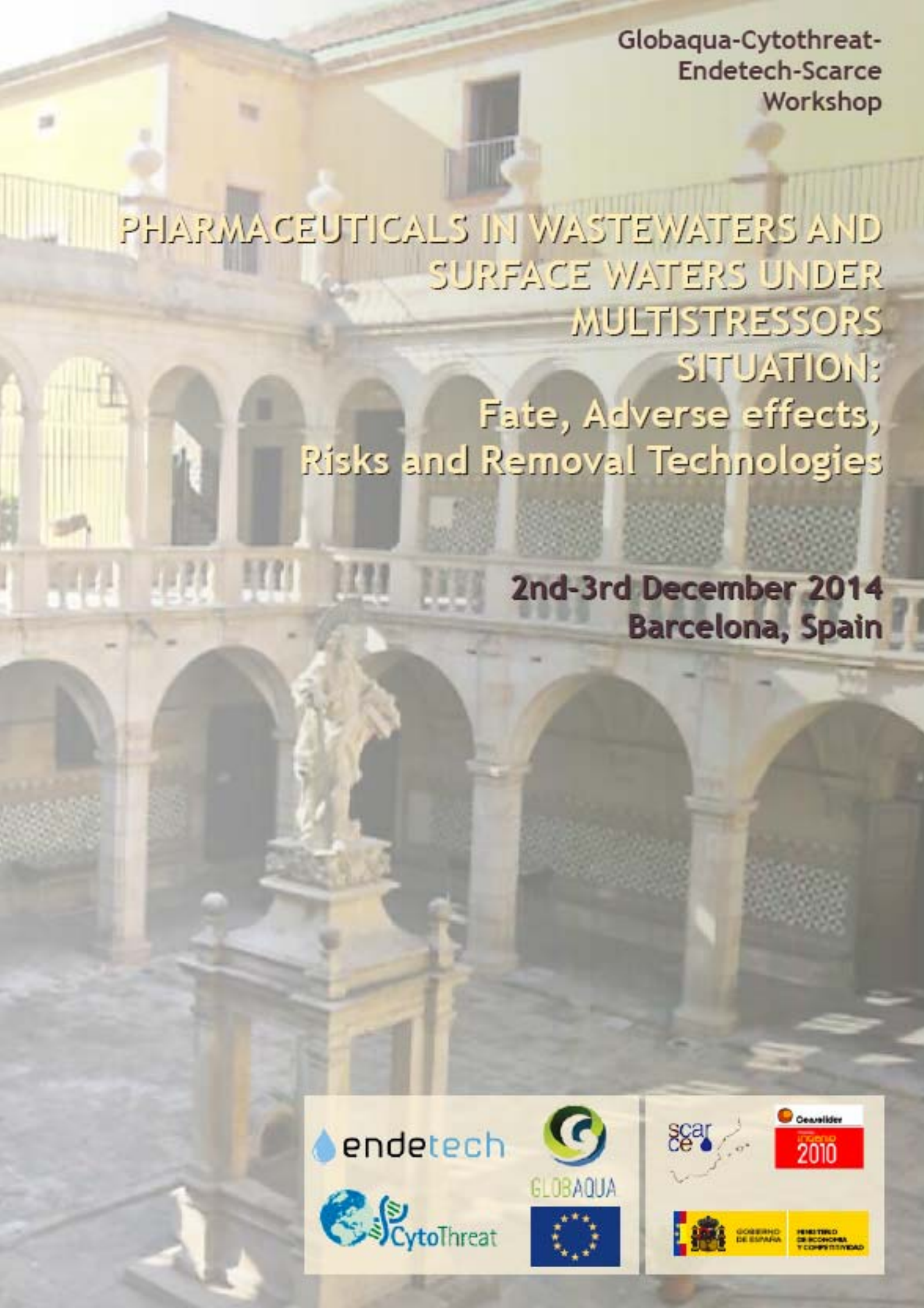
Globaqua-Cytothreat-
Endetech-Scarce
Workshop

# PHARMACEUTICALS IN WASTEWATERS AND SURFACE WATERS UNDER MULTISTRESSORS SITUATION: Fate, Adverse effects, Risks and Removal Technologies

**2nd-3rd December 2014**
**Barcelona, Spain**



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# PHARMACEUTICALS IN WASTEWATERS AND SURFACE WATERS UNDER MULTISTRESSORS SITUATION: Fate, Adverse effects, Risks and Removal Technologies

## 2-3 December 2014, Barcelona, Spain

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Book of abstracts of the Globaqua-Cytothreat-Endetech-Scarce Workshop
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Editors: Laia Sabater and Damià Barceló Cullerés
With contributions of all conference participants

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# Preface

The purpose of the Workshop is to cluster the research on pharmaceuticals residues carried out in three European projects and one Spanish project. They are described below:

GLOBAQUA (603629-ENV-2013-6.2.1) aims at identifying the prevalence, interaction and linkages between stressors, and to assess their effects on the status of freshwater ecosystems in order to improve water management practice and policies. The project assembles a multidisciplinary team of 23 scientific institutions, as well as water authorities and river basin managers, and includes experts in geomorphology, biology, chemistry, hydrology, modelling, socio-economics and policy advocacy.

CYTOTHREAT (ENV.2010.1.2.2-2) is a research project that deals with the occurrence and fate of anticancer drugs and their transformation products, as well as potential impact of selected anticancer drugs and their mixtures on aquatic organisms and humans, in order to define possible risks and to support introduction of appropriate regulation.

ENDETECH (European Union FP7-ENV-2011-Eco-innovation.Project 282818) program promotes the development of a novel eco-efficient environmental technology whose use can substantially contribute to the reduction of polluting emissions. The global objective of the program is to contribute to cleaning water bodies by improving the treatment of wastewater before it reaches the environment. The removal of pharmaceutical pollutants and endocrine disruptors in urban, hospital, and/or industrial effluents will be an important step towards the regeneration of pristine rivers, lakes but also groundwater reservoirs.

SCARCE (Consolider-Ingenio 2010 CSD2009-00065) is a multipurpose project that aims to describe and predict the relevance of global change impacts on water availability, water quality and ecosystem services in Mediterranean river basins of the Iberian Peninsula, as well as their impacts on the human society and economy.

The three EU projects funded under the European Union's Seventh Framework Programme together with SCARCE from the Consolider Programme (Spanish Ministry of Economy and Competitiveness), have joined the efforts to prepare a unique Workshop in which pharmaceuticals will be considered from a multidisciplinary perspective. The Workshop is organized in form of oral talks, posters and discussions, where experts from different fields will share their knowledge.

The Workshop that covers the problems associated with the occurrence, treatment and risk of pharmaceuticals will be carried out in four sessions: 1) Analysis of pharmaceuticals in waste waters and hospital effluents, 2) Fate and behavior of pharmaceuticals in Waste Water Treatment Plants and River water, 3) Remediation Technologies and 4) Risk assessment.

Partners from the four projects will be presenting their most recent research achievements. The results and conclusions of this Workshop will be highly valuable not only for the scientific community involved in pharmaceutical residue research but also to policy makers, wastewater treatment plants operators and newcomers who want to learn more about state-of-art pharmaceutical contamination in European water resources.

We would like to thank to all participants and especially to EU funding through the three projects (603629-ENV-2013-6.2.1-Globaqua, ENV.2010.1.2.2-2 and European Union FP7-ENV-2011-Eco-innovation.Project 282818) and to Spanish Ministry of Economy and Competitiveness for SCARCE project (Consolider-Ingenio 2010 CSD2009-00065). Institut d'Estudis Catalans is also acknowledged for the excellent facilities of the Conference room.

Wishing you an enjoyable stay in Barcelona

D. Barceló, L. Sabater, M. Filipič and P.A Bandinelli

Barcelona, November 2014

# Final Programme

## Tuesday, 2nd December 2014

**9.00 – 9.30** **Registration**

**9.30 – 10.00** **Welcome**
Damià Barceló[1,2], Metka Filipič[3], Miren López de Alda[1] and Sara Rodriguez-Mozaz[2]
[1]Water and Soil Quality Research Group, IDAEA-CSIC, Barcelona, Spain
[2]Catalan Institute of Water Research, ICRA, Girona, Spain
[3]National Institute of Biology, Ljubljana, Slovenia

**10.00 – 10.30** **Opening Session**
*The need for catchment management of pharmaceuticals: the role of STPs*
*Nikolaos Voulvoulis*
*Centre for Environmental Policy, Imperial College London, UK*

### Session I: Analysis of pharmaceuticals in waste waters and hospital effluents

*Chairperson: Metka Filipič*

**10.30 – 11.00** **Analysis of 17 anti-cancer drugs and metabolites in municipal and hospital wastewaters from Spain: study of their behaviour during sample filtration, occurrence, and environmental risk.**
Noelia Negreira[1], Miren López de Alda[1] and Damià Barceló[1,2]
[1]Water and Soil Quality Research Group, Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Barcelona, Spain
[2] Catalan Institute for Water Research (ICRA), Girona, Spain

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

### Session I: Analysis of pharmaceuticals in waste waters and hospital effluents

*Chairperson: Miren López de Alda*

**11.30 - 12.00** *Tracing pharmaceuticals and their transformations from hospital effluents to surface waters*
*Bozo Zonja[1], Sandra Perez[1], Miren Lopez de Alda[1] and Damia Barcelo[1,2]*
*[1] Water and soil quality research group, IDAEA-CSIC, Barcelona, Spain*
*[2]Catalan Institute of Water Research, ICRA, Girona, Spain*

**12.00 – 12.20** **What is going on with glucoronides of pharmaceuticals in wastwwater treatment? Application of suspect screening approach**
Sandra Pérez[1], Bozo Zonja[1], Peter Eichhorn[1] and Damià Barceló[1,2]
[1]Water and soil quality research group, IDAEA-CSIC, Barcelona, Spain
[2]Catalan Institute of Water Research, ICRA, Girona, Spain

**12.20 – 12.40** **Interlaboratory exercise on cytostatic drugs in aqueous samples**
Marjeta Česen[1,2] Tina Kosjek[1,2], Noelia Negreira[3], Miren Lopez deAlda[3], Laura Ferrando-Climent[4], Lucie Blahova[5], Tung Viet Nguyen[6], Mohamed Adahchour[7], Achim Ruebel[8], Neville Llewellyn[9], Janez Ščančar[1,2], Srdjan Novaković[10], Vesna

Mislej[11], Damia Barcelo[3,4] and Ester Heath[1,2]
[1]Jožef Stefan Institute, Ljubljana, Slovenia
[2]International postgraduate School Jožef Stefan, Ljubljana, Slovenia
[3]IDAEA-CSIC, Barcelona, Spain
[4]Catalan Institute for Water Research, Girona, Spain
[5]Masaryk University, RECETOX, Brno, Czech Republic
[6]National University of Singapore, Singapore, Singapore
[7]Hoofd R&D, H.J.E. Wenckebachweg, Amsterdam-Duivendrecht
[8]IWW Water Centre, Muelheim, Germany
[9]CEH Lancaster, Lancaster Environment Centre, Bailrigg, UK
[10]Institute of Oncology Ljubljana, Ljubljana, Slovenia
[11]Central Wastewater Treatment plant Ljubljana VO-KA, Ljubljana, Slovenia

**12.40 – 14.30** **Lunch**

## Session II: Fate and behavior of pharmaceuticals in Waste Water Treatment Plants and River Water

*Chairperson: Marina Isidori*

**14.30 - 15.00** *Occurrence of pharmaceuticals in Iberian rivers: modelling and prioritization*
*Maja Kuzmanović[1], Antoni Ginebreda [1], Victoria Osorio[1], Sandra Pérez[1], Mira Petrović [2,3] and Damià Barceló[1,2]*
*[1]Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Barcelona, Spain*
*[2]Catalan Institute for Water Research (ICRA), Girona, Spain*
*[3]Catalan Institution for Research and Advanced Studies (ICREA), Barcelona, Spain*

**15.00 – 15.30** *CytoThreat and its Contribution to our Understanding of the Fate of Cytostatic Pharmaceuticals in the Environment*
*Ester Heath[1,2], Tina Kosjek[1,2], Marjeta Česen[1,2], Janez Ščančar[1,2], Božo Žonja[3], Noelia Negreira[3], Miren Lopez deAlda[3] and Damia Barcelo[3]*
*[1]Jožef Stefan Institute, Ljubljana, Slovenia*
*[2]Jožef Stefan International Postgraduate School, Ljubljana, Slovenia*
*[3]Spanish Council for Scientific Research (CSIC), Institute of Environmental Assessment and Water Research (IDAEA), Barcelona, Spain*

**15.30 – 15.50** **Persistence of wastewater-related xenobiotics during transport along an urban river segment**
Gaëlle Guillet[1], Marc Schwientek[1], Hermann Rügner[1], Bertram Kuch[2] and Peter Grathwohl[1]
[1]WESS, University of Tübingen
[2]ISWA, University of Stuttgart

**15.50 – 16.10** **Wide coverage of pharmaceuticals and PCPs for environmental analysis. Turia River as a study case**
Eric Carmona[1], Vicente Andreu[2] and Yolanda Picó[1]
[1]Environmental and Food Safety Research Group, University of Valencia, Burjassot, Spain
[2]Desertification Research Centre — CIDE (CSIC-UV-GV), Moncada, Spain

**16.10 - 16.40** **Poster session/Coffee break**

## Flash Poster Presentation

*Chairperson: Sandra Pérez*

**16.40 – 17.40** **Poster Presentations**

**21.00** **Joint Dinner**

## Wednesday, 3rd December 2014

### Session III: Remediation Technologies

*Chairperson: Ester Heath*

**9.00 – 9.30** *Laccase-grafted ceramic membranes in an enzymatic membrane reactor for the degradation of recalcitrant pharmaceuticals in wastewaters*

*Matthias. de Cazes, R. Abejon, Marie-Pierre Belleville and José Sanchez-Marcano*
*Institut Européen des Membranes (IEM), UMR 5635 (CNRS-ENSCM-UM2), Université Montpellier 2, Montpellier, France*

**9.30 – 10.00** *Enzymatic degradation of endocrine disrupting compounds: Application to real wastewater*

*Martin Wagner[1], Dennis Becker[1], Sara Rodriguez-Mozaz[2], Marta Llorca[2], Saulo Varela della Giustina[2], Damià Barceló[2], Matthias de Cazes[3], José Sanchez[3], Marie-Pierre Belleville[3], Rob Schoevaart[4], Sally Bayer[5], Rico Czaja[5], Olivier Couillerot[6], Pierre-Alain Bandinelli[6], Jean de Gunzburg[6] and Jörg Oehlmann[1]*
*[1]Goethe University, Department Aquatic Exotoxicology, Frankfurt am Main, Germany*
*[2]Catalan Institute for Water Research, ICRA, Girona, Spain*
*[3]Institut Européen des Membranes (IEM), Université de Montpellier II, Montpellier, France*
*[4]ChiralVision BV. J.H. Oortweg 2, Leiden, The Netherlands*
*[5]C-LEcta GmbH, Leipzig (Germany)*
*[6]Da Volterra, Paris, France*

