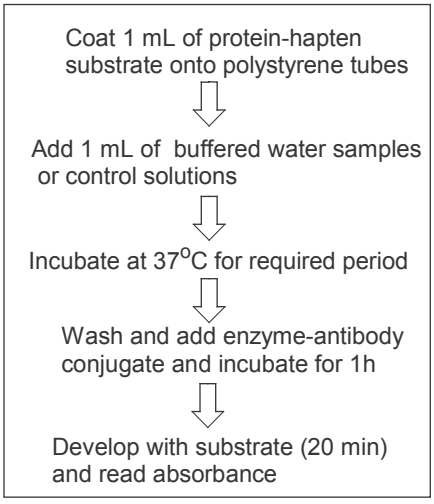

Figure 3: A flow chart showing the main steps of the assay protocol.

Tests with commercial protease preparations showed varied but generally high activity. Higher activities were found with certain microbial protease preparations where sub nano units of enzyme activities could be detected. Figure 4 shows an example of a protease dose response graph. It demonstrates a detection limit of less than $1x10^{-9}$ units of enzyme unit.

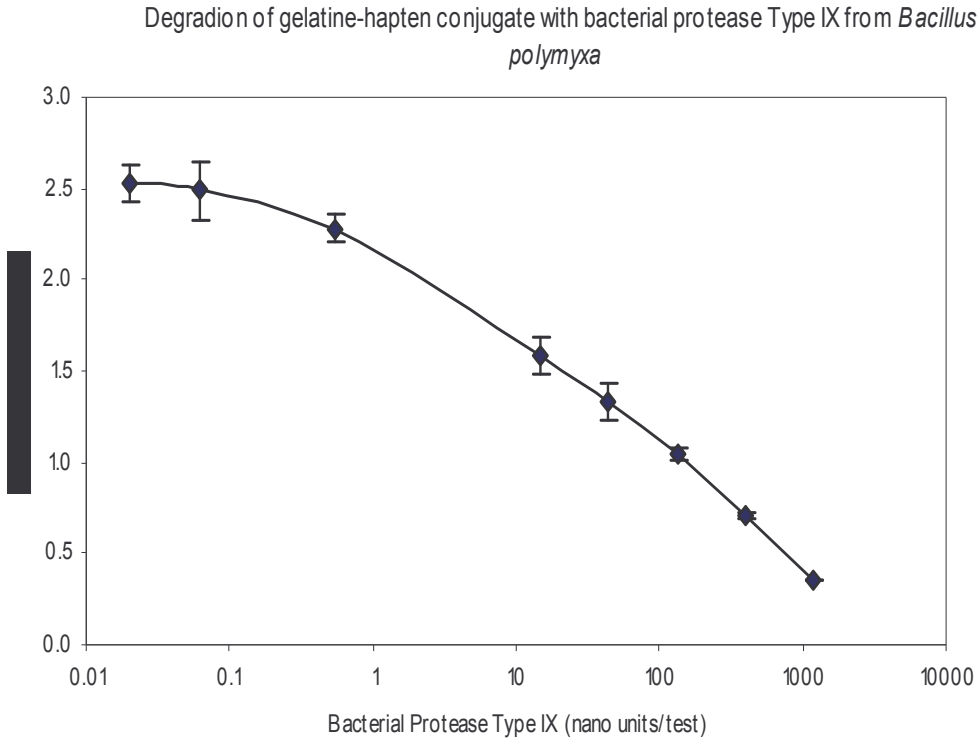

Figure 4: A dose response graph of Bacterial Protease type IX from Bacillus polymyxa.

Initial tests with samples collected during October and November from The Thames showed that bacterial protease activity could be easily detected by the described assays. River samples which were shown to contain approximately 30 CFU/mL (3000 CFU 100 mL^{-1}) by standard microbial culture methods produced maximum signal effect in this new test (Fig 5). Tests on micro-filtered river samples showed no activity thus indicating that the observed protease activity is associated with intact microbial cells.

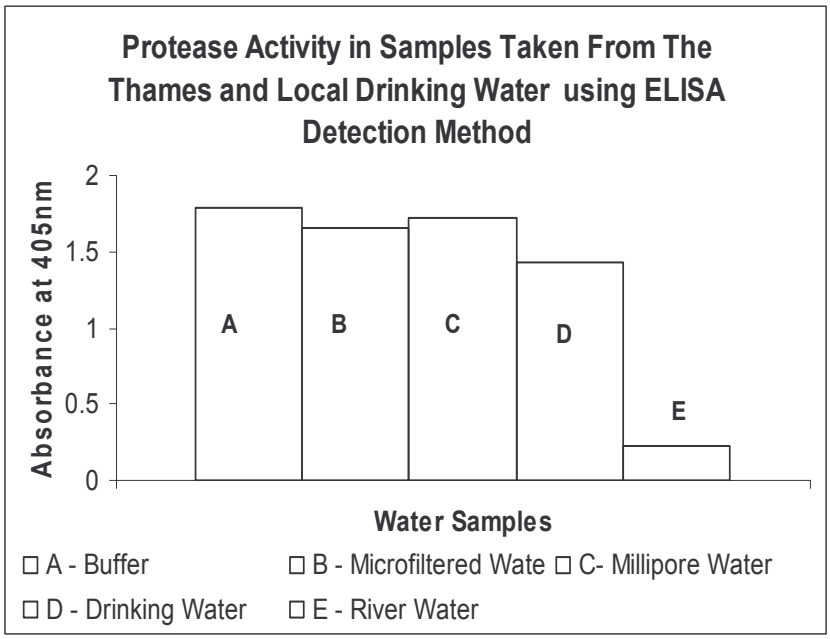

Figure 5: Results of tests carried out on samples from The Thames and local tap water. Test on microfiltered river samples showed no detectable activity.

Tests on river samples over a period of weeks showed varied activity (1000 to 30,000 CFU 110 mL^{-1}) with slight increases following rainfall in the area (which is known to cause sewage overflow into the river). Specific culture medium tests with glucuronide substrates showed that some10% of the detected CFUs were *E.coli.*

The described protease-dependent tandem method presents a new approach to detecting bacteria in biological samples. The method is extremely sensitive, relatively rapid and convenient. This procedure is especially suitable for screening low levels of bacteria in rivers and drinking water bodies.

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COMPOUND SPECIFIC ISOTOPE ANALYSIS (CSIA) TO CHARACTERISE DEGRADATION PATHWAY AND TO QUANTIFY IN SITU DEGRADATION OF FUEL OXYGENATES AND OTHER FUEL DERIVED CONTAMINANTS

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Benzene, toluene, ethylbenzene and xylenes (BTEX) are groundwater pollutants of particular concern, since these compounds represent a significant health risk due to their high abundance and toxicity. Fuel oxygenates (commonly ethers and alcohols) were used as octane enhancers since more than 2 decades of which methyl tertiary butyl ether (MTBE) is by far the most commonly used oxygenate. As a result of this extensive use, and due to its high water solubility, considerable mobility and slow degradation rates, MTBE has become one of the most frequently detected volatile organic compounds in groundwater (1). MTBE is currently substituted by ethyl tertiary butyl ether (ETBE) in Europe due to tax incentives for the application of biomass-derived ethanol which is synthesized to produce the ethyl group of ETBE. It can be expected that ETBE will be one of the emerging fuel derived contaminants in Europe in the future because production and consumption of ETBE will increase.

Only biodegradation leads to a decrease of BTEX and fuel oxygenate concentrations in groundwater coupled to a sustainable reduction of their mass. Therefore, the evaluation of in situ biodegradation is essential for the implementation of groundwater management strategies such as Monitored Natural Attenuation (MNA). In recent years, compound-specific stable isotope analysis (CSIA) has become a tool for characterizing and assessing in situ biodegradation of organic pollutants in contaminated aquifers. This concept relies on the fractionation of stable isotopes occurring during the microbial degradation of the contaminants leading to an enrichment of heavier stable isotopes in the residual fraction of a pollutant. Thus, the observation of isotope ratio shifts for carbon, hydrogen or other elements that are involved in the breakage or generation of chemical bonds during the initial step of microbial transformation can be used as an indicator for the in situ biodegradation. CSIA makes use of kinetic isotope fractionation processes upon biodegradation leading to an enrichment of heavy isotopes (^{13}C and ^{2}H) in the residual fraction. For quantitative assessment of in situ degradation the compound specific isotope fractionation factor (α) is needed which is obtained in controlled laboratory studies (2, 3).

The kinetically controlled breaking of chemical bonds may give a characteristic isotope fractionation pattern depending on the biochemical degradation pathway. Therefore, the correlation of carbon and hydrogen fractionation may be used to characterize the degradation pathway in field studies. This concept making use of the fractionation of carbon and hydrogen isotopes has been proposed as scheme to decipher and evaluate biodegradation processes and has been introduced to the literature as two dimensional isotope analyses (2D-CISA) (4).

