

1ST INTERNATIONAL CONFERENCE ON RISK ASSESSMENT OF PHARMACEUTICALS IN THE ENVIRONMENT

SEPTEMBER 8-9, 2016 | PARIS - FRANCE



Abstract book

Livre des résumés



ACADÉMIE NATIONALE DE PHARMACIE

SANTÉ PUBLIQUE - MÉDICAMENT - PRODUITS DE SANTÉ - BIOLOGIE - SANTÉ ENVIRONNEMENTALE

*Fondée le 3 août 1803 sous le nom de Société de Pharmacie de Paris
Reconnue d'utilité publique le 5 octobre 1877*

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ICRAPHE 2016

*Under the High Patronage
Under the Honorary Sponsorship of*

Ségolène ROYAL, Minister of Environment, Energy and Sea

Marisol TOURAINE, Minister for Social Affairs and Health

Cloître des Cordeliers - 21 rue de l'École de Médecine 75006 PARIS

PROGRAM

8 SEPTEMBER

8h30 **Reception**

9h00 **Opening session**

Ségolène ROYAL, Minister of Environment, Energy and Sea (to be confirmed)

Benoît VALLET, General Director of Health, Ministry for Social Affairs and Health

Claude MONNERET, President of the French Academy of Pharmaceutical Sciences

9h30 **Plenary conference by Maria NEIRA**, Department of Public Health, Environmental and Social Determinants of Health, WHO

10h00 **Oral Session "Hazard identification - adverse effects in human and environment"**

"Ecotoxicological characterization of pharmaceuticals during regulatory assessments: state of the art, options for improvement"

Speaker and Moderator, Dr Thomas BACKHAUS, Professor for Ecotoxicology and Chemical Risk Assessment at the University of Gothenburg (Sweden) and leader of the Center for Future Risk Assessment and Management Strategies

Communications:

- 10h40: **Impact of psychotropic pharmaceuticals on freshwater organisms**, J.-Y. MAZZITELLI, Biochimie et Toxicologie des Substances Bioactives (BTSB, EA7417)-Institut National Universitaire Champollion, Albi, France

- 11h00: *Toxic and genotoxic effects of the residues of anticancer drugs in aquatic organisms*, M. FILIPIC, Department of Genetic Toxicology and Cancer Biology-National Institute of Biology (NIB) - Ljubljana, Slovenia
- 11h20: *Analysis of toxicological effects of atenolol, caffeine, lidocaine and oxytetracycline on *Raphidocelis subcapitata* and *Chlorella vulgaris* algae*, Z. PROCOPIO, Glasgow Caledonian University, Glasgow, United Kingdom
- 11h40: *Combination effects of endocrine active pharmaceuticals in wastewaters using the Calux reporter-gene assay*, H. BIELAK, IWW Water Centre, Mülheim, Germany
- 12h00: *Mixtures effect of antibiotics on soil microbial nitrogen processes*, V. DAVID, AgroParisTech, INRA- ECOSYS (Ecologie fonctionnelle et éco-toxicologie des agro-écosystèmes), Versailles, France

12h30 pm **Lunch**

BREAK AND POSTER EXHIBITION

2h00 pm **Oral Session “Exposure assessment”**

“Pharmaceuticals as environmental contaminants: analysis and levels in the environment”

Speaker and Moderator, Dr Ettore ZUCCATO, Department of Environmental Health Sciences-Mario Negri Institute for Pharmacological Research, Milan, Italy

Communications:

- 2h40 pm: *Innovation in effect monitoring of pharmaceuticals - in vitro, in vivo, in environment*, J. BACHMANN, Federal Environment Agency (UBA), Dessau-Roßlau, Germany
- 3h00 pm: *New rapid cell-based assays for β -blocker and nonsteroidal anti-inflammatory drug determination in wastewater effluents*, F. FREY, Steinbeis Transfer Center for Applied Biological Chemistry, Mannheim, Germany
- 3h20 pm : *A novel approach for determining the uptake of pharmaceuticals and personal care products from sediment at the landscape scale*, L. CARTER, Environment Department, University of York, York, United Kingdom

3h40 pm BREAK AND POSTER EXHIBITION

- 4h10 pm: *Next-Generation Sequencing to highlight community changes in river biofilms linked to pharmaceutical loads from a wastewater treatment plant*, T. CHONOVA, INRA, UMR CARTELE, Thonon-les-Bains, France
- 4h30 pm: *Prioritisation of veterinary pharmaceuticals prior to a monitoring campaign: Case of Brittany, an intensive husbandry area*, L. CHARUAUD, École des Hautes Études en santé publique (EHESP)-Laboratoire d'Étude et de Recherche en Environnement et Santé (LERES), Rennes, France

5h00 pm **End of the first day: closing remarks**

7h30 pm **Gala Dinner**

9 S E P T E M B E R

9h00 **Oral Session “Risk assessment”**

“Pharmaceuticals in the environment: are they posing unacceptable risks?”

Speaker and Moderator, Alistair BOXALL, Professor of Environmental Science at the University of York, York, United Kingdom

Communications:

- 9h40: *Health risk assessment of pharmaceuticals in drinking water in France*, P. PANETIER, ANSES-Agence nationale de Sécurité sanitaire de l’Alimentation, de l’Environnement et du Travail, Paris, France
- 10h00: *Assessing effects of pharmaceuticals on aquatic ecosystems*, I. ROESSINK, Alterra, Wageningen, Netherlands
- 10h20: *Occurrence of pharmaceuticals in hospital wastewaters and assessment of their associated environmental risk and hazard: a Spanish case study*, M. LOPEZ DE ALDA, Department of Environmental Chemistry-Institute of Environmental Assessment and Water Research (IDAEA), Barcelona, Spain

10h40 BREAK AND POSTER EXHIBITION

- 11h10: *Critical evaluation of different inputs for the estimation of pharmaceuticals exposure seeking an improved environmental risk assessment*, A. PEREIRA, University of Coimbra, Faculty of Pharmacy-LAQV, REQUIMTE, Group of Bromatology, Pharmacognosy and Analytical Sciences, Coimbra, Portugal
- 11h30: *Human and ecotoxicological potential impact of pharmaceutical and personal care products from USEtox™ life cycle impact assessment characterization factors*, R. IRUSTA-MATA, Department of Chemical Engineering and Environmental Technology, University of Valladolid, Valladolid, Spain
- 11h50: *People thinking about pharmaceutical risk – health and environment: evidence from noPILLS*, P. TEEDON, Glasgow Caledonian University, Glasgow, United-Kingdom
- 12h10: *Investigation of the implications for Ireland of emerging standards on pharmaceuticals in receiving waters*, N. ROWAN, Bioscience Research Institute-Athlone Institute of Technology (AIT), Athlone, Ireland

12h30 pm **Lunch**

BREAK AND POSTER EXHIBITION

2h30 pm **Session “Risk management”**

Speaker and Moderator, Dr Klaus KÜMMERER, Professor of Sustainable Chemistry and Material Resources-Institute of Sustainable and Environmental Chemistry-Leuphana University, Lüneburg, Germany

3h00 pm: **Round table** – **Maria NEIRA** (Department of Public Health, Environmental and Social Determinants of Health, World Health Organisation); **Jean-Marc VIDAL**

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(Specialised Scientific disciplines Department, European Medicines Agency, London, United Kingdom); **Shane A. SNYDER** (Department of Chemical and Environmental Engineering, University of Arizona, Tucson, USA); **Miquel PARAIRA FAUS** (Aiguës de Barcelona and AQUADOM (Agbar group), Barcelona, Spain); **Sandrine SOURISSEAU** (Environment and Health Department-VEOLIA Environnement Recherche et Innovation, Poissy, France)

5h00 pm **closing remarks**



MINISTÈRE
DE L'ENVIRONNEMENT,
DE L'ÉNERGIE
ET DE LA MER

MINISTÈRE
DES AFFAIRES SOCIALES
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PROGRAMME

8 SEPTEMBRE

8h30 **Accueil**

9h00 **Session d'ouverture**

Ségolène ROYAL, Ministre de l'Environnement, de l'Énergie et de la Mer (à confirmer)
Benoît VALLET, Directeur Général de la Santé, Ministère des Affaires Sociales et de la Santé

Claude MONNERET, Président de l'Académie nationale de Pharmacie

9h30 **Conférence plénière : Maria NEIRA, Directeur du département Santé Publique et Environnement - Organisation Mondiale de la Santé**

10h00 **Session « Identification des dangers, effets néfastes chez l'homme et sur l'environnement »**

« Caractérisation écotoxicologique des résidus de médicaments dans le cadre de l'évaluation réglementaire : état de l'art, options d'amélioration »

Conférencier et modérateur Dr Thomas BACKHAUS, Professeur d'écotoxicologie et d'estimation des risques chimiques à l'Université de Gothenburg (Suède), Directeur du « Center for Future Risk Assessment and Management Strategies »

Communications :

- 10h40: **Impact of psychotropic pharmaceuticals on freshwater organisms**, J.-Y. MAZZITELLI, Biochimie et Toxicologie des Substances Bioactives (BTSSB, EA7417)-Institut National Universitaire Champollion, Albi, France
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- 12h00: *Mixtures effect of antibiotics on soil microbial nitrogen processes*, V. DAVID, AgroParisTech, INRA- ECOSYS (Ecologie fonctionnelle et éco-toxicologie des agro-écosystèmes), Versailles, France

12h30 **Déjeuner**

PAUSE ET VISITE DES POSTERS

14h00 **Session « Estimation des expositions »**

« Les résidus de médicaments en tant que contaminants de l'environnement : analyse et niveaux dans l'environnement »

Conférencier et modérateur, Dr Ettore ZUCCATO, Department of Environmental Health Sciences - Mario Negri Institute for Pharmacological Research, Milan, Italie

Communications:

- 14h40: *Innovation in effect monitoring of pharmaceuticals - in vitro, in vivo, in environment*, J. BACHMANN, Federal Environment Agency (UBA), Dessau-Roßlau, Germany
- 15h00: *New rapid cell-based assays for β -blocker and nonsteroidal anti-inflammatory drug determination in wastewater effluents*, F. FREY, Steinbeis Transfer Center for Applied Biological Chemistry, Mannheim, Germany
- 15h20 : *A novel approach for determining the uptake of pharmaceuticals and personal care products from sediment at the landscape scale*, L. CARTER, Environment Department, University of York, York, United Kingdom

15h40 PAUSE ET VISITE DES POSTERS

- 16h10: *Next-Generation Sequencing to highlight community changes in river biofilms linked to pharmaceutical loads from a wastewater treatment plant*, T. CHONOVA, INRA, UMR CARTELE, Thonon-les-Bains, France
- 16h30: *Prioritisation of veterinary pharmaceuticals prior to a monitoring campaign: Case of Brittany, an intensive husbandry area*, L. CHARUAUD, École des Hautes Études en santé publique (EHESP), Laboratoire d'Étude et de Recherche en Environnement et Santé (LERES), Rennes, France

17h00 **Conclusion de la journée**

19h30 **Dîner de gala**

9 S E P T E M B R E

9h00 **Session « Estimation des risques »**

« Les résidus de médicaments dans l'environnement : un risque inacceptable ? »

Conférencier et Animateur, Alistair BOXALL, Professor of Environmental Science at the University of York, United Kingdom

Communications:

- 9h40: *Health risk assessment of pharmaceuticals in drinking water in France*, P. PANETIER, ANSES-Agence nationale de Sécurité sanitaire de l'Alimentation, de l'Environnement et du Travail, Paris, France
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- 10h20: *Occurrence of pharmaceuticals in hospital wastewaters and assessment of their associated environmental risk and hazard: a Spanish case study*, M. LOPEZ de ALDA, Institute of Environmental Assessment and Water Research (IDAEA), Barcelona, Spain

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- 11h10: *Critical evaluation of different inputs for the estimation of pharmaceuticals exposure seeking an improved environmental risk assessment*, A. PEREIRA, University of Coimbra, Faculty of Pharmacy-LAQV, REQUIMTE, Group of Bromatology, Pharmacognosy and Analytical Sciences, Coimbra, Portugal
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- 12h10: *Investigation of the implications for Ireland of emerging standards on pharmaceuticals in receiving waters*, N. ROWAN, Bioscience Research Institute-Athlone Institute of Technology (AIT), Athlone, Ireland

12h30 **Déjeuner**

PAUSE ET VISITE DES POSTERS

14h30 **Session « Gestion des risques »**

Conférencier et animateur, Klaus KÜMMERER, Professor of Sustainable Chemistry and Material Resources; Leuphana University, Lüneburg, Germany

15h **Table ronde** : **Maria NEIRA** (Directeur du département Santé Publique et Environnement-OMS) ; **Jean-Marc VIDAL** (Agence Européenne des Médicaments, Département des disciplines scientifiques spécialisées-UK) ; **Shane A. SNYDER** (Department of Chemical and Environmental Engineering, University of Arizona, Tucson, USA) ; **Miquel PARAIRA FAUS** (Directeur et Chef de laboratoire, AIGUËS DE BARCELONA-Espagne), **Sandrine SOURISSEAU** (Responsable du Département Environnement et Santé, VEOLIA Environnement Recherche et Innovation-France)

17h00 **Conclusions**

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IMPACT OF PSYCHOTROPIC PHARMACEUTICALS ON FRESHWATER ORGANISMS.

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Background and objective : The aquatic organisms are frequently exposed to a wide diversity of chemical compounds suspended in water or adsorbed on substrates. These compounds such as pharmaceuticals are often found in the environment from either industrial or domestic polluted effluent. Most of chemicals, notably psychotropic pharmaceuticals, are very persistent even after biotic and abiotic treatment in environment and waste water treatment plant (WWTP). Among the aquatic biodiversity, the molluscs are broadly studied for their position in the food chain (primary consumer), but also because of their low celerity. *Radix balthica* is a freshwater mollusc present in many French rivers, allowing easy sampling of this snail for eggs collection. The flatworms including *Schmidtea polychroa* belongs to benthic invertebrate are an ecotoxicological model. *Dugesiiidae* is a family broadly studied, first for their ability of regeneration and secondly for their range in the food chain (secondly consumer). The aim of this study was, to evaluate the toxicity of four psychotropic drugs from four different therapeutic classes and three chemical families. Oxazepam (anxiolytic, benzodiazepine), carbamazepin (antiepileptic, benzodiazepine), cyamemazin (neuroleptic, phenotiazin) and sertraline (antidepressant, Inhibitor Selective of Reuptake of Serotonin (ISRS)) have been selected because of their frequencies and their presence in the French WWTP effluents.

Methods and results : Pharmaceuticals toxicity was evaluated on *R. balthica* embryos using hatching success and hatching delay on a concentration range from environmental concentration to 100µg/L. For instance, these indicators showed an effect from 1µg/L to 100µg/L for oxazepam. A differential RNAseq method and a RT-qPCR validation were released on *R. balthica* exposed of oxazepam at 10µg/L and 0.815µg/L or to reconstituted water for control. Statistical analyses have shown 144 contigs differentially expressed for the 10µg/L condition. Among these differentially expressed contigs, we have highlighting several pathways impacted. Pharmaceutical impact was also evaluated on the movement, reproduction ability and regeneration integrity of *Schmidtea polychroa*. Only the movement and the reproduction results showed significant differences to the control. The RT-qPCR study is in progress for targeting the transcriptomic responses relating to the phenotypic responses.

Discussion and conclusion : These results revealed that oxazepam impact the hatching success, fecundity rate and locomotor behaviour of studied aquatic invertebrates at low concentrations. This drug impacts also expression of many genes encoding proteins involved in fundamental cellular functions as differentiation, proliferation, metabolism... Perturbation of these cellular functions could explain macroscopic effects on development, reproduction and displacement.

TOXIC AND GENOTOXIC EFFECTS OF THE RESIDUES OF ANTICANCER DRUGS IN AQUATIC ORGANISMS

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Background and objective : Residues of anticancer drugs represent new and emerging pollutants in aquatic environments. Many of these drugs interfere with genetic material and cell signalling, and it has been postulated that they can cause adverse effects in aquatic ecosystems. The aim of our studies was to explore ecotoxicological properties of four highly consumed anticancer drugs (5-fluorouracil (5-FU), cisplatin (CDDP), etoposide (ET) and imatinib mesylate (IM)) with different mechanisms of chemotherapeutic action in experimental models with aquatic organisms from different trophic levels.