**10.00 – 10.30** *Enzymatic treatment of wastewater for the removal of antibiotics. Identification of transformation products of target antibiotics*

*Sara Rodriguez-mozaz[1], Marta Llorca[1], Saulo Varela della Giustina[1], Dennis Becker[2], Martin Wagner[2], Jörg Oehlmann[2], Matthias de Cazes[3], José Sanchez[3], Marie-Pierre Belleville[3], Rob Schoevaart[4], Sally Bayer[5], Rico Czaja[5], Olivier Couillerot[6], Pierre-Alain Bandinelli[6], Jean de Gunzburg[6] and Damià Barceló[1,7]*
*[1]Catalan Institute for Water Research, ICRA, Girona, Spain*
*[2]Goethe-University Frankfurt am Main - Institut für Ökologie, Evolution & Diversität Abteilung Aquatische Ökotoxikologie, Frankfurt am Main, Germany*
*[3]Institut Européen des Membranes (IEM), Université de Montpellier II, Montpellier, France*
*[4]ChiralVision BV. J.H. Oortweg 2, Leiden, The Netherlands*
*[5]C-LEcta GmbH, Leipzig, Germany*
*[6]Da Volterra, Paris, France*
*[7]Water and Soil Quality Research Group, Department of Environmental Chemistry, IDAEA-CSIC, Barcelona, Spain*

**10.30 – 10.50** **Enzymatic Biotransformation of Endocrine-Disrupting Compounds: Bisphenol A and Ethynylestradiol by Laccase from *Pycnoporus sanguineus* fungi**

Rodrigo Garcia-Morales[1,2], Karen Gomez-Mariscal[1], C Orona-Navar[1], Melissa M. Rodríguez-Delgado[1], Carlos Hernandez-Luna[3], Roeb García-Arrazola[1], E. Torres[4], Diana Cardenas-Chávez[1], Jürgen Mahlknecht[1],

Roberto Parra[1] and Nancy Ornelas-Soto[1]
[1]Cátedra de Bioprocesos Ambientales, Centro del Agua para América Latina y el Caribe, Tecnológico de Monterrey, Monterrey, Mexico
[2] Universidad Juárez Autónoma de Tabasco. Tabasco, Mexico
[3]Laboratorio de Enzimología, Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, Ciudad Universitaria San Nicolás de los Garza NL, Mexico
[4]Posgrado en Ciencias Ambientales, Benemérita Universidad Autónoma de Puebla, Puebla, Mexico

**10.50 – 11.10** **Emerging organic contaminant removal in hybrid constructed wetlands for treatment of wastewater in decentralized areas**

Cristina Ávila[1], Joan García[1], Isabel Martín[2] and Josep Maria Bayona[3]
[1]GEMMA-Group of Environmental Engineering and Microbiology, Universitat Politècnica de Catalunya-BarcelonaTech, Barcelona, Spain
[2]Foundation Centre for New Water Technologies (CENTA). Seville, Spain
[3]Department of Environmental Chemistry, IDAEA-CSIC, Barcelona, Spain

**11.10 - 11.40** **Poster session/Coffee break**

## Session IV: Risk assessment

*Chairperson: Antoni Ginebreda*

**11.40 – 12.10** *Biofilms facing co-occurring stressors in river ecosystems: potentialities and constraints*

*Sergi Sabater[1,2], Natàlia Corcoll[1], Albert Ruhi[1], Belinda Huerta[1], Mira Petrovic[1], Sara Rodriguez-Mozaz[1], Damià Barceló[1,3] and Vicenç Acuña[1]*
*[1] Institute of Aquatic Ecology, University of Girona*
*[2] Catalan Institute for Water Research, ICRA, Girona, Spain*
*[3] IDAEA-CSIC, Barcelona, Spain*

**12.10 – 12.30** **Environmental Risk Assessment of Pharmaceuticals - Effects on Fish in a Swedish River**

Anna Furberg[1], Rickard Arvidsson[1], Maria Florberger[2] and Sverker Molander[1]
[1]Environmental Systems Analysis, Energy & Environment, Chalmers University of Technology, Gothenburg, Sweden
[2]Golder Associates, Gothenburg, Sweden

**12.30 – 12.50** **Cyclophosphamide and ifosfamide in aqueous environment: analysis and ecotoxicity**

Marjeta Česen[1,2], Tina Kosjek[1,2], Tina Eleršek[3], Boris Kompare[4], Metka Filipič[3] and Ester Heath[1,2]
[1]Jožef Stefan Institute, Ljubljana, Slovenia
[2]Jožef Stefan International Postgraduate School, Ljubljana, Slovenia,
[3]National Institute of Biology, Ljubljana, Slovenia
[4]Faculty of Civil and Geodetic, University of Ljubljana, Ljubljana, Slovenia

**12.50 – 13.10** **Analyses of synergistic and antagonistic effects of cytostatic drugs in the Tradescantia micronucleus assay**

Miroslav Misik[1], Clemens Pichler[1],Armen Nersesyan[1], Siegfried Knasmüller[1] and Michael Kundi[2]
[1]Institute of Cancer Research Department of Internal Medicine I, Medical University of Vienna, Austria
[2]Medical University of Vienna, Institute of Environmental Hygiene, Medical University of Vienna, Austria

**13.10 – 14.30** Lunch

## Session IV: Risk assessment

*Chairperson: Martin Wagner*

**14.30 – 15.00** *Can transcriptome changes predict adverse effects of exposure to cytostatics in zebrafish?*
*Špela Baebler[1], Ana Rotter[1], Tina Demšar[1], Matjaž Novak[1,2], Bojana Žegura[1], Róbert Kovács[3], Katalin Bakos[3] and Metka Filipič[1]*
*[1]National Institute of Biology, Ljubljana, Slovenia*
*[2]Szent István University, Department of Aquaculture, Gödöllő, Hungary*
*[3]Institute of Ecological Engineering, Maribor, Slovenia*

**15.00 – 15.30** *Risk assessment of selected anticancer drugs*
*Siegfried Knasmüller[1], Michael Kundi[2], Marina Isidori[3], Zoran Gačić[4], Verica Garaj[5], Miren López de Alda[6], Akos Horvath[7], Ester Heath[8] and Metka Filipič[9]*
*[1]Institute of Cancer Research Department of Internal Medicine I, Medical University of Vienna, Austria*
*[2]Inst. of Environmental Health, Medical University of Vienna, Austria*
*[3]Seconda University Degli Studi di Napoli, Italy*
*[4]Institute for Multidisciplinary Research, Belgrade, Serbia*
*[5]Institute for Medical Research and Occupational Health, Zagreb, Croatia*
*[6]Agencia Estatal Consejo Superior De Investigaciones Cientificas, Barcelona, Spain*
*[7]Dep. of Agriculture, Szent Istvan University, Hungary*
*[8]Jozef Stefan Institute, Ljubjana, Slovenia*
*[9]National Institute of Biology, Ljubjana, Slovenia*

**15.30 – 15.50** **Acute and chronic toxicity of cytostatic drugs in zebrafish**
Ákos Horváth[1], Róbert Kovács[1], Zsolt Csenki[1], Katalin Bakos[1], Béla Urbányi[1], Goran Gajski[2], Marko Gerić[2], Verica Garaj-Vrhovac[2] and Metka Filipič[3]
[1]Department of Aquaculture, Szent István University, Gödöllő, Hungary
[2]Institute for Medical Research and Occupational Health, Zagreb, Croatia
[3]National Institute of Biology, Ljubljana, Slovenia

## Closure of the meeting

**Good-Bye including Poster Award and Final remarks**

**End of meeting**

## Posters

### Session I: Occurrence and fate of Pharmaceuticals in the aquatic environment, soil and waste waters treatment plants

**1.- Evaluation of exposure and uptake of pharmaceuticals in fish from the Llobregat river**

Jaume Aceña[1], Pilar Campos[1], Lluís Benejam[2], Sandra Pérez[1] and Damià Barceló[1,3]
[1]Water and Soil Quality Research Group, IDAEA-CSIC, Barcelona, Spain
[2]Department of Environmental Sciences, University of Vic, Vic, Spain
[3]Catalan Institute of Water Research, ICRA, Girona, Spain

**2.- Preliminary study of pharmaceuticals compounds by different technologies used in WWTPs in the Región de Murcia. Prediction of consumption data**

Jose Manuel Guillén[1], Jose Javier Padilla[1], Carmen Fernández-López[1], Gabriel Caravaca[1], Agustín Lahora[2] and John Parsons[3]
[1] UCAM Catholic University of Murcia, Murcia, Spain
[2] ESAMUR, Regional Entity for Sanitation and Wastewater Treatment in Murcia Region, Murcia, Spain
[3] IBED Institute for Biodiversity and Ecosystem Dynamics, Amsterdam, The Netherlands

**3.- Eutrophication and emerging pollutants in a Brazilian tropical reservoir: Relationship and spatial distribution**

Julio C. López-Doval[1], Cassiana C. Montagner[2], Gisela Umbuzeiro[3] and Marcelo L. Pompeo[1]
[1]Universidade de São Paulo, Departamento de Ecologia, Lab. de Ecologia
[2]Universidade Estadual de Campinas, Instituto de Química
[3]Universidade Estadual de Campinas, Faculdade de Tecnologia

**4.- Preliminary study of stability of 8 common pharmaceuticals in soil**

Mª Rosa Pino[1], Jonatan Val[1], Ana Mª Mainar[2] and Elisa Langa[1]
[1]Universidad San Jorge, Instituto de Medio Ambiente, GIMACÉS
[2]Universidad de Zaragoza, Instituto de Investigación en Ingeniería de Aragón (I3A), GATHERS

**5.- Simultaneous determination of sixteen uv filters in environmental surface waters with solid phase extraction and liquid chromatography-tandem mass spectrometry**

Serena Stampachiacchiere, Anna L. Capriotti, Chiara Cavaliere, Giorgia La Barbera, Patrizia Foglia and Salvatore Ventura
Dipartimento di Chimica, Università degli Studi di Roma, Rome, Italy

### Session II: Removal Technologiess in in Waste Water Treatment Plants and River water

**6.- Highlights of the DEGRAPHARMAC project in the treatment of hospital wastewater (veterinary and human) with fungal reactors**

Marina Badia-Fabregat[1], Francesc Castellet[1], Carles Cruz-Morató[1], Ernest Marco-Urrea[1], Paqui Blànquez[1], Montserrat Sarrà[1], Gloria Caminal[2], Teresa Vicent[1], Daniel Lucas[3], Laura Ferrando[3], Marta LLorca[3], Meritxell Gros[3], Sara Rodríguez-Mozaz[3] and Damià Barceló[3,4]
[1]Departament d'Enginyeria Química, Escola d'Enginyeria, UAB, Bellaterra, Spain
[2]Unitat Associada de Biocatàlisi Aplicada IQAC-CSIC, Escola d'Enginyeria, UAB, Bellaterra, Spain
[3]Catalan Institute for Water Research, ICRA, Girona, Spain
[4]Departament de Química Ambiental, IDAEA-CSIC, Barcelona, Spain

**7.- Removal of selected sulphonamides during the ozonation process in the presence and absence of bicarbonates**

Natalia Lemańska-Malinowska[1] and Viggo A. Bjerkelund[2]
[1]Sielsian University of Technology, Gliwice, Poland
[2]Norwegian University of Science and Technology, Trondheim, Norway

**8.- Can we trust in Managed Aquifer Recharge (MAR) to deal with emerging contaminants present in reclaimed water?**

Marta Hernández[1], Oriol Gibert[1,2], A. Castañeda[1], Xavier Bernat[1], Karsten Nödler[3] and Tobias Licha[3]
[1]CETaqua, Water Technology Center, Cornellà de Llobregat, Spain
[2]Chemical Engineering Department, Technical University of Catalonia, Barcelona, Spain
[3]Department Applied Geology, Geoscience Centre of the University of Göttingen, Göttingen, Germany

## Session III: Toxicity and Risk assessment

**9.- Cytotoxicity and genotoxicity of imatinib mesylate in zebrafish liver (ZFL) cells, human hepatoma (HepG2) cells and human peripheral blood lymphocytes (HPBLs)**

Matjaž Novak[1,2], Bojana Žegura[1], Goran Gajski[3], Marko Gerić[3], Verica Garaj Vrhovac[3] and Metka Filipič[1]
[1]National Institute of Biology, Department of Genetic Toxicology and Cancer Biology, Ljubljana, Slovenia
[2]Ecological Engineering Institute d.o.o., Maribor, Slovenia
[3]Institute for Medical Research and Occupational Health, Zagreb, Croatia

**10.- Toxic and genotoxic effects of BAC and its binary mixtures with four cytostatics in the crustacean *Ceridodaphnia dubia***

Alfredo Parrella[1], Michael Kundi[2], Margherita Lavorgna[1], Emma Criscuolo[1], Chiara Russo[1] and Marina Isidori
[1]Dipartimento di Scienze e Tecnologie Ambientali, Biologiche e Farmaceutiche, Seconda Università di Napoli, Caserta, Italy
[2]Institute of Environmental Health, Center for Public Health, Medical University of Vienna, Vienna, Austria

**11.- Impact of *in vivo* and *in vitro* exposure to 5-fluorouracil, cisplatin, etoposide and vincristine sulphate on dna damage in haemocytes of freshwater mussels *Unio pictorum* AND *Unio tumidus***

Stoimir Kolarević[1], Zoran Gačić[2], Margareta Kračun-Kolarević[3], Jovana Kostić[1,2], Karolina Sunjog[1,2], Momir Paunović[3], Jelena Knežević-Vukčević[1] and Branka Vuković-Gačić[1]
[1]University of Belgrade, Faculty of Biology, Chair of Microbiology, Center for Genotoxicology and Ecogenotoxicology, Belgrade, Serbia,
[2]University of Belgrade, Institute for Multidisciplinary Research, Belgrade, Serbia
[3]University of Belgrade, Institute for Biological Research ¨SinišaStanković, Belgrade, Serbia

**12.- Effect of 5-FU on transcriptomic level in different zebrafish models**

Matjaž Novak[1,4], Bojana Žegura[1], Špela Baebler[2], Ana Rotter[2], Róbert Kovács[3], Katalin Bakos[3] and Metka Filipič[1]
[1]National Institute of Biology, Department of Genetic Toxicology and Cancer Biology, Ljubljana, Slovenia
[2]National Institute of Biology, Department of Biotechnology and System Biology, Ljubljana, Slovenia
[3]Szent István University, Department of Aquaculture, Gödöllő, Hungary
[4]Ecological Engineering Institute d.o.o., Maribor, Slovenia