For this work we analyzed carbon and hydrogen isotope fractionation factors for benzene, toluene and fuel oxygenate to characterize and quantify in situ biodegradation processes. A highly contaminated aquifer in Zeitz, Germany, was selected to apply CISA to characterize and quantify the in situ biodegradation of benzene.

The carbon and hydrogen isotope fractionation associated to microbial benzene degradation was investigated in laboratory experiments with anaerobic enrichment cultures from the field site and aerobic pure cultures. In agreement with recently published studies, our results showed that aerobic and anaerobic benzene degradation lead to nearly the same carbon isotope fractionation but to significantly different hydrogen isotope fractionation. Hence, it is possible to distinguish between aerobic and anaerobic benzene biodegradation in the field by means of carbon and hydrogen isotope ratios analyses. Based on multi-level sampling, the vertical and horizontal distributions of carbon and hydrogen isotope ratios for benzene were detected within the contaminant plume in Zeitz (5). Our field data provided evidence for degradation of benzene especially at the fringe of the BTEX plume. Changes in carbon isotope ratios were used to quantify the in situ biodegradation of BTEX using the Rayleigh concept.

Furthermore, we investigated whether the 2D-CISA can be used to characterize the benzene in situ biodegradation process. By doing so it could be shown that the biodegradation of benzene occurred under anoxic conditions (Fig. 1).

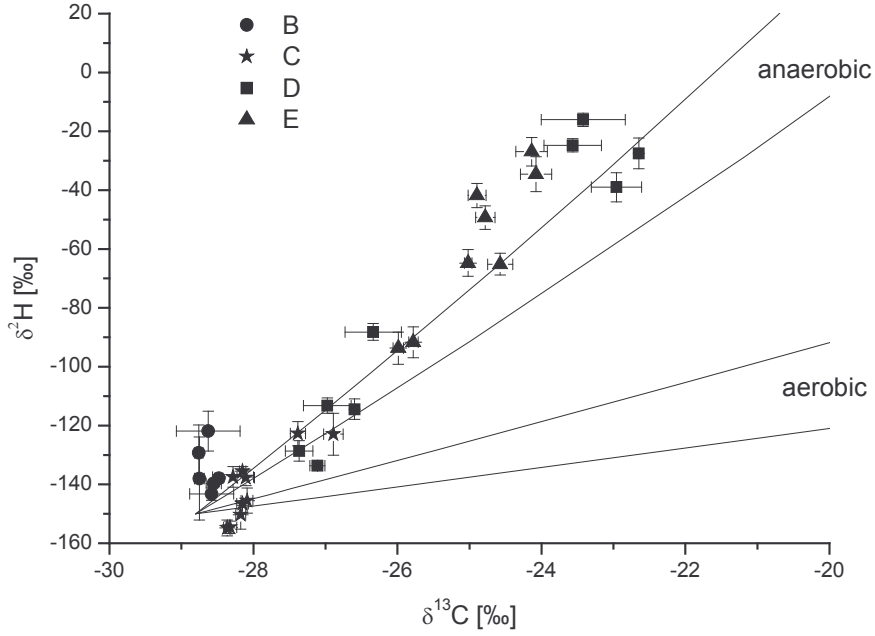

Fig. 1: Carbon and hydrogen isotope ratios of benzene measured at the various sampling depths of a monitoring transect (B - E) down gradient the contamination source in Zeitz. Solid lines show the development of isotope pattern due to aerobic and anaerobic benzene degradation calculated from published enrichment factors and the isotope signature of the contaminant source using the Rayleigh concept (5).

Although the correlation of hydrogen and carbon isotope fractionation pattern show a certain overlap in case of aerobic MTBE degradation complicating the in situ characterization (6), 2D-CISA was found to be robust to characterize the in situ

biodegradation of benzene. In summary, recent investigations show the potential of CSIA for monitoring and characterising in situ biodegradation in contaminated aquifers.

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BIOAVAILABILITY OF ORGANIC CONTAMINANTS IN SEDIMENT AND SOIL – EMERGING INSIGHTS

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Assessment of the environmental risks of emerging compounds requires information on their sorption to soil and sediment and the influence this has on their bioavailability. The bioavailability of non-polar organic contaminants is determined by how strongly they are bound to organic matter in soil and sediment. Research over the last few years has revealed that binding to different fractions of organic matter is responsible for the variations in kinetics of sorption of non-polar organic chemicals. Soot and charcoal (black carbon) bind many hydrophobic pollutants so strongly that they enter the water phase only very slowly and therefore have a low bioavailability. Polluted sediments in particular often have significant black carbon contents and as a consequence contain significant amounts of hydrophobic chemicals that are not accumulated by sediment organisms or degraded by sediment microorganisms. For example, under field conditions, the black carbon-sorbed fraction may be in the order of 80-90% of the total content of planar hydrophobic contaminants such as PAHs, PCDDs and non-ortho-substituted PCBs. This fraction shows extremely slow uptake by sediment organisms and similarly slow microbial degradation and therefore forms a non-bioavailable residue. This fact should be taken into account in a realistic assessment of the environmental risks of these contaminants.

Recent results suggest that sorption to black carbon may also be important for a wider range of contaminants than planar hydrophobic contaminants and is significant for e.g. hexachloroethane and even some pesticides. Therefore this process is likely to be important for the more hydrophobic emerging compounds. Other emerging compounds are more polar and may need new concepts to be developed that include sorption to inorganic components in soil and sediment and the effect of this process on bioavailability.

DESIGN AND APPLICATION OF A SET OF ANALYSIS OF A BATTERY OF BIOMARKERS AS A NEW ACHIEVEMENT IN DREDGED MATERIAL CHARACTERIZATION AND MANAGEMENT

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The disposal and relocation of dredged material and sediments in estuaries and marine ecosystems mostly occurs with maintenance dredging operations for coastal harbours and waterways. Depending on their origin, the sediments and dredged material can be highly contaminated with various chemicals. The contaminants may be toxic to flora and fauna at the dumping site either directly as associated with the sediment or after resolubilization in the water column. Contaminated sediments suppose a risk to aquatic life, human health, and wildlife throughout the world. There is an overwhelming amount of evidence that demonstrates that chemicals in sediments are responsible for toxicological and adverse effects. Frequently, the chemicals causing these effects are present in the sediment as mixtures of organic, metal, and other types of contaminants, including emerging contaminants.

Identification of toxicants in sediments is useful in a variety of contexts. Adverse environmental effects have been found concerning to the analysis of sediment, resulting in international parties and protocols for the environmental management of these dredged sediments. In order to avoid these adverse effects, the disposal of dredged material is controlled in the areas that belong to the OSPAR and Helsinki Conventions (Noth Sea, North-East Atlantic, Baltic Sea) proposed the guidelines to control the disposal of sediment. The dredged material disposal is managed in the countries that belong to the OSPAR and Helsinki Convention. These conventions only provide recommendations for this activity and are not included in a regulatory framework. Therefore, each country has adopted these recommendations for dredged material characterization and management and developed their regulatory guidelines, which are mainly based in physico-chemical characterization of the sediment. This kind of characterization has allowed the derivation of numerical Sediment Quality Guidelines (SQGs) which are widely utilized. These guidelines recommend the use of TIER testing approaches, which include a first physical and chemical determination of the dredged material, including grain size, contaminants and nutrients. If the information obtained it is not enough to performe a final assessment, the analysis of the potential ecotoxicological effects of the sediment is required. Although, toxicity studies are explicity mentioned in the dredged material guidelines of the OSPAR and Helsinki, they do not lay down which ecotoxicological tests have to be conducted and are not sill included in a decision-making framework for dredged material management (Nendza, 2002). In this context, different approaches have been conducted in order to develop numerical Sediment Quality Guidelines as a tool to support dredged material and sediment management and to implement policies and regulatory strategies (Casado-Martínez et al., 2006).

Spain has been party of MARPOL, OSPAR (Nothestern Atlantic) and Barcelona (Mediterranean Sea) since 1974 and 1976, respectively. However, at time in Spain,

there were no regulations to characterize the dredged material and to control its disposal. The first document regarding the characterization and control of dredged material was published in 1994 (DelValls et al., 2004), *Recommendations for the management of dredged material* in ports of Spain, RMDM (CEDEX, 1994). Either the Spanish RMDM nor proposal for initial tier testing for characterization of dredged material used by different regulatory agencies (USEPA, Environment Canada, Environment Australia and Dutch Agencies) were based on a chemical approach. Nevertheless, each country has performed particular guidelines in order to manage the sediment and dredged material. All these SQGs guidelines can be used to asses individual chemicals by comparing the chemical concentration with the limit concentrations or to estimate the probability of acute sediment toxicity and to determine the possible biological effect of combined toxicant groups by calculating mean quotients for a large range of contaminants (Long et al., 1998).