Methods and results : Acute and chronic toxicity of the four drugs was determined in algae (*P. subcapitata*), cyanobacteria (*S. leopoliensis*), crustacea (*D. magna* and *C. dubia*) and zebrafish (*Danio rerio*). In crustacea and in zebrafish genotoxic potential of the four drugs was determined with the comet assay and micronucleus test. In the two generation study with zebrafish also a whole genome transcriptomic analysis of liver samples of F1 generation of fish exposed to 5-FU has been performed. In crustacea and zebrafish the four drugs showed low acute toxicity. Relatively high toxicity was observed in the reproduction assays with algae, cyanobacteria and crustacea. In crustacea all four cytostatics induced a dose dependent increase in DNA damage at concentrations lower from those that inhibited reproduction. In zebrafish, in a two-generation study, the exposure to 5-FU (0.01, 1.0 and 100 µg/L) did not affect their survival, growth and reproduction; however, histopathological changes were observed in the liver and kidney, along with genotoxic effects, at all 5-FU concentrations. Increases in DNA damage were significant in the liver and blood cells, but not in the gills and gonads. In erythrocytes, a significant, dose-dependent increase in frequency of micronuclei was observed at all 5-FU concentrations. Whole genome transcriptomic analysis of liver samples of F1 generation zebrafish revealed dose-dependent increases in the number of differentially expressed genes, including up-regulation of several DNA-damage-responsive genes (i.e. pak 2a, 2b; gadd45 ab; xrcc5) and oncogenes (i.e., jun, myca).

Discussion and conclusion : The studies demonstrated that in aquatic organisms 5-FU, CDDP and IM induced genotoxic effects at low, for environmental occurrence relevant concentrations. These findings indicate that residues of certain anticancer drugs may pose threat to aquatic organisms, and that data from chronic exposure tests focusing on specific effects (i.e. genotoxic, reprotoxic...) are needed to determine the significance of the presence of these drugs in the environment. The research leading to these results received funding from the EC's FP7/2007-2013 under grant agreement no. 265264 (Cytotoxreat).

ANALYSIS OF TOXICOLOGICAL EFFECTS OF ATENOLOL, CAFFEINE, LIDOCAINE AND OXYTETRACYCLINE ON RAPHIDOCELIS SUBCAPITATA AND CHLORELLA VULGARIS ALGAE

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Background and objective : Increased levels of pharmaceuticals and personal care products (PPCP) in hydric resources have become a global issue based on its high persistency in the water even after treatment. To understand the potential toxic impacts produced by PPCP in the environment, two algae, *Chlorella vulgaris* and *Raphidocelis subcapitata*, were evaluated. Atenolol, caffeine, lidocaine and oxytetracycline were selected from those proposed by Pharmaceutical Input and Elimination from Local Point Sources project as key substances.

Methods and results : Algae were pre-cultured in Jaworski's Medium. Synthetic wastewater was prepared as described in OECD 303. All experiments were performed in a test solution containing 90% JM: 10% synthetic wastewater (v/v). Drug toxicity effects were tested at eight different concentrations between 0.003 & 2.000 mg l⁻¹ depending on substance; and each was evaluated individually over a period of 96h, at 20°C, 50% humidity, agitated at 120rpm and light routine of 12h at 3500lux. Photosynthesis efficiency (PE) and cell concentrations was analysed after drug incubation, employing the ToxY-PAM Analyzer and Micro Counter 1100, respectively. The most significant effect was observed when *Chlorella* was exposed to atenolol. This drug increased the algae growth rate from 24h incubation onwards, with a maximum of >50% at 72h. The same was detected in *Raphidocelis* culture at a reduced intensity after 48h and only in the four most concentrated samples. Additionally, atenolol in the *Chlorella* medium stimulated PE; and inhibited around 20% PE activity in *Raphidocelis*, at 96h and 0.047 mg l⁻¹. Caffeine suppressed the PE capacity in both cultures. *Chlorella*'s growth was stimulated in lower dilutions of caffeine, but inhibited by higher concentrations. Meanwhile, *Raphidocelis* growth appeared to be repressed by caffeine. Lidocaine did not show effects on PE or on the growth of *Chlorella*. However, after 96h incubation with *Raphidocelis*, this algae suffered an inhibition around >10% on PE and >20% on growth. Oxytetracycline affected only *Chlorella* growth, which, at 48h, were stimulated at >10% levels.

Discussion and conclusion : Growth capacity was highly affected by atenolol and caffeine in both organisms. The experimental drug-induced enhancement in growth rate and photosynthesis can potentially lead to imbalances in the aquatic ecosystem, as algae overgrowth can be detrimental to other species survival. Lidocaine and oxytetracycline did not show real effect on PE or growth rate; nonetheless, there is still cellular stress responses that need to be evaluated. This study indicates specific events in the algae life cycle that can be affected by drug contaminants and at what concentrations.

COMBINATION EFFECTS OF ENDOCRINE ACTIVE PHARMACEUTICALS IN WASTEWATERS USING THE CALUX REPORTER-GENE ASSAY

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Background and objective : National and international directives (e.g. EU water framework directive) demand the evaluation of potentially harmful chemicals, including endocrine active substances, with suitable test methods to achieve a good quality of water bodies. Recently, the European Commission consulted experts to define criteria for the identification of endocrine disruptors (press release, 09/2014). Those initiatives reflect the increasing attention on endocrine active substances (EAS) in the last few years. However, most EAS in cosmetics or pharmaceuticals still are not restricted. Some pharmaceuticals are known to have a biological activity already at very low concentrations (in picomolar range), thus are of specific interest in toxicological evaluations. Such substances are widely used in cancer therapy. Because EAS cannot be completely removed during waste water treatment, these substances are released into surface water mainly through the waste water treatment plant (WWTP) effluents and have been shown to affect aquatic organisms, and potentially humans. Not only estrogens, but also androgens, gestagens, and other steroid hormones can influence the endocrine system of organisms. Some observed effects induced by EAS are masculinization of female snails, vitellogenin production in male fish, feminization of reptiles, and reproduction disturbance of birds. Compared to estrogen active compounds, the amount of androgen active substances excreted by humans is 10 to 100-fold higher and can also be released into the environment through WWTP effluents, hence should be considered in wastewater assessment. However, in contrast to estrogenic substances, there is only little known about effects of androgens in the environment. Also, studies on the interaction of different EAS in complex water samples, e.g. waste water, are still rare. Therefore, the aspect of hormonal combination effects was investigated in this project. The main objective is the investigation of additive as well as inhibitory effects of substance mixtures in different waste waters regarding endocrine activity. It is of special interest in which way estrogenic and anti-estrogenic, androgenic and anti-androgenic EAS influence each other and act in cellular systems. Effect-directed analysis with bioassays (here i.e. Calux tests) is a useful tool to determine the presence of EAS based on the measured biological effect, thereby allowing the determination of a specific endocrine activity as sum parameter. This is of particular advantage when unknown transformation products are built in a mixture or in cases when chemical analyses show compound signals which cannot be correlated to a specific toxic effect. So, bioassays are an ideal addition to chemical analyses, giving the possibility to assess the potential risk of a tested water sample.

Methods and results : In order to detect possible combination effects present in complex water samples, four pharmaceuticals with a known endocrine activity were added to wastewater samples from different WWTP influents in a defined concentration (i.e. their EC₅₀ values, determined before). The substances and their main effects were 17 β -ethinylestradiol (EE2, estrogenic), toremifene (anti-estrogenic), 17 α -methyltestosterone (MT, androgenic) and bicalutamide (anti-androgenic). The individual effect of each substance as well as the effect of mixtures in three different wastewaters (hospital and municipal) was analyzed using Calux (chemically activated luciferase gene expression) assays to determine the respective endocrine activity. The ER-Calux measures the activation of the estrogenic receptor, the AR-Calux the activation of the androgenic receptor in genetically modified human cell lines (here: U2-OS), while the anti-assays detect the inhibition of the estrogenic and androgenic receptor, respectively. Cells were exposed to the prepared samples for 24 hours in a test-specific dilution of 1:10 in medium. The results of a luminescence measurement after exposure enable the quantification of the endocrine activity in relation to a reference substance as equivalent concentrations (EQs) or relative activities (percent of the maximum response of the reference). The wastewaters are denoted as A, B and C. All original wastewater samples showed an average relative estrogenic activity of 19-29% (estradiol EQ mainly below LOQ) and an androgenic activity between 1-5% (1.5 - 6 DHT EQ ng/L). No inhibition of the estrogen receptor was detected in the wastewaters, while anti-androgenic activity was observed in samples B and C. In wastewater A the estrogenic activity was increased by 30% by adding EE2 (1.8 ng/L), and decreased by 20% when toremifene was added (5x10⁴ ng/L). The combination of EE2 and toremifene, and also of all four substances, had a decreasing effect on estrogenicity by 5%. Bicalutamide and MT in a mix (4x10⁵ ng/L, 3.9x10² ng/L) slightly increased the estrogenic response by 8%. In water B and C the same tendency was observed,

except for bicalutamide and MT which had no influence on the estrogenic effect. However, in no sample the estrogenic activity was removed completely and only in some cases, when toremifene was added, the response was below 10%. The same substance concentrations were applied in the anti-ER-Calux. In all wastewaters the addition of toremifene led to an anti-estrogenic activity up to 97% of the reference (tamoxifen) response, the combination with EE2 decreased this effect by around 40%. The androgenic activity of all wastewaters was very low. The response was modified especially by the addition of MT, the activity increased by 20-25%. This effect was only slightly weakened when bicalutamide was also applied. Bicalutamide, EE2 and toremifene as single substances barely modified the androgenic activity of the wastewaters, but had different influences in anti-androgenic testing. In wastewater A, which was originally not anti-androgenic, bicalutamide led to an androgen-receptor inhibition (49% of max. flutamide response) while with all other substances no anti-androgenic effect was observed. Likewise, in wastewater B and C bicalutamide enhanced the inhibitory effect, while MT eliminated the anti-androgenic response in both cases. EE2, toremifene and mixtures had different influences on wastewater B and C, respectively. The addition of EE2 and toremifene as single substances decreased the anti-androgenic response in wastewater B slightly, but increased the response in wastewater C. However, their combination in both cases led to a decrease of the activity almost by half. The combination of MT and bicalutamide had an elimination effect only in water C, in combination with EE2 and toremifene however, in both cases the anti-androgenicity was removed.

Discussion and conclusion : The comparison of four different endpoints in the Calux assay revealed that municipal as well as hospital wastewater covers several endocrine activities but with different intensities, which may be changed in combination with other endocrine substance. It has been already shown that there is an additive effect when several estrogenic active compounds are applied in the ER-Calux. Moreover, in 2014 Ihara et al. approved that anti-estrogenic compounds have a masking effect on estrogenic substances. The last observation was approved in the present study when a mixture of an agonist and the antagonist was applied in one assay. Independent from the wastewater matrix, estrogenic activity was decreased when the anti-estrogenic toremifene was added to the sample. In the same way, the anti-androgenic activity of bicalutamide inhibited the androgenic response of methyltestosterone, although in a lower level. These results allow the conclusion that masking effects are of significant importance in the assessment of complex water samples, especially in case when some substances are eliminated leading to a higher overall effect in the wastewater due to remaining bioactive substances, e.g. during wastewater treatment. Although mostly a similar trend of the activities after substance addition was observed, the intensity of the activation and inhibition, respectively, differs between the wastewaters. So, it is important to notice that a quantification of specific activities which are induced by EAS cannot be transferred from one wastewater to another, but need to be assessed for each sample. Furthermore, the results reveal that from the presence and concentration of a certain EAS in a mixture alone, no direct conclusions can be drawn about the overall biological effect of the sample, which emphasizes the importance of further investigations on effect-based analysis tools in addition to instrumental chemical analyses.

MIXTURES EFFECT OF ANTIBIOTICS ON SOIL MICROBIAL NITROGEN PROCESSES

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Background and objective : During the last two decades, the environmental concern regarding the antibiotics has increased considerably. The persistence of both antibiotics and antimicrobial resistances in the environment has become a major human health and research issue. However their ecotoxicological impacts on microbial ecosystem functions involving in biogeochemical processes, are still not well-understood (Roose-Amsaleg & Laverman, 2016). Furthermore, the environmental risk assessment, based on existing guidelines for other chemicals, may overlook adverse effects of antibiotics on environmental microorganisms and related functions (Boxall et al., 2012), due to the lack of representativeness of ecotoxicological tests for bacterial toxicity in guidelines for environmental risk assessment of antibiotics. In addition, while ecosystems are contaminated by mixtures of chemicals rather than individual substances, toxicity mixture effects of antibiotics on key microbial processes have been not investigated (Brandt et al., 2015). The scope of this study was to unravel the mixture toxicity of antibiotics on soil microbial nitrification and denitrification processes, to improve knowledge of suitable ecotoxicological endpoints, for risk assessment.

Methods and results : The tested antibiotics were belonged to several classes (tetracycline, sulfonamide, macrolide, fluoroquinolone) with various modes of action. The effects of each antibiotic individually and as mixtures were assessed across dose-response approaches (performed with R software, DRC package) on potential nitrification in slurry bioassay (NF EN ISO 14238) and denitrification (Roose-Amsaleg et al., 2013) of soil microbial communities. Antibiotic mixtures were built-up in order to test additivity, based on Toxic Unit (TU) ratio, in addition to other mixtures close to environmental surveys (literature data). The antibiotic exposure was verified by measuring the concentrations of antibiotics at the beginning and the end of the experience. Complementary investigations were realized on community structure parameters of specific N-cycling microbial guilds with molecular approaches, to help us in identifying key actors involving in structure – function relationships (species interaction). The first results demonstrated strong differences of magnitude effect among each antibiotic individually, in relation with the mode of action of AB and different levels of sorption on soil components, influencing their bioavailability in the nitrification bioassay. The results of the experimental approaches were analyzed regarding mathematical modeling interpretation based on the concepts of concentration addition or independent action. In many cases, independent action concept better predicted mixture toxicity of different classes of antibiotics, harboring different mode of action. The responses of structural parameters of microbial guilds were observed at higher concentration than the functional endpoints. The decreases of abundances of several microbial groups were correlated with those of the respective activities.

Discussion and conclusion : Ecotoxicological effects were recorded at very low concentrations, in our bioassays, based on model microbial communities, in optimal growth conditions. These conditions are not representative of the bulk soil conditions but can correspond to some microbial hot-spots in functional domains, in soils (i.e. rhizosphere). Whilst many previous studies have mainly focused on normalized single species bioassay, significant well-designed studies, improving the integration of ecotoxicological endpoints at the community level, should grab the existing gap on understanding and environmental assessment of mixture impact on microbial ecosystem processes. The archeal nitrifiers / bacterial nitrifiers ratio seems to be related to the magnitude of nitrification responses, only for some antibiotic and the mixture (Konopka et al., 2015). These results can be explained by the lower sensitivity of Archaeae to certain antibiotics, notably those targeting the bacterial cell-wall components.

INNOVATION IN EFFECT MONITORING OF PHARMACEUTICALS – IN VITRO, IN VIVO, IN ENVIRONMENT

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Background and objective : Residues of pharmaceuticals occur in raising concentrations in the environment, e.g. due to incomplete elimination by wastewater treatment. Currently, the environmental monitoring of pharmaceuticals still focusses on the analytical detection of single substances. Very limited exceptions are published, for example, effect monitoring of synthetic steroids in UK river fish species or investigations of mollusc populations at European coastlines. The established chemical substance monitoring seems to be incapable to measure all groups of pharmaceuticals with the same mode of action simultaneously – because of technical or monetary reasons. In this context new approaches of biological effect monitoring are under discussion and investigation. The aim of the German research project “EffPharm” (2013-2016) was to develop and validate a mode of action based in vitro cell assay that indicates any interaction of compounds with a specific receptor target, and thus, can be used as a prognostic tool for possible effects in wildlife organisms.

Methods and results : Diclofenac and metoprolol were used as model substances because of their environmental and regulatory relevance. Both pharmaceuticals have specific modes of action - non-steroidal anti-inflammatory drug acting via cyclooxygenase inhibition and beta blocking on adreno receptors respectively. As new effect monitoring tools, mode of action based in vitro cell assays were developed for both pharmaceutical groups. Biosensor cell lines were constructed to be sensitive to pharmaceutical target interaction and able to generate a signal which can be quantified, for instance, by fluorescence. In laboratory and field experiments, aquatic invertebrates and fish were exposed to single substances, mixtures, and wastewater. Furthermore, aquatic communities were investigated in mesocosms under semi-field conditions. In these exposure experiments, the effect endpoints, e.g. behaviour or reproduction, are characterised by a specific population relevance and therefore are of regulatory relevance. Additionally, biomarker studies were conducted with aquatic invertebrates and fish. These test results were used to justify the suitability of the mode of action based in vitro assays to conclude on effects relevant for populations of wildlife species.