**13.- Exposure to binary mixtures of four anticancer drugs in crustaceans: chronic and genotoxic effects**

Alfredo Parrella[1], Michael Kundi[2], Margherita Lavorgna[1], Emma Criscuolo[1], Chiara Russo[1] and Marina Isidori
[1]Dipartimento di Scienze e Tecnologie Ambientali, Biologiche e Farmaceutiche, Seconda Università di Napoli, Caserta, Italy
[2]Institute of Environmental Health, Center for Public Health, Medical University of Vienna, Vienna, Austria

**14.- Genotoxic effect of 5-fluorouracil in zebrafish after chronic exposure to low doses in a two-generation study**

Kovács Róbert[1], Csenki Zsolt[1], Bakos Katalin[1], Urbányi Béla[1], Horváth Ákos[1], Garaj-Vrhovac Vera[2], Gajski Goran[2], Gerić Marko[2], Noelia Negreira[3], Miren López de Alda[3], Damià Barceló[3,4], Ester Heath[5], Tina Kosjek[5], Bojana Žegura[6], Matjaž Novak[6,7], Irena Zajc[6], Špela Baebler[6], Ana Rotter[6], Živa Ramšak[6] and Metka Filipič[6]
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[5]Institute Jožef Stefan, Ljubljana, Slovenia
[6]National Institute of Biology, Ljubljana, Slovenia
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**15.- *In situ* assessment of DNA damage in *Branchiura sowerbyi* Beddard, 1892 (Oligochaeta: Tubificidae) from the Sava river using comet assay**

Margareta Kračun-Kolarević[1], Stoimir Kolarević[2], Ana Atanacković[1], Jovana Kostić[2,3], Zoran Gačić[3], Momir Paunović[1] and Branka Vuković-Gačić[2]
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**16.- Methodology approach using effect-based monitoring tools to link water quality and pollutants**

Julián Blasco[1], Neus Roig[2,3], Ignacio Moreno-Garrido[1], Jordi Sierra[2,4] , Elena Nieto[1], Martí Nadal[2], Miriam Hampel[1], Elena Pérez Gallego[5] and Marta Schuhmacher[2,3]
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# Oral presentations

# The need for catchment management of pharmaceuticals: the role of STPs

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The EU Water Framework Directive (WFD) aims to stabilise and enhance the chemical and biological status of water quality through phasing out and reducing priority pollutants. Even though pharmaceuticals are not currently considered as priority substances under the WFD, many pharmaceutical compounds are ubiquitous in environmental matrices and ecotoxicological data suggests that there are concerns for aquatic and terrestrial ecosystems. Catchment management plans based on accurate source assessment are therefore required for reducing source emissions should pharmaceuticals become priority pollutants in the future. Catchment level management uses geographical and hydrological boundaries as a framework for detailed assessment of diffuse and point source pollution. This paper focuses on emissions of human and veterinary pharmaceuticals from anthropogenic sources, at a catchment level, that require more holistic and integrated decision making to reduce environmental quality impacts. Typically, pharmaceuticals consumed by humans are excreted in urine and faeces as parent compounds or metabolites and enter the sewage system, reaching sewage treatment plants (STPs) where they are partially removed before being discharged into surface water. Therefore there is an increased need to improve the performance of municipal STPs in high density urban centres that discharge in sensitive catchments. As pharmaceuticals can reach the environment through waste disposal also, from a catchment management perspective, a holistic approach is more appropriate. For example the introductions of a medicine reuse scheme in primary care (most likely one which redistributes hormones) is recommended as a way to address pharmaceutical waste. However, significant advances in the control and verification of medicine quality are first required if reuse is to prove feasible, in practice, at a national level. Such an approach aims to help both industry and society to promote a better understanding of the environment at a local level and deliver positive and sustained outcomes for the water environment. Local collaboration and more transparent decision-making when both planning and delivering such activities will further facilitate this approach.

# Analysis of 17 anti-cancer drugs and metabolites in municipal and hospital wastewaters from Spain: study of their behaviour during sample filtration, occurrence, and environmental risk.

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The presence and impact of anticancer drugs in the aquatic environment is largely unknown. In the present study, the occurrence of 13 cytostatics and 4 metabolites was investigated in wastewater samples from various wastewater treatment plants (WWTPs) and from a large hospital from Spain. The studied compounds belonged to the classes of (i) alkylating agents, (ii) antimetabolites, (iii) plant alkaloids and other natural products, (iv) cytotoxic antibiotics and related substances, and (v) other antineoplastic agents. For analysis, an automated multi-residue method based on on-line solid phase extraction-liquid chromatography–tandem mass spectrometry (SPE-LC-MS/MS) was developed. In the study of the previous filtration step of the samples, some compounds were observed to be strongly adsorbed to nylon filters, while cellulose acetate filters appeared to be the best option. Ten out of the 17 target compounds, namely, methotrexate (MET), ifosfamide (IF), cyclophosphamide (CP), irinotecan (IRI), doxorubicin (DOX), capecitabine (CAP), tamoxifen (TAM) and the metabolites endoxifen (OH-D-TAM), hydroxytamoxifen (OH-TAM) and hydroxypaclitaxel (PH-PAC), were found in the samples analysed at levels between 2 ng $L^{-1}$ (for MET) and 180 ng $L^{-1}$ (for TAM). Some of these compounds were observed to be efficiently removed after wastewater treatment, e.g. MET, DOX and IRI, whereas other compounds, such as TAM, CP and IF remained largely unaltered. The aquatic environmental risk associated to the detected compounds was also assessed.

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# Tracing pharmaceuticals and their transformations from hospital effluents to surface waters

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Pharmaceuticals undergo biotransformation in humans which produce metabolites with related but modified chemical structures. Their physico-chemical properties can also be altered compared to their parent compounds. They are metabolized by a variety of drug-metabolizing enzymes such as cytochrome P450 (CYP), UDP-glucuronosyltransferase (UGT), and glutathione S-transferases (GST) which are present in the human liver at high abundance.

Once the pharmaceuticals are excreted from the human body both the unchanged fraction of the parent compounds and their human metabolites are discharge into the sewer. Consequently, they enter the wastewater treatments plants (WWTPs) where another set of biotransformations can occur in the secondary treatment (eg. activated sludge). Finally, the fraction not removed during the treatment cascade eventually ends up in the aquatic environment where it can undergo photochemical degradation deemed the principal transformation process in surface waters.

This talk will address the different approaches of evaluating the transformations that some pharmaceuticals undergo. The *classical* approach to evaluation is the concept that first a set of lab-scale experiments is performed to determine the degradation products which are identified using high resolution mass spectrometry (HRMS). Following the identification, a method is created in order to detect those transformation products (TPs) in the aquatic environment (if any). An example of *classical* approach to evaluate the degradability of compounds will be shown using the photodegradation of the antiviral zanamivir.

In contrast to this *classical* approach, recent introduction of very sensitive high-resolution MS platforms and the development of specific software essential for efficient and automated processing of MS data have allowed so-called suspect analysis. It holds a great promise in terms of affording a far more comprehensive overview of tracing transformations through the use of suspect and non-target analytical methods. Example of this will be a suspect screening of iodinated contrast media photodegradates and their occurrence in surface waters. This approach will shows a time-effective analysis where only those TPs detected in surface water samples were prioritized based on their detection. Based on this prioritization, only dose detected were identified, isolated (when possible) and included in the list of target compounds for quantitative analysis. This type of analysis will present the detection-based prioritization as a crucial step to reduce the number of transformation products to be identified and thereby reducing costs and time in the subsequent target analysis.

Non-target example which will be discussed will focus on evaluating biotransformations of an anticonvulsant lamotrigine. The results will show that while the parent compound is resistant to degradation, it is an indirect source of structurally-derived compounds. Those transformations of lamotrigine were detected in the aquatic environment and are produced via its metabolism and its metabolite transformations in WWTPs.

**Acknowledgements**

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# What is going on with glucoronides of pharmaceuticals in wastwwater treatment? Application of suspect screening approach

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Recent studies on the burden of contaminants in wastewater influents (WWi) have revealed the presence of a plethora of bioactive compounds including pharmaceuticals. However, pharmaceuticals are a special class of contaminants because some of them enter in the WWi already transformed. They undergo biotransformations in humans which lead to metabolites with different chemical structures and physico-chemical properties than their parent compounds (pharmaceuticals). They are metabolized by a variety of drug-metabolizing enzymes such as cytochrome P450 (CYP), UDP-glucuronosyltransferase (UGT), and glutathione S-transferases which are present in the human liver at high abundance. After excretion of pharmaceuticals in conjunction with their human metabolites and their discharge into the sewer, they enter in wastewater treatments plants (WWTP) and they can be also biotransformed by secondary treatments. Some authors have been proposed that some glucuronides of the pharmaceuticals can be cleavaged in the WWTP treatment. However, little evidence has been published as regards metabolic pathways in complex microbial communities of the glucuronides of pharmaceuticals like those encountered in the aeration tank of the activated sludge treatment. Determination of polar organic contaminants in wastewaters commonly relies on a compound-specific approach by low-resolution mass spectrometry (MS) on triple-quadrupole instruments in which sample preparation protocols and subsequent quantitative determinations are optimized in regards of sample extraction efficiency and analytical sensitivity for a predefined set of analytes. In contrast to this classical target analysis, so-called suspect analysis is holding great promise in terms of affording a far more comprehensive overview of the contamination of urban wastewaters, and of any other environmental sample of great complexity. The path towards successful non-targeted analysis has been paved by recent introduction of very sensitive high-resolution MS platforms and the development of specific software essential for efficient and automated processing of MS data. Not only can such data be examined for the presence of any known pharmaceuticals (using elemental composition, isotope pattern and spacing as well as chromatographic retention behavior as criteria for compound identification) but reported or predicted metabolites can be included in the search algorithms. In a previous work[1], we have detected by chance some non-predicted transformation products of lamotrigine glucuronide in WWe using FISH program. Against this background, the present study aimed at investigating the biodegradation of some selected glucuronides (lamotrigine-N2-glucuronide, sulfamethoxazole-N1-glucuronide, propranolol-O-glucuronide, tamazepam-O-glucuronide, diclofenac-acyl-glucuronide and atorvastatin-O-glucuronide) under controlled laboratory settings in order to gain further insight into the biodegradability and metabolic pathways of the selected compounds. The samples from the biodegradation studies were screened for the presence of stable intermediates and these were characterized by Q-exactive-Orbitrap-MS of unusual microbial transformation products. Differences in occurrence patterns of several glucuronides of pharmaceuticals have been observed in influent and effluent samples. Further examination of mass spectral data of N-glucuronides has revealed the presence of closely related compounds in a wastewater-dependent manner. Lab-scale biodegradation studies with mixed liquor have demonstrated the conversion of the N-glucuronides of sulfamethoxazole and lamotrigine into various species; identification of these transformation products is under way. In this work, the applicability of suspect analysis for the screening of target pharmaceuticals and their glucuronides as well as their identified TPs in wastewaters was evaluated. Following generic sample preparation on solid-phase extraction sorbents with different selectivities, extracts were analyzed by ultra performance liquid chromatography (UPLC) coupled to electrospray high-resolution MS on a Q-Exactive instrument. Detection of novel transformation products in real sewage samples has highlighted the usefulness of HR-MS suspect screening in combination with biodegradation studies.

[1] Zonja et al. Anal Chem (in preparation)

# Interlaboratory exercise on cytostatic drugs in aqueous samples

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The objective of this study, carried out within the EU FP7 project CytoThreat, was to perform an interlaboratory comparison on the determination of cytostatic drug residues in surface water, hospital wastewater and wastewater treatment plant effluent. This kind of comparison is essential in the absence of certified reference materials, if laboratories are to have confidence in their analytical abilities. After an extensive search for partners in this task, nine laboratories worldwide confirmed their participation. The compounds selected included cyclophosphamide, ifosphamide, 5-fluorouracil, gemcitabine, etoposide, methotrexate and cisplatinum, while sample preparation methods included solid phase extraction (SPE) for sample preconcentration and the use of surrogate/internal standards for quantification. Chemical analysis was performed by either liquid or gas chromatography (LC or GC) coupled to mass (MS) and most frequently tandem mass (MS/MS) spectrometry, except for cisplatinum that was determined by inductively coupled plasma (ICP-MS). Laboratory performances were evaluated using z-score values, mean and median values, standard deviations, repeatability and reproducibility. According to submitted results only cyclophosphamide, ifosfamide, methotrexate and etoposide were included in the statistical evaluation. Overall, sample preparation was satisfactory. The smallest absolute differences between spiked values and the participant results were observed in surface waters. Repeatability was highest for methotrexate in all matrices and for three laboratories using LC-MS/MS (CV $\leq$ 12 %). Overall reproducibility was poor (CV: 27 % - 143 %) with the exception of methotrexate in a spiked hospital waste water (CV: 8 %).

# Occurrence of pharmaceuticals in Iberian rivers: modelling and prioritization

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Occurrence and potential risk of pharmaceuticals in environment become an issue of increased concern over the last years. Pharmaceuticals compounds have been detected in different environmental compartments worldwide generaly at low concentrations (ng/l) [1-3]. However, due to their everyday use and continuous release into environment they are almost omnipresent pollutants and the chronic exposure of wildlife to them might be a realistic biodiversity threath[4].

Because of their low levels found in the environment and reasonably low acute toxicity it is not expected they would pose a risk of acute effects in most of the areas. However, considering that pharmaceutical compounds are bioactive chronic environmental effects are still unknown for majority of these compounds. For the purpose creating a more comprehensive picture of the environmental risk of the pharmaceuticals is necessary.

In this study we assessed the ecotoxicological risk of 80 pharmaceuticals together with 120 other chemical compounds of possible concern that were detected in 4 Iberian river basins (Llobregat, Ebro, Júcar and Guadalquivir). The sampling was performed in two campaigns in 2010 and 2011with different river flow and meteorological conditons.

Comprehensive data set was gathered from the SCARCE-Consolider project.

The ecotocixological risk was estimated by toxic unit approach (TU) for three standard test species (algae, invertebrates and fish). For the priroiritzation purpose *ranking index* (RI) [5] was developed which is based on the frequency of compounds TU being in the certain importancy rank along the concerning river basin. It considers both the toxic units of the compound and their distribution in the studied area. Rank frequencies $f_x$ expressed as the fraction of sites (as a percentage) in the river basin where TU of the compound belongs to the specific rank are determined. The higher the RI the compound is considered as of higher priority for the studied river basin.

The results of risk assessment showed there was no risk of acute effects of studied pharmaceuticals in neither of studied river basins since they were not found in high ranks of toxic units. However, chronic effects can not be excluded at the many sites along river basins. The sensitivity to detected pharmaceuticals was ranked algae>invertebrates>fish. Serotonin reuptake inhibitor sertraline was the pharmaceutical ranked highest according to RI.