The use of these Sediment Quality Guidelines, alone, has been widely discussed and different and important limitations as a tool for the assessment and management of sediment and dredged material have been stated. The complex matrix of dredged material places limitations on the use of chemical analytical methods alone for estimating the bioavailability and the toxicity of contaminants present (DelValls et al., 2004). These values only permit the characterization of the sediment in a predictable way, they are not site specific and they do not take into consideration the bioavailability and effects of the contaminants present in the sediment. The majority of countries take into account the total concentration of arsenic and metals (Cd, Cr, Cu, Hg, Ni, Pb, and Zn) but a more limited number of countries take into account their speciation, and emerging contaminants present in the sediment unknown and known as phthalates, brominated flame retardants (BFRs), nonyphenols, octyphenols, pesticides, pharmaceutical and personal care products (PPCPs), which exhibit potential harmful effect in the environment (Gagné et al., 2006); some of them are defined as priority substances in the Water Framework Directive (WFD), nevertheless, are hardly included in the legal frameworks of European countries as criteria for dredged material.

The complex matrix of dredged material is a limitation in the use of chemical characterization alone in the assessment and management of sediment and dredged material. There is an increasing interest in taking into consideration the impact of contaminants on sediment biota and its dependence with contaminants bioavailability. However, it is difficult to estimate the bioavailable fraction in sediments. Several approaches have been tried with various levels of success (e.g. extraction with weak acids, sequential selective extraction methods or the acid volatile sulphide approaches) (Riba et al., 2004). However, biological testing is becoming widely accepted for characterizing the chemical hazards in dredged material, and for providing information to support the process of evaluating the impact of the dredged material. By exposing relevant organisms under controlled conditions to samples of the material to be dredged and then measuring toxicological effects (e.g. mortality or reduced growth) and/or the bioaccumulation of contaminants in tissues , estimates can be made in the chemical hazards present (DelValls et al., 2004). In this sense, different countries have developed toxicity methods applicable to whole sediment, sediment elutriate, sediment suspension, porewater and /or sediment extract. The scientific community has been developing science –based tools to identify sediments that are impaired and, ultimately, to support effective management decisions and priorities for dealing with contaminated sediments. The different toxicity tests can mainly be grouped in pore water and whole sediment exposure tests. These tests are conducted using benthic organisms such as

amphipods, polychaetes, algae and fish juveniles. Different agencies and governmental bodies from the Netherlands, Canada, Australia, USA, UK and Spain recommend sediment bioassays as part of a tier-testing approach. These bioassays mainly include acute and sublethal responses (e.g., growth and reproduction) and bioaccumulation. The use of different species of organisms associated with the sediment as bioindiators allows, for example, the possibility of estimating the effect of sediment ingestion. The role of sediment ingestion, which should not be underestimating, has been identified as an important route of uptake of polycyclic aromatic hydrocarbons (PAHs) by deposit-feeding benthic organisms, as shown in experiments with polychaete worms.

The use of both chemical and ecotoxicological analysis integrated into a tier testing-approach based on a Weight of Evidence (WOE) is the most powerful tool for determining the hazards associates with contaminants bound to dredged materials (DelValls et al., 2004). Nevertheless, the toxicity tests recommended nowadays, do not take into consideration the exposure and effects of emerging contaminants, unknown or difficult to analyze and that could produce potential adverse effects in the biota. Besides, there is a necessity to determine other biological effects not associated with mortality, reproduction or growth, reversible or irreversible that could produce mutagenesis, carcinogenesis, sex change or other effects affecting the structure of ecosystems.

Therefore, lately efforts are focused in the inclusion of the assessment of sublethal endpoints (biomarkers) in acute and long-term bioassays for dredged material characterization and management. Incorporating other lines of evidence (chronic toxicity tests, biomarkers…) will avoid the lack that exists when assessing emerging contaminants and the uncertainties of their effects and when looking for more sensitive responses in the assessment of dredged material.

Recently the biomarker approach has been incorporated into several pollution monitoring programs in Europe and the USA. Likewise, different methods for biological effect measurement have been evaluated in a series of practical workshops organized by the International Council for the Exploration of the Sea (ICES) and the Intergovernmental Oceanographic Commission (IOC), such as those in the North Sea (Stebbing and Dethlefsen, 1992. The United Nations Environment Programme has founded a biomonitoring programme in the Mediterranean Sea including a variety of biomarkers (UNEP). Recently biomarkers have also been included in the Joint Monitoring Programme of the OSPAR convention where Portugal and Spain are members. Nevertheless, the biomarkers approach has not been included in the guidelines for the management and monitoring of dredging and disposal activities. The current guidelines for the control of these activities are based on the several approaches which take into account chemical measurements, analysis of benthic communities and toxicity tests. Very few have already been studied about the utility of the use of biomarkers. Most of the regulation agencies comment the validation of them for this propose and their effectiveness in the new guidelines. Nevertheless, there are still some aspects that need to be defined before recommending them widely.

Several methodologies can reveal both the “exposure” to specific classes of chemicals or different “effects” experienced by the organisms (ICES). An important advantage of biomarkers in assessing the impact of dredged materials is the inherent capability to detect early occurrence of various stress conditions within the organism and monitor the temporal progression (or regression) of the disturbance of various

levels of the biological organization (Regoli et al., 2002). In this sense, some authors have been developed different studies for the application of the use of biomarkers to assess the impact of dredged materials. Most of the studies are focused in the determination of the activity of biotransformation enzymes, antioxidant enzymes and biochemical indices of oxidative damage. Detoxification enzymes metabolize the xenobiotic compound into a more water soluble form, which can be excreted from the body more easily than the parent compound (Lech and Vodicnik, 1985). Some xenobiotics compounds including aromatic diols and quinines, nitroaromatics, aromatics hydroxylamines, bipyridyls and certain transition metal chelates (Rodríguez-Ortega et al., 2003) after the biotransformation in the organisms oxyradical are produced. Defense systems that tend to inhibit oxyradical formation include antioxidant enzymes. If this oxyradical formation is not inhibited it could lead to enzyme inactivation, lipid peroxidation, DNA damage and, ultimately, cell death (Winston and Di Giulio, 1991).

Biomarkers have come of age, and there is now enough scientific evidence to apply biomarkers in a regulatory framework provided the essence of this regulation is a weight of evidence approach. Biomarkers may be applied to modern pollution problems such as chronic exposure (Galloway, 2006).

Biomarkers applied in a tier-testing approach for sediment management could allow the performance of more sensitive SQGs for dredged material assessment and management. Here it is discussed their inclusion in a tier- testing approach, starting with screening biomarkers together with chemical characterization on TIER I. Then, it is advised the determination, on TIER II, of oxidative stress responses (cytochrome P450 enzymes, lipid peroxidation…) and metallothionein like-proteins (MTLP) as biomarkers of exposure to organic and metallic contaminants, together with biomarkers of effect (genotoxicity, endocrine disruption, inmunotoxicity…). Finally, it is proposed the verification of these responses *in situ* assays on a TIER III.

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HOURLY DETERMINATION OF C_6-C_{12} ATMOSPHERIC VOCs USING AN AUTOMATIC ON-LINE GC-MS SYSTEM

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There are a large number of atmospheric volatile organic compounds (VOCs) in ambient air. Although they are in a very low concentration, it is very important to identify and measure them because they are precursors of ozone and fine particles, they contribute to the climate change and they have adverse effects on human health. Concretely, several of them are listed as toxic in the Air Quality Guidelines of the Word Health Organization (2000).

To study the temporal evolution of VOCs in ambient air, the use of automatic gas chromatography systems has grown rapidly in recent years. In 1997, an online gas chromatograph (GC), with a dual-column system and twin flame ionization detectors (FID) was installed in the ETSI of Bilbao (Northern Spain) to attempt systematic monitoring of these compounds.

Nowadays, mass spectrometer detectors (MSD) of the latest generation can achieve a sensitivity comparable to FID. Moreover, they give the chance of positive identification of new compounds. Thus solves some analytical problems observed in previous works using a GC-FID for the continuous measurement of VOCs: coelution of compounds (specially those ones with more than 7 atoms of carbon) and the instability of the retention times, which lead to the incorrect identification of chromatographic peaks. The use of a specific detector allows improving the identification of compounds and increasing the number of VOCs, including some toxic compounds that could not be identified with the GC-FID, but are very important in urban and industrial atmospheres.

During the last year on-line VOCs measurements have been done in the ETSI of Bilbao using an auto GC-MSD. The system has been optimised for systematic, unattended analysis of 49 VOCs (C_6-C_{12}) in urban air, every hour, 24 times a day, with sensitivities down to 0.1 ppbv. After several years of experience measuring with an auto-GC-FID, the lightest compounds (between 2 and 6 atoms of carbon) have been perfectly identified.

In the present work, the setting-up of the system, the identification of new compounds and the main results, for those compounds which are considered toxic, are presented. In addition, for the treatment and validation of the big amount of information generated, specific methods of processing and interpretation have been developed, taking into account the special characteristics of the system: on-line sampling without pretreatment of the sample.