Discussion and conclusion : In addition to the development of the mode of action based in vitro assays, results for the eco-toxicological investigations in laboratory as well as in mesocosms and wastewater treatment bypass systems will be presented and discussed here. Furthermore, the complex of regulatory problems and background leading to the “EffPharm” project will be explained. Finally, the possibility of future applications of such cell based assays in different regulatory contexts (e.g. pharmaceuticals, plant protection products) as well as in environmental science will be addressed.

NEW RAPID CELL-BASED ASSAYS FOR β -BLOCKER AND NONSTEROIDAL ANTI-INFLAMMATORY DRUG DETERMINATION IN WASTEWATER EFFLUENTS

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Background and objective : Pharmaceutically active compounds like β -blockers and nonsteroidal anti-inflammatory drugs (NSAIDs) are two important groups of emerging environmental contaminants. The use of these pharmaceutical compounds is supposed to rise dramatically in the future due to demographic changes. A number of studies revealed the abundance of these compounds in the environment as their removal from wastewater remains frequently inefficient. Consequently, a biologically active mixture of pharmaceuticals as well as their mostly unknown metabolites and transformation products is regularly discharged into surface waters. This biologically active mixture of pharmaceuticals could potentially reach groundwater, and may influence even drinking water. Environmental monitoring of β -blockers and NSAIDs is still based upon target analysis. However, such kind of monitoring is incapable of encompassing all groups of biologically active compounds that exhibit the same mode of action (MOA). In the frame of the German research project “EffPharm” (2013-2016) we developed two recombinant sensor cell lines which express MOA-based sensing and reporting units. These allow for live-cell visualization of β -blocker and NSAID activities by measuring immediate fluorescence signal changes. The objective was to develop rapid in vitro tests to monitor all active compounds and metabolites with the same mode of action at once. In the long run, these MOA-based tests are envisaged to be implemented in environmental monitoring programmes e.g. in the context with the characterization of the efficiency of new wastewater treatment purification technologies.

Methods and results : For the sensitive and specific measurement of β -blocker activities a fluorescence resonance energy transfer (FRET)-based cell line was developed to monitor β -adrenoceptor binding by providing an optical signal for receptor inhibition. The primary action of β -blockers could thus be measured in the concentration range of the lowest observed metoprolol effect concentrations (LOECs) reported for the most sensitive species (10-100 nmol/L). A cell line expressing a genetically encoded fluorescent redox sensor was developed to monitor inhibition of cyclooxygenase and thus NSAID activity. Due to the high test sensitivity this NSAID activity could be measured directly in the effluent of wastewater treatment plants. β -blocker and NSAID activities measured in wastewater treatment plant effluents were compared to the concentration of metoprolol and diclofenac, determined by standard MS analysis.

Discussion and conclusion : In this work we have developed novel β -blocker and NSAID live cell imaging assays for MOA-based evaluation of water quality. So far, MOA-based reporter gene assays have been most abundantly used for the evaluation of water quality and the effectiveness of water treatment. Such reporter gene assays generate a signal at the downstream end of a long signal transduction pathway allowing side effects by interfering substances to potentially occur at every step along this cascade. In contrast to reporter gene assays, which respond to an analyte only after 12-48 hours, our live cell imaging technology based on genetically encoded fluorescent biosensors leads to immediate fluorescence signal changes in a time range of just seconds. Adapting such methods to construct cell lines that generate immediate fluorescent signals following a pharmaceutical-target interaction should therefore substantially advance MOA-directed analysis of biological activities due to pharmaceuticals in environmental samples.

A NOVEL APPROACH FOR DETERMINING THE UPTAKE OF PHARMACEUTICALS AND PERSONAL CARE PRODUCTS FROM SEDIMENT AT THE LANDSCAPE SCALE

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Background and objective : The degree of chemical uptake into aquatic organisms is highly dependent on species traits, physico-chemical properties of the test compound and characteristics of the test environment. It has been estimated that between 85 and 95 % of pharmaceuticals are ionisable and therefore one environmental factor which is likely to be important in determining uptake of these substances is pH. The pH of water bodies and sediments in the natural environment can vary significantly, with pH values in surface waters ranging from 2.2-9.8 and in sediments ranging from 3.4-7.6. It is therefore possible that the uptake (and effects) of these substances could vary significantly across the landscape. A few studies have documented how the bioaccumulation and toxicity of ionisable chemicals in the water column varies is effected by the pH of the test media. However, the sediment compartment has received much less attention even though a number of pharmaceuticals and personal care products are known to have high affinity for aquatic sediments.

Methods and results : In this work we present and evaluate an approach for estimating the uptake of two ionisable pharmaceuticals (diclofenac and fluoxetine) and one ionisable personal care product (triclosan) in water-sediment systems using data from batch sorption studies and water-only uptake exposures under a range of pH conditions. Using a combination of sediment sorption coefficients (K_d), water concentrations and predicted kinetic rates at pH 7.67 it was possible to model the internal concentration of the selected test compounds in the oligochaete *Lumbriculus variegatus* exposed to spiked natural sediment. Comparison of the model predictions with measured concentrations of the study compounds in *L. variegatus* exposed to sediment showed that for diclofenac and fluoxetine there was remarkably good agreement between model predictions and measured data. For triclosan, however, modelled predictions over time were within a factor of five of the measurements. These differences are partially explained by the fact sediment ingestion is known to play a role in the uptake of triclosan while this is not an important uptake route for the diclofenac and fluoxetine.

Discussion and conclusion : Our results indicate that by using data from water only uptake studies, sorption studies and physico-chemical properties, it is possible to predict ionisable chemical uptake into *L. variegatus* in natural sediment systems. The approach therefore offers the potential for estimating the uptake and effects of ionisable pharmaceuticals across a wide spatial scale, accounting for differences in pH and sediment type observed across the landscape. The approach could therefore be invaluable for better characterising the potential risks of pharmaceuticals and other ionisable compounds to aquatic systems. Our future work will focus on evaluating the approach using a wider range of test substances and sediment types. This work has received support from the EU/EFPIA Innovative Medicines Initiative Joint Undertaking (iPiE grant n° 115735)

NEXT-GENERATION SEQUENCING TO HIGHLIGHT COMMUNITY CHANGES IN RIVER BIOFILMS LINKED TO PHARMACEUTICAL LOADS FROM A WASTEWATER TREATMENT PLANT

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Background and objective : Hospital wastewaters (HWW) contain wider spectrum and higher concentrations of pharmaceuticals than urban wastewaters (UWW). Despite this, they are usually discharged in sewers without pre-treatment and co-treated with the UWW. Traditional wastewater treatment plants (WWTP) are not designed to remove specific hospital contaminants. Hence urban and hospital treated effluents may still contain residues of pharmaceuticals, as well as their metabolites, which are then released into the recipient aquatic environment and might cause environmental risk. Therefore, a better understanding of the effect of pharmaceuticals in the environment is required. Biofilms are effective “biological sensors” for assessing the environmental effects of pharmaceuticals due to their ability to respond rapidly to physical, chemical and biological fluctuations by changes in their structure and composition. Molecular fingerprinting approaches gave interesting although rough insights in microbial community composition changes linked to pharmaceuticals. The use of Next-Generation Sequencing (NGS) allows us to improve our knowledge by giving a deeper and more accurate characterization of changes in the community structure. Furthermore, due to NGS we are able to identify species and associate them to functional patterns. This is helpful to describe specific adaptations of bacterial and algal communities to the presence of pharmaceuticals and thereby disentangle multiple stress effects in environmental studies.

Methods and results : The study site and sampling protocol have been previously described in Chonova et al.(2016). Briefly, environmental biofilms were colonized (6 times from Feb to July 2014) at the output of a wastewater treatment plant 1/ in separately treated effluents from urban and hospital wastewaters and 2/ in the recipient river (River Arve, French Alps) up- and downstream from the WWTP output. A joined monitoring of environmental abiotic factors (SIPIBEL observatory) and pharmaceuticals was performed. Integrated exposure of 14 pharmaceutical compounds belonging to 7 therapeutic classes was studied through chemical catchers (POCIS). NGS, using PGM Ion Torrent technology, was used for in-depth characterization of the composition of diatoms and bacterial communities in the biofilms through a metabarcoding approach based on rbcL and 16S rRNA amplicons, respectively. The sequencing data was analyzed with the MOTHUR software. Multivariate statistical analyses were performed to disentangle links between NGS-inventories, abiotic environmental factors and pharmaceutical loads.

Discussion and conclusion : As expected, the hospital treated effluents (HTE) exhibited higher overall loads of pharmaceuticals than the urban treated effluents (UTE). However certain therapeutic classes of pharmaceuticals were found in higher concentrations in the UTE (e.g. NSAIDs). The treated effluents exhibited much higher concentrations of all measured pharmaceuticals than the recipient river. Concentrations of pharmaceuticals in the river were higher downstream the WWTP output. NGS sequencing showed strong differences between microbial biofilm communities developed in UTE and HTE. Differences in the river between communities up- and downstream from the WWTP discharge were visible as well. Biofilm communities differed in their diversity and composition not only according to the sampling site, but also to the sampling season. However local similarities were stronger than seasonal ones, revealing the strong impact of the wastewater effluents in shaping the environmental communities. Finally, the multivariate analysis found significant co-structure between community composition assessed through NGS and environmental conditions (including pharmaceutical compounds, nutrients, meteorological data, etc.) which suggested that communities were strongly influenced by the pollution caused by pharmaceuticals. However, other environmental factors also had effect on the microorganisms leading to clear seasonal gradient in the community composition. Our results clearly showed that assessment of community composition using NGS, coupled to integrated measurement of pharmaceuticals, is efficient to highlight in the environment the disturbing effect of pharmaceuticals on natural microbial communities.

PRIORITISATION OF VETERINARY PHARMACEUTICALS PRIOR TO A MONITORING CAMPAIGN: CASE OF BRITTANY, AN INTENSIVE HUSBANDRY AREA

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Background and objective : Pharmaceuticals residues are nowadays of growing concern, especially in aquatic environments. Numerous researches have been conducted on human pharmaceutical residues, while occurrence of veterinary pharmaceutical residues remains still largely unknown. Brittany is a region subjected to high animal husbandry pressures. Veterinary pharmaceuticals residues can enter the environment directly or indirectly during pastures or spreading of animal manure on soils. Thus, aquatic environments in Brittany are sensitive spots, potentially contaminated by a lot of veterinary residues. The project aims at realizing an overview of the contamination of raw water resources subjected to a strong agricultural pressure, and into drinking water obtained from those resources. The first step was to perform a prioritization of veterinary pharmaceuticals, to select the most susceptible to reach and to be detected in the aquatic environment.

Methods and results : A preliminary list composed of 73 veterinary pharmaceuticals was used. Four criteria were considered to realize the prioritization. First, the potential to reach the environment which was decomposed into the animal target (aquaculture or livestock or pets), the administration route (topical application versus other routes), the usage and veterinary practices in Brittany and the behavior during storage of manure and slurry prior to spreading into land. Secondly, the metabolism was investigated. According to the metabolic rates, it was decided to consider the parent compound or the main metabolite or both. The third element to be investigated was the fate in the environment and ability of the molecules to run off or leach from soil to water, and their behavior (biodegradation, photolysis, sorption to sediments...) once in water resources. The last criterion corresponds to the analytical feasibility. This prioritization allows a classification in groups of different levels of criticity toward the contamination of water resources.

Discussion and conclusion : Prioritization was a complex task when concerning veterinary pharmaceuticals, indeed there is a lack of information concerning the environmental fate of the veterinary pharmaceuticals residues. Some antibiotic families were extensively studied, but information is still scarce for antiparasitic drugs or anticoccidians for example, while those veterinary medicines are widely used in intensive livestock areas like Brittany. Then, this work highlights the fact that the choice of veterinary molecules regarding their potential to reach the aquatic environment has to be performed at a regional or local scale. Indeed, the usage of veterinary medicines can differ deeply between regions and even between veterinarians.

HEALTH RISK ASSESSMENT OF PHARMACEUTICALS IN DRINKING WATER IN FRANCE

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Background and objective : Since 2006, the French Agency for food, environmental and occupational health and safety (ANSES) works on health risk assessment linked to exposure by drinking water to pharmaceuticals. To do that, ANSES developed a prioritization strategy, to identify the most relevant human and veterinary medicines to study in French drinking water. On this basis, ANSES run a national campaign of analyses for determining the occurrence of pharmaceuticals in drinking water. Among the 44 molecules investigated, 18 were detected at least once, 5 of which being detected below the quantification limits. A new and specific method for health risk assessment for pharmaceuticals in drinking water was developed and applied to 6 pharmaceuticals found in drinking water: carbamazepine, ibuprofen and ketoprofen (human medicines) and danofloxacin, florfenicol and tylosin (veterinary drugs).

Methods and results : The method consists of eight steps: 1- physical and chemical characterization; 2- relevant metabolites; 3- relevant transformation products; 4- exposure assessment through drinking water; 5- characterization of biological effects; 6- selection of one human toxicity reference value among existing values when available, otherwise using published toxicological data from MAA or scientific publications. In situations with a lack of data, the minimum therapeutic dose (MTD) could be used adding a supplementary uncertainty factor of 10, or in the worst condition, the threshold of toxicological concern (TTC); 7- determination of a drinking water guideline limit value; 8- health risk assessment for the parent substance, its active metabolites and/or its transformation products when data are available. The health risk assessment for carbamazepine and 10-11-epoxycarbamazepine, danofloxacin, tylosin, florfenicol, ibuprofen and ketoprofen, at levels found in French drinking water, reveal a negligible risk. For metabolites or transformation products of danofloxacin and of ibuprofen, the health risk assessment could not be achieved due to lack of suitable data.

Discussion and conclusion : For both human medicines and veterinary drugs, adverse effects are known at therapeutic doses. Despite the preclinical toxicological studies reported in the MAA dossiers, it remains difficult to find data for establishing a robust human chronic toxicity value, because such data are either confidential or inexistant. The use of therapeutic doses is impossible for veterinary drugs and for metabolites and/or transformation products. As for human medicines, the minimum therapeutic dose might not be efficient to protect specific population such as children, so that an additional uncertainty factor is necessary. The conference will present the method, results obtained and strengths and weaknesses of the proposed method.

ASSESSING EFFECTS OF PHARMACEUTICALS ON AQUATIC ECOSYSTEMS

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Background and objective : Nowadays pharmaceuticals are measured frequently in surface water monitoring schemes. These substances reach surface waters via waste water treatment plants (WWTP) and are distributed throughout the receiving water system, usually in low concentration. The effect these substances at field relevant concentrations might have on the aquatic environment, however, is largely not known. To study the potential effects of pharmaceuticals, several laboratory experiments were conducted varying from single species behavioural assays to indoor microcosm (using a small ecosystem) testing.

Methods and results : To study potential effects of pharmaceuticals originating from a WWTP, effluent was collected and stored cold and dark. In the laboratory, 16 microcosms were installed containing a small aquatic community comprising *Elodea* sp. (plant), *Lumbriculus variegatus* (worm), *Physella* sp. (snail), *Asellus aquaticus* (crustacean) and *Daphnia magna* (cladoceran). Four microcosms were filled with 12L effluent, four with 12L control water and four were filled with 12L control water and received a spike of a mix of selected pharmaceuticals. The mix comprised metformin, guanylurea, metoprolol, sotalol, atenolol, irbersartan, hydrochlorothiazide, diclofenac and carbamazepine. In addition, single species behavioural assays were conducted using the Multispecies Freshwater Biomonitor (MFB) exposing *Gammarus pulex* (crustacean) to fluoxetine, ibuprofen, carbamazepine and CTAB. Exposure to fluoxetine and ibuprofen in the 10-100 ng/L range showed a significant decrease in *Gammarus* activity compared to controls. Interestingly, this response disappeared at higher test concentrations (1 ug/L – 1mg/L). This pattern was also observed for carbamazepine, although not statistically significant. CTAB evoked an overall decrease in *Gammarus* activity at increasing concentrations. In both the effluent and the mix spiked microcosms, however, no negative impact on test organism populations could be detected. In the microcosms receiving effluent the populations had actually increased most.

Discussion and conclusion : Apparently, the exposure in the microcosms to pharmaceuticals at effluent relevant concentrations did not cause effects on the survival on the tested populations of *Lumbriculus*, *Physella*, *Asellus* and *Daphnia*. The excess of nutrients from the effluent seems actually to favour the populations, resulting in increases in abundance compared to the control. In contrast, MFB assays with *Gammarus*, another macrocrustacean, showed that at low concentrations effects on behaviour could be detected which again disappeared at higher test concentrations. These results indicate that pharmaceuticals do not seem to follow the standard (eco)toxicological rules where an increase on concentration shows and increase in effects but have much more subtle effect windows. In addition, pharmaceuticals evoke effects on other endpoints than the traditional survival endpoint. Although more subtle, an impact on behavioural parameters can be just as detrimental to populations as toxicity. Such effects, however, will be difficult to observe in model ecosystems where only a part of the aquatic community is present. To have a proper understanding of the impact of pharmaceuticals on a system, model ecosystems should at least comprise processes as competition and predation in order to be able to translate behavioural effects to actual impacts on populations.