In 2010, the pharmaceuticals were found in low toxic units at almost 50% and 40 % of the sites, in Llobregat and Ebro, respectively, mainly downstream from WWTP or close to urban and industrial areas. In Júcar and Guadalquivir were found in low TU only at few sampling sites. However, in 2011 the sites with low TU of pharmaceuticals was significantly higher, almost 100%. This is of special interest due to low flow conditions that were characteristic for that rather dry year. This is expected to become even more frequent situation in Mediterranean area in the context of global climate change together with its following increasment of risk of micropollutants [6] which is evident already.

Finally to have a more quantitative insight on the influence of hydrological factors, some representative pharmaceutical compounds have been modeled in order to capture the effects of variable river flow discharge on their observed concentrations.

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# CytoThreat and its Contribution to our Understanding of the Fate of Cytostatic Pharmaceuticals in the Environment

Ester Heath[1,2], Tina Kosjek[1,2], Marjeta Česen[1,2], Janez Ščančar[1,2], Božo Žonja[3], Noelia Negreira[3], Miren Lopez deAlda[3] and Damia Barcelo[3]

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In 2011 a critical review (1) was published on the existing literature regarding the analysis of pharmaceutically derived cytostatic compounds in the environment. Cytostatics are a broad group of mostly organic compounds possessing a diverse range of physico-chemical properties. These differences and the fact that they are present in the environment in trace amounts make their determination in complex matrices a major challenge. Prior to 2011 the research conducted on environmental cytostatics focused mainly on the analysis of hospital effluents and the active anti-cancer substances, rather than on actual environmental samples and cytostatic's human metabolites and/or environmental transformation products, which could also contribute to their overall toxicity.

At the outset of the EU FP7 CytoThreat project, which began in 2011 we promised to research the chemodynamics of cytostatics in natural waters and to deliver more data on the occurrence and fate of these compounds, including their human metabolites and environmental transformation products. This has required the development of advanced analytical methods making use of state-of-the-art analytical tools including the latest separation methods and cutting edge analytical instrumentation, which were available within the project. The presentation will show an overview of CytoThreat contribution (2-14) in the field of analysis of cytostatic compounds in environmental samples.

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8.Ščančar et al: Speciation of platinum based chemotherapeutic drugs in environmental water samples by HILIC-ID-ICP-MS (submitted to STotEn)
9.Česen et al.: Method development for trace analysis of selected human metabolites of cytostatics cylophosphamide and ifosfamide, their occurrence in aqueous environment and formation during biological treatment and treatment with advanced oxidation processes (in preparation).
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11.Kosjek et al. Aerobic activated sludge transformation of vincristine: Identification of biotransformation products vincristine (in preparation).
12.Negreira et al. Study of the reactivity of cytostatics in water with free chlorine by ultra-performance liquid chromatography- hybrid quadrupole - Orbitrap - tandem mass spectrometry (in preparation).
13.Negreira et al. Degradation of vinca alkaloids in chlorinated water and identification of their transformation products by ultra-high performance liquid chromatography - hybrid quadrupole-Orbitrap - tandem mass spectrometry" (in preparation).
14.Isidori et al: Toxicological and chemical characterisation of hospital and municipal wastewaters from Spain and Slovenia (in preparation)

# Persistence of wastewater-related xenobiotics during transport along an urban river segment

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Xenobiotics are increasingly produced by industrial processes and introduced into the environment. Many of them are not completely eliminated by conventional waste water treatment plants (WWTP) and enter the receiving waters by WWTP outfalls or combined sewer overflows. In many cases, little is known about their toxicity, persistence and transport behavior in aquatic systems.

In this study, the behavior of selected organic pollutants along a 4 km long urban river segment was studied by an experimental approach during last summer 2013. The Steinlach River in southwest Germany with a total catchment area of 140 km² receives treated wastewater from a WWTP a couple of kilometers upstream of its confluence with the Neckar River. In its further course, the river channel is largely straightened and does not receive any larger tributaries. For this segment, a detailed mass balance was determined over a complete 24 h cycle. To this end, 2 h composite samples (sampling interval: 15 min) were taken using automated samplers at the upstream and downstream ends of the segment, respectively, and analyzed in the lab.

A model-based analysis of the data demonstrated, on the one hand, that substances were persistent to a variable degree during the transport along the river segment. On the other hand, transformation processes seemed to be dependent on the time of day. The investigated compounds could be separated into a conservative (e.g. the anticonvulsant carbamazepine and the phosphorous flame retardants TCPP und TDCPP) and a reactive group. The latter comprised substances that were eliminated mainly during daytime (e.g. the disinfectant triclosan and the phosphorous flame retardant TDCPP) and others that were transformed as well during nighttime (e.g. the synthetic fragrance HHCB and the pharmaceutical oxcarbazepine). A likely explanation is the variable sensitivity to photodegradation. Another sampling campaign could be performed last winter, yielding similarities and differences in observed behaviours.

Next steps will be a more detailed investigation of the processes involved and the factors regulating them. Moreover, the sampling strategy shall be further improved, aiming at a reduced time demand while maintaining high level of accuracy.

# Wide coverage of pharmaceuticals and PCPs for environmental analysis. Turia River as a study case

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The occurrence and fate of 54 pharmaceuticals, including illicit drugs, and personal care products (PCPs) in waste water, surface water and in sediments of the Turia River Basin (Valencia, Spain) was studied in a punctual sampling carried out in October of 2013. For this, a liquid chromatography tandem mass spectrometry (LC–MS/MS) method was developed for the determination of these substances by electrospray (ESI) in either, negative (NI) or positive (PI) ionization mode. In NI mode, ammonium fluoride in the mobile phase improved ionization efficiency by an average increase in peak area of 5 compared to ammonium formate or formic acid. Furthermore, all studied compounds were detected and their concentration was waste water > surface water > drinking water. PPCPs were in waste water treatment plants (WWTPs) influents up to 7.26 μg $L^{-1}$, dominated by ibuprofen, naproxen and 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THCOOH). WWTPs were highly effective in removing most of them, with an average removal rate of > 90%. PPCPs were still detected in effluents in the 6.72–940 ng $L^{-1}$ range, with the THCOOH, triclocarban, gemfibrozil and diclofenac as most prevalent. Similarly, diclofenac, gemfibrozil, ibuprofen, naproxen and propylparaben were detected quite frequently from the low ng $L^{-1}$ range to 7 μg $L^{-1}$ in the surface waters of Turia River. These results confirm previous studies carried out in the area with a limited number of acidic compounds[1]

The extraction of these compounds from solid matrices, such as sediments, soils and sludges is still a pending issue. QuEChERS was an appropriate methodology only for some of the selected analytes. Thus, different sample preparation techniques were compared and also the analytical method of choice (methanolic extraction followed by SPE clean up) was optimized. Ibuprofen, methylparaben, salicylic acid and tetrahydrocannabinol (THC) were at concentrations up to 0.85 ng $g^{-1}$ d.w. in sediments. The discharge of WWTP as well as of non-treated waters to this river is a likely explanation for the significant amount of PPCPs detected in surface waters and sediments. The presence of pharmaceuticals in several environmental compartments raises concern about the possibility of ecosystem and human health effects from pharmaceuticals in water and sediment.

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# Laccase-grafted ceramic membranes in an enzymatic membrane reactor for the degradation of recalcitrant pharmaceuticals in wastewaters

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Traces of pharmaceutical pollutants are a threatening ecological issue as pharmaceutical compounds are designed to be biologically active at very low concentrations in humans and animals. These compounds are relatively resistant to classical wastewater treatment techniques and their long term effects are so far unpredictable and undocumented. Among pharmaceuticals, antibiotics like tetracycline (TC), are frequently present in treated wastewaters and could become an important public health problem in a near future. Enzymatic membrane reactors (EMRs) can be considered as an original way to eliminate these recalcitrant pollutants in mild conditions [ref 1].

The objective of this work is the degradation of TC from aqueous solutions in an EMR with laccase-grafted ceramic membranes. The active membranes were prepared according to a 3-step procedure which involves the porous ceramic support coating with a gelatin solution, the cross-linking and activation with glutaraldehyde and finally the enzyme grafting [ref 2]. The interest using porous ceramic supports is that they can be regenerated and reused for further immobilization once the enzymes have lost their activity. The active membranes were prepared using as supports 0.2µm and 1.4 µm monochanel membranes from Pall-Exekia and 0.2 µm 7 channels membranes from Tami Industries. Active membranes were firstly characterized by SEM. Results show that 0.2 µm membranes present a thin continuous gelatin-grafted enzyme layer at the surface while for 1.4 µm membranes the layer was discontinuous and pores entrance is still visible. TC degradation experiments were carried out with 20 ppm tetracycline solutions in osmosed water. For this purpose, 15 cms length enzymatic membranes were placed in a pilot unit. The configuration used was in a batch mode by recirculating the retentate in the feeding tank. The runs were carried out in cycles by periodically replacing the TC solution with a fresh substrate solution and the TC concentration was analyzed continuously by HPLC. An average tetracycline degradation rate of 120 $mg.h^{-1}.m^{-2}$ was reached with a 0.2 µm porous support while this value was up to 280 $mg.h^{-1}.m^{-2}$ with 1.4 µm porous membrane. In this latter case it is possible that enzymes were not only immobilized on the surface but also within the pores, enabling a higher surface of contact with the substrate. It is important to note that no enzymatic activity drop could be observed after 5 cycles of reaction (for a total of 100 hours) proving that activity of enzymatic membranes is relatively stable.

The research leading to these results has received funding from the European Union Seventh Framework Programme (FP7/2007-2013) under ENDETECH grant agreement n°282818. This work is a collaboration between Da Volterra (France), C-Lecta (Germany), ChiralVision (Netherlands), European Membrane Institute (France), Catalan Institute for Water Research (Spain) and Goethe University (Germany).

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# Enzymatic degradation of endocrine disrupting compounds: Application to real wastewater

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Micropollutants (e.g., pharmaceuticals, hormones, and industrial chemicals) are continuously emitted into the environment in low concentrations. Concerns have been raised regarding their specific toxicity (e.g., endocrine disrupting compounds, EDCs), and their limited degradation during conventional wastewater treatment.

While most advanced wastewater treatment technologies rely on resource-intense oxidative or adsorptive processes (ozonation, activated charcoal), the EU-funded project ENDETECH develops a novel biotechnological approach to degrade micropollutants. Specific enzyme libraries were screened to identify promising candidates that remove these pollutants effectively. The enzymes are optimized and immobilized for application in bioreactors. Priority targets for enzymatic degradation are antibiotics, hormones, and EDCs. Every step within the project is accompanied by *in vitro* bioassays for endocrine effects (e.g., estrogenicity, androgenicity) and mutagenicity. This is to evaluate the degradation of effects and assess potential new effects caused by the generated transformation products.

Several enzymes were discovered; those ones from fungi seemed to be the most promising ones (i.e., laccases from *T. versicolor, M. thermophila*). The degradation of antibiotics in ultrapure water was already successful. For instance tetracycline is degraded by 78% after 18 h treatment with fungal laccases. As proof of concept we aim at demonstrating the feasibility of enzymatic degradation under "real world" conditions with actual wastewater. In experiments with endocrine active hospital wastewater we observed a significant reduction of androgenicity and estrogenicity. For example, the latter was reduced by 80% after 24 h laccase treatment. This demonstrates the degradation of unspecific endocrine activity and the applicability of enzymes in wastewater. Further, we investigate the degradation of mixtures of known EDCs in ultrapure water and in real wastewater. As a final point, we will compare our bioassay data with data from chemical analysis.

Taken together, these results demonstrate that enzymatic degradation of micropollutants in wastewater is feasible. This technology can be an efficient and eco-friendly alternative or addition to existing wastewater treatment technologies. Finally, our studies demonstrate that accompanying the development of a new technology by parallel ecotoxicological analysis helps to decrease investment efforts and increase the acceptance of the new technology.

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# Enzymatic treatment of wastewater for the removal of antibiotics. Identification of transformation products of target antibiotics

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Some chemical contaminants present in wastewaters, such as antibiotic residuals, are not efficiently removed by conventional treatment technologies and they can thus be found in the natural environment. The chronic exposure of organisms to these compounds can pose a threat to them since long term effects are scarcely described and difficult to predict. In addition, the presence of traces of antibiotics in the environment could induce the development of antibiotic-resistant pathogens, posing a risk not only to aquatic environment but also to human health. The global objective of the ENDETECH EU-project is to develop a technology that aims at eliminating recalcitrant pharmaceutical pollutants (including antibiotics) in wastewaters generated from drug manufacturing sites, households, hospitals and animal farms. An innovative ENzymatic DEcontamination TECHnology, based on the use of different enzymes, can be applied to inactivate target pollutants in such effluents. In this work, degradation studies using enzymatic decontamination processes developed within the frame of the project are presented. Special attention is paid to the formation of transformation products of target antibiotics during such processes, since in occasions, these emerging compounds might pose an equal or higher risk to the environment than the parent compounds. Within the project, different enzymes have been tested for the inactivation of selected compounds, such as cyclines or macrolides, both, at laboratory scale and then extrapolated to a batch reactor system. During these studies, the presence of the selected compounds (*i.e.* tetracycline and erythromycin) as well as the generation of any transformation product during the degradation process was assessed. In addition, the antibiotic activities for the tested compounds as well as the possible endocrine disruption have been evaluated along the biodegradation experiments. The different degradation processes showed the capabilities of the enzymatic decontamination as a complementary technology when the common removal process is not enough. For example, the degradation of tetracycline antibiotic by laccase enzyme from *Trametes versicolor* reached *c.a.* 78% after 18h of exposure without the presence of mediators at laboratory scale. When this process is transferred to a batch reactor, the degradation arrived to 60% (after 24h) for the enzymes immobilized in membranes while the degradation with free enzymes did not exceed the 30%. Another process optimized within the project has been the degradation of erythromycin by EreB esterase enzyme without the presence of mediators. In this case, the degradation of this compound reached the 50% after 16h of exposure. In these two examples the antibiotic activities were assessed along all the experiments decreasing *c.a.* 100%. As regards to the transformation products (TPs) detected during the experiments, different structures were postulated after the analysis of the samples in a high resolution mass spectrometer Orbitrap.