MICROWAVE-ASSISTED EXTRACTION AND ULTRASOUND EXTRACTION TO DETERMINE POLYCYCLIC AROMATIC HYDROCARBONS IN NEEDLES AND BARK OF *PINUS PINASTER* AND *PINUS PINEA*

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Pine trees can act as biomonitors for the occurrence of a wide range of persistent organic pollutants in the environment, mainly through the retention properties exhibited by the waxy layer of their needles. The worldwide presence of different pine species allows not only a very representative estimation of the levels of POPs but also comparative data to establish bioaccumulation or transport patterns between different locations. Pine bark has also been studied for some organic compounds, but is mostly recognized for trapping metallic elements.

However, extraction and analysis of pine needles and bark require effective extraction and clean-up procedures to retain the target compounds and eliminate waxy compound and other interferences. Hence, many approaches are continuously being attempted in search of faster, cleaner and reliable analytical methodologies.

The main objective of the current work is to test the efficiency of microwave-assisted extraction (MAE) and ultrasound extraction (USE) followed by alumina cartridge solid-phase extraction (SPE) clean-up for the determination of the 16 EPA polycyclic aromatic hydrocarbons (PAHs) in pine needles and bark. PAHs are ubiquitous priority pollutants resulting from natural and anthropogenic sources and possess carcinogenic and mutagenic properties. *Pinus pinaster* and *Pinus pinea* trees were chosen, once they are the two main pine species in Portugal and are also common in the Mediterranean area.

Two sample masses (1 g and 5 g) and two PAHs spiking levels (20 ng/g and 100 ng/g) are used for both needles and bark of each species. The two methods are compared not only in terms of validation parameters (detection limits, precision, recovery) but also though the PAHs levels detected in samples from 8 natural sites (4 in Portugal and 4 in Catalonia). Analysis and quantification is performed by gas chromatography–mass spectrometry (GC-MS) in SIM mode, using deuterated PAHs as internal standards.

AN AUTOMATED TECHNIQUE FOR THE CONTINUOUS MONITORING OF ORGANIC MATERIAL CONCENTRATIONS AND TOTAL TOXICITY IN WATER SAMPLES: REAL WORLD APPLICATIONS OF THE BOD-TOXICITY MICROBIAL BIOSENSOR

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A new automated technique for the real time detection of Biochemical Oxygen Demand (BOD) and Total Toxicity has been developed by Biosensores, S. L and has been objectively assessed by the scientific community (EU Concerted Action - BIOSET). The BOD-Toxicity Microbial Biosensor (BOD-TOX) uses the respirometric activity of a stable population of sensor microorganisms to measure the quantity of organic material in a water sample. The double calibration of the device during each analytical cycle, with a standard solution of known BOD_5, has been shown to facilitate the detection and quantification of toxic effects using changes in the rate of respiration of a microorganism population. 'On-line' measures of BOD, using the BOD-Toxicity Microbial Biosensor, correlated positively with standard BOD_5 assays (r=0.98). The device has been shown to detect toxic events not only in the laboratory but in the field. The BOD-TOX prototype has been used to monitor the BOD and toxicity of WWTP and River waters and its long-term viability is demonstrated by 3 years of uninterrupted, real time monitoring, 'in situ'. Field experiments carried out on the Rio Jarama show a high level of utility for this device. BOD-TOX has been demonstrated to be a viable and efficient online monitor of Total Toxicity and BOD and is a proven rapid, accurate and low cost (0.8€) per cycle, including both BOD and Total Toxicity measurements) automated and portable alternative to conventional methods for monitoring organic matter in water. The BOD-Toxicity Microbial Biosensor is a good example of the transition of a biosensor technology, from laboratory development, to a robust device with 'real world' applications.

IMPROVEMENTS IN DETERMINATION OF LEAD IN ROUMANIAN WINES BY ELECTROTHERMAL ATOMIC ABSORPTION SPECTROMETRY

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This paper proposes a method for the determination of lead in Roumanian wines. A electrothermal atomic absorption spectrometry technique using transverse heated graphite atomizers and comparatively, deuterium lamp and Zeeman-effect background correction were applied.

The analysed samples were prepared prior to measurement by using a microwave digestion system in a closed vessel. The graphite furnace parameter were optimised, namely pyrolysis and atomization temperature.
Because of the low amount of the lead in wine, it is preferable to perform the analysis directly on the samples, without any former processing. The digestion of the sample using macrowaves is reccomanded because of the complex matrix of the wine, but this methods requires a 5 times diluting of the sample. For this reason a preconcentrating of the sample was necessary.

The performances of the method in terms of precision and sensitivity are described.

DEVELOPMENT, CALIBRATION AND FIELD VALIDATION OF PASSIVE SAMPLING DEVICES FOR MONITORING EMERGING CONTAMINANTS IN AQUATIC ENVIRONMENTS

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There is an urgent need for the development and validation of cost-effective technologies that can be adopted for routine monitoring of surface waters to provide reliable data on levels of specific pollutants. Currently the most commonly used method for measuring pollutants is spot (bottle) sampling but this provides ony a snapshot of the situation at the moment of sampling. Alternative methods include continuous, on-line monitoring systems, sensors, and passive samplers. These approaches provide a more representative picutre of average conditions over a preriod of days to months. Passive sampling is based on free flow of analyte molecules from the sampled medium to a receiving phase in a sampling device, as a result of a difference between the chemical potentials of the analyte in the two media. Sampling proceeds without the need for any energy sources other than this chemical potentialdifference. Passive samplers allow the measurement of time-weighted average concentrations of freely dissolved contaminants in water over time periods up to several weeks.

We show several passive sampling devices with a design that can be adapted to monitor many classes of emerging polutants e.g. endocrine disrupting compounds, polar and non-polar pesticides and organometallic compounds in aquatic environments. One versatile passive sampler design, called Chemcatcher, is based on the diffusion of analytes through a semipermeable membrane to a sorbent disk. The device uses a common design of a sampler body with interchangeable receiving phases and diffusion membranes depending on the application. Another device, MESCO (membrane-enclosed sorptive coating), is a miniature sampling system that combines sampling with solventless pre-concentration in polydimethylsiloxane material. The sampler enables direct analysis of the accumulated contaminants by thermodesorption coupled on-line to GC, thereby avoiding time-consuming sample preparation and clean-up. We show examples of calibration and field validation of various sampler configurations and discuss their applicability for monitoring of emerging pollutants in aquatic environments.

SIMULTANEOUS DETERMINATION OF THREE NITROIMIDAZOLES AND THEIR METABOLITES IN EGGS BY PRESSURIZED FLUID EXTRACTION (PFE) AND LIQUID CHROMATOGRAPHY- TANDEM MASS SPECTROMETRY

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Nitroimidazoles had been widely used as veterinary drugs for the treatment of coccidiasis in poultry. These antiprotozoal compounds are potentially carcinogenic, toxic and mutagenic [1], and for this reason nitroimidazoles have been classified into Annexe IV (banned substances) of the Council Regulation (EC) 2377/90 [2].

A new method has been developed to simultaneous determination of three nitroimidazoles (Dimetridazole (DMZ), Ronidazole (RNZ), Metronidazole (MNZ)) and their metabolites Dimetridazole-OH (HMMNI) and Metronidazole-OH (MNZOH)) in eggs. The proposed method includes pressurized fluid extraction (PFE) [3], liquid-liquid partition, solid phase extraction (SPE) clean-up on an aminopropyl (NH2) cartridge, and final LC-MS-MS detection.

PFE factors such as solvent, time and temperature have been optimised, and for the SPE step different cartridges were investigated. The determination was achieved by liquid chromatography (LC) coupled to positive electrospray ionisation tandem mass spectrometry (ESI-MS-MS). The ion source settings including capillary temperature, seath gas pressure, auxiliary gas pressure and spray voltage, were optimized using a design of experiments (DOE) approach [4]. The optimized method was validated according to Commission Decision 2002/657/EC[5]. The procedure gives recoveries between 93 and 108% with relative standard deviations (RSD) that ranged from 8 to 20%. The decision limit (CCα) ranged between 0.50 μgkg^{-1} for RNZ and 1.4 μgkg^{-1} for MNZ, and the detection capability (CCβ) varied from 0.73 μgkg^{-1} for RNZ up to 1.5 μgkg^{-1} for MNZ.