OCCURRENCE OF PHARMACEUTICALS IN HOSPITAL WASTEWATERS AND ASSESSMENT OF THEIR ASSOCIATED ENVIRONMENTAL RISK AND HAZARD: A SPANISH CASE STUDY.

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Background and objective : Hospital wastewaters (HWWs) are a relevant source of pharmaceuticals (and their metabolites and transformation products) in the water cycle. However, to date, there are still important knowledge gaps with respect to the occurrence of various therapeutic groups in HWWs, the relative contribution of HWWs to the total load of pharmaceuticals entering wastewater treatment plants (WWTPs), and the associated environmental risks and hazard. In this context, the main objective of the present work was to shed some light into these questions by monitoring various classes of PhACs in HWWs in Spain.

Methods and results : Analysis of the target compounds in the HWWs (collected daily from the hospital) was carried out with a method based on solid phase extraction (SPE) and liquid chromatography-tandem mass spectrometry (LC-MS/MS). Assessment of the risk was based on measured environmental concentrations (MECs) and Predicted No Effect Concentrations (PNECs). Environmental hazard was assessed by calculating the persistence, bioaccumulation and toxicity (PBT) Index for each target compound. The results of the study showed the presence of most of the compounds investigated at concentrations varying between a few ng/L and 2 mg/L. The highest concentrations corresponded to the iodinated contrast media (ICM) iomeprol, the analgesic and anti-inflammatory (AAF) acetaminophen, the diuretic furosemide, and the antibiotics (ABs) ofloxacin and trimethoprim. The highest hazard quotients (HQs), above 10, in the HWW samples were obtained for the AAFs acetaminophen, diclofenac, ibuprofen and naproxen, the ABIs clarithromycin and ofloxacin, and the β -blocker propranolol, although only ibuprofen would show a HQ > 1 after considering dilution and degradation processes. The environmental hazard assessment yielded maximum PBT index values of 9 for the AAFs diclofenac and ibuprofen and the ABI clarithromycin.

Discussion and conclusion : Some pharmaceuticals found to be present in HWWs show potential to cause negative effects on aquatic organisms in the receiving water bodies and should be subject to control and attenuation measures. The approach presented can be useful to prioritize compounds and adopt appropriate mitigation policies. Acknowledgements The authors would like to thank the financial support provided by the Spanish Ministry for Economy and Competitiveness through the Carlos III Health Institute with the program "Projects on Health Research 2014-2016 FIS (PI14/00516)", the European Commission through the project SOLUTIONS (contract 603437), and the Generalitat de Catalunya (Consolidated Research Groups "2014SGR418-Water and Soil Quality Unit" and 2014SGR291-ICRA65). General Management of Hospital is acknowledged for allow the authors to take the samples. Merck is acknowledged for the gift of LC columns.

CRITICAL EVALUATION OF DIFFERENT INPUTS FOR THE ESTIMATION OF PHARMACEUTICALS EXPOSURE SEEKING AN IMPROVED ENVIRONMENTAL RISK ASSESSMENT

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Background and objective : The presence of human pharmaceutical in the environment has raised a worldwide concern. Due to their increased consumption and their pharmacokinetic properties, they can enter into aquatic systems mainly through wastewater treatment plants (WWTPs). Based on this knowledge, the European Medicines Agency (EMA) guideline on environmental risk assessment (ERA) of medicinal products for human use came into force in 2006, predicting the possible environmental impact of new marketing authorisations. Therefore, the aim of the present work was to discuss an approach for the estimation of the predicted environmental concentrations (PECs), based on EMA guideline, concerning the most consumed pharmaceuticals in Portugal. Our goals were also to evaluate uncertainties in PEC calculations, comparison with measured environmental concentrations (MECs) and the predicted no effect concentrations (PNECs), adopting the best suited model and suggesting solutions to strengthen the European Union legislation.

Methods and results : An ERA for 16 of the most consumed pharmaceuticals in Portugal, embracing 5 therapeutic groups, was developed for wastewater effluents (WWE). The PECs were based on the EMA guideline on risk assessment, followed by the incorporation of several refinements (consumption data; population; percentage of excretion; WWTPs removal efficiencies; amount of wastewater per inhabitant per day (WASTEW_{inh})), resulting in five different equations. The best approach was selected by inverse modelling, comparing these results with WWE MECs collected from 6 recent Portuguese studies. Finally, the risk assessment was performed comparing the PECs and MECs, with the PNECs. The results showed that, concerning the penetration factor (F_{pen}), the default value used by EMA (0.01), was surpassed by 9 pharmaceuticals. National consumption and excretion data (equation 3) were the two most critical parameters for PEC calculations, providing close agreement with MECs for the majority of the pharmaceuticals (Fig.1). The risk quotient (RQ) between PECs and PNECs was higher than 1 for 7 pharmaceuticals (similar results were obtained replacing the PECs with MECs) (Fig.2).

Discussion and conclusion : Observing the data on the above mentioned refinements and the precautionary principle, suggestions were made for improvement on the ERA for new marketing authorizations, changing the default value of the F_{pen} to 0.04 and the WASTEW_{inh} to 133L. Moreover, this evaluation should be performed for pharmaceuticals already available in the market using this consumption and excretion based prediction model. Comparing our PECs with PNECs, a RQ were found up to 231, posing all three trophic levels at risk. Additionally, ERA should incorporate the risk-benefit analysis, a key step for the risk management.

HUMAN AND ECOTOXICOLOGICAL POTENTIAL IMPACT OF PHARMACEUTICAL AND PERSONAL CARE PRODUCTS FROM USETOX™ LIFE CYCLE IMPACT ASSESSMENT CHARACTERIZATION FACTORS

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Background and objective : In the last years pharmaceutical and personal care products (PPCPs) have been found at different level of concentrations in all environmental compartment (air, water and soil), and many of their impacts are still unknown or they are under analysis. One tool used for estimated the potential impact of PPCPs in the environment is the life cycle assessment (LCA). PPCPs are being increasingly included in LCA studies since they have evidenced ecological and human adverse effects and due to their presence in different environmental compartments, wastewater facilities and industry. In environmental LCAs, characterization factors (CFs) -alternatively referred to as equivalency factors- are used to determine the relative importance of a substance to toxicity related impact categories, such as human toxicity and freshwater ecotoxicity (Huijbregts et al. 2005a). In this sense, the USEtox™ model is a powerful tool endorsed by the UNEP/SETAC Life Cycle Initiative to calculate CFs which can be used in life cycle impact assessment (LCIA) and comparative risk assessment (Huijbregts et al., 2010a). Despite the large number of substances which have been included in the USEtox™ database (more than 3000 in the USEtox™ organic database 1.01) a small amount of PPCPs have been considered (approximately less than 2% of the organic database corresponds to this group of compounds). Accordingly, the CFs of many PPCPs have not yet been calculated. The main goal of this work is presenting the CFs estimates, using the USEtox™ model, of 27 worldwide used PPCPs, to incorporate these values in LCIA studies or to generate impact score rankings.

Methods and results : In this work, an impact score ranking is proposed for 49 PPCPs using the new CFs calculated, the CFs already available and also the data of PPCPs occurrence in the environment in Spain from a previous study (Ortiz et al., 2013). PPCPs from 14 different therapeutic classes have been considered in this study: analgesic/antipyretic, Angiotensin converting enzyme inhibitor, angiotensin receptor blockers, antibiotics, antidepressants, antiepileptics, anxiolytics, blood lipid regulators, cytostatics/cancer therapeutic, H2 blocker, hormones, Platelet inhibitor, non-steroidal anti-inflammatory drugs (NSAIDs)/antirreumatics, X-ray contrast media and PCPs. Physicochemical properties, degradation rates, bioaccumulation, ecotoxicity and human health effects were collected from experimental data, recognized databases or estimated by EPI Suite™. The input parameters required by USEtox™ program were: molecular weight (MW), partition coefficient between octanol and water (Kow), partition coefficient between organic carbon and water (Koc), Henry law coefficient at 25°C (KH), vapor pressure at 25°C (Pvap), solubility at 25°C (Sol), degradation rate in air (KdegA), degradation rate in water (KdegW), bioaccumulation factor of the chemical (BAF), water ecotoxicity (chronic and acute) and human carcinogenic and non-carcinogenic effects. Emission of PPCPs to continental freshwater compartment showed the highest CFs for human effects (ranging from 1E-9 to 1E-3 cases kgemitted-1), following by emissions to air (1E-9 to 1E-5 cases kgemitted-1), soil (1E-11 to 1E-5 cases kgemitted-1) and sea water (1E-12 to 1E-4 cases kgemitted-1). CFs of the affectation of freshwater aquatic environments were the highest from emission to continental freshwater (from 1 to 1E4 PAF m3 d kg-1) due to the direct contact between the source of emission and the compartment affected, followed by soil (from 1E-1 to 1E4 PAF m3 d kg-1), air (from 1E-2 to 1E4 PAF m3 d kg-1) and the lowest were continental sea water CFs (from 1E-28 to 1E-3 PAF m3 d kg-1)

Discussion and conclusion : PPCPs with the highest impact scores are hormones, antidepressants, fragrances, antibiotics, angiotensin receptor blockers and blood lipid regulators, which have been already found in other ranking scores. In this study most antibiotics are located in the top 20 of the ecotoxicity impact score, and for human toxicity impact score, azithromycin and levofloxacin are in the top 10. Although it is not surprising that some of the compounds studied in this research occupy the top ranking (by previous researches) even their CFs was not known. The estimation of new CFs should be continued, either for compounds that are already marketed as for the new ones. These results, not available until now, are useful to perform better LCIA's incorporating these pollutants in such studies or for assessing single hazard/risk environmental impact assessments.

PEOPLE THINKING ABOUT PHARMACEUTICAL RISK – HEALTH AND ENVIRONMENT: EVIDENCE FROM NOPILLS

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Background and objective :As part of the INTERREG IVb Programme noPILLS the team at GCU undertook investigation of perceptions and behaviours associated with pharmaceutical use and disposal amongst the Scottish public. The aim of this work was to explore individuals’ attitudes to ‘medicine’ use; perceptions of ‘risk’; relationships with prescribers and the decision factors that came into play when disposing of unwanted medicines as well as the environmental-awareness impacts they displayed.

Methods and results : The paper explores the findings of these qualitative investigations in 5 Scottish communities where individuals demonstrated complex relationships with medicinal use influenced by a range of factors: cultural, familial, professional advice as well as by independent information searching. There is evidence of considerable variability in the associated practices resulting from these, often conditioned by the perceived ‘trustworthiness’ of key agents in the system and frustrations at confused or confusing messages about risk (side effects etc). Belief systems played an important role in determining use but also in disposal, where individuals had an acute sense of ‘safety’ with respect to both appropriate use and of (possible) human health or environmental impacts. However, there is evidence that in many cases this behaviour was misconceived or at least contrary to recommended practice. The information associated with this, in itself, was often seen to be vague or not easily accessible.

Discussion and conclusion : The paper concludes that where policy makers wish to intervene in this area their responses need to be nuanced whether they are addressing use (prescription etc) or (appropriate) disposal. Because, whilst many people appeared to demonstrate risk aversion, others showed a degree of ambivalence with respect to both use and disposal risks. Whilst there is considerable good intention evidenced in the research by members of the public there is also confusion in the minds of many as to what is ‘best practice’ in avoiding risky behaviours.

INVESTIGATION OF THE IMPLICATIONS FOR IRELAND OF EMERGING STANDARDS ON PHARMACEUTICALS IN RECEIVING WATERS

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Background and objective : Water is essential for all human activities. Drinking and food preparation, support of the natural environment and a growing economy all require a healthy and secure water supply. Unfortunately there are significant pressures on this fragile resource. To ensure both the preservation of healthy waters and the restoration of unhealthy waters, it is critical that such harmful pressures be addressed by researchers. The aim of this project was thus to provide a baseline study investigating pollution of Irish waters with three potential hazardous pharmaceutical compounds, diclofenac, 17-beta-estradiol, and 17-alpha-ethynylestradiol. Pharmaceutically active chemicals PhACs (PhACs) include the active ingredients in pharmaceuticals and their metabolites/transformation products. These pollutants most commonly enter waterways via human use of medications, followed by excretion and incomplete removal at municipal wastewater treatment plants (WWTPs). There is increasing concern about the continuous release of PhACs into the aquatic environment. The Water Framework Directive (WFD) -the main piece of European legislation for protecting and improving water quality -has thus put forward new legislation. In accordance with Article 8b of Directive 2013/39/EU, diclofenac (an anti-inflammatory drug), 17-beta-estradiol (E2) and 17-alpha-ethynylestradiol (EE2) (a natural and synthetic estrogenic hormone) have been added to a so called “watch list.” These three PhACs will receive Union-wide monitoring, which will determine whether or not they are added to the priority substances listed by the WFD. Thus the objective of this one-year desk study was to investigate the implications for Ireland of the addition of these three PhACs to the priority substances list.

Methods and results : First, a comprehensive systematic literature review was conducted to evaluate the current state of European knowledge on these three PhACs. After screening 3,952 potentially relevant articles, an EU-wide database of 1,268 publications on diclofenac, E2 and EE2 was created. A bibliographic analysis (an analysis of published research) of the publications in the database revealed that water-related research on these compounds has increased steadily from 1995-2015. The literature review found that European surface water concentrations of diclofenac are typically below the proposed environmental quality standard (EQS) of 100 ng/l, but that exceedances occasionally occur. E2 and EE2 surface water concentrations are typically below 50 ng/l and 10 ng/l respectively, but these values greatly exceed the proposed EQSs for these compounds (0.04 and 0.035 ng/l respectively). Most importantly, while current laboratory-based analytical chemistry methods are sufficiently sensitive for the detection and quantification of diclofenac, these methodologies require increased sensitivity for E2 and EE2. Next, in order to evaluate the mobility of these PhACs in an Irish context, existing Irish data were collated and used to map monitoring locations, frequency, and where possible, concentrations of the compounds that exceed proposed EQSs. This novel mapping exercise revealed that based on the data extracted from the literature and mapped in this report, it appears that the majority of Irish surface waters may not exceed WFD-proposed EQS values for diclofenac, E2 and EE2, but that point sources of pollution could lead to occasional hotspots that exceed these limits. These predictions, however, are based upon the use of very limited data and are especially uncertain for the estrogens because of the problems associated with detecting such low concentrations of these compounds. In alignment with national standards, this project also created the first Irish-specific, semi-quantitative risk assessment model for identifying WWTPs that pose high environmental risks related to these PhACs. A case study was carried out to evaluate this model and potential improvements suggested. Future developments of the model could allow further benchmarking with national and European risk assessment standards. Finally an easily digestible toolkit was created for the implementation of control measures at WWTPs in regards to the PhACs of interest. This work concluded that diclofenac is resistant to conventional wastewater treatment, while E2 and EE2 have high removal rates via biodegradation or sorption to organic matter. The effectiveness of advanced treatments was discussed; however the most recent literature indicates that the environmental benefits of these treatments may not outweigh costs.

Discussion and conclusion : Overall this report provides an understanding of the state of research on diclofenac, E2 and EE2 in aquatic matrices in Europe and nationally. It demonstrates that more Irish monitoring data on

PhACs are needed, and stresses the importance of preventing the contamination of waterways with this harmful class of emerging pollutants. Acknowledgement to Environmental Protection Agency Ireland for funding this important research.

INVESTIGATION OF THE EFFECT OF ENVIRONMENTAL PARAMETERS ON THE TOXICITY OF TREATED DOMESTIC WASTEWATER.

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Background and objective : One of the major challenges facing the wastewater treatment industry is the prevalence of micro-pollutants. These organic substances occur in the range of a few ng or $\mu\text{g/l}$ and include pharmaceutically active compounds. While some micro-pollutants are effectively removed by traditional wastewater treatment plants (WWTP) many are not biodegraded or adsorbed onto sludge enabling them to pass unchanged into the rivers and other watercourses with the treated wastewater. Contamination of surface and ground waters by these compounds has been recognised in a number of countries as of environmental concern. Ecotoxicological assessment of this type of pollution has the major advantage over the traditional chemical methods by considering the effect of all biological active pollutants in the effluent and additionally allowing for modification of the impact by other constituents.