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# Enzymatic Biotransformation of Endocrine-Disrupting Compounds: Bisphenol A and Ethynylestradiol by Laccase from *Pycnoporus sanguineus* fungi

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Contaminated waters by micropollutants like endocrine disruptors represent one of the most urgent issues to solve. The inefficient removal system from Wastewater Treatment Plants and their continuous and not regulated discharge into the environment has spread its presence to superficial waters, groundwaters and drinking waters. Unfortunately, there is no available technique that absolutely assures the removal of micropollutnts from waters. Several methods have been developed in order to biodegrade these chemicals. Some of them are photodegradation, electrolysis and carbon adsorption. However, these methods result expensive and limited. Enzymatic oxidation by fungal laccases offers a promising alternative for efficient, sustainable removal of organic pollutants in water. In this work the biocatalytic ability of laccases from the fungus *Pycnoporus sanguineus* CS43 was evaluated. A filtered culture supernatant (laccase cocktail) evidenced an enhanced biotransformation capability to remove common endocrine-disruptor compounds (EDCs): Bisphenol A and 17-α-Ethynylestradiol. A removal of around 89-100% was achieved for both EDCs in synthetic samples (10 ppm) after enzymatic treatment with 100 UL-1. The reaction rates were determined and adjusted to the Michaelis-Menten model. In comparison with purified laccases, the use of a laccase cocktail represents a more sustainable and less costly process in comparison to pure enzymes for water treatment. As far as we know this is the first study on enzymatic degradation of target EDCs by a laccase cocktail from any strain of *Pycnoporus sanguineus*. Further work will focus on the treatment of real groundwater samples coming from northwestern Mexico.

# Emerging organic contaminant removal in hybrid constructed wetlands for treatment of wastewater in decentralized areas

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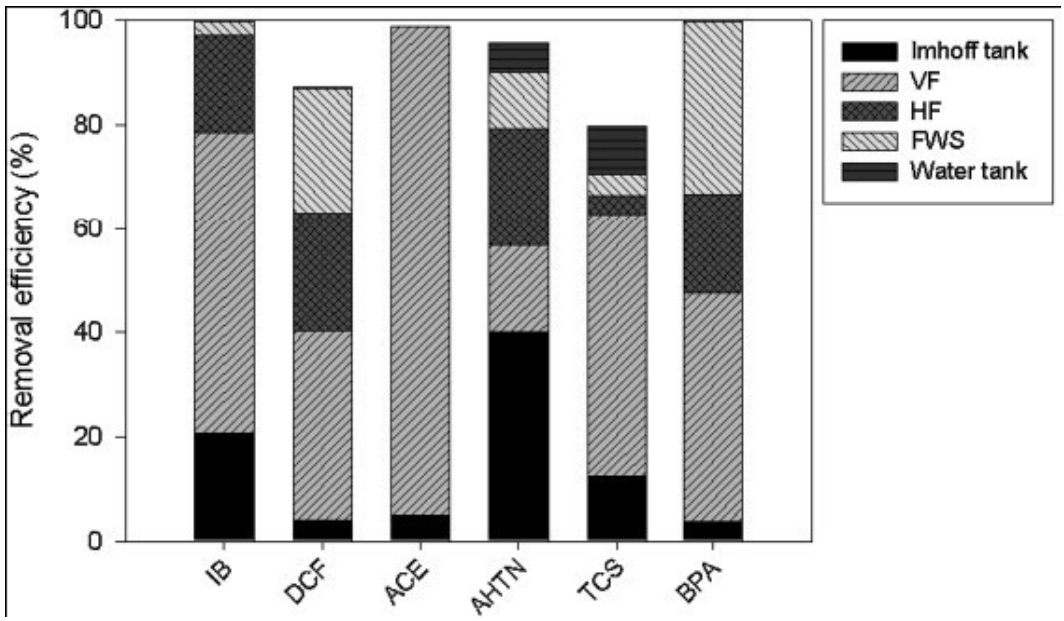
The occurrence of emerging organic contaminants (EOCs) in poorly treated wastewater and eventually in other watercourses constitutes nowadays an increasing concern worldwide due to their possible toxicological effects to the environment and living organisms. The application of reclaimed water could pose unknown undesirable effects to the environment, and to this regard, constructed wetlands (CWs) constitute a low-cost, easy-to-operate alternative for wastewater treatment and thus the occurrence and behavior of EOCs in this type of treatment systems should be further studied (Ávila et al., 2014).

The scope of this study was to evaluate the efficiency of a full-scale hybrid CW system on the removal of EOCs (i.e. Ibuprofen -IB-, diclofenac -DCF-, acetaminophen -ACE-, ethinylestradiol, tonalide -AHTN-, oxybenzone, triclosan -TCS-, bisphenol A -BPA-) from a combined sewer effluent. The hybrid treatment system was part of a larger pilot-scale treatment plant that received the wastewater from 2500 P.E. from the municipality of Carrión de los Céspedes (Seville,Spain) together with the runoff collected in a combined sewer system. The treatment line consisted of an Imhoff tank followed by a 317 m2 vertical subsurface flow CW (VF), a 229 m2 horizontal subsurface flow CW (HF) and a 240 m2 free water surface CW (FWS) in series. They were all planted with Phragmites australis and received an average flow of 14 m3 d-1. The VF received an average organic loading rate of about 6 g BOD5 m-2 d-1 and a hydraulic loading rate of 44 mm d-1. The final effluent was collected in a 20 m3 open-air water tank acting as a raft for irrigation. Effluent 24-h composite samples of the different treatment units were grabbed once a week (n = 8) from May to June 2011. They were transported to the laboratory in 250 mL amber glass bottles and kept refrigerated at 4ºC until analysis (sample holding time < 1 day). Samples were analysed for EOCs as described elsewhere (Matamoros and Bayona, 2006).



The experimental system appears as an integrated approach capable of accomplishing a good treatment of a combined sewer effluent, while achieving a great removal of EOCs. This reinforces the idea of hybrid CWs as very robust systems for wastewater treatment and reuse in small communities.

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# Biofilms facing co-occurring stressors in river ecosystems: potentialities and constraints

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The arrival of chemicals is a main stressor that co-occur with others in many human-disturbed environments. Potential outcomes of these stressors can also be multiple, since the prevalence of one or the other (complicated by the respective stressors'intensity) differ in human-disturbed environments. Stressors might be ranked according to their associated energy and frequency of occurrence, so their effect on the receptors (organisms, communities, ecosystems) can also be ranked. In river ecosystems pharmaceuticals co-occur with water flow alterations, habitat modification, nutrient or organic matter excess, as well as with biological (community simplification, loss of biodiversity, local extinctions, invasive species) situations. Within this complex reality, understanding the effect of pharmaceutical products is necessary to prevent and predict harmful consequences in the environment. Biofilms are a complex mixture of microbial autotrophs (algae, cyanobacteria) and heterotrophs (bacteria, fungi), and also include metazoans (microfauna and meiofauna). All of these biological elements coexist within a polymeric matrix that shows in itself specific physico-chemical characteristics. Biofilms develop at the interphase between water and sediments, and therefore occupy the forefront of the response of receptors to chemicals and other stressors arriving to the river. Pharmaceuticals in many instances are reaching biofilms continuously, and therefore the effects may be chronic, and may even potentially transmit to upper trophic levels. Effects of pharmaceutical products on biofilms can be multiple, depending on the mode-of-action. Bactericides such as triclosan have high effect on the bacterial component (according to the bactericide effect) of biofilms, but also on algae, since photosynthetic efficiency was inhibited with increasing triclosan concentrations, and other photosynthetic mechanisms were also affected. Therefore observed effects were beyond the target organisms, and stress that algae and bacteria separately may provide different outputs than the one by the biofilm. Biofilms accumulate pharmaceutical products as a result of chronic inputs. In an experiment monitoring the WWTPs dynamics after entering a river system, biofilm accumulated PHACs. Composition and concentrations of PHAC-EDC's in biofilm were significantly correlated to those in water, with 3 PHAC's (Diclofenac, Gemfibrozil, Venlafaxine) and 1 EDC (TBEP) in common. Some of the products accumulated in the biofilm were also observed in higher trophic levels. PHAC-EDC's accumulated by the herbivore Ancylus (scraper) were marginally correlated to those in water (Ancylus-water $\rho = 0.89$, $P = 0.068$) and biofilm (Ancylus-biofilm $\rho = 0.89$, $P = 0.080$). Diclofenac and Ibuprofen did not accumulate in the herbivore but indeed in some filterer organisms (12-183 ng $g^{-1}$) showing that biofilm inputs are relevant but not the only ones in operation in the river system. Another study has shown the effect of pharmaceuticals on biofilms (laboratory streams) under simulated drought or continuous flow . The effects of pharmaceuticals on the biofilm were extremely different in one or the other hydrological condition. The impact of flow interruption was in itself much higher than the one solely exerted by pharmaceuticals, and biofilms under flow return conditions (after drought) showed different response to pharmaceuticals than those non-affected by the hydrological interruption. Flow intermittency modulated the effects of chemicals differently on algae than on bacteria. The algal community became more sensitive to short-term exposure of pharmaceuticals (lower EC50 value), while the bacterial community became less sensitive to short-term exposure of pharmaceuticals (higher EC50) when exposed to water intermittency. The study highlighted that the environmental risk of pharmaceuticals was high, but different depending on the flow regime, as well as the target organisms (autotrophs vs heterotrophs). These examples show that responses between pharmaceutical products and the biota are non-linear. Biological and environmental complexity needs to be accounted for when considering the evaluation of effects of these products on the ecosystem.

# Environmental Risk Assessment of Pharmaceuticals - Effects on Fish in a Swedish River

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*Purpose* The aim of this study was to assess exposure and chronic effects on fish from down-the-drain pharmaceuticals in the Swedish Göta Älv river. Both effects of individual pharmaceuticals and mixture effects were evaluated.

*Methods* Spatially resolved exposure of pharmaceuticals was modelled with the Geo-referenced Regional Environmental Assessment Tool for European Rivers (GREAT-ER) and mixture effects were calculated using the concept of concentration addition as a conservative first tier. A habitat comparison was performed by comparing the locations with high concentrations of pharmaceuticals to the locations of known habitats in the Göta Älv river for the fish *Salmo salar* and *Salmo trutta*. The modelling results were also validated by comparison with measurements of concentrations performed in other Swedish rivers. In total, 12 pharmaceuticals were studied; diclofenac, propranolol, carbamazepine, ethinylestradiol, ibuprofen, metoprolol, gemfibrozil, estradiol, paracetamol, sertraline, verapamil and estrone.

*Results and discussion* The results of the modelling showed that the predicted pharmaceutical concentrations in the Göta Älv river, when considered individually, generally did not cause adverse chronic effects to fish. An exception was gemfibrozil, which may cause adverse chronic effects to fish in some parts of the river. However, when mixture effects were considered, the results showed that adverse chronic effects might occur in parts of the river. The risk ratios for the mixture were relatively high (between 0.61 and 0.81) in almost all parts of the river. The two parts of the Göta Älv river catchment with the highest concentrations of the studied pharmaceuticals, were also the parts with known habitats for the studied fish species. The validation of the modelling results showed that the predicted pharmaceutical concentrations in the Göta Älv river were much lower than measured concentrations in other Swedish waters. The reasons for this were probably the large difference in both the number of people connected to the WWTPs, affecting the quantity of emitted pharmaceuticals, and the differences in dilution of the WWTP effluents in the respective surface water recipients. There are uncertainties in the results from this study due to the lack of data, especially in chronic toxicity data for the assessment endpoints *Salmo salar* and *Salmo trutta*. At the same time, the exposure of pharmaceuticals in the Göta Älv river are most probably underestimated in this study, due to the exclusion of certain sources of pharmaceuticals, having implications for the interpretation of the results.

*Conclusions and perspectives* The results serve as an indication of risk for chronic effects to fish in the Göta Älv river and of the importance of considering mixture effects. Further studies providing site-specific measurements would be required in order to validate these results.

# Cyclophosphamide and ifosfamide in aqueous environment: analysis and ecotoxicity

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Cyclophosphamide (CP) and ifosfamide (IF) are widely prescribed pharmaceuticals in chemotherapy. Their increasing consumption raises questions concerning their occurrence and possible harmful effects once in the environment. This study reports on development of analytical method for determination of CP, IF and their metabolites in wastewaters, the occurrence of investigated compounds in the Slovene aquatic environment, the removal of parent compounds from wastewaters (WW) under biological and advanced treatments and finally the assessment of possible ecotoxic effects of investigated compounds. The analytical method for determination of investigated compounds [1,2] included SPE extraction with HLB Oasis cartridges for CP, IF and ketocyclophosphamide (keto-CP), and ENV+, carboxycyclophosphamide (carboxy-CP) and N-dechloroethylcyclophosphamide (N-decl-CP). Derivatization process for parent compounds was performed using trifluoroacetic anhydride with ethyl acetate, whereas for metabolites N-(tert-butyldimethylsilyl)-N-methyltrifluoroacetamide with 1% tert-butyldimethylchlorosilane and acetonitrile were used. Analysis was performed using GC/MS. Results on the occurrence of selected compounds showed that all investigated compounds were detected in hospital wastewaters, whereas CP was found in wastewater treatment plant influents and effluent. Biological removal of CP and IF in flow-through bioreactors with attached-growth biomass on Mutag BioChip™ carriers gave an average removal of 41.2 ± 11.8 % and 18.2 ± 11.3 % for CP and IF, respectively. The highest removal efficiencies for CP and IF were achieved by advanced oxidation processes (AOPs) including $UV/O_3$ with 5 g $L^{-1}$ and were 98.8 ± 0.7 % and 94.0 ± 2.4 %, respectively. Coupling of biological treatment to selected AOP resulted in 99.7 ± 0.1 % and 99.8 ± 0.0 % for CP and IF, respectively. To our knowledge, this is the first study that reports removal efficiencies of  99 % of the studied compounds from wastewater. Toxicity evaluation of parent compounds and selected metabolites included studies on CP and IF with two phytoplankton species: alga *Pseudokirchneriella subcapitata* and cyanobacterium *Synechococcus leopoliensis* (3 - 320 mg $L^{-1}$). Results revealed NOEC values of both compounds towards both species above 320 mg $L^{-1}$. Further, we tested the toxicity of selected metabolites towards *S. leopoliensis* (3 - 320 mg $L^{-1}$). Results revealed NOEC values of N-decl-CP and keto-CP above 320 mg $L^{-1}$, whereas $EC_{50}$ for carboxy-CP was 21.1 mg $L^{-1}$. The results indicate that parent compounds' toxicity data are not sufficient for risk assessment and for the evaluation of cytostatic residues in the environment mixtures of parent compounds and their metabolites/transformation products should be studied.