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USING DECISION TREE ANALYSIS AND GIS IN MODELLING (SEMI)VOC EMISSIONS AT THE EUROPEAN SCALE

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Emissions of chemicals to air, surface water and soil are important in human and environmental risk assessment of chemicals. In this study focus is on Volatile and semi-Volatile Organic Compounds (VOCs and semi-VOCs), which cover a large group of chemicals predominantly found in industrial processes and in many consumer products. Some emissions are point sources and can give rise to high local concentrations, hot-spots, in adjacent surface water, soil and the surrounding air. Other emissions are diffuse and cause emissions that are widely distributed in the environmental compartments. Emission quantification, or emission inventories, is the natural starting point of the life-cycle analysis of VOCs. Large emissions are often related to the production, formulation and processing and can be found from direct use of the chemical, use as intermediate in the production of other chemicals, use of products containing the chemical and disposal of the chemical and products. The complex nature of chemical emission patterns makes quantification of emissions very uncertain, and in many cases the predominant uncertainties in a risk assessment are indeed related to the uncertainties of the emission inventories. The basic features of emission estimation are:

1) Identification of chemicals to be included
2) Quantification of production and use amounts
3) Distribution of chemicals to products and activities
4) Quantification of emission factors for each chemical and use
5) Specification of location of releases, and mapping

Important and comprehensive work has already been performed for 78 single chemicals by the EU member states and coordinated by the European Chemicals Bureau. This work includes all the above criteria and is compiled in risk assessments reports (RARs). The approach used in this study is to look at the detailed results in the RARs, and examine how to utilise them in order to predict emissions of not assessed chemicals in the most reliable and transparent way. Furthermore quantification of use amounts in industry and consumer products are found from Eurostat and the Nordic Products Database (SPIN). Selected parameters such as vapour pressure, logKow and water solubility are found from e.g. IUCLID. With respect to mapping, industrial emissions will be assigned to locations described in the European Pollutant Emission Register (EPER). Wide-dispersive emissions related to public/household use and to other use delivering uncontrolled exposure are mapped accordingly:

a) Emissions from use in households are correlated with population density or urban areas
b) Emissions from industrial activities are correlated with CORINE industrial land use or more specific information if available

c) Emissions from urban areas and professional workers are correlated with CORINE land cover, urban categories
d) Emissions from transportation are correlated with traffic density maps

The output is thus a quantification and spatial distribution of emissions, which will support the selection of risk scenarios on a European scale.

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STUDIES OF LARGE ORGANO - METALLIC POLLUANTS USING ELECTRON SPECTROSCOPY

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In the Environmental Chemistry our research is tackling various kinds of polluants existing in water, which are necessary for the preservation of the environment, and on high-performance separation and analysis methods, as well as on the decomposition and processing techniques to deal with refractory chemical substances present in the water. These pollutants are divided into two categories, the first one includes metallic ions such as Nickel, Cadmium, Ferric, Chromium, Cuppric ions. The second one includes organic pollutants such as aliphatic and aromatic amines like, phenyl alanine, tryptophane, arginine, amino-2-phenol and N-N-dimethyl formamide, as well as the non-metal, iodine. Formations of complexes and their behaviour are also included in this work.

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APPLICATION OF HPLC AND ELISA METHODS FOR MONITORING OF SELECTED EMERGING POLLUTANTS IN SLOVAKIAN SURFACE WATERS

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The use of on-line solid-phase extraction coupled to liquid chromatography and diode array detection (SPE-HPLC-DAD) for analysis of selected "emerging" pollutants in surface waters in Slovakia is presented. The substances from the group of emerging pollutants which are monitored at national level in Slovakia are: phthalates, nonylphenols, octylphenols and bisphenol A. For the enrichment of these substances, the SPE microcoluns packed with styrene-divinylbenzene copolymer was used. To increase the selectivity and sensitivity of detection for alkylphenols and bisphenol A, the efluent from DAD detector was connected to the fluorescence detector. In the past years much attention was devoted to presence of bezothiazole in Slovakian surface waters. This substance is the main accelerator of vulcanisation in rubber indurtry. The production of benzothiazole and its derivatives is specific for Slovakia. Benzothiazole is not toxic, but affect the sensorial properties of water. Its degradation products - benzothiazolecarboxylates are auxine active. For the monitoring of benzothiazole again the reverse-phase HPLC-DAD method was used. Other types of emerging pollutant are some polar pesticides. One of these pesticides is glyphosate, which is organophosphorous derivatives of glycine. Glyphosate is widely used total herbicide, with high solubility in water. Trace determination of this herbicide is complicated due to its low molecular weight and absention of chromophores. In our laboratory we apply the in-sample pre-column derivatization and on-line SPE-HPLC-DAD-FLD method. The 9-fluorenylmethylchloroformate was used as the derivatizing agent. The cyanobacteria, also known as blue-green algae, are a major group of bacteria that occur throughout the world and may concentrate on the surface as blue-green "scums". Some species of cyanobacteria produce toxins, as hepatotoxins (e.g. microcystins), neurotoxins (e.g. anatoxins). These cyanobacterial toxins are also identified as emerging pollutants. Sensitive method for detection of the cyanobacterial toxins - microcystins was developed and evaluated. Microcystins were analysed in water samples and in biomass by HPLC-DAD. Enrichment of toxins from water samples was realized in off-line mode using SPE columns. Also comparison of HPLC method with alternative ELISA method for the analysis of microcystins was done. MCYST plate kit uses polyclonal antibodies, which bind both microcystins and MCYST-LR-enzyme conjugate. Antibodies which bind the toxins components are immobolized to the inside of the test wells. The ELISA method provides toatal concentration of microcystins.The microcystins in Slovakian lakes were detected as microcystins -RR, -YR and -LR; the -RR and -LR types were the main components of cyanotoxin in the lakes.

RESPONSE SURFACE METHODOLOGY FOR THE MICROWAVE ASSISTED EXTRACTION OF INSECTICIDES FROM SOIL SAMPLES

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A rapid analysis of pesticide residues in soil samples is required for environmental analyses and for the evaluation of laboratory experiments such as pesticide degradation or leaching in soil columns. A response surface methodology has been used for the optimization of the extraction procedure of deltamethrin, □-cypermethrin, diazinon, dimethoate and malathion in soil using microwave assisted extraction (MAE). These insecticides, for which a single extraction method is envisaged, belong to pyrethroid and organophosphorus families, and present a wide range of physicochemical properties, such as water solubilities (from 20 g L^{-1} to <0.2 μg L^{-1}) or hydrophobicities (log K_{ow} values range from 0.704 to 6.94). The design of experiments for dealing with the optimization and understanding of the system performance can provide information about rates of output response changes, as well as indicate interactions between the factors implied with a minimum number of experiments to reach the objectives as quickly and as accurately as possible with the appropriate precision. Different factors have been optimised with this methodology: soil/extraction solvent proportion, water addition to the extraction solvent, ramp time, final temperature and duration of temperature plateau. The MAE extractions were carried out with ethyl acetate as the extraction solvent and continuous agitation during the process. The analysis of the extracts, after centrifugation and evaporation to dryness, was carried out by gas chromatography with electron capture detector. Under the experimental conditions the factors water addition and the proportion soil/extraction solvent had the greatest effect on pesticide responses. For final temperature and plateau time the interaction was the strongest. An interaction between soil/extraction solvent proportion and final temperature was also found. The behaviour of dimethoate and malathion, not stable to heating, was different from the rest of compounds and was affected by final temperature of extraction. Multiple responses variables create difficulty because when optimal for a response it may not be optimal for other responses. In this case, the response surface methodology was appropriate for the optimization of the extraction procedure since it explored the relationships between the extraction conditions and one or more pesticide recoveries to obtain an optimal response.

EVALUATION OF PESTICIDES POLLUTION IN THE IRRIGATION AND DRAINAGE CHANNELS OF THE EBRO RIVER DELTA DURING THE GROWING SEASON OF RICE USING CHEMOMETRIC AND GEOSTATISTICAL METHODS

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Different data sets obtained in the quantitative determination of seventeen selected pesticides collected in water samples of a network of irrigation and drainage channels in the Ebro river delta (Catalonia, NE Spain), were analyzed by chemometric and geostatistical methods. Samples were taken at fourteen locations during the main growing season of the rice crop cultivation, from May to August 2005. Chemometric methods allowed the investigation of both spatial and temporal distributions of the main pollution patterns due to the application of pesticides in the region under study. A first pesticide contamination pattern coming from the Ebro river was differentiated from a second more specific pesticide contamination pattern coming from the delta water drainage channels, collected from the rice fields. The seasonal peak in the use of this more specific rice pesticide source was observed in July. During this month, the effect of repumping was clearly apparent, when there was not enough water into the irrigation channels and already polluted water was reused to irrigate the fields. Coupling the results of chemometric data analysis with the use of geostatistical methods is shown to be a useful procedure to underline the more significant spatial and monthly variations of the main pesticide contamination patterns, taking into account the particular geographical structure of the area under study.