Methods and results : The influence weather and standard UK wastewater processes (trickling filter and activated sludge treatment) on the environmental risk potential of treated domestic wastewater were evaluated using a bank of test organisms. The toxicity of river water before and after modification by effluent together with the discharge from the WWTP were assessed using inhibition of *Aliivibrio fischeri* bioluminescence; reduction of the effective quantum yield of photosystem II of *Raphidocelis subcapitata* and immobilisation of *Daphnia magna*. Samples were collected over two four-day periods at both WWTP representing dry and wet weather sampling conditions and comprised grab samples (river); 24 hour composite and 2 hourly samples (WWTP). Chemical characterisation of the samples was also carried out using standard parameters (pH, COD, BOD, etc).

Discussion and conclusion : During periods of low rainfall toxicity peaked during the late afternoon and was lowest overnight. As expected rainwater dilution of the WWTP influent reduced the toxic response demonstrated by all three test organisms at all sampling locations however this reduction was not consistent. There was no association between standard chemical parameters (such as COD; BOD; nitrate levels) and toxicity measured using the three organisms, suggesting that other agents are responsible for the inhibitory responses observed. Micro-pollutants identified in the samples are being considered as potentially responsible for this toxicity.

DEVELOPMENT OF A REPRODUCTIVE TOXICITY BIOMARKER BY FLOW CYTOMETRY. APPLICATION TO THE DETECTION SENSITIVITY OF WINDOWS DURING CHEMICAL CONTAMINATION UNDER CONTROLLED CONDITIONS.

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Background and objective : Chemical contamination of the environment is a recognized phenomenon. Some of contaminants have the capacity to interact with the reproductive process and it seems important to develop biomarkers target on this physiological function. The first objective was to develop a new tool to characterize the degree of sexual maturity of a freshwater bivalve; the zebra mussel (*Dreissena polymorpha*) based on analysis of DNA content of germ cells by cell cycle using flow cytometry and called Index of Sexual Maturity (IMS). This technique would faster analysis than histology which is classically used for reproduction study. The second objective of this study was to apply this tool to determinate if there is during spermatogenesis a period where cells would be more sensitive to a contaminant, carbamazepine. Two concentrations were selected: 0.5 and 50 µg/L. Three key periods of spermatogenesis were identified to realize exposure: resting – meiosis – maturity stage.

Methods and results : For the first part of this work, gonads of mussels were dissected and separated in two parts. The first part is allowed for histological analysis (with conventional protocol) and second part for cytometry analysis. Gonads are dissociated before being stained by a DNA intercalant agent. Results are then read by flow cytometry. Results are translated with a scoring system. At each cell cycle step is attributed a coefficient which is correlated with the sexual maturity degree. $IMS = ((\% \text{ sub-haploid cells} * 5) + (\% \text{ haploid cells} * 4) + (\% \text{ G2-M cells} * 3) + (\% \text{ S cells} * 2) + (\% \text{ G0-G1 cells} * 1)) / 100$ These analyses were realized at different moment of year and Flow cytometry analysis has highlighted different periods of spermatogenesis. Histological analyses have showed similar results. For the second part of this study, mussels were exposed to carbamazepine for 4 weeks in the lab at three defined periods. Bioaccumulation of carbamazepine in soft tissues of mussels was also performed. Gonads were dissected and analyzed by flow cytometry as described upper. Results of bioaccumulation have revealed the presence of carbamazepine in soft tissues of mussels. Results have showed no solvent effect on the degree of sexual maturation. IMS results have showed that the spermatogenesis chronology is overall the same as in environmental conditions. However, it seems that carbamazepine at tested concentrations would have a minor impact on spermatogenesis dynamic and maturation of germ cells.

Discussion and conclusion : The development of the IMS proved effective for evaluating the degree of sexual maturity in zebra mussel. This technique is accurate, fast and would permit to overcome histological analysis of gonads to determine spermatogenesis and associated disturbances.

EFFECTS OF CARBAMAZEPINE IN MYTILUS EDULIS IN A SHORT AND LONG-TERM EXPOSURE

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Background and objective : Among the most concerning contaminants worldwide are pharmaceuticals, since they are widely consumed by humans and largely used in aquaculture and agriculture. Generally, drugs end up in the environment mainly through municipal wastewater in concentrations ranging from low ng/L to µg/L since many of these compounds are not readily degraded in sewage treatment plants (WWTPs). However, despite the low concentrations in water, the continuous discharges of these compounds into the aquatic ecosystems raises concerns on their toxic effects on aquatic living organisms. Carbamazepine (CBZ) has been identified as a major contaminant found in diverse aquatic systems. Carbamazepine is an antiepileptic, relatively lipophilic, with an octanol/water partition coefficient of 2.2, and is consistently found at relatively high concentrations in WWTPs influents and effluents, surface waters, groundwater and even in treated drinking water, with concentrations ranging from 0.03 to 11.6 µg/L. Although a variety of pharmaceutical compounds, including carbamazepine, have been detected in the environment, few studies have addressed their impact on non-target species. Thus, the objective of the present study was to evaluate the acute and chronic toxicity of CBZ induced in the mussel *Mytilus edulis* when exposed to environmentally relevant concentrations of this drug. For this a set of biochemical markers was used, including energy related and oxidative stress parameters.

Methods and results : After acclimatization under laboratory conditions (temperature 18°C, photoperiod 12 light : 12 dark, salinity 25 g/L) mussels were exposed for 96h and 28 days to four concentrations of CBZ (0.3, 3.0, 6.0 and 9.0 µg/L) plus control (0.0 µg/L). For each condition 4 containers were used, with 6 organisms per container. As during the acclimatization period, organisms were maintained in artificial seawater (25 g/L). Biomarkers indicators of energetic reserves (electron transport system, glycogen, protein content), antioxidant (glutathione reductase, GRed; superoxide dismutase, SOD; catalase, CAT) and biotransformation (Glutathione S-transferases, GSTs) enzymes activity and cellular damage (lipid peroxidation, LPO) were determined. The concentration of CBZ in mussels and water was quantified using the immunoassay ELISA. The results obtained showed that, when comparing to control conditions, both under acute and chronic exposures mussels increased their LPO levels only at the lowest CBZ test concentration but with the increasing of exposure concentrations a decrease on LPO levels was recorded. Along the increasing exposure gradient mussels tend to maintain or even decrease their antioxidant (SOD, CAT, GRed) activity when exposed for 96h and 28 days to CBZ. The results obtained also showed that the activity of biotransformation enzymes (GSTs) was maintained (acute exposure) or increased (28 days). The present findings further revealed that, both under acute and chronic exposures, mussels increased their metabolic activity with the increase of exposure concentration, which was accompanied by a decrease on their energy reserves (glycogen and protein).

Discussion and conclusion : The results obtained showed that mussels were able to prevent CBZ accumulation which was accompanied by an increase on their metabolic activity and expenditure of energy reserves. The increase of GSTs, especially after 28 days of exposure, can be explained as the enhanced requirement for biotransformation waste products resulting from the potential cellular damage due to the CBZ exposure. Due to their capacity to prevent CBZ accumulation with the increasing of exposure concentrations, both after 96h and 28 days, mussels did not show cellular damages (measured by LPO levels) or increase on antioxidant defenses (SOD, CAT, GRed), especially noticed at higher exposure concentrations (3.0, 6.0, 9.0 µg/L).

THE IMPACTS OF ANTIHISTAMINES ON THE MUSSEL SPECIES MYTILLUS EDULIS: BIOCHEMICAL ALTERATIONS INDUCED AFTER CHRONIC EXPOSURE TO CETIRIZINE

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Background and objective : The high consumption of a variety of pharmaceuticals by an exponentially growing world population resulted in their ubiquity in the environment. Furthermore, due to their incomplete removal in waste water treatment plants (WWTPs), with removal efficiencies below 10% for some substances, pharmaceuticals are continuously introduced in aquatic systems. For these reasons, and because pharmaceuticals might preserve their biological activity when in the environment, with potential impacts to aquatic wildlife, over the last years increasing attention has been paid to understanding the impacts of these contaminants in aquatic ecosystems, especially on inhabiting organisms. Antihistamines are among the most commonly used pharmaceuticals worldwide, which. They are mainly used in the treatment of allergic diseases. One of these drugs is cetirizine (CTZ), a second-generation antihistamine, characterized by reliable and consistent inhibition of histamine-induced allergic reactions. After use, CTZ is excreted from the human body in its unaltered and active form, 50% of the ingested cetirizine being released. In order to assess CTZ effects when released into aquatic systems, this study used a set of oxidative stress-related biomarkers to evaluate the possible alterations induced in the mussel *Mytilus edulis* after a long-term exposure to environmentally relevant concentrations of cetirizine.

Methods and results : The chronic toxicity test (28 days) was performed by exposing the mussels to four concentrations of CTZ (0.3, 3.0, 6.0 and 12.0 $\mu\text{g/L}$) plus control (0.0 $\mu\text{g/L}$ of CTZ). Biomarkers such as indicators of energetic reserves, antioxidant and biotransformation enzymes and cellular damage were determined. The results obtained revealed that, with increasing CTZ concentrations, an increasing oxidative stress occurs. Organisms were able to increase their energy reserves along the increasing exposure gradient but only at the highest exposure concentration significantly higher metabolic activity (assessed by the activity of electron transport system) was observed.

Discussion and conclusion : The present study showed that mussels exposed to an increasing CTZ concentration gradient increased their lipid peroxidation (LPO) levels. Accompanying this trend, the activity of catalase showed a remarkable increase indicating that mussels antioxidant defenses were enhanced when in the presence of CTZ but were not enough to prevent the increase in LPO. The gain in protein content along the exposure concentration gradient may indicate that mussels were able to increase the amount of enzymes, namely antioxidant and biotransformation enzymes, to fight against the stress caused by CTZ. Glycogen content increased along the increasing exposure gradient demonstrating the capacity of *M. edulis* to preserve this energy reserves under stressful conditions. The metabolic activity was maintained up to the highest tested concentration (12.0 $\mu\text{g/L}$) showing that mussels may boost their metabolism only at a given concentration level to fight against the stress caused by CTZ. Overall, the present study showed that a chronic exposure to environment relevant CTZ concentrations induced oxidative stress in the mussel *M. edulis*, which is in accordance with previous studies developed by others authors with different *Mytilus* species exposed to various pharmaceuticals.

EXPLORING RESPONSES OF MARINE MUSSEL TO THE ANTI-INFLAMMATORY DRUG DICLOFENAC

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Background and objective : Human pharmaceuticals, such as nonsteroidal anti-inflammatory drugs (NSAIDs), are an emerging environmental threat to marine organisms. Bioaccumulation along with possible ecotoxicological effects may be of concern, as shown by the inclusion of diclofenac (DCF) in the first watch list by the EU Water Framework Directive. In humans, NSAIDs act through inhibition of cyclooxygenase (COX) conversion of arachidonic acid into prostaglandins (PGs). Even if there have been studies on the physiological effects of NSAIDs on aquatic organisms, relatively little is known regarding their potential alteration of PG levels in non-target organisms. The objective of this study was thus to investigate whether DCF affects PG levels in the marine mussel *Mytilus galloprovincialis*.

Methods and results : One experiment was carried out whereby marine mussels were exposed for 72 h to 1 and 100 µg/L DCF. A specific and sensitive analytical method relying on liquid chromatography hyphenated to high resolution tandem mass spectrometry (LC-HRMS/MS) was developed to quantify DCF accumulation in marine mussel tissues along with the formation of DCF metabolites. The developed method could also clearly identify and quantify COX products, i.e. prostaglandins PGD₂ PGE₂, and PGF₂α levels and be used to assess their modulation following DCF exposure. Measured concentrations of DCF in water and mussel tissues allowed determining a low bioconcentration of 27 L/kg which is consistent with DCF possible biotransformation in organisms, supported by the detection of hydroxy-diclofenac metabolites in exposed organisms and water. PGs quantification in non-exposed and exposed mussels revealed basal PGE₂ concentrations ranging from under the limit of detection (LoD) to 202 µg/kg dw, whereas PGD₂ was always found below the LoD. The most interesting result was a downward trend in the PGE₂ concentration observed between non-exposed mussels and those exposed to 1 µg/L DCF. This decrease was confirmed and statistically significant for exposure to 100 µg/L (see documentation attached) suggesting the MoA of DCF could be partially conserved in mussels. PGF₂α globally ranged from 90 to 518 µg/kg dw. No difference was observed for PGF₂α levels between controls and exposed organisms.

Discussion and conclusion : DCF was found to be weakly accumulated; its biotransformation in mussel was demonstrated. Regarding the prostaglandin levels measured in this study, they appear to be up to 1000-fold lower than those measured in the freshwater mussel *Perna viridis* using HPLC-UV [1]. However, the use of a confirmatory technique such as LC-HRMS/MS along with analogous deuterated standard lead to confidence in the results even if they are lower than those previously reported in the literature. Mussels exposed for 72 h to 100 µg/L DCF showed a significant decrease in PGE₂ levels. This is the first time that a direct link between NSAIDs and COX-products is established in mussel. Previous reports reported similar conclusions in fish [1, 2]. Further experiments need to be conducted to understand i) how DCF modulates PG production, and ii) the implications for PG-related physiological functions, such as osmotic regulation and spawning. [1] Chakraborty K et al., 2014. *Journal of Functional Foods*. 7:527 – 540. [2] Morthorst JE et al., 2013. *Comparative Biochemistry and Physiology - Part C: Toxicology & Pharmacology* 157, 251–257. [3] Bhandari K et al., 2011. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*. 153, 251 – 257. Funding support was obtained from the The French National Research Program for Environmental and Occupational Health of Anses (2015/1/091).

ENVIRONMENTAL OCCURRENCE AND EFFECTS IN FISH OF LIGANDS OF THE PROGESTERONE RECEPTOR: CURRENT PROGRESS OF THE FRENCH NATIONAL PROJECT PROOFS

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Background and objective : To date, most studies on endocrine disrupting compounds have focused on estrogenic compounds with a particular attention to natural (estradiol, estrone, estriol) and synthetic estrogens (17 α -ethinylestradiol). However, there is a now emerging evidence for the occurrence of other natural and synthetic steroids in aquatic systems. Among them, the potential risk posed by synthetic progestins on aquatic species has been recently pointed out. These compounds are widely used in human medicine, especially in oral contraceptives and hormone replacement therapy. However, their occurrence, fate and effects have been poorly investigated. To fulfill these gaps, the national ANR project PROOFS aimed at investigating i) the mode of action of a broad range of progestins and their effects in fish and ii) the occurrence of (anti)-progestagenic activities in the aquatic environment.

Methods and results : A broad range (24) of currently used progestins were screened using in vitro and in vivo reporter gene assays for their capacities to interact with human (h) or zebrafish (zf) nuclear progesterone receptor (PR) and to deregulate the tissue-specific expression of steroidogenic genes in zebrafish embryos. By using human reporter cell lines stably expressing either hPR or zfPR, we revealed marked interspecies differences on the ability of progestins to activate/antagonize PRs. In transgenic cyp19a1b-GFP zebrafish embryos, progestins derived from 19-nor-testosterone induced brain aromatase in radial glial cells through an ER-dependent mechanism. Finally, in vitro screening of a bank of environmental samples (including more than 100 wastewater effluents and surface waters) allowed the quantification of (anti)progestagenic activities, with noticeable zfPR agonistic activities in waste waters at several sites

Discussion and conclusion : The PROOFS project provides an extensive (eco)toxicological characterization of a large number of currently used (anti-)progestin pharmaceuticals on key molecular targets. We showed marked differences between hPR and zfPR in vitro and ii) early developmental effects in brain of zf embryos. Furthermore, environmental bioanalysis revealed fish-specific activities in the aquatic ecosystems. The ANR PROOFS also depicted for the first time (anti)progestagenic activities in aquatic samples showing wastewaters as a source of contamination by zf-active compounds that still need to be identified. Overall, this study supports the need to further characterize ecotoxicological risks posed by progestins

EU DEMEAU PROJECT: PRACTICAL APPLICATION OF IN-VITRO BIOASSAYS IN WATER QUALITY ASSESSMENT

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Due to increasing number of chemicals (including pharmaceuticals) in surface waters and the analytical challenges of monitoring and identifying each pollutant often present at low dose levels, the need for an innovative and more comprehensive water quality and safety monitoring framework is recognized. Bioassays are promising tools to provide solution for such challenges, as they can be applied as early warning systems and are capable of integrating the combined toxic potency of all compounds present.

Through various case studies the usability of bioassays to measure combined effects of emerging and unknown water pollutants were demonstrated and suggested within the EU funded project “DEMEAU”.