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# Analyses of synergistic and antagonistic effects of cytostatic drugs in The Tradescantia micronucleus assay

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Cytostatic drugs are among the most toxic pharmaceuticals which are produced. Many of them cause damage of genetic material; therefore, it was postulated that their release in the environment may cause adverse effects. We tested the induction of DNA damage of the most widely used cytostatics in higher plants in a previous study (Misik et al. 2014) and found positive effects at doses which exceed the environmental concentrations by several orders of magnitude. However, it can not be excluded that synergistic effects take place when combinations of drugs are present in environmental compartments. Therefore, we analyzed the effects of binary mixtures of 5-fluorouracil (5FU), cisplatin (CisPt), etoposide (Et) and imatinib mesylate (IM) in Tradescantia micronucleus assays. These drugs cause DNA damage via different modes of actions, therefore we analyzed their combined effects with Bliss independence as reference model. Isobolograms were calculated on the basis of results obtained with the individual drugs. Subsequently, three optimally discriminating endpoints were established. The results of experiments with dual combination show that not only Bliss independence but also synergistic and antagonistic interactions take place between the drugs. For example, a clear antagonistic effect was found in a binary mixture of 5FU + Et, while mixtures containing a IM + CisPt, IM + Et and IM +5Fu acted synergistically. The effects depended strongly on the concentrations of individual drugs. Taken together, our findings show that the behavior of the drugs in the environment can not be predicted solely on analyses of the toxic/genotoxic properties of individual compound but that in some cases synergistic effects occur which may amplify their adverse impact on the ecosystem level.

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# Can transcriptome changes predict adverse effects of exposure to cytostatics in zebrafish?

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Cytostatics are, due to their mechanism of action, classified as carcinogenic, mutagenic, carcinogenic and/or toxic to reproductive systems and it can be assumed that they can elicit these effects in exposed non-target aquatic organisms. Changes in gene expression can be related to either adaptive processes or are the indicator for toxic effects and can indicate harmful impacts of chemicals even in cases where classical toxicological endpoints show no obvious adverse effects. Therefore gene expression markers are valuable tools for testing for potential adverse effects in water systems.

In the framework of the Cytothreat project we have established a methodology for transcriptome analysis and obtained transcript signatures of most consumed cytostatic drugs in zebrafish (*Danio rerio*) *in vitro* and *in vivo* experimental systems. The transcriptome response to short treatment with 5-fluorouracil (5-FU) and imatinib mesylate in *in vitro* systems (cell line ZFL and embryos) was relatively weak and gene expression markers that could predict adverse effects could not be identified.

On the other hand, continuous exposure of zebrafish to environmentally relevant concentrations (0.01 µg/L and 1 µg/L) of 5-FU caused dramatic gene expression changes in the liver of F1 generation of treated fish. Genes involved in regulation of cell growth, signalling and response to stress were up-regulated while genes involved in developmental processes were down-regulated. Along with the primary effects of the cytostatic, such as inhibition of protein synthesis, we observed deregulation of a number of genes involved in DNA damage response and oncogenesis. The observed gene expression changes can be linked to the DNA damage, micronuclei formation and histopatological alterations in liver and kidney which observed in the same experiment which all corroborate the conclusion that chronic exposure to environmental residual 5-FU can affect water ecosystems.

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# Risk assessment of selected anticancer drugs

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Cytostatic drugs are among the most potent chemicals which are produced. Many of them act via damage of the genetic material, therefore, the release these compounds may affect the fertility of organisms in the environment and may cause adverse effect the stability of ecosystems. We investigated the acute toxic and genotoxic properties of the most widely used cytostatics (5-fluorouracil, 5FU; cisplatin, CDDP; etoposide, ET and imatinib mesylate, IM) in a variety test systems and indicator organisms, i.e. in bacteria, lower and higher plants, crustacea, molluscs, fish and selected cell lines. The drugs were tested in acute and chronic toxicity tests and furthermore in various genotoxicity assays (i.e. single cell gel electrophoresis assay, SCGE; gene mutation tests and micronucleus (MN) assays). The concentrations which were required to cause significant effects in different experimental models were in general 2 to 5 orders magnitude higher as the predicted environmental concentrations (PEC and refined PEC). However, the in crustacean and zebrafish after chronic, multigenerational exposure the genotoxic effects were found at low, for environmental exposure relevant doses. CDDP caused pronounced DNA damage (comet formation) in *Daphnia magna* at the levels ≥ 10 ng/l. Also in zebrafish exposure to 5-FU caused DNA damage, micronuclei formation, changes in gene expression in liver as well as histopathological alteration in liver and kidney at concentrations ≥ 10 ng/l. Additional experimental experiments were conducted to assess synergistic and/or antagonistic interactions between individual drugs. Therefore, binary mixtures of the cytostatics were tested and the results were compared to those obtained with individual chemicals under identical conditions. Mostly additive effects were observed, however in some cases, evidence for synergistic effects was found, for example in MN assays with Tradescantia (IM+CDDP, IM+5FU and IM+ET) and in toxicity experiments with algae (*Pseudokirchneriella subcapitata* and *Synechococcus leopoliensis*, 5-FU+CDDP, 5-FU+IM) and IM+CDDP in *Ceriodaphnia dubia*. These findings indicate that the environmental risks caused by release of mixtures of cytostatics may be higher as those caused by individual cancer drugs. The release of the compounds calculated on the basis of consumption levels, leads to environmental concentration which are below those which cause adverse effects. The general assumption, that the release of drugs does not have a negative impact on ecosystems are supported by chemical analyses of waste waters. However, it is notable that amounts which were detected in untreated waters from oncological wards and hospitals may cause adverse effects. Results obtained with Tradescantia, Crustaceae and zebrafish show that indeed in a few cases genotoxic effects are detectable at concentrations which were detected in these water samples. This latter findings underline the importance of chemical monitoring and of removal of the drugs by waste water treatment.

# Acute and chronic toxicity of cytostatic drugs in zebrafish

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The acute and chronic effects of 4 cytostatic drugs – 5-fluorouracil (5-FU), cisplatin (CI), etoposide (ET) and imatinib mesylate (IM) – on zebrafish (*Danio rerio*) were investigated. Acute tests were carried out in a static system in accordance with the OECD guideline 203 for adult fish and the guideline 236 for fish embryos (FET test) in order to find the LC50, LOEC and NOEC values of the four cytostatics. Early-life Stage Toxicity Test on zebrafish was conducted according the the OECD guideline 210 using the cytostatics 5-FU and IM in a semistatic system with the objective of investigating the sub-chronic effects of the cytostatic on fish. Finally, two-generation tests were carried out using 5-FU starting with a parent generation (F0) treated for 2 weeks, continued with a treatment of an entire F1 generation for 7 months and finished with an F2 generation treated according to the OECD guideline 210. In adult fish, the cytostatics 5-FU and ET did not pass the limit test, thus, are considered non-toxic. In case of cisplatin, LC50 was calculated at 64.5 mg/l, LOEC at 65 mg/l while NOEC 50 mg/l, whereas in case of IM, LC50 was calculated at 70.8 mg/l, 100 mg/l was LOEC and 70 mg/l was NOEC. In the FET test, LC50 of 5-FU at 72 hours post fertilization (hpf) was 2441.6 mg/l. In case of CI, LC50 was 349.9 mg/l at 48 hpf and it progressively decreased to 81.3 mg/l at 120 hpf. In addition, CI caused a significant delay in the hatch of larvae. In case of ET, LC50 was 421.9 mg/l at 96 hpf while at 120 hpf it was 363.9 mg/l. LC50 values of imatinib mesylate (IM) were: 48 hpf: 158.3 mg/l , 72 hpf: 141.6 mg/l, 96 hpf: 118.0 mg/l and 120 hpf: 65.9 mg/l. In the Early-life Stage Test with 5-FU, embryonic deformities were not detected during the tests. Regarding mortalities, the 10 mg/l concentration can be considered as LOEC, as statistically significant difference in mortalities was detected in this group alone. Concerning dry body weight, body length and condition factor, 1 mg/l is the LOEC. In case of IM, the highest tested concentration (10 mg/l) can be considered LOEC for mortalities, however, the treatment did not have an effect on the other investigated parameters of fish (dry and wet weight, body length). In the two-generation test using 5-FU, significant genotoxic effects of the tested cytostatic on F1 generation fish was observed even in the lowest concentration (10 ng/l). Generally, the treatment had no effect on the mortality of fish in any of the treated generations, however, it affected the growth of fish in F1, reflected in body weight and length. Thus, all tested cytostatics had a toxic effect on zebrafish, although, with significant variation according to the age of fish, length of exposure and concentration of the substance.

# Poster sessions

# Evaluation of exposure and uptake of pharmaceuticals in fish from the Llobregat river

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In the last decade, the occurrence of pharmaceuticals in the rivers has been reported over the world. In Spain, several studies indicate that Llobregat river is a high polluted river basin (1, 2). The presence of drugs cause concerns about the potential adverse effects on exposed wildlife. Although some studies have been determined pharmaceuticals in fish, still very little is known about their metabolism in these organisms. Therefore the main goal of the present work is to determine the occurrence of drugs and their metabolites in the different fish tissues from the highly impacted river. To this end, a sampling campaign was designed to collect two fish species, *Barbus graellsi* and *Cyprinus carpio*, from the Llobregat River. First, for the rapid evaluation of pharmaceutical exposure fish bile was analyzed because the concentration of drugs or their metabolites could be roughly 1000 times higher than the concentration found in the surrounding surface waters (3). So, bile samples were analyzed directly after dilution by ultra-high performance liquid chromatography coupled to high-resolution mass spectrometry (UPLC-HRMS) on the Q Exactive (Orbitrap) system. In order to evaluate the accumulation of drugs fish muscle was analysed as follows: fish muscle homogenates were extracted with 8 mL of methanol/0.1M acetic acid by sonication prior analysis by UPLC-HRMS. The accurate mass measurements obtained by HRMS allowed to screen for suspected drugs and their metabolites. This approach has allowed the detection of several plausible phase I and phase II metabolites in fish bile. The detection of pharmaceuticals in muscle demonstrated the uptake of drugs from water and their accumulation. In accordance with the literature, psychiatric drugs have been the most commonly detected drugs in fish.

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# Preliminary study of pharmaceuticals compounds by different technologies used in WWTPs in the Región de Murcia. Prediction of consumption data

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Preliminary study of pharmaceuticals compounds by different technologies used in WWTPs in the Región de Murcia. Prediction of consumption data

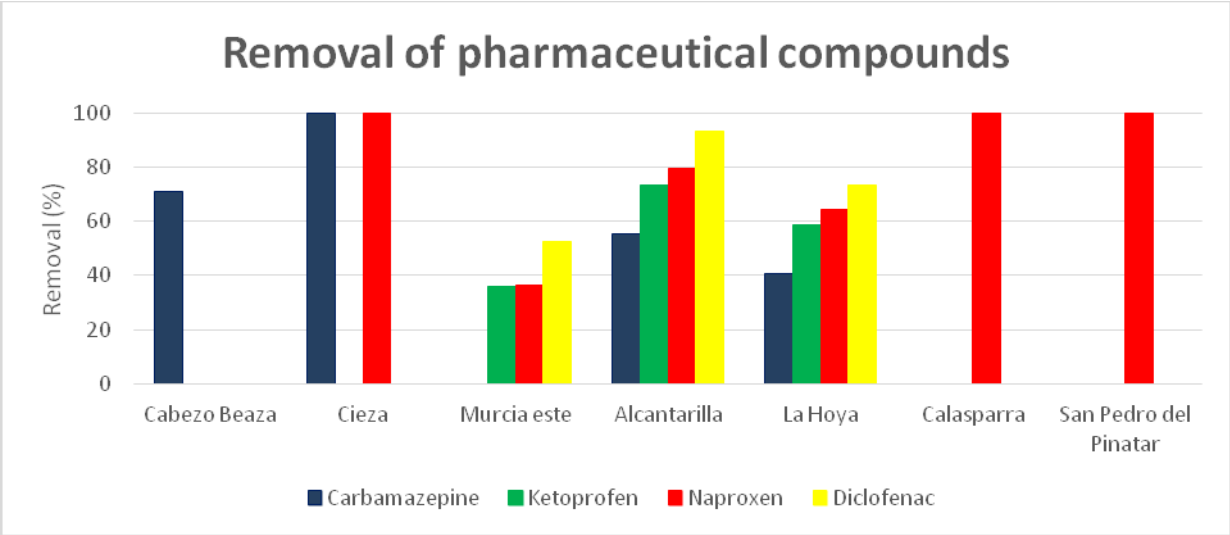
The presence of certain pharmaceuticals in grounds and surface waters is a serious environmental problem as these compounds are biologically active and could affect non-targeted and potentially susceptible species. The occurrence of pharmaceuticals in the environment indicates incomplete removal of these drugs from municipal wastewater treatment plants (WWTPs).

The first objective of this proposal is to identify and quantify four representative pharmaceutical compounds in 12 WWTPs throughout the Región of Murcia to know the influence of these compounds in the acuatic ecosystems. The second objective is to evaluate the relative efficiency of different technologies in eliminating these four pharmaceuticals compounds and finally to verify if the averaged concentrations of pharmaceuticals in the studied WWTPs could be extrapolated from commercial sales and consumption data.

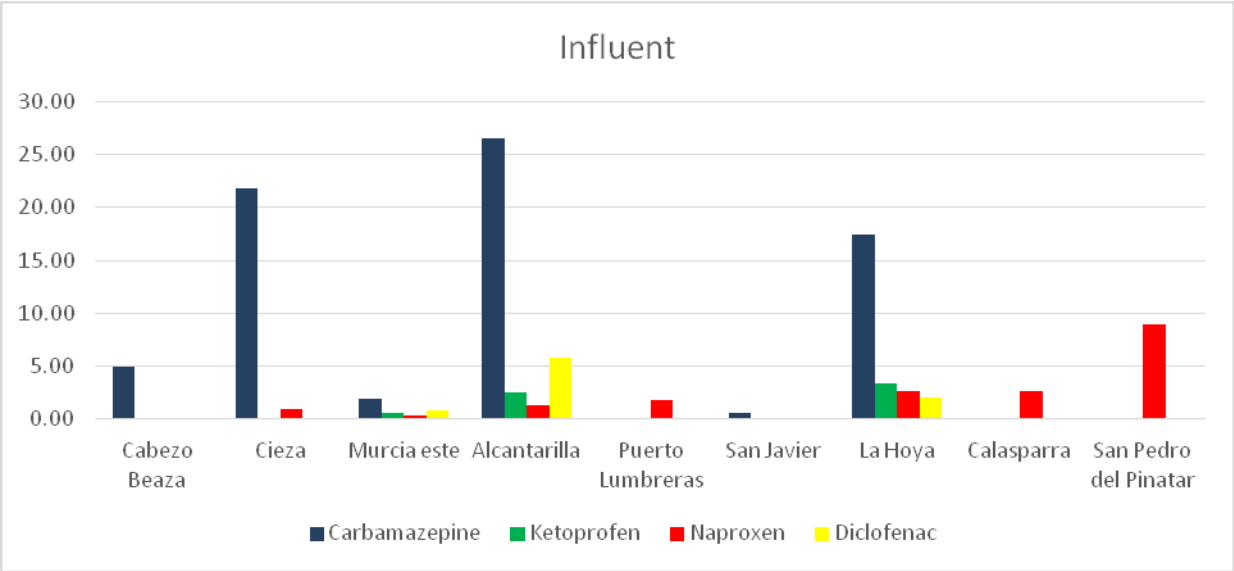
Influents and effluents wastewater in 12 WWTPs were sampled on a weekly basis during four consecutive weeks. Pooled samples were collected over a period of 24 hours in automated samplers. Compound concentrations were quantitatively determined with HPLC-DAD.