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SYNERGISTIC AND ANTAGONISTIC EFFECTS OF C_{ORG} AND NO_3^- IN DENITRIFICATION ACTIVITY FROM A WETLAND SOIL IN THE BASQUE COUNTRY

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INTRODUCTION

Nitrous oxide (N_2O) is a long-lived trace gas contributing to the enhancement of the greenhouse effect and depletion of the stratospheric zone layer (Bouwman, A. F., 1990). It is a potent greenhouse gas, perhaps, 200-300 times more effective than CO_2 (IPCC, 1990). Soil and wetland zones denitrification is one of the most important sources of natural nitrous oxide emissions specially those places that receive N subsidies from agricultural drainage. (Lowrance et al., 1995). Previous studies have shown that the physical-chemical characteristics of Salburua wetland non-saturated soil make possible the denitrification process: important clay cover, very low hydraulic gradient and organic matter rich wetted soils (Sanchez-Perez et al 1995, Ruiz et al 2004).

The biological denitrification activity changes according to the organic carbon composition and the nitrate content of the soil, and there can exist both antagonistic and synergistic effects (Jørgensen et al., 2004, Svensson et al., 1998).

We measured in laboratory the rates of N_2O emission using the C_2H_2 block technique (Yoshinari and Knowles, 1976) in soil samples collected from Zurbano wetland located in the East Sector of the quaternary aquifer of Vitoria-Gasteiz (Basque Country), which was designated by Basque Government as a Nitrate Vulnerable Zone according to the 91/676/CEE European Directive.

METHODOLOGY

The sampling of soils was carried out in Zurbano pool in Salburua wetland (Vitoria-Gasteiz) in February of 2006. The soil is mainly formed by clay and silt. Groundwater level is very close to the surface; the soil is wholly wetted and scarcely drained. Two horizons of soil were used, the most superficial horizon (0-20 cm) and other subsuperficial (45-65 cm).

The sets of vials were used for measuring the enzyme activities (denitrification potential with C_2H_2 block technique) after the addition of different amounts of nitrate (0, 50, 100 y 200 mg NO_3 L^{-1}) and substrate (1 mg glucose) to 10 g of soil samples. The evolution of CO_2 and N_2O in serum vials each during the seven days of experimentation, were determined with gas chromatograph.

RESULTS

The addition of different amounts of nitrate increases the N_2O emissions in all soil samples. The soil the more nitrate quantity was added the higher values of nitrous oxide were measured: 54.8 mg N_2O kg dry soil^{-1} in 50 mg NO_3 L^{-1} treatment; 155.3 mg N_2O kg dry soil^{-1} in 200 mg NO_3 L^{-1} treatment. The increase in N_2O generation rate is more accused in soils with greater content in organic matter. The average nitrous oxide generation values after substrate addition was two times higher than the initial values without glucose, however it could be measured in some cases values that were three times higher than the original ones (without glucose 181.7 mg N_2O kg dry soil^{-1}, with glucose 553.7 mg N_2O kg dry soil^{-1} d^{-1}).

However the CO_2 generation rates showed different evolution patterns according to the nitrate treatment. In some soil samples microbial respiration rates were inhibited (with 200 mg NO_3 L^{-1} 62% less intense than with no nitrate) whereas in other cases it was stimulated (with 200 mg NO_3 L^{-1} 68% higher than with no nitrate). The evolution of CO_2 generation rate shows an increase in all treatments after glucose substrate addition, from a maximum of 28682.5 mg CO_2 kg dry soil^{-1} in the treatment with substrate to a minimum of 770.9 mg CO_2 kg dry soil^{-1} without substrate.

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EC FP6 COORDINATION ACTION (CA) ON RISK-BASED MANAGEMENT OF THE WATER-SEDIMENT-SOIL SYSTEM AT THE RIVER BASIN SCALE (RISKBASE)

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Introduction: In RISKBASE, leading European scientists and representatives of major, European stakeholder groups will review and synthesise the outcome of EC RTD Framework Program projects, and other major initiatives, related to integrated risk assessment-based management of the water/sediment/soil system at the river-basin scale. The synthesis will lead to the development of integrated risk assessment-based management approaches enabling the prevention and/or reduction of the negative impacts caused by human activities on that system.

Deliverables: 1) An overarching concept, generic approach and guiding principles to integrated risk based management of river basins, 2) Recommendations towards evolution and implementation of risk based management in national and community policies and towards implementation in management, and 3) A proposal for the European research agenda related to risk based management.

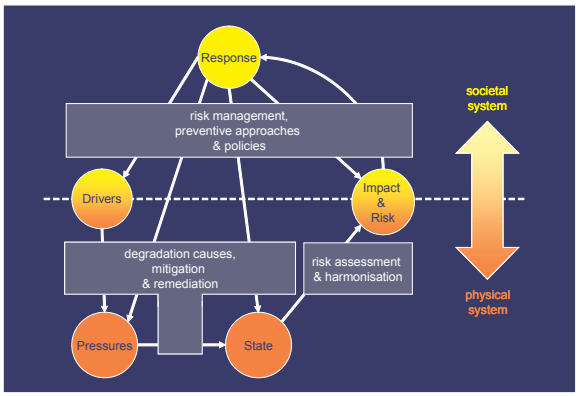

Fig. 1: RISKBASE project structure.

Working modus: Based upon ample experience in previous EC CAs, Thematic Networks and/or Accompanying Measures, a simple project structure is chosen (see figure 1), with only a minimum number of Work Packages (WP). Each WP will be managed by one WP-leader, supported by a few other partners (contractors) in the project. The WPs will organise several workshops dedicated to specific issues related to risk based management at the river-basin scale. Furthermore, RISKBASE will annually organise a General Assembly (GA) and will make use of EUGRIS as web-based information exchange structure. The workshops, GA and the website will be open to all who are interested and willing to contribute to achieve the RISKBASE goals and objectives.

Furthermore, during the project, each WP will select core-team members to assist the WP-leader in reviewing, synthesising and then reporting of the outcome of WP-workshops. Thus an open, transparent and flexible structure is created ensuring the integration of all essential knowledge, expertise and experience in order to make RISKBASE a success.

Project duration: 36 months (start: 1 September 2006; end: 31 August 2009)

Topic addressed: RISKBASE (Contract No. 036938 GOCE) addresses topic II.2.1 “Integrated risk based management of the water-sediment-soil system at river-basin scale”. This is a topic under the thematic sub-priority area “Global Change and Ecosystems” in the 4th FP6 call for proposals, call identifier: FP6-2005-Global-4.

TOXICITY ASSESSMENT OF FLUORINATED ALKYL COMPOUNDS USING *Vibrio fischery*

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Fluorinated alkyl compounds (FACs) are found in trace amounts in many environmental compartments proceeding from different areas of production including stain repellents, paper coatings, polymers, and surfactants. This, along with the highly persistent and bioaccumulative nature of PFOS, presents a concern for possible effects in aquatic ecosystems. In recent years various analytical methods have been developed for the analysis of FACs in environmental samples. But many questions about human exposure, toxicity, and ecotoxicology remain for these emerging contaminants.

The objective of this study was to determine the toxicity vs. *Vibrio fischeri* of most common fluorinated alkyl compounds found in aquatic environments. *Vibrio fischeri* was selected as reference test specie because it is a standardized organism for acute aquatic ecotoxicity.

The FACs selected in this study were: Pentadecafluoro-octanoic acid (PFOA) (CAS:335-67-1), Heptadecafluorooctanesulfonic acid (PFOS) (CAS: 1763-23-1), Tridecafluoro-heptanoic acid (PFHpA) (CAS: 375-85-9), and Tricosafluorododecanoic acid (PFDoA) (CAS: 307-55-1).The effective concentration producing the 50% of bioluminescence inhibition (EC_{50}%) and the toxicity units (*TU*) using *Vibrio fischeri* for each compound were determined.

In addition, a preliminar sinergism study was carried out measuring the effect produced by mixtures of these compounds in different proportions.

The toxicity studies were carried out using a new developed portable device AbraTox Camera, and the results were compared with those using the EN-ISO procedure.
Finally, the results obtained with *Vibrio fischery* were compared with those using representative freshwater organisms, such as *Daphnia magna*.

EXAMINATIONS OF PHYSICAL AND CHEMICAL PROPERTIES OF SEDIMENTS TOWARDS ADSORPTION OF SELECTED ANTIPHLOGISTIC DRUGS

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Sediments contaminated with organic and inorganic compounds resulting from human activities are recognized as a world-wide problem. The contaminants can harm both the environment and human health. Certain amount of pharmaceuticals and their residues, among other contaminants, enter the aquatic environment and eventually may occur in drinking water. Because of their environmental persistence they may avoid degradation in sewage and drinking-water treatment plants.

The occurrence and environmental behaviour of pharmaceuticals in water and sediments has been little investigated in Poland. Sediment is one of the key components of the aquatic ecosystem.

The aim of the study was to determine the chemical and physical properties of sediments taken from the Dobczyce drinking water reservoir in southern Poland, which supplies about 1 million inhabitants of the city of Krakow with drinking water.

A preliminary step will be further investigation of the sediments' adsorptive abilities towards selected drugs: ibuprofen, salicylic acid, diclofenac. The study should answer the following questions: conditions of accumulation of the drugs in sediments, methods for of their extraction, their determination in sediment samples, and the current state of their occurrence in river and lake sediments.