The presentation

- i) explains how (*in vitro*) bioassays can provide cost-effective means for safety evaluations of water samples and investigation of activity profile of single chemicals,
- ii) introduces a selection criteria system that helps to compile a relevant bioassay panel for the effect-based screening of drinking, surface and waste waters,
- iii) briefly provides the basis of drinking and environmental water trigger values elaboration,
- iv) summarizes the main results and conclusions of the demonstration studies.

The following studies will be presented:

1. Establishment of bioassay trigger values

The need for trigger values is internationally acknowledged and within the project a proposal to derive human health related trigger values has been reported. Ecotoxicological trigger values are being currently developed.

2. Selection criteria to assess the suitability of bioassays

A recent inter-laboratory study described the application of a broad panel of *in vitro* bioassays to waste, recycled, storm, surface and drinking waters. Each type of water showed a characteristic bioanalytical profile with particular groups of toxicity pathways. Since there are numerous bioassays available for these pathways, we prepared a criteria system to assess the suitability of the respective bioassays and selected the most promising techniques for water quality assessment.

3. Bioscreening of Managed Aquifer Recharge (MAR) samples

Various types of MAR sources from two sampling campaigns were subjected to screening in the selected bioassay panel for their toxicity profile and investigate the impact of micropollutants present in these samples. The study revealed the importance of endocrine, oxidative stress and photosynthesis inhibition pathways, and showed differences between the samples collected in two sampling campaigns.

4. Ecotoxicological evaluation of wastewater treatment

The efficiency of ozonation and various post-treatments to reduce ecotoxicological effects still occurring in the conventionally (biologically) treated wastewater was assessed in this study. The investigations revealed that the ozone treatment resulted in significantly improved water quality in the majority of bioassays as compared to effects measured in the conventionally treated wastewater. In a few assays, partially higher effects after ozonation occurred, but could be removed by suitable post-treatments.

NEW DEVELOPMENTS IN ESTROGEN AND EDC MONITORING AND REGULATORY OPTIONS FOR WATER QUALITY MANAGEMENT

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Background and objective : With the publication of the European Commission Implementing Decision EU 2015/495, three steroidal estrogens, namely 17α -ethinylestradiol, 17β -estradiol and estrone, have been included in the so-called “watch list” of the Water Framework Directive. Before applying more demanding chemical analytical methods to quantify these substances, we recommend to screen environmental samples for the presence of estrogenic activity. In vitro bioassays, among the different possible applications, are able to detect estrogenic activity in environmental mixtures in a cost-effective way. In the context of the Working Group “Chemicals” and as a follow-up of the Science-Policy Interface activity, an approved international project aims at: • Promoting reliable screening methods for the monitoring of endocrine disrupting compounds (EDCs) in wastewater and surface waters • Harmonizing monitoring strategies for EDCs options across Europe as well as data interpretation methods • Linking reliable effect-based tools with regulatory needs and cost-efficient chemical monitoring.

Methods and results : This project involves 25 research organisations and environmental agencies from 12 different countries. So far, 16 surface water and 17 wastewater samples have been collected across Europe, which are being analysed using the following effect-based and chemical analytical methods: • High end chemical HPLC MS-MS analysis (JRC, BfG, Swiss Centre for Applied Ecotoxicology) • ER-Calux (BDS) • MELN (INERIS) • BG1Luc4E2 + ER-GeneBLAzer (UFZ) • Hela 9903 (RECETOX) • Yeast Estrogen Screen assays (BfG) • T47D-Kbluc assay (RWTH Aachen) First results for waste water assessment show that effect-based methods can effectively quantify chemical pressures and mixture risks. Cumulative risk quotients derived from chemical measurements in wastewater samples were highly correlated with risk derived from measured biological estradiol equivalent concentrations.

Discussion and conclusion : Promising first results demonstrate that water quality assessment can progress from a purely analytical approach to an effect-based monitoring, from single substance to known and unknown mixture assessment, and from in vitro screening to population relevant risk assessment. We will further investigate the comparability of methods and thus contribute to the European Community Strategy on Endocrine Disruptors. Results will assist water managers in devising a strategy to monitor water bodies regarding EDCs. Moreover the project supports the work programme activity of the Common Implementation Strategy (CIS) of the WFD for the period 2016-2018 in which it is foreseen to use the best available methods to detect and evaluate mixtures of pollutants and to link the chemical and ecological status of water bodies.

ACTIVE UPTAKE OF PHARMACUTICALS IN THE ENVIRONMENT BY NON-TARGET ORGANISMS – AN ENVIRONMENTAL AND FOOD SAFETY RISK

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Background and objective :Pharmaceutical residues in the environment (e.g. wastewater sewage sludge, surface water, drinking water, manure), their fate and adverse effects to non-target organisms has gained much attention, particularly exposure and effects towards aquatic organisms due to high loading in waste water effluents. Even though a great number of pharmaceuticals are not necessarily persistent by definition, they are characterized as pseudo-persistent since the discharge into the environment is constant and higher than their degradation. The majority of pharmaceuticals are both water soluble and persistent, and many are bypassing conventional treatment systems. Positively charged pharmaceuticals are attractive to negatively charged organic matter, however, their transfer to sewage sludge have been overlooked/underestimated in previous risk assessment related to application of sewage sludge to soil for food cultivation due assumed low transfer of water soluble organic compounds to sludge. For instance, the dicationic metformin is found in 8 mg/kg dry weight in sludge (Færøyene) and 750 ng/L in recipient water. The objective of our presentation is to highlight the possibilities of active uptake of Pharmaceuticals from the environment to non-target organisms and to discuss how this should be taken in account for in risk assessments.

Methods and results : The poster will be based on experimental uptake studies (one published, one under performance). Recently accumulation of an anti-diabetic pharmaceutical, metformin, in oily rape seeds (*Brassica napus* and *Brassica rapa*) was reported, 15-70 times higher than in cereals. A new plant uptake experiment with metformin has been performed and will results will be included in the poster.

Discussion and conclusion : The hypothesis that metformin, a small molecule with structural similarity to natural plant compounds e.g. guanidine and arginine, is “mistaken” as natural plant organic-N compound and translocated via transporters into seeds was put forward. Other pharmaceuticals of interest is amino acid-like structures e.g. mesalazine. The effects of metformin on the expression of important enzymes involved in steroidogenesis (cyp11A and 3 β -hydroxysteroid dehydrogenase: 3 β -hsd) and the cholesterol transporter steroidogenic acute regulatory (StAR) protein, and CYP17 activity in mammalian system has been reported. Recently, it was demonstrated that exposure of fish to environmentally relevant concentrations produced endocrine disrupting effects. Most pharmaceuticals functions by interfering with human/animal target proteins (receptors, transporters, ion-channels and enzymes). When taken up by non-target organisms, pharmaceutical may interfere with proteins structurally related to the human/animal drug targets and result in a harmful outcome. There is a huge knowledge gap regarding pharmaceuticals and their exposure and effects on non-target organisms. Can structure-similar pharmaceuticals be taken up actively by non-target organisms – and if so – will this results in a higher risk of adverse effects?

EVALUATION OF PREDICTIVE MODELS FOR SORPTION AND UPTAKE OF PHARMACEUTICALS

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Background and objective : The majority of pharmaceuticals in use are ionisable and may become charged at environmentally relevant pH values. Most existing models for estimating the fate and uptake of organic compounds have been developed for neutral organic compounds and are therefore probably not appropriate for ionisable compounds. For example, the uptake of ionisable compounds by organisms has been previously explored in aquatic compartment; where studies have demonstrated differences in uptake and toxicity of charged chemicals in comparison to chemicals in their unionised state. The aim of this study, therefore, was to collate data on the sorption and uptake of ionisable compounds and use these data to evaluate the applicability of existing predictive models for these endpoints.

Methods and results : A database has been built which has collated data (endpoint and associated metadata) from previous studies on the uptake and sorption of ionisable chemicals (pharmaceuticals and pesticides) in aquatic systems. The database includes information on acidic and basic molecules and covers a range of fish and invertebrate species and matrix types (sediment type, water with different pH values). Using this database, relationships between contaminant physicochemical properties (e.g. hydrophobicity), uptake parameters and bioconcentration factors (BCFs) and sorption behaviour have been explored. In addition, a number of previously published models have been evaluated using the collated literature to see if they can accurately predict ionisable chemical sorption and uptake, this will help towards future model development, specifically for predicting ionisable chemical uptake.

Discussion and conclusion : Previously published models for predicting chemical uptake into aquatic organisms are available for neutral chemicals but it is unknown whether these will work for ionisable substances. Considering that a large proportion of pharmaceuticals are ionisable chemicals, it is import to elucidate whether models previously developed for neutral chemicals are suitable, and if not, what properties are key in predicting ionisable chemical uptake including organism traits and environmental characteristics (e.g. pH). This information will be invaluable in future model development and in the design of experimental testing programmes to support model development. This work has received support from the EU/EFPIA Innovative Medicines Initiative Joint Undertaking (iPiE grant n° 115735).

ECOTOXICOLOGICAL IMPACT OF SULFAMETHOXAZOLE ON NITROGEN CYCLING MICROBIAL COMMUNITIES, IN AGRICULTURAL AMENDED SOIL.

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Background and objective : The fate and effects of human and veterinary antibiotics in the environment have been the subject of intense investigation for nearly two decades. While the occurrence and persistence of antibiotics and antimicrobial resistance in environment have become major human health and research issues, their impacts on microbial ecosystem processes (i.e. nutrient recycling) are not well-understood. The growing interest for organic wastes recycling as soil amendments, can lead to various diffuse antibiotic contaminations, requiring an ecotoxicological assessment of such agricultural practices. The environmental risk assessment (ERA) methodologies for antibiotics have been developed based on standard ecotoxicology tests existing in guidelines for other chemicals, but such regulatory assessment has been questioned (Boxall et al., 2012). Actually, the modes of action of antibiotics are mainly specific for bacteria, but bacterial toxicity tests play a very minor role during the ERA of antibiotics (Kümmerer et al., 2004; Brandt et al., 2015). Consequently, this may overlook adverse effects of antibiotics on microbial ecosystem functions. Thus, the current ecotoxicological studies should aim at bridging the gap existing in the understanding and assessment of ecotoxicological impact of antibiotic on microbial ecosystem processes. The scope of this work aims at identifying microbial community-based tests and endpoints to improve targeted protection of key microbial soil processes. It also unravels the diversity – function relationships and the edaphic factors controlling antibiotic bioavailability, which are involved in the resilience of soil microbial functioning, following application of contaminated amendments. This study investigated the effects of sulfamethoxazole (SMX) on microbial nitrogen transformations.

Methods and results : A dose-effect approach was performed with different doses of SMX added to different organic wastes (compost of sewage sludge - green waste (SGW), farmyard manure (FYM)), before their mixing with soil, in microcosms. The final concentrations of SMX in amended soils ranged from 0.022 to 2.22 mg kg⁻¹ dw, with control soil microcosms amended with SGW or FYM without SMX. Nitrogen forms and nitrification and denitrification activities were determined, after 8, 28 and 84 days following amendments. Abundances and composition of specific microbial guilds (ammonia oxidizing bacteria and archae and some denitrifiers) were determined with molecular tools. The total and available concentrations of SMX were extracted with organic and aqueous solutions, respectively, and then quantified by UHPLC-MS/MS.

Discussion and conclusion : Only the nitrification activity was adversely impaired by SMX, following a dose-response pattern, in SGW amended soils, but not in FYM amended soils. These effects had short-term outcomes for nitrogen nutrient dynamic from 0.22 mg SMX kg⁻¹ dw, in SGW amended soils. The chemical availability of SMX did not show strong differences between the SGW or FYM amended soils and failed to explain the difference in SMX impact on microbial activities. Actually, the bacteriostatic toxicity of SMX, was only detected on growing microbial populations, such as in SGW amended soils, where high NH₄⁺ levels did not limit the growth of nitrifier bacteria. Denitrification activity was only slightly affected by high SMX doses, in SGW amended soils. A higher diversity and functional redundancy of denitrifier microorganisms can explain this stronger resistance of denitrification to SMX compared to nitrification. In addition, the magnitude of SMX effects on nitrification slightly decreased at long-term (day 84) compared to short-term (days 8 and 28), in accordance with the decrease of SMX availability, in SGW amended soils. Analytical chemistry (See presentation Goulas et al.) improves our understanding of the functional resilience of microbial communities, by the assessment of the exposure to antibiotics. Furthermore, some modifications of community composition also contributed to the functional resilience following the initial antibiotic stress, as previously observed by Ollivier et al., 2013. This work reveals that narrow niche processes (nitrification) appeared as a sensitive indicator of SMX effects on soil microbial functioning, while broad scale processes (denitrification) was more resistant.

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Also, this study highlighted the necessity to include the assessment of antibiotic chemical availability and ecological characteristics of microbial communities (resistance and resilience) in ERA methodologies.

ECOTOXICITY OF THE ANTIHISTAMINIC DRUG CETIRIZINE ON STRESS BIOMARKERS OF THE CLAM *RUDITAPES PHILIPPINARUM*

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Background and objective : Pharmaceuticals are applied worldwide for the the treatment and prevention of human and animal diseases. The high consumption and the low degradation rates in wastewater treatment plants (WWTPs) are the main reasons for the environmental contamination with pharmaceutical drugs. Cetirizine (CTZ) is an antihistaminic found in diverse water bodies (WWTP effluents, surface waters, groundwater and treated drinking water) with concentrations ranging from low ng/L to mg/L. CTZ is used for the treatment of seasonal allergic reactions which results in the seasonal variation in the concentrations found in the environment. The main concern of the presence of CTZ in the environment is based on its biological activity and thus, the possibility of toxic effects on non-target organisms, such as bivalves. However, only few works have studied the effects of this drug on aquatic organisms. Thus, the present study aimed to evaluate the chronic effects of environmentally relevant concentrations of CTZ on the edible clam *Ruditapes philippinarum*, through the use of a battery of physiological and biochemical biomarkers.

Methods and results : The chronic toxicity test (28 days) was performed by exposing the clams to four concentrations of CTZ (0.3, 3.0, 6.0 and 12.0 µg/L) plus control (0.0 µg/L of CTZ). Biomarker indicators of energetic reserves (glycogen and protein content), antioxidant (superoxide dismutase (SOD) activity) and biotransformation (glutathione S-transferases (GSTs) activity) enzymes and cellular damage (lipid peroxidation, LPO) were determined. The results obtained showed a decrease of glycogen and protein content at lower concentrations (0.3 and 3.0 µg/L), followed by an increase at higher CTZ concentrations (6.0 and 12.0 µg/L). Moreover, at 6.0 and 12.0 µg/L the effects of CTZ resulted in higher cellular damage (LPO), accompanied by higher activity of antioxidant (SOD) and biotransformation (GSTs) activity.

Discussion and conclusion : Results showed that, at lower concentrations, clams spent glycogen reserves to face the stress induced by CTZ. However, at higher CTZ concentrations (6.0 and 12.0 µg/L) the increase in glycogen content may reveal that this energy reserve is not being expended into any defense mechanism. The increase in protein content may indicate an induction of protein synthesis involved in detoxification processes and thus be related with the increase in SOD activity. Although the activity of SOD is increased at higher concentrations, LPO is also increased, indicating that despite of the effort of the antioxidant system, the production of reactive oxygen species is still exerting toxic effects on clams. Overall, CTZ caused a stress response in the clams *R. philippinarum* after exposure to environmentally relevant CTZ concentrations, especially at 6.0 and 12.0 µg/L. These results are possibly a response to the high accumulation of CTZ at higher exposure concentrations. The results obtained in this work are in accordance with previous studies assessing the toxic effects of other pharmaceutical drugs in *R. philippinarum*.

SYNTHETIC PROGESTINS ACTIVATE HUMAN AND ZEBRAFISH NUCLEAR PROGESTERONE RECEPTOR IN VITRO

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Background and objective : As compared to (xeno-)estrogens, natural and synthetic ligands of the progesterone receptor (PR) have been scarcely studied on aquatic organisms while the potential risk posed by environmental progestins has been recently pointed out. However, there is still a lack of data to accurately characterize the hazards posed by these compounds. In that respect, the capacity of synthetic progestins to interact with fish nuclear progesterone receptor was poorly investigated. In the present work we aimed at assessing potential endocrine disruption of these compounds towards zebrafish nPR and to compare their effects with that of human nPR to identify possible interspecies differences.