WWTPs with Conventional activated sludge (CAS) systems and Sand filters (SF) + Lamella clarifier (L) + Ultraviolet disinfection (UV) resulted in removal efficiencies of above 50% of the majority of the compounds detected, indicating low charge of these compounds in aquatic ecosystems. In most cases, the averaged concentrations of pharmaceuticals in the studied WWTPs are above of estimated prediction and therefore could not be extrapolated from commercial sales and consumption data.

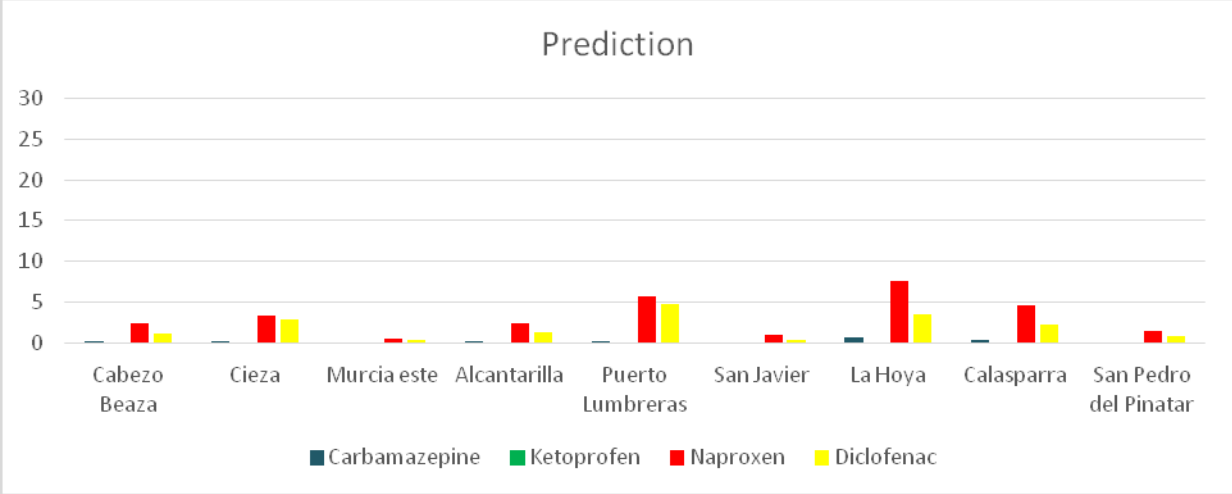

*Figure 1: WWTPs selected*




*Graphic 1. Removal of pharmaceutical compounds in WWTPs selected*




*Graphic 2. Compounds measuring in the influent of WWTPs selected*




*Graphic 3. Prediction of pharmaceutical compounds in WWTPs selected*

**Acknowledgements**

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# Eutrophication and emerging pollutants in a Brazilian tropical reservoir: Relationship and spatial distribution

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Due to the inputs of untreated urban sewages, urban reservoirs of the Metropolitan Area of São Paulo (SP, Brazil) have serious problems of eutrophication, with loss of biological diversity and blooms of toxic cyanobacteria that increase the cost of water treatment for human consumption. Eutrophication in Brazil is even more problematic than in other countries because of the tropical characteristics of these reservoirs (high temperature over the year and high and constant solar irradiation). Guarapiranga reservoir is one important source of the São Paulo's Metropolitan Area. The basin soil is highly populated and mainly used for human activities. With diffuse inputs of urban sewages, this reservoir has been classified as eutrophic or hyper-eutrophic. Correlation between eutrophication and the presence of emerging pollutants (EPs) such as pharmaceuticals has been previously described in Iberian reservoirs. The objective of this work is to study possible correlations of EPs with eutrophication and spatial pattern.

Following a head-to-dam gradient, 3 areas with different degrees of human impact (A, B and C) were studied in February 2014. We sampled water from 3 sites in each area, at 1 m deep. Physical and chemical data were collected to evaluate the eutrophic state. Samples were also analysed for pharmaceuticals, endocrine disrupters, illicit drugs and pesticides using a LC/MS/MS ESI. To find patterns based on physical parameters and nutrients or based on EPs, both groups of data were analyzed separately by PCA. Possible correlation (Spearman) between nutrients and EPs concentrations was also studied. Trophic state, based on total P, ranged from mesoeutrophic (area A) to eutrophic (B and C). One pharmaceutical, seven endocrine disrupters and 12 pesticides were detected in the samples but only 4 EPs (bisphenol A, cocaine, benzoilecognine, caffeine) and 7 pesticides were quantified. While the eutrophy followed a head-to-dam gradient suggesting biological processing of nutrients in the reservoir, EPs did not show a specific pattern, and were detected even in mesotrophic areas, suggesting diffuse inputs and higher environmental persistence. In addition to nutrient inputs, EPs are threatening water quality in this tropical reservoir. Monitoring programs for control of water quality are mainly focused in the monitoring of the trophic state, but this work suggests that EPs have a different behavior in environment and require different monitoring strategies. But more data are required to confirm this hypothesis.

# Preliminary study of stability of 8 common pharmaceuticals in soil

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The global impact of pharmaceuticals in terrestrial ecosystems still remains unclear. Although these substances are generally less persistent than classical persistent organic pollutants, their continuous release and their concentration in the soil matrix makes them semi-persistent (Daughton and Ternes, 1999) so these substances deserve special attention as environmental contaminants. The metabolic and environmental degradation of these compounds produce a huge variety of metabolites and degradation products that increase the complexity of the problem. However, studies on the ecotoxicological effects of pharmaceuticals in soil biota are especially scarce.

Studies about pharmaceutical toxicity in soil systems have focused primarily on higher organisms such as plants (D'Abrosca et al., 2008; Feito et al., 2013; Furtula et al., 2012) and much less on soil invertebrates. Soil is used as a substrate in these ecotoxicological experiments, so it is important to know what extent the soil could affect the initial chemical structure of our compounds over the assay time. If a certain compound is degraded early and high toxicity values are found, a clear relation between the original drug and its toxic effects could not be established, and the toxic effects could have come from its degradation products. The aim of this study was to measure the persistence in soil of seven pharmaceuticals belong to two therapeutic families on which the scientific community has focused its attention because of the drugs' expected environmental impact from their extensive consumption and their systematic presence in the environment, namely blood lipid-lowering and antibiotics. We studied five blood lipid-lowering agents (bezafibrate, gemfibrozil, atorvastatin, simvastatin and lovastatin) and three antibiotics (sulphamethoxazole, trimethoprim and tetracycline). The presence of the test drugs was evaluated after 14 days in artificial OECD soil substrate consisting of industrial fine sand, sphagnum peat, and kaolin clay in a 7:1:2 ratio, respectively. The detection of the studied pharmaceuticals in the collected supernatants was performed in a Shimadzu-LC 20AD HPLC equipped with a diode-array detector (SPD-M20A) and a Waters Novapack-C18 column (15 cm × 3.9 cm and a 4 μm particle size). The absorbance was registered at all UV-VIS wavelengths. Analysis of chemical degradability of the pharmaceuticals shows that blood lipid-lowering agents seem to be much more stable than antibiotics, with bezafibrate being the only degraded drug (a new peak appears on the chromatogram before the standard retention time) at the end of the test. The three antibiotics, were found to be degraded with tetracycline completely degraded in 14 days. Among the entire group of tested drugs, tetracycline is the only one with no presence at all at the end of the experimental time. These results suggest the need for systematic studies of stability when soil toxicity studies are conducted to ensure that the effects of toxicity detected are due to the original product and not because of its degradation products.

**Acknowledgements**
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# Simultaneous determination of sixteen UV filters in environmental surface waters with solid phase extraction and liquid chromatography-tandem mass spectrometry

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In the last years, there is an increasing attention of both public institutions and researchers toward the so-called "emerging contaminants". Among them, the commercial category of the UV filters include various and heterogeneous chemical classes. UV filters are present as ingredients in personal care products, such as sunscreen products, creams, cosmetics, lipsticks, hair sprays, shampoos etc., to protect skin and hair from the negative effects of sunlight. As pharmaceuticals, due to their large use, they are continually released and accumulated into the environment. Residues of more polar organic UV filters have been found in all kinds of water matrices. Moreover UV filters, especially those which have the highest hydrophobic character, are expected to end up in sediments from water and sewage sludge during wastewater treatments. Recently, several studies have been carried out on the toxicity and potentially dangerous consequences of the occurrence of these compounds on the aquatic environment. Some of these studies have also indicated their potential estrogenic activity [1].

The concentrations of these compounds in environmental matrices, both solid and liquid, are estimated ranging from few to several thousands of ng $L^{-1}$ or ng $g^{-1}$. So there is a necessity to employ sensible and selective analytical methodologies for their determination and quantification in these types of environmental matrices [2].

The aim of this work was to develop and validate a highly sensitive multiresidue method based on solid phase extraction, employing graphitized carbon black as sorbent material, followed by UPLC/ESI-MS/MS for the analysis of sixteen UV filters and environmental degradation products with a wide range of physicochemical properties [3]. After a thorough investigation of SPE and LC-MS/MS conditions, it permits the enrichment and determination of a multiclass of these compounds, between the most commercially used, in a single methodology. Recoveries above 70% were obtained for all the analytes from lake water samples. A survey on few water samples from three lakes in the area of Rome showed that some of these UV filters are present in small amount.

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# Highlights of the DEGRAPHARMAC project in the treatment of hospital wastewater (veterinary and human) with fungal reactors

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The use of ligninolytic fungi is a promising alternative to treat recalcitrant effluents. White-rot fungi have a very powerful enzymatic system, which comprises highly unspecific extracellular and intracellular enzymes, and production of free radicals. *Trametes versicolor* or its enzymes alone have been successfully applied in the treatment of paper mill effluents and effluents containing dyes among others, normally under sterile conditions. However, its application to degrade pharmaceuticals and personal care products (PPCPs) in real effluents has not yet been reported.

In this project the fungus *T. versicolor* is applied for the first time to treat hospital effluents. Human hospital effluent comes from the pipe lines of Dr Trueta hospital (Girona, Spain) and veterinary hospital effluent was sampled in the pipe lines of a veterinary university hospital in Universitat Autònoma of Barcelona (Bellaterra, Spain). A complete characterisation of each effluent was necessary, both in physicochemical terms and pharmaceutically active compounds concentrations. In total, 64 compounds belonging to 19 different therapeutically groups were analysed. Firstly, fungal viability experiments for sterile effluents were performed in order to identify possible lack of nutrients. Moreover some pharmaceuticals were selected for an in-depth individual study. These assays were carried out to study the individual degradation of target compounds, to determine their fungal metabolites, to identify the enzymes involved in the first degradation steps and to elucidate the degradation pathways.

A 1.5 L fungal air-pulse fluidized bioreactor was used to treat the effluents under different operational conditions (sterile/non-sterile and batch/continuous) in view of a possible future implementation. *T. versicolor* was inoculated in pellets form. Fungal activity was monitored in terms of enzymatic activity, glucose consumption and COD removal, among other parameters. Concentrations of pharmaceutically active compounds were also analysed at different sampling points. In addition to these analysis, toxicity of the samples taken along the treatment were determined (Microtox and RYA assay). The results point out the importance of an external addition of nutrients and the control of aeration for an efficient removal of contaminants by the inoculated fungus. The importance of conjugation and deconjugation processes is also highlighted in this project. They are a restriction in the assessment of PhACs degradation in real effluents due to the absence of conjugates in the analytical methods and, at the same time, conjugates are important intermediate metabolites in the fungal degradation of the selected contaminants. The initial PhACs concentration into the bioreactor was 5617 µg/L in batch treatment and the overall load elimination was 53%, for human hospital effluents. For veterinary effluents was 67 µg/L and the overall elimination was 66%. In a continuous treatment of hospital veterinary effluent at 3 days HRT, the PhACs removal was 44%.

**Acknowledgements**

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# Removal of selected sulphonamides during the ozonation process in the presence and absence of bicarbonates

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The main purpose of this study was to compare the efficiency of selected sulphonamides oxidation by ozone in the aqueous solution in the presence and absence of bicarbonates.

For the experiment two pharmaceutical substances from sulphonamides group were selected: sulfadiazine and sulfamethazine. Initial concentrations of compounds used in studies were equal $2*10^{-5}$ M/L for sulfadiazine and $1.8*10^{-5}$ M/L for sulfamethazine.

Studies were carried out in aqueous solution, mimicking tap water, at stable conditions: pH ~7.0 and temperature18-20$^0$C. In experiments with bicarbonates the concentration of $5.7*10^{-3}$ $M_{HCO3-}$/L was used.

Ozone was applied in three different doses: $2*10^{-5}$ $M_{O3}$/L, $6*10^{-5}$ $M_{O3}$/L and $1*10^{-4}$ $M_{O3}$/L.

The loss of investigated compounds concentration in time was performed by HPLC.

The most efficient removal of both substances from sulphonamides group was achieved by dose of $1*10^{-4}$ $M_{O3}$/L in the presence of bicarbonates. Percentage removal of both compounds at this conditions reached 100 % in 10 seconds.

With the same ozone dose but in the absence of bicarbonates removal of sulfadiazine and sulfamethazine was around 81% and 87 %, respectively.

Based on the results obtained from this investigation it can be concluded that the presence of a naturally occurring bicarbonates in tap water enhance removal of selected substances from sulphonamides during ozonation process. The most efficient dose of ozone used in these studies is located in the lower limit of economical dosage rates for drinking water.

# Can we trust in Managed Aquifer Recharge (MAR) to deal with emerging contaminants present in reclaimed water?

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Managed Aquifer Recharge (MAR) is a widely-used technique in Europe, but the role and function of MAR in terms of emerging pollutant removal is still uncertain. The European co-funded DEMEAU project (7th Framework Programme) aims at demonstrating how MAR can be considered a feasible possibility to deal with emerging contaminants in reclaimed water. This work presents results of column experiments using reclaimed water to assess the removal of target emerging pollutants under denitrification conditions. Sandy material filtration has been compared with enhanced conditions, using a compost-made organic layer at the column inlet. Results showed a significant increase of removal in the enhanced system for some of the target pollutants.