ENVIRONMENTAL ANALYSIS OF FLUORINATED ALKYL SUBSTANCES BY LIQUID CHROMATOGRAPHY-(TANDEM) MASS SPECTROMETRY. A REVIEW.

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Fluorinated alkyl substances (FASs) are widely distributed contaminants that have been found in many environmental, human, and biological samples throughout the world. Perfluorochemicals are used in many industry and consumer products, such us polymers and surfactants, because they have unique useful properties (they are stable, chemically inert and generally non reactive). However, these compounds have also found to be toxic, persistent and bioacumulative. The family of FASs includes the perfluoroalkyl sulfonates [perfluorooctane sulfonate (PFOS; $C_8F_{17}SO_3^-$) and related chemicals, such as N-methyl and N-ethyl perfluorooctanesulfonamidoethanol, and also short- and long-chain perfluoro sulfonate acids], the perfluoroalkyl carboxylates [perfluorooctanoate (PFOA; $C_7F_{15}COO$-) and fluorotelomer alcohols (FTOHs; $F(CF_2CF_2)_{n(2-5)}CH_2CH_2OH$)], and the short- and long-chain perfluoroalkyl acids [e.g. perfluorodecanoic acid (PFDA)].

In the last years various analytical methods have been developed for the analysis of FASs in environmental samples. Most of these methods are based on the use of liquid chromatography coupled to mass spectrometry (LC-MS) or tandem mass spectrometry (LC-MS/MS) since this is considered the technique of choice. This technique, especially when working in the SRM mode, allows limits of detection in the picogram or low nanogram per liter range in the case of water samples, in the pg-ng/mL range in the case of biological samples, and in the pg-ng/g range in the case of solid environmental samples, after fairly simple pretreatment procedures. RP-C18 and perfluorinated RP-C8 are the LC stationary phases that provide the best chromatographic separation. The analysis of most perfluorinated surfactants is better achieved by ESI in the negative ionization mode. The main problems encountered in the environmental analysis of these analytes are the abscence of reliable standards (they are complicated mixtures of isomers that often contain impurities), the existence of ion suppression due to matrix effects, and contamination during all stages of the analytical procedure.

This poster reviews the various LC-(tandem)MS methods described so far for the analysis of FASs in water, sediment, sludge, air and biota samples. It provides information on the main experimental conditions used for sample pretreatment and for analysis as well as the most relevant problems encountered and the limits of detection achieved.

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DESIGN AND PERSPECTIVES OF THE USE OF A BATTERY OF BIOMARKERS FOR THE ADVERSE EFFECT ASSESSMENT OF NEW EMERGING POLLUTANTS: PHARMACEUTICALS AND PERSONAL CARE PRODUCTS (PPCPS)

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The effluents are a major source of many environmental contaminants, including polyaromatic hydrocarbons, pesticides, surfactants, steroids and metals. Recently, pharmaceuticals and personal care products (PPCPs) have been identified as an emerging class of potential pollutants for the aquatic environment. Indeed, non-prescription drugs such as analgesics and stimulants, nonsteroidal anti-inflammatory agents, and prescription drugs such as carbamazipine (CBZ), have been found at ng/L to ug/L levels in municipal wastewaters (Boyd et al., 2003).

To date, EU and US legislation include specific guidelines for assessing the environmental exposure to veterinary medical substances and risk assessment procedures of the exposure to medical substances for human treatment. The proposed directives prescribe that a risk assessment should be part of the approval procedure of new substances. But it seems that this legislation is not of high concern, since only few new medical substances in recent years, to the authors' knowledge, have been subjected to a complete risk assessment where a battery of appropriate ecotoxicological tests were included. Therefore, there is a demanding necessity of proposing and validating appropriate toxicity tests which could include the determination of biomarkers and their developing under the laboratory and in situ conditions. The specific redox reactivity of PPCPs forms the basis for their respective biological (therapeutic) effects, metabolism, elimination, and toxicity. Exposure to these products can after the oxidative state of cells and thereby increase oxidative stress. Although PPCPs act at various tissues, most of them are metabolized in the liver (hepatocytes) by five cytochrome P450s from the CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP2A4 families in humans, CYP2C9 and CYP3A4 representing the major isoenzymes for drug metabolism (Stresser et al., 2000).

The study of the cytotoxic effects of these compounds through the determination of the activities of the cytochromes P450s enzymes involved in the metabolism of these drugs has been demonstrated to be sensitive biomarkers of exposure to PPCPs and to provide an estimation of pollutant bioavailability. Together with the evaluation of pollutant bioavailability, early biological responses can be assessed with the determination of lipid peroxidation and DNA damage in bioindicator species (Gagné et al., 2006). In these studies, laboratory to field extrapolation becomes crucial. The design and validation of this battery of biomarkers should be tested in the laboratory with the extract of the effluent and the PPCPs and in the field with the exposure to the stream.

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TENAX EXTRACTION AS A TOOL TO EVALUATE THE AVAILABILITY OF BROMINATED FLAME RETARDANTS IN SEDIMENTS.

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The majority of current experiments for assessing the risk present in the aquatic environment from sediment are based in the determination of the total quantity of certain contaminants in this matrix, and their accuracy is interpreted in chemical terms. However, not all the chemicals present in the medium are available for the uptake or transformation by living organisms. Due to increasing contact time the (bio)available fraction of the chemicals decreases[1].

Solid Phase Extraction (SPE) with Tenax® or cyclodextrin have been used to measure the bioavailable fraction of Hydrophobic Organic Contaminants (HOC) that can rapidly desorb from sediment. The sorption of pollutants to these adsorbents is so strong that the concentration of them in the water decreases to values near to zero, so that the phase equilibrium between water and sediment is shifted to the water. SPE during a certain period has been proven several times to correlate well with the fraction that a certain organism can uptake or degrade from sediment. They have been used for PAHs, PCBs and other pollutants of concern as DDT[2,3].

In this study we applied by the first time the Tenax extraction for some emerging pollutants that are used as Brominated flame retardants (BFRs) in a broad range of consume products. They are bioaccumulating, persistent and ubiquitous. Disruption of thyroid hormones, deficiencies in neuronal development and cancer have been proved for some of them in long time of exposure.

A solid phase extraction with Tenax was performed in 6 consecutive steps up to 432 hours at 20ºC. For that purpose, we have used the method described by Cornelissen[2] with some modifications. The wet equivalent of 2g dry weight of sediment was mixture with 40 mL of an aerobic growth medium composed of water plus nutrients, 0,5 mL of $HgCl_2$ (for preventing microbial degradation) and 2 g of Tenax TA in a separation funnel and shaken. After each selected period the Tenax was separated from the rest of the mixture and 2 g of new Tenax was added. The Tenax isolated each time and the residual sediment were analyzed. Besides, a total extraction of the sediments was performed in order to compare it with the fractions desorbed during each interval. Both the exhaustive extraction from the sediment and the extraction from the Tenax were performed by Microwave Assisted Extraction followed by a liquid-liquid extraction with water. The concentrated extracts were

analyzed by Gas Chromatography coupled with Mass Spectrometry and quantified by Internal Standard method.

Results show that the measured flame retardants are present in the sediment in the available as well as residual fraction after Tenax extraction, showing that the bioavailability concept is also valid for these types of compounds. The thyroid hormone effect of the specific Tenax extracted fractions will be reported upon later.

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SOLID PHASE EXTRACTION STRATEGIES AS APPLIED TO THE EXTRACTION OF SULFONAMIDE ANTIMICROBIALS FROM DIFFERENT WATER SAMPLES.

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Sulfonamides are one of the most commonly used antimicrobials in humans and animals. They act as bacteriostatic agents, being able to inhibit bacterial growth .During the last years, the concern regarding the potential presence of these antibiotics in the environment has increased considerably, due mainly to their high excretion rates (after their intake by humans or livestock)[1] and their high water solubilities, which convert them in highly persistent pollutants in the environment. Furthermore, sulfonamide antibiotics are metabolized to a considerable and varying extent in the human body, (e.g. by acetylation and hydroxilation) and these metabolites, together with the not assimilated parent substance, are also excreted mainly via urine. The retransformation of N4-acetylsulfamethazine to the active sulfamethazine in manure storage has already been shown by Berger et al [2], suggesting a similar cleavage of other N4-acetylated sulfonamides.