Methods and results : Two human cell lines co-expressing either human PR (hPR) or the zebrafish PR (ZfPR) and luciferase gene, namely HELN-hPRB and U2OS-zfPR cells, were developed and characterized using promegestone (R5020) as a reference agonist ligand. R5020 induced luciferase activity in both cell lines in a concentration-dependent manner. These effects were completely blocked by co-exposing the cells with mifepristone (RU486), a potent PR antagonist. A large set of natural and synthetic progestins (25) was screened in the two cell lines. All of the compounds except the natural zebrafish progestin $17\alpha,20\beta$ -dihydroxy-4-pregnen-3-one (DHP) activated the hPR. About half of the progestins induced a maximum effect of 100% when compared to R5020 while the other half partially induced luciferase activity. In contrast to cells expressing hPR, progestins behaved very differently with the zfPR since only five of them were active on zfPR. Interestingly, we found that DHP strongly activated zfPR but not hPR. To assess whether some progestins were partial agonists, they were coexposed with R5020. Half of them antagonized the activity induced by R5020 on hPR and are thus partial hPR agonists. All the active progestins on zfPR antagonized R5020 and are therefore partial zfPR agonists.

Discussion and conclusion : Two new luciferase reporter cell lines were developed and characterized, providing novel information regarding the activity of a large set of progestins on the zfPR. These models allowed us to highlight major interspecies differences. These results support the need to further determine the effects of zfPR agonist progestins on PR-dependent physiological processes in order to characterize the hazards posed by progestins in fish.

IN VITRO TOXICITY PROFILING OF THE WFD PRIORITY SUBSTANCES AND A SELECTION OF PHARMACEUTICALS MONITORED IN EUROPEAN SURFACE WATERS

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Background and objective : A selection of pharmaceuticals from the EU DEMEAU project and all substances listed in the Annex X of the Water Framework Directive (WFD) (in total ~90 compounds), which are routinely or occasionally monitored in European surface waters, were screened within this study using a panel of in vitro reporter gene assays. The 24 CALUX bioassays covered a wide range of toxic endpoints from endocrine disruption, through oxidative stress, genotoxicity to xenobiotic- and lipid metabolism.

Methods and results : The aim of the study was the following: □ Toxicity profiling¹ of the routinely/occasionally monitored substances in European surface waters □ Identification of the toxic pathways picked up by these substances □ Comparison of the toxicity pathways induced by the tested substances with relevant toxicity pathways for water quality assessment (suggested by various case studies²⁻⁴) □ Demonstrate the advantageous use of effect-based bioanalytical methods for high throughput toxicity screening of large set of compounds.

Discussion and conclusion : The screening revealed that □ the majority of the tested compounds were active in at least one of the bioassays performed. The screening resulted in no distinct toxicity profile, however, revealed the importance of endocrine - (particularly the activation of the estrogen alpha-, anti-androgen, anti-progesterone receptors), genotoxicity and xenobiotic metabolism-related pathways of the routinely monitored substances; □ this primary effect profile is consistent with only part of the profile observed in water samples indicating that not all substances are picked up by the compound panel of the current monitoring programs. In other words, realistic water samples may contain other compounds inducing various toxic effects (e.g. receptor activation) not covered by WFD listed substances or other monitored substances.

EFFECT OF PHARMACEUTICAL RESIDUES ON THE ANAEROBIC DIGESTION PROCESS

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Background and objective : Pharmaceutical residues (PR) generally originate from the urban- or built-environments and may reach the natural aquatic environment at concentrations up to $\mu\text{g/L}$. Although these concentrations are unlikely to affect human health, they can cause chronic exposure damage to aquatic organisms and a few are included as watch-list substances in the Water Framework / Priority Substances Directives. Some pharmaceuticals are totally or partially removed in Wastewater Treatment Plants. However, PRs may have an effect on any biological processes taking place in these treatment plants. Anaerobic digestion (AD), a common tertiary treatment in the UK, is a complex biological process where different microorganisms take part and many organic or inorganic compounds can inhibit it. Thus, the principal aim of this work was to investigate the effect of PRs on the AD process. Crucially, as the majority of PRs of concern are found in the aqueous phase, this report will focus on the direct anaerobic treatment of wastewater.

Methods and results : The anaerobic biodegradability of the wastewater and different pharmaceutical residues were tested in batch experiments, using a simple and effective methodology based on the UNE-EN standard ISO 11734. This enabled the rapid assessment of bacterial inhibition and biomethanization potential of the organic residues and estimation of the amount of biogas produced. To study the influence of different AD design parameters, several operating conditions were tested: synthetic wastewater (SWW) with organic carbon loadings of 0.2, 0.5 and 1g organic C of substrate per volatile solids content in the inoculum and SWW with two different pharmaceuticals mixtures (Table 1). The low and high pharmaceuticals concentrations were established based on the concentrations reported in the literature. Additionally, caffeine, lidocaine and carbamazepine were studied separately in SWW at 2 different concentrations (1 and 50 $\mu\text{g/L}$, 0.02 and 0.3 $\mu\text{g/L}$ and 1 and 3 $\mu\text{g/L}$ respectively) in order to evaluate their individual effect on AD.

Discussion and conclusion : The biogas production increased in association with increasing organic loading rates of the SWW (Figure 1). However, the average degradation of the substrate was similar after almost 40 days of incubation for all the samples (39, 43 and 41% for 0.2, 0.5 and 1 rates respectively), indicative of a lack of inhibition of microorganisms regardless the organic loading added. Different degradation patterns were observed in syringes with low and high pharmaceuticals concentrations in SWW (Figure 2). Samples with a high pharmaceuticals concentrations exhibited initially high biogas production, which declined after 3 days, achieving only a 25% of organic degradation after 12 days. However, the biogas production was not inhibited when the pharmaceuticals concentration was low. In addition, after 12 days, the degradation rate was higher in these samples than in those without pharmaceuticals, 41 and 34% respectively. • Additional results are in the process of being analysed. Data for COD and pharmaceutical removal will be made available shortly. • The batch experiment with different pharmaceuticals concentrations is still under testing. Based on the results, it can be concluded that a low concentration of a mixture of the 11 selected pharmaceuticals may favour AD process, increasing the wastewater degradation. On the other hand, when the concentration of these pharmaceuticals was high, the system appears to be inhibited as the degradation rates obtained were lower. Caffeine, lidocaine and carbamazepine individually appeared not to affect the AD process.

INFLUENCES OF SLUDGE PRETREATMENTS AND THE ANTIBIOTIC EXISTENCE ON THE BIOCHEMICAL METHANE PRODUCTION POTENTIAL OF THE WASTEWATER SLUDGES

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Background and objective : The anaerobic wastewater sludge digestion with the application of various pretreatments can be effective both for the antibiotic removal from the sludge and for the organic content reduction of the sludge so that it can be safely disposed. This study investigates the effects of sludge pretreatment on the removal of antibiotics and the biochemical methane production potential of antibiotic containing wastewater sludges. In the study, persulfate (S₂O₈²⁻) and hydrogen peroxide (H₂O₂) pretreatments are used for the degradation of the antibiotics from the sludge, and the microwave irradiation was also applied to sludge samples together with peroxide and persulfate pretreatments (S₂O₈+MW, and H₂O₂+MW). The remaining sludge after the pretreatments will be anaerobically digested to treat waste activated sludge and produce energy from biomass in form of biogas.

Methods and results : The waste activated sludge and the inoculum were mixed in 100 mL serum bottles at a food to microorganism (F/M) ratio of 1. All reactors were incubated at 37°C for 30 days. Total gas production and gas composition analyzed periodically during the digestion period of batch reactors. Initial and final sludge characteristics were analyzed according to Standard Method. When gas production stopped, the reactors were shut down and reactor contents analyzed to see the effect of pretreatments and antibiotic existence on final sludge characteristics.

Discussion and conclusion : The results of the study showed that applying pretreatments to the wastewater sludges removed the antibiotics and enhanced the biogas production considerably.

RISK ASSESSMENT AFTER INTERVENTION: EFFECT OF DIFFERENT TREATMENT TECHNOLOGIES ON THE RISK ASSOCIATED WITH PHARMACEUTICALS IN WASTEWATER

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Background and objective : The effluent quality of different treatment steps in a full-scale hospital wastewater treatment plant (HWWTP) was assessed with respect to eco-toxicological effects and the occurrence of 72 micropollutants.

Methods and results : The HWWTP consisted of sequential and parallel combinations of the advanced treatment steps membrane bioreactor (MBR), ozonation, powdered activated carbon adsorption and sand-filtration (Fig. 1). Figure 1. Flow scheme of the HWWTP including sampling points. The applied eco-toxicological test battery included two on-site (flow-through) and three off-site acute and chronic in vivo tests and, additionally, one in vitro test (Tab. 1). The test battery is characterized by different trophic levels, by acute and chronic toxicity tests as well as different toxicological endpoints (Tab. 1). It was designed according to recent recommendations for the use of bio-analytical tools in water quality assessment [1]. Table 1. Overview of the applied bio-analytical test battery.

Discussion and conclusion : Reliable steady state conditions of the activated sludge system were ensured by a 6 month-period of stable operation prior to the start of the comprehensive sampling campaign for toxicity assessment [5]. Figure 2 indicates a stable biomass concentration as well as almost complete C and N removal by the MBR over long-term. Figure 2. Left: Biomass concentration in the bioreactor; Right: Removal rates for COD, BOD, N and P (without chemical P removal). By MBR treatment the growth inhibition of the green algae *Desmodesmus subspicatus* was significantly reduced (approx. 50% at 1:1 dilution). Nevertheless, the MBR effluent was still toxic to *D. subspicatus* (24% growth rate inhibition) and the snail *Potamopyrgus antipodarum* (18% mortality), slightly enhanced the reproduction of the snails (Fig. 3) and showed distinct anti-estrogenic activity in the YAES. To a certain degree this is in line with the chemical analysis of a broad set of 72 micropollutants - mainly pharmaceuticals and musk fragrances - showing a pronounced substance-specific elimination. For about half of the detectable micropollutants the elimination by the primary treatment step (MBR) was less than 50%. Even if most of these compounds could be eliminated below the limit of detection by the advanced treatment steps ozonation as well as powdered activated carbon adsorption there were still toxic effects detectable. Four bioassays (*P. antipodarum* reproduction test, *L. variegatus* toxicity test, *D. subspicatus* growth inhibition test and *D. magna* population test) evidently showed an increased toxicity after the ozonation of the suspended solids free MBR effluent (Fig. 3). These are strong indicators of adverse effects induced by transformation products being generated during ozonation. It was also observed, that the reversibility of these effects by a subsequent post-treatment was much less pronounced in comparison to published data for the ozonation of secondary effluent from municipal wastewater treatment plants [2, 3, 4]. This might be explained by the challenge to establish an effective biologically active sand-filter following double-stage disinfection (MBR, ozonation). Figure 3. *Lumbriculus variegatus*. Dry biomass per replicate (A). *Potamopyrgus antipodarum*. Total number of embryos per female (B). Green algae *Desmodesmus subspicatus*. Mean growth rate of 3 independent tests (C). Abbreviations: C, control; F, after MBR; O, after ozonation; OS, after sand filtration and ozonation; SEM, standard error of the mean; Significant differences to C are indicated with asterisks inside bars, between the treatment groups with asterisks above bars (t-test : □, p<0.05, □□, p<0.01, □□□, p<0.001; A: n=5, B: n=30-44; C: n=3). Even if non-specific bioassays prevent direct cause-and-effect relations with certain micropollutants chemical analysis support the interpretation of the observed mixture effects within this study. Furthermore, due to the use of non-enriched original wastewater samples as well as on-site flow-through systems the loss of insufficiently extractable polar transformation products can be excluded.

SCIENCE-BASED APPROACHES TO PRIORITISE THE ENVIRONMENTAL RISKS POSED BY LEGACY HUMAN MEDICINAL PRODUCTS

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Background and objective : There are approximately 1500 Active Pharmaceutical Ingredients (APIs) in use today. However, the environmental risk of only a small proportion of these APIs has been assessed. Our ability to detect trace levels of pharmaceuticals in environmental matrices is also outpacing our capacity to assess these data within the context of environmental risk. Consequently, there is a real need for scientifically sound and pragmatic approaches to prioritize legacy APIs based on potential environmental risk for additional experimental testing or assessment under established regulatory guidelines. Over the past decade, several prioritization approaches have been proposed, primarily by the research community, for use on APIs and these have been used to identify substances for further scrutiny. These approaches vary in the methodologies used: some are exposure-based, some hazard-based and some take a risk-based approach; the majority of approaches have focused on aquatic exposures with only a few considering potential impacts in terrestrial and benthic environments; few have considered exposure of birds and mammals; and general approaches have been restricted to specific geographical regions and environmental compartments. As existing experimental data are only published for a small proportion of pharmaceuticals in use, most prioritization processes rely on existing data or information on read across from the human pharmacology and pre-clinical safety data and/or predictive models such as quantitative structure-activity relationships (QSARs). While our knowledge around human to environment read-across has increased over the past few years, there are still many uncertainties. The applicability of many of the predictive models that have been applied is questionable, particularly given that the majority of APIs are ionogenic in nature while most predictive models in use have been developed for neutral organic compounds.

Methods and results : Within the Innovative Medicines Initiative (IMI) project iPiE we have reviewed the strengths and weaknesses of the existing approaches that have been used to prioritise the environmental risks and hazards of human APIs. While many of the previous prioritization tools present useful concepts for prioritization, given the limited scope of many schemes and the reliance on models that have not validated for APIs or which may be completely inappropriate, there is a need for a more holistic and scientifically robust approach. Based on previous experiences, we would argue that such a prioritisation approach should (i) be primarily risk-based but also consider persistence, bioaccumulation and toxicity concerns within the risk-ranking, (ii) focus on patient use in a manner that captures changes in patient use over time; (iii) consider the aquatic, benthic and terrestrial environments; (iv) address standard acute, chronic and mode of action specific endpoints; (v) combine predictive and experimental approaches; (vi) maximise the use of preclinical data; (vii) be tiered and flexible; and (viii) be validated with appropriate quality assurance metrics.

Discussion and conclusion : This presentation outlines a potential approach, that meets the criteria described above, that could be employed by the iPiE project. This approach pulls together some of the approaches that have been used previously but is much more holistic in terms of the compartments and risk-endpoints considered. It is our expectation that the approach could be parameterised and applied to different geographical regions to reflect differences in the API emission pathways to different compartments into the natural environment, levels of treatment, treatment connectivity and characteristics of the receiving environment. The approach advocates the use of pharmacology and toxicology data in combination with in silico, in vitro and in vivo models and information on species traits (e.g. on presence/absence of receptors) to derive effect concentrations for apical and non-apical effects in different taxonomic groups in aquatic and terrestrial systems. Similarly, API usage data and MEC data are used alongside ADME information and in silico and in vitro data to estimate the concentrations of an API in different environmental media (surface waters, sediments, soils, organisms and plasma). Assays advocated using the approach could include in vitro gill cell uptake (Stott et al., 2015; Schnell et al., 2015) and in vitro hepatic metabolism (Baron et al., 2012). To operate the approach, a range of parameters will be required. In this presentation, we will make a first assessment of whether these parameters are likely to be available for APIs in use and, if not available, provide an assessment of the availability of good predictive models/approaches for a particular parameter. Potential useful data that might help to derive selected

parameters will also be identified. This is very much a first analysis but we envisage that this will provide a basis to promote discussions across the IMI iPiE project and the broader stakeholder community to ensure that the method development work is focused and delivers the approaches needed for prioritization of legacy APIs in the future. References 1. Baron, M.G., Purcell, W.M., Jackson, S.K., Owen, S.F., Awadhesh N. Jha. 2012. Towards a more representative in vitro method for fish ecotoxicology: Morphological and biochemical characterisation of 3-dimensional spheroidal hepatocytes. *Ecotoxicology* 21 (8), 2419-2429. 2. Schnell, S., Stott, S.C., Hogstrand, C., Wood, C.M., Kelly, S.P., Pärt, P., Owen, S.F., & Bury N.R. (2015). Procedures for the reconstruction, primary culture and experimental use of rainbow trout gill epithelia. *Nature Protocols* (Accepted Sept 2015). 3. Stott L.C., Schnell S., Hogstrand C., Owen S.F., Bury N.R. (2015) A primary fish gill cell culture model to assess pharmaceutical uptake and efflux: Evidence for passive and facilitated transport. *Aquatic Toxicology* 159, 127–137. This work has received support from the EU/EFPIA Innovative Medicines Initiative Joint Undertaking (iPiE grant n° 115735)

TOWARDS A VIRTUAL FISH ECOTOXICOLOGY LABORATORY

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Background and objective : All new active pharmaceutical ingredients (API) must undergo an environmental risk assessment (ERA) before being authorised. Currently tens of thousands of fish are used worldwide as part of API ERAs. Development of predictive in silico models already has the potential to significantly reduce animal use (3Rs) and reduce R&D costs around the ERA of pharmaceuticals. These models, when combined with recently developed in vitro bioassays, can be used to prioritise risk prospectively. The objective of our work is to develop an evidenced-based approach that uses data derived from in silico and in vitro models to predict the movement of an API from the patient through to aquatic systems, the subsequent impacts on the ecosystems and to determine risk to both apical end-points (e.g. impacts on fish reproduction and growth) and non-apical end-points (e.g. effects on behaviour).

Methods and results : Our approach will characterise the movement of an API from the patient, through the wastewater and river systems, into fish tissues and predict the apical and non-apical effects on individuals and populations. By understanding the pathway from patient to effect, it will be possible to develop an optimum experimental testing strategy for an API. While the focus will be on APIs, we anticipate that the system will also be applicable to other bio-active molecules, such as pesticides, biocides and consumer products e.g. printing ink to replace and refine the application of animals in regulatory toxicology studies.

Discussion and conclusion : The aim of our work is not purely to replace animal models with equivalent in silico and in vitro models; rather, it is to produce better systems that more accurately predict efficacy, safety and environmental effects and to refine the planning/execution of in vivo studies, thus reducing the overall number of tests needed in fish. In the EU, an estimated 200,000 fish per year are used in in vivo toxicology tests and in the USA, 2-6 million, for all chemicals. The range of technologies that will be developed includes computational and mathematical methods to produce evidenced in silico and in vitro models. We anticipate that our work will increase the development and commercialisation of non-animal technologies and testing systems that better predict human and animal responses to, and the environmental effects of, chemicals and new molecular entities, including human and veterinary APIs, on ecosystems.

ANALYSIS OF TRACE LEVELS OF PHARMACEUTICALS AND DRUG OF ABUSE IN DRINKING, INLAND SURFACE AND WASTEWATER USING ON-LINE ENRICHMENT AND LC-MS/MS

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Background and objective : For some time now it has been raising interest in the analysis of pharmaceuticals and drug abuse (Petrovic, 2005). These compounds along with personal care products (PCP) are among those who have been called "emerging contaminants". Pharmaceuticals and drugs of abuse are continuously discharged to the environment as a result of manufacturing processes, the disposal of products that have expired or not used and the excretion of ingested pharmaceuticals and drugs of abuse. As they are from anthropogenic origin, their analysis not only can be useful to monitor the environment, but also the health and drug consumption patterns of a city (Bones, 2007). Several studies to determine their concentrations in the environment have been conducted (Borton, 2007). All of them highlighted the need for liquid chromatography with mass spectrometry detection, due to the complexity of the matrices that are studied (Hao, 2006). Since these analytes are commonly found in low concentrations, almost all of the methods in the literature contemplate a stage of sample pre-concentration (Thurman, 2010). Usually this stage is "off-line" prior to the injection of the sample. In our case we have developed an on-line solid phase extraction enrichment step (on-line SPE), which allows the injection of a relatively large sample volume (2 ml) which is pre-concentrated on a mini-column and then eluted and injected into the chromatograph. This system coupled to LC-MS/MS, allows great process automation as well as the levels of sensitivity and selectivity required for pharmaceutical and drug of abuse determination.

Methods and results : An Agilent 1200SL binary pump (Agilent, Palo Alto, CA, USA) (Pump 2. Figure 1.1) was coupled to a hybrid triple quadrupole / linear ion trap mass spectrometer (3200 QTRAP, Applied Biosystems-Sciex, Foster City, CA, USA) operated with a orthogonal ESI interface in positive ionization mode (Figure 1.2). On-line sample enrichment was performed using an Agilent 1200 pump and a Strata-X cartridge (2 x 20mm, 25µm) from Phenomenex (Torrance, CA, USA) (Pump 1. Figure 1.1). This equipment also includes a PAL autosampler (CTC Analytics, Switzerland). Chromatographic separation was performed on a reversed-phase column Zorbax Eclipse XDB-C18 (50 x 4.6 mm, 1.8µm) from Agilent (Figure 1.1). In this work it was difficult to find completely free of pharmaceutical and drug of abuse wastewater samples. Based on this experience and the literature, it is very likely that most of the samples to be analyzed for these parameters present some of them. The validation of the developed technology, on-line SPE coupled to LC-MS/MS was carried out fortifying the various matrices at several concentration levels. For pharmaceuticals in drinking water was fortified at the limit of quantification (0.010 µg/L) (n=4), inland surface water at a medium level (0.070 µg/L) (n=4) and wastewater at the limit of quantification (0.20 µg/L) (n=4) and at high concentration level (1000 µg/L) (n=4). For drugs of abuse in drinking water was fortified at the limit of quantification (0.002 µg/L) (n=4), inland surface water at a medium level (0.005 µg/L) (n=4) and wastewater at high concentration level (1000 µg/L) (n=4). The results of the validation study are shown in table 1.1. The accuracy and precision shown are the maximum values obtained in the different matrices and levels fortified. We can see that in all cases the accuracy was above 85% and in most cases much better. The precision was in all cases better than the 13%. Uncertainties of the method were calculated and in all cases it was less than 25%. The limits of quantification (LOQ) were set at 0.010 µg/L for most pharmaceuticals and 0.002 µg/L for most drugs of abuse in drinking and inland surface water (Table 1.1). In Wastewater, due to the complexity of the sample, a previous dilution was needed, so the LOQ were set at 0.20 µg/L for pharmaceuticals and 0.040 µg/L for most drugs of abuse in wastewater (Table 1.1). The limits of detection LOD were calculated as three times the standard deviation of the results at the fortified level of 0.010 µg/L, and they are much lower than LOQ (table 1.1). Afterwards, a study was made (Boix, 2015) comparing the results of inland surface and wastewater samples analyzed with this validated on-line method and a direct injection UHPLC-MS/MS method validated by Boix. The results showed very good correlation between both methods, proving the robustness of the developed methodology. Table 1.1 Results of the validation study of Drinking, Inland Surface and Wastewater samples. Limit of Detection (LOD), Limit of Quantification (LOQ), Uncertainty (U), Accuracy (A) and Precision (P) are shown. Pharmaceuticals and drugs of abuse Drinking Water Wastewater Drinking and inland surface Water Wastewater U A P LOD (µg/L) LOD (µg/L) LOQ (µg/L) LOQ (µg/L) (%) (%) (%) Clarithromycin 0.001 0.05 0.010 0.20 25 92 8 Enalapril 0.002 0.03

0.010 0.20 25 92 6 Enrofloxacin 0.003 0.02 0.010 0.20 25 94 10 Erythromycin 0.003 0.03 0.010 0.20 25 96 10
 Flumequine 0.003 0.04 0.010 0.20 25 89 10 Ofloxacin 0.003 0.04 0.010 0.20 25 89 9 Pantoprazole 0.001 0.05
 0.010 0.20 25 90 7 Pefloxacin 0.004 0.09 0.015 0.30 25 88 9 Sarafloxacin 0.005 0.07 0.010 0.20 25 89 13
 Sulfamethoxazole 0.001 0.05 0.010 0.20 25 91 8 Venlafaxine 0.003 0.06 0.010 0.20 25 92 9 Ciprofloxacin 0.003
 0.05 0.010 0.20 25 85 9 Furaltadone 0.001 0.04 0.010 0.20 25 93 6 Norfloxacin 0.002 0.02 0.010 0.20 25 94 10
 Oxolinic Ac. 0.002 0.07 0.010 0.20 25 91 10 Nalidixic Ac. 0.002 0.01 0.010 0.20 25 91 9 4-Aminoantipyrine
 0.003 0.03 0.010 0.20 25 85 13 Acetaminophen 0.003 0.11 0.020 0.40 25 92 9 Naproxen 0.005 0.10 0.020 0.40
 25 93 10 Ketoprofen 0.003 0.06 0.010 0.20 25 95 9 Diclofenac 0.002 0.04 0.010 0.20 25 93 7 Amphetamine
 0.0003 0.024 0.005 0.100 25 92 8 Benzoylcegonine 0.0003 0.012 0.002 0.040 25 93 11 Cocaethylene 0.0003
 0.009 0.002 0.040 25 90 8 Cocaine 0.0003 0.010 0.002 0.040 25 93 8 MDMA 0.0003 0.007 0.002 0.040 25 93 6

Discussion and conclusion : We conclude that it can be performed the analysis of pharmaceuticals and drugs of abuse in drinking, inland surface and wastewater accurately, precisely and with very low limits of quantification by the proposed method of large volume injection with on-line enrichment and tandem mass spectrometry detection. Furthermore, this method is able to determine some substances belonging to the Watch list for Union-wide monitoring in the field of water policy pursuant to Directive 2008/105/EC (Diclofenac, Erythromycin, Clarithromycin), and many others that could be incorporated.

HOW TO SCREEN HUNDREDS OF PHARMACEUTICAL RESIDUES IN A SINGLE ANALYSIS OF WATER SAMPLES

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Background and objective : Pollution of environmental waters by pharmaceutical residues is a real public health concern which implicates the screening of a large number of emerging pollutants for the control of water quality. However, the available analytical methods are generally limited to a predetermined and restricted list of molecules. The development of novel, fully automated pollutant screening methods are thus highly desirable.

Methods and results : A library of 750 molecules representative of the principal classes of pesticides and drugs found in the environment was build. A database gathering information on fragmentation spectra, retention time and exact mass was created. The confrontation between these database and the information of pollutants detected in water samples then allows the identification and semi-quantification of more than 700 molecules in a single analysis of only 5 ml of water. The developed screening method is based on integrated sample preconcentration and liquid chromatography coupled to high resolution mass spectrometry (SPE-UHPLC-HRMS). Analyses of surface and ground water showed a high diversity of emerging pollutants. Up to 60 pharmaceutical residues and metabolites were detected in a study on water from French rivers. In 40 ground water samples from different area in France, the number of emerging pollutants could reach 37. A study on tap water samples collected in and around Paris showed the presence of an average of 16 pollutants per sample with concentrations below the 0.1 µg/L, the European Union limit for drinking water.

Discussion and conclusion : A new multi-residues method allowing the detection and semi-quantification of more than 700 molecules, among which half were drug residues and their metabolites was developed. To the best of our knowledge, it is one of the most extensive in terms of pharmaceuticals in water. The development of an in-house database allows the reliable identification of the detected pollutants. The studies of various waters showed the presence of a large number of pharmaceuticals and their metabolites. Given these preliminary results, it would be interesting to evaluate at a larger scale different water resources in order to characterize more thoroughly their pollution state. If this screening method has the potential to contribute significantly to mapping of known and emerging water pollutants, the acquired data could as well be used for post-target and non-target analysis of chemical fingerprints.

DEVELOPMENT OF LC-MS/MS METHODS TO STUDY DICLOFENAC AND SOME TRANSFORMATION PRODUCTS IN BIVALVE, FISH, SEDIMENT AND WATERCRESS. APPLICATION TO A CONTAMINATED MODEL ECOSYSTEM

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Background and objective : Pharmaceuticals are ubiquitously found in the aquatic environment, mainly at trace levels, however long-term exposure can have negative impacts on biotic communities due to the intrinsic biological activity of these drugs. Diclofenac (DCF) is one of the most frequently detected human pharmaceuticals in water and it has recently been included on the “watch” list of the European Union. Nevertheless little data deals with the the detection of this substance and its transformation products in aquatic organisms. In this context, analytical methodologies based on LC-MS/MS analysis, after a QuEChERS or an ASE extraction, were developed to quantify traces of DCF along with nine of its biotic and abiotic transformation products in bivalve, fish, sediment and watercress collected from lotic mesocosm experiments.

Methods and results : The analytical protocol was optimized and validated for each matrix of interest separately: sticklebacks, zebra mussels, sediment and watercress. Uncontaminated matrices were used for the optimization steps, they corresponded to the matrices introduced into the mesocosms before the contamination with DCF. Considering the great diversity of transformation products and metabolites, a focus was made in this study on chemically stable and commercially available substances. The developed methods were next applied to samples collected after a 6-months exposure in mesocosms where DCF was continuously introduced at three concentrations (5; 0.5 and 0.05 µg/L).

Discussion and conclusion : The QuEChERS extraction was implemented for all matrices, except for sediment where best results were obtained with ASE extraction. DCF was quantified in zebra mussels and sediment for both highest exposure conditions and in watercress solely for the highest concentration. In sticklebacks DCF resulting concentration was inferior to our quantification limits probably due to rapid depuration as fishes were collected one week after the end of experiments. Among the selected metabolites, 4’OH-DCF was the most often quantified : it was observed in sticklebacks, sediment and watercress for the highest exposure concentration. The metabolites DCF-lactam and 5-OH-DCF were also measured as well as the 2-indolone either in sediment, watercress or bivalves. In this presentation, the optimization of the extraction and clean-up steps will be described. The results of the quantification of DCF, along with nine of its degradation products and metabolites, in organisms and watercress collected from mesocosms will be presented.

POCIS PASSIVE SAMPLERS DEPLOYMENT TO MONITOR VENLAFAXINE AND THEIR TRANSFORMATION PRODUCTS IN MARINE ENVIRONMENT.

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Background and objective : Pharmaceuticals have come into focus due to their continuous release into the environment and their biological activity. Venlafaxine (VEN) is an antidepressant drug prescribed for the treatment of clinical depression and anxiety disorders. After human consumption, VEN and its metabolites are excreted in wastewater and enter the sewage treatment where they are partially removed. VEN has been regarded as a potential tracer of emerging contaminants in surface water due to its poor elimination (about 40%) in wastewater treatment (WWTP) and its high resistance to biodegradation. In human, VEN is mainly metabolized in its active metabolite O-desmethylvenlafaxine (ODV) (30%). Other minor pathways lead to the formation of four metabolites, N-desmethylvenlafaxine (NDV), N,O- didesmethylvenlafaxine (NODDV), N,N- didesmethylvenlafaxine (NNDDDV) and N,N- didesmethyl-O-desmethylvenlafaxine (NNDDODV) (Figure 1). Regarding its aquatic fate, recent studies have indicated the presence of VEN in WWTP effluent [1], surface water [2] and even drinking water [3]. A recent study has reported bioaccumulation of VEN and metabolites in the vicinity of a WWTP outfall [4]. However, the occurrence of these substances in the marine environment still poorly documented [5]. The objectives of this work were to evaluate the occurrence and concentration of VEN and its metabolites in the marine environment. Thus, this study included (i) the development and validation of an analytical procedure for the semi quantification of VEN and its metabolites sampled with POCIS (ii) the determination of the uptake rates (Rs) of target compounds in salt water; (iii) the characterisation of contamination by these emerging contaminants and their transformation products in coastal areas receiving WWTP discharges.

Methods and results : Passive samplers Polar organic chemical sampling (POCIS) were used for qualitative and semi-quantitative evaluation of target compounds. POCIS were deployed near two pipeline outfalls during two-year survey monitoring, from January 2011 to January 2013 in the French Mediterranean coast, in the Gulf of Lion. The first pipeline outfall was submarine (300 000 inhabitants connected to this WWTP) and the second a surface pipeline outfall (1 million inhabitants connected to this WWTP). In total, eleven monitoring campaigns in the first sampling site and four monitoring campaigns in the second sampling site were conducted. Three sites were selected as reference sites. The extraction procedure of the POCIS was based on a previously developed method [6]. Analysis was performed with liquid chromatography coupled to high resolution mass spectrometry (LC-MS/MS). Target analytes were detected at trace levels, with detection limits lower than 19 pg/L, except for NNDDDV (107 pg/L). In order to measure the uptake of venlafaxine and their metabolites in POCIS sampler, the sampling rate (Rs, L/d) of each compound was measured in a laboratory calibration study. Marine water free of target compounds was spiked at 5 µg/L of each compound and the concentration in water was measured during 7 days. The extraction and analysis protocol was similar to POCIS extraction. The objective was to measure the decline in sea water concentrations (Cw) compared to the initial concentration (C0) over exposure time ($\ln[C_w/C_0]$) using a model described previously [7]. The sorption of VEN and its metabolites in the POCIS showed a linear pattern over a period of 7 days. The determination coefficients (R²) were higher than 0.8320 for all studied compounds and statically significant. Rs values varied from 0.199 for ODV to 0.672 for NDV. In marine water, all target compounds were detected in every sampling. VEN and ODV were the most frequently detected, 88% and 67% respectively in site 1 and 94% for both in site 2. Maximum total concentrations (sum of all compounds) were 542 pg/L in site 1 (May 2012, West from the outfall) and 513 pg/L in site 2 (September 2012, North from the outfall). Concentrations observed in