# Cytotoxicity and genotoxicity of imatinib mesylate in zebrafish liver (ZFL) cells, human hepatoma (HepG2) cells and human peripheral blood lymphocytes (HPBLs)

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Imatinib mesylate (IM) is a selective tyrosine kinase inhibitor, which is used for treatment of chronic mylelogenous leukemia and gastrointestinal stromal tumour, with high specificity towards tumour cells and less toxicity to non-target normal cells. IM is one of the most widely used anticancer drugs in European countries. After therapeutic use IM is subjected in the body to metabolic degradation and can be excreted into aquatic environment by urine or faces as a mixture of metabolites and the unchanged parent compound, where it can harm non-target organisms. In addition, also healthy humans can be unintentionally exposed i.e. health care workers or through potable water drawn from contaminated supplies. There is some limited evidence that IM is genotoxic and that it can harm non-target environmental organisms. In this study we evaluated cytotoxicity and genotoxicity of IM in experimental model with zebrafish liver cell line (ZFL), human hepatoma HepG2 cells and human peripheral blood lymphocytes (HPBLs). After exposing the cells to graded doses of the drug for different periods of time we examined their viability with the MTT assay and by differential staining with acridine orange and ethidium bromide, genotoxicity by measuring DNA strand breaks with the comet assay and chromosomal damage with the cytokinesis-block micronucleus cytome assay (CBMN). IM induced time and dose reduction of cell viability in all three cell lines. The cell lines showed different sensitivity towards IM. Induction of DNA strand breaks and micronuclei formation was determined at non-cytotoxic doses. In HepG2 and ZFL cells IM induced dose and time dependent increase of DNA strand breaks with ZFL cells being slightly more sensitive than HepG2 cells. In the isolated HPBLs IM at selected concentrations did not induce DNA strand breaks. Increase in micronuclei formation (MNi) was detected in ZFL cells and HPBLs, but not in HepG2 cells. In HPBLs also increase of nucleoplasmic bridges (NPBs) and nuclear buds (NBUDs) was detected. Our results contribute to the increasing evidence that IM is genotoxic.

The research leading to these results has received funding from the EC's FP7/2007-2013 under the grant agreement no. 265264 (Cytothreat).

# Toxic and genotoxic effects of BAC and its binary mixtures with four cytostatics in the crustacean *Ceridodaphnia dubia*

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Benzalkonium chloride (BAC), a quaternary ammonium compound, is commonly used for the disinfection of surfaces in medical care applications as well as in some industries (Sütterlin et al., 2008). BAC persists in the environment and hardly degrades (Perez et al., 2009) so that it has been detected in effluents from different European hospitals at concentrations ranging from 0.05 to 6.03 $mgL^{-1}$ (Kümmerer et al., 1997) and in surface waters at concentration in the order of $\mu gL^{-1}$ (Ferrer and Furlong 2001). Pharmaceuticals are present in the aquatic environment and they may interact with disinfectants such as BAC, producing effects at concentrations that could be different from those of single compounds. In this context, anti-neoplastic drugs play an important role because of their potential for interfering with the structure and functions of DNA in exposed organisms also at low concentrations. The combined long term toxic potential and genotoxicity of 5-fluorouracil [5-FU], cisplatin [CDDP], etoposide [ET] and imatinib mesylate [IM]) were investigated testing their binary mixtures with BAC on *Ceriodaphnia dubia*. The isobologram method (curves in the plane of doses of binary mixtures expected to result in equal effects given the reference model) was applied to obtain the optimal combined dosage regimen. The combined effects were assessed using two distinct effect percentages. Direct statistical comparison, by analysis of variance of single and combined effects under the assumption of Bliss independence, allowed to accept or reject the independency hypothesis. Results of BAC tested as single compound showed a median chronic toxicity effect in the order of tenths of $\mu gL^{-1}$ while genotoxicity occurred at concentrations in the order of $ngL^{-1}$. Chronic combined experiment results showed an antagonistic effect at higher effect percentage for all BAC binary combinations, except with IM. Heteroadditivity was found for all mixtures at lower effect percentage. The genotoxicity combined results showed antagonistic effect for BAC+ET and BAC+CDDP at the two effect percentages while heteroadditivity was shown by BAC+IM and BAC+5-FU at both effects selected. These results highlighted that single compounds even at low concentrations, when act simultaneously, may lead to serious overall effects.

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# Impact of *in vivo* and *in vitro* exposure to 5-fluorouracil, cisplatin, etoposide and vincristine sulphate on dna damage in haemocytes of freshwater mussels *Unio pictorum* AND *Unio tumidus*

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The impact of 5-Fluorouracil (5-FU), Cisplatin (CP), Etoposide (Eto) and Vincristine sulphate (Vin) on DNA damage level was studied *in vivo* and *in vitro* on haemocytes of freshwater mussels *Unio pictorum* and *U. tumidus* using alkaline comet assay. For *in vitro* experiments, two different approaches were applied: i) isolated haemocytes were treated for 30 min in physiological solution, ii) primary culture of haemocytes was treated for 22h. For *in vivo* experiments, mussels were exposed in static system for 72h. $CdCl_2$ was used as positive control. The level of DNA damage was analyzed by Comet Assay IV software. $CdCl_2$ induced significant increase in DNA damage *in vitro* and *in vivo*.
Treatments *in vivo* with 5-FU, Eto and Vin resulted in significant increase of DNA damage. Genotoxic potential was also detected *in vitro* for Vin after 30 min of exposure, and Eto after 22h. CP did not induced increase of DNA damage *in vivo* or *in vitro,* but post treatment with hydrogen peroxide indicated existence of DNA crosslinks.
Lack of genotoxic effects of cytostatics in isolated haemocytes can be attributed to short period of exposure (30 min), while the lack of the effects in primary cultures can be assigned to the mechanisms of action of these drugs.
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# Effect of 5-FU on transcriptomic level in different zebrafish models

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The fluoropyrimidine 5-fluorouracil (5-FU) is one of the most frequently used cytostatics for systemic and local treatment of many types of cancer. 5-FU belongs to a family of antimetabolites and is cell-cycle specific to S phase of cell division. The drug exerts its anticancer effects through incorporation of its metabolites in lieu of pyrimidine bases, and inhibition of normal DNA synthesis/replication by inhibiting thymidylate synthetase. After therapeutic use, 5-FU and its metabolites can be released into aquatic environment via hospital and municipal effluents where they can harm non-target organisms. Due to its non-selective mode of action, practically all eukaryotic organisms are vulnerable to DNA damage. Alterations in gene transcription represent primary response of the organism to the exposure to various xenobiotics, that occur prior to the onset of physiological and biological changes. The aim of this study was to evaluate the changes in gene transcription induced in zebrafish (*Danio rerio*) liver after long term exposure (4 months old F1 generation) to 5-FU (10 ng/L and 1 µg/L) and two *in vitro* models; zebrafish embryos (24h exposure to 10 and 100 µg/L), and zebrafish liver cells (ZFL) (72 h exposure to 10 ng/L, 10 µg/L, 1 mg/L). The results showed that 5-FU treatment of embryos caused hardly any transcriptional changes (137 deregulated genes), showing low sensitivity of this experimental model. On the other hand, in ZFL cells significant deregulation of gene expression (5827 deregulated genes), was observed at the highest tested concentration (1 mg/L) while at environmentally more relevant concentrations (10 ng/L and 10 µg/L) hardly any changes were observed. In contrast to short-term exposure experiments, in liver of chronically exposed adult F1 zebrafish transcriptional deregulation of gene expression was observed already at the lowest tested concentration (10 ng/L; 1879 deregulated genes). Analysis of unique and common differentially expressed (DE) genes (regardless of concentration used) has shown that cells and F1 liver treated with 5-FU share 1251 DE genes, meanwhile all three experimental models share just 5 DE genes. The results obtained with the pathway analysis of the gene expression changes indicate that long term *in vivo* exposure can be used for the prediction of potential adverse effects of environmentally relevant concentrations of 5-FU. However, we did not identify common DE that could be used as specific indicator of exposure and effects of 5-FU in the short term *in vitro* assays.

The research leading to these results has received funding from the EC's FP7/2007-2013 under the grant agreement no. 265264 (Cytothreat).

# Exposure to binary mixtures of four anticancer drugs in crustaceans: chronic and genotoxic effects

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Pharmaceutical residues found in the aquatic environment generally occur as mixtures. The knowledge about toxicological effects of pharmaceutical mixtures is rather limited although they might reveal different effects than single compounds (Fent et al., 2006; Galus et al., 2013). The concentrations of pharmaceuticals in the aquatic systems depend on their prevalence of use, human metabolism, possible biotic and/or abiotic transformations, and on the effectiveness of wastewater treatment plants. In this context, also anti-neoplastic drugs play an important role. Although their concentrations in environmental media are often lower than those of other pharmaceuticals, their potential for interfering with the structure and functions of DNA in exposed organisms is likely higher, even more when they occur as complex mixtures. Furthermore, as most of anti-neoplastic drugs act on the same biological target but often with different modes of action, it is difficult to predict their interaction in mixtures. For this reason, the aim of the present study was to investigate the combined long term toxic and genotoxic potential of four anti-neoplastic drugs (5-fluorouracil [5-FU], cisplatin [CDDP], etoposide [ET] and imatinib mesylate [IM]) testing their respective binary mixtures on two primary consumers of the freshwater aquatic chain with similar taxonomy: *Daphnia magna* and *Ceriodaphnia dubia*. The combined toxicities and genotoxicity were assessed using two distinct effect sizes that should be observed if Bliss independence holds. Direct statistical comparison by analysis of variance of single and combined toxicities under the assumption of Bliss independence allowed to accept or reject the independency hypothesis (Katsnelson et al., 2011). Chronic toxicity data (Parrella et al., 2014) and genotoxicity data, previously evaluated in *D. magna* and *C.dubia* from exposures to single anti-neoplastic drugs, were extracted to obtain a standardized format of results for all experiments; the isobologram method (curves in the plane of doses of binary mixtures expected to result in equal effects given the reference model) was applied to obtain the optimal combined dosage regimen. The long-term results showed that the additivity predicted was confirmed for all mixtures both in *D. magna* and in *C. dubia*, except for IM+ ET and IM+CDDP in *D. magna* and for ET+CDDP and ET+5-FU in *C. dubia* which at the highest concentrations showed an antagonistic interaction. A synergic tendency was found testing IM+CDDP on *C. dubia* at the lowest concentration selected. Comet combined results showed in *D. magna* an antagonistic interaction for IM+5-FU. In *C. dubia,* additive effect was found for IM+CDDP and IM+5-FU.
Thus, the ecotoxicological data evaluated in this study show not only a potential environmental risk of anticancer drugs, especially considering their heteroadditivity effects, but also the necessity to integrate predicted statistical models with experimental data to establish the real environmental impact of such compounds.

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# Genotoxic effect of 5-fluorouracil in zebrafish after chronic exposure to low doses in a two-generation study

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The residues of anti-neoplastic drugs are new and emerging pollutants in aquatic environments. Many of these drugs are genotoxic and it has been postulated that they may cause adverse effects in aquatic ecosystems. Here we present the results of the first investigation of genotoxicity of 5-fluorouracil, one of the most extensively used anti-neoplastic drugs in cancer therapy, in a two generation study with zebrafish (*Danio rerio*). The exposure of fish to 5-FU (0.01, 1 and 100 µg/L) was initiated with adult fish (F0) and continued through hatch of F1 and F2 generation till 33 post fertilization day. The exposure to 5-FU did not affect the survival, growth and reproduction of fish, however the histopathology changes in liver and kidney and genotoxic effects, determined in F1 generation fish, were observed at all tested concentrations. The increase in DNA damage determined with the comet assay was significant only in liver, but not in gills gonads and blood cells. However, with the micronucleus assay a significant, dose dependent increase in frequency of micronuclei was observed in erythrocytes at all concentrations of 5-FU. The whole genome transcroptomic analyses of the liver samples of F1 generation fish revealed dose dependent increase in the number of differentially expressed genes including up-regulation of several DNA damage responsive genes and oncogenes (i.e. jun, myc). Although chronic exposure to environmentally relevant concentration of 5-FU did not affect viability and reproduction of exposed fish it cannot be excluded that it may lead to degenerative changes, including cancer, which over the long term exposure of several generations may affect fish populations and consequently the aquatic ecosystems. The results contribute to better understanding of the consequences of chronic exposure of fish to low concentrations of anti-neoplastic drug.

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# *In situ* assessment of DNA damage in *Branchiura sowerbyi* Beddard, 1892 (Oligochaeta: Tubificidae) from the Sava river using comet assay

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Aquatic oligochaete, *Branchiura sowerbyi* (Beddard, 1892) is a cosmopolitan tubificid species commonly found in organically enriched freshwater environments. These aquatic worms inhabit river and lake sediment where they feed by decomposing organic matter. *B. sowerbyi* is characterized by limited mobility and could be used as effective indicator organism. Collecting of *B. sowerbyi* was done monthly during 2014 on the Sava River (sampling site Duboko). Duboko is situated downstream of city Obrenovac (50.000 inhabitants). The site is under the influence of two major pollution sources. One is the Kolubara River (under the influence of various pollution pressures, including wastewaters from town Obrenovac) and the other is thermal power plant "Nikola Tesla" which is situated few kilometres upstream. Suspensions of cells (haemocytes and coelomocytes) were used for the assessment of genotoxicity by comet assay. As reference the value of Comet response obtained from individuals acclimatized in controlled aquarium conditions was used. For each sampling images of 200 nuclei were analyzed with a fluorescence microscope and analysed using the software Comet Assay IV. During the period of investigation the wider area of investigation was affected by a huge flooding (May 2014) when entire city of Obrenovac was evacuated and consequently there was not wastewater discharges. Therefore, in May and June one source of pollution was excluded. Comparing with reference value, the significant increasing of DNA damage was recorded during examination period, except in May and June. Also, microbiological quality of water was assessed each month. High correlation with faecal indicators indicates that integrity of DNA molecule of *B. sowerbyi* is affected by urban pollution.

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# Methodology approach using effect-based monitoring tools to link water quality and pollutants

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Water Framework Directive (WFD) is the main legislative piece of EU in relation to water quality. According to this Directive, the assessment of water status is based on the link between chemical and ecological status, although in some cases are not in coherence. To resolve this problem, ecotoxicity tests have been proposed as useful tools (Roig et al., 2013; 2014). Moreover, the use of effect-based monitoring tools has been mentioned in the context on the Common Implementation Strategy (CSI) 19 (EU, 2011), 25 (EU, 2010) and 27 (EU, 2014). Current chemical monitoring is focused on regulated substances, which involves that many substances are not considered and their contributions to toxicity are lacking. Among these substances, pharmaceuticals due to their pseudo persistence and chronic inputs represent a risk for environment. Due to sediment is able to integrate stream pollution for a long time and may act as both sink and source of contaminants for the water column; it has advantage over other environmental compartments to assess the environmental quality. This approach has been employed in the Ebro River to evaluate water surface quality (Roig et al., 2014) although the relative contribution of different pollutants for the whole toxicity was not assessed. The aim of this work is develop a general methodology which allows to identify the main pollutants and to evaluate the relative contribution of them to the toxicity in order to share these results with managers and stakeholders to improve management tools.

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