MATRICES

In this study, we have developed a highly selective method for the determination of nine sulfonamides and one acetylated metabolite in four different water matrices: groundwater, surface water and wastewater (influent and effluent of a wastewater treatment plant). The different water samples (400 ml, 200 ml for WWTP influent water) were cleaned up and preconcentrated by solid phase extraction (SPE) using three different strategies: HLB cartridges (Hydrophilic-Lipophilic Balance Sorbent reversed-phase sorbent), MCX cartridges (Mixed-mode Cation-eXchange and reversed-phase sorbent; highly selective for basic compounds) and a tandem MCX-HLB extraction. Samples were previously spiked with a mixture of the analytes to get a final concentration in the extract of 0.5 μg/mL. A surrogate was also added at the same concentration. Besides, depending on the strategy followed, H_2SO_4 concentrated was used to lower the pHs of the samples. After SPE, cartridges were eluted and the corresponding extracts evaporated and reconstituted in water-acetonitrile (75:25). The final extracts were analyzed by liquid chromatography tandem mass spectrometry, using electrospray in the positive ionization mode (LC-(ESI)-MS/MS) and multiple reaction monitoring (MRM).

D4-Sulfamethoxazole and D4-Sulfathiazole were the chosen surrogate and instrumental internal standard, respectively. However, the quantification was carried out following external calibration due to the irregular signals obtained from the chosen analytes mentioned previously.

The highest recoveries obtained in the different water matrices were those corresponding to the HLB Oasis cartridge strategy. The recoveries from the tandem SPE were also quite good, but showing generally higher variation coefficients between replicates, being the results from the MCX extraction the ones showing the

lowest recoveries. In reference to the different matrices, recoveries from the surface water samples were the highest, over 65 % and a deviation coefficient between 1 and 11% for the HLB cartridges. Water samples from the influent of the WWTP showed the lowest recoveries and, as expected, a strong influence of ion suppression in this matrix. Regarding the method performance, detection limits for the analytes ranged from 1 to 10 ng/mL in surface water up to 5 to 65 ng/mL in influent water samples, being the LODs for groundwater and WWTP effluent in between.

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APPLICATION OF A NEWLY DEVELOPED, HIGHLY SENSITIVE METHOD, BASED ON PRESSURIZED LIQUID EXTRACTION AND ANALYSIS BY LIQUID CHROMATOGRAPHY-ELECTROSPRAY-TANDEM MASS TO THE ANALYSIS OF TWENTY-TWO MEDIUM TO POLAR PESTICIDES IN SEDIMENTS OF THE LLOBREGAT RIVER BASIN

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This work reports the occurrence of twenty-two medium to polar pesticides, analysed by liquid chromatography-electrospray-tandem mass spectrometry (LC-MS/MS) in sediments of the Llobregat river basin. A total of twenty-four sediment samples were collected at four selected sites of the Llobregat river and 3 selected sites of the Anoia river (a tributary of the Llobregat river) during three different sampling campaigns, which were performed in June 2005, October 2005 and June 2006, to account for possible geographical and seasonal variations. Samples were extracted with a mixture of acetone:methanol (1:1) by pressurized liquid extraction (PLE). PLE extracts were purified by solid phase extraction using Carbograph Extract-Clean Column SPE cartridges (1000mg, 15mL; Alltech). Analysis of the purified extracts was performed by LC-MS/MS using a hybrid quadrupole-ion trap instrument provided with an electrospray interface operating in the selected reaction monitoring (SRM) mode. 2,4-D, bentazone, fenitrothion, MCPA, mecoprop and propanil were analysed in the negative ion mode and deisopropylatrazine, desethylatrazine, simazine, cyanazine, atrazine, terbutylazine, malathion, diazinon, dimethoate, chlortoluron, isoproturon, diuron, linuron, alachlor, metolachlor and molinate were analysed in the positive ion mode. Two SRM transitions were recorded per compound. The method showed satisfactory precision and accuracy with relative standard deviations lower than 20 % and recovery percentages higher than 50% for most compounds. Method detection limits (DLs) were between 0.034 ng/g (for propanil) and 6.7 ng/g (for alachlor). All pesticides investigated, except for diuron, propanil and bentazone, were present at very low levels, in the pg/g or low ng/g range, in the sediments investigated and the levels found did not differ significantly between sampling sites or campaigns. The method developed is well suited for the routine monitoring of medium to polar pesticides in sediments and its application to this and other solid environmental samples such as soils, may help establishing quality criteria in future regulations.

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LC-MS- ION TRAP DETERMINATION OF NATURAL AND SYNTHETIC ESTROGENS IN DRINKING WATER

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During the last decades, the ever increasing number of organic compounds which has been detected in surface and groundwaters has raised concern about the contamination of drinking water resources. Since it is impossible to monitor every substance that may be present in the surface water, it is necessary to define major targets of interest for water resources protection by focusing on substances that might be able to enter the drinking water supply. A wide variety of pollutants has been reported as dangerous for humans and wildlife and they are classified as ‘‘endocrine-disrupting compounds’’ (EDCs) after their biological effect. The aim of this work is to develop a simple method for the determination of hormones, either natural or synthetic (estradiol-17b, ethinylestradiol, estrone) which can be implemented in automated systems for drinking water control. The method is based on SPE and LC-MS determination with a Ion Trap analyzer.

A SPE protocol, based on C18 phase and methanol recovery, has been developed and validated. The concentrations of estrogens in different elution fractions have been determined showing that the elution of adsorbed compounds occurred in a bimodal way. This evidence suggests that two mechanisms of adsorption are active on C18 phase. The chromatographic method is based on a water-methanol (80:20) isocratic separation on a phenyl-hexyl column.

The ion-trap MS detector allows to obtain an acceptable level of sensitivity for estrogen compounds (about 1-5 ppb by direct injection) with a good selectivity due to MS-MS scanning mode and a linear response in a 5-1000 ppb concentration range.
Validation tests have been performed with fortified drinking water samples at 20 ppt. Since a concentration factor of 1000 has been used, a method LOD of 5 ppt has been estimated. Recoveries were always better than 80% and reproducibilities ranged from 4% at 200 ppt to 20% at 5 ppt, showing that accuracy and precision of the method are acceptable for drinking water monitoring purposes.

The analysis of different matrices, such as surface waters, was accomplished by using isotopic dilution correction with deuterated standards, which helped to overcome ionization suppression effect due to the matrix.

Preliminary tests with a triple quadrupole instrument showed that the sensitivity of the method can be improved from10 to 50 times.

This work has been carried out under the framework of workpackage 2.5 of AQUATEC project, a national program about the improvement of a technological approach for the protection of drinking water supply. The final aim of the workpackage is to develop an on-line LC-MS system for the monitoring of emerging organic pollutants in water used as drinking water supply.

AUTOMATED IN-TUBE SPME COUPLED TO LIQUID CHROMATOGRAPHY FOR THE DETERMINATION OF PHTHALATES IN ENVIRONMENTAL WATER SAMPLES

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Phthalates esters are synthetic compounds used as polymer additives in plastics, rubber, cellulose and styrene production [1]. Due to the widespread use of phthalates, they are considered as ubiquitous environmental pollutants. The phthalates can have adverse effects on human health, they can be considered endocrine disrupting compounds by means their carcinogenic action. The European Union has published a list of priority substances with a potential endocrine disrupting action, which includes di-n-butyl phthalate (DBP) and di-2-ethylhexyl phthalate (DEHP). As DEHP is the most widely used phthalate, it was incorporated in the list of priority substances in the field of water policy established by the EU and the World Health Organization (WHO), the guideline value was established at 8.0 µg/L in fresh and drinking waters [2, 3].

In order to study their impact in the environment, reliable quantification methods are required. The determination of phthalates in water samples generally requires a preconcentration technique, such as liquid-liquid extraction or solid-phase extraction (SPE), followed by gas chromatography (GC) or liquid chromatography (LC). These sample preparation methods often result in high blank values due to phthalates present in chemical and plastic accessories [4]. The use of solid-phase microextraction (SPME) provides extraction and preconcentration in one step, besides this is a solvent-free extraction technique. This sample preparation method has been used for the determination of phthalates, in some works it was coupled to LC or to GC.

In-tube SPME is a relatively new microextraction and preconcentration technique, which can be easily coupled to LC. This technique, using a coated open capillary as the SPME device, allows the automation of the process, so it saves the analysis time and provides better precision, accuracy and sensitivity relative to off-line manual techniques. In this work, we report contamination levels of DBP and DEHP in water samples by means an automated procedure based on a simple in-tube SPME configuration. It was coupled to LC with a rapid column and DAD system. To our knowledge, there are not published methods which carry out an automated in-tube SPME procedure to determine phthalates in water simples [5, 6]. A 80-cm length capillary coated with 95% polydimethylsiloxane and 5 % polydiphenylsiloxane was employed to carry out the extraction and preconcentration of dibuthyl phthalate (DBP) and di-2-ethylhexyl phthalate (DEHP). The proposed conditions have been applied to determine those compounds at low ppb levels ($\leq$ 250 ng/mL) in aqueous samples. The limits of detection achieved were 1 µg/mL for DBP and 5 µg/mL for DEHP. The selectivity was evaluated by processing other phthalates as well as different families of organic compounds commonly found as pollutants in environmental water samples. None of those compounds interfere with the analytes. Data on the application of the described method to the analysis of different water samples collected in different two seasons are presented.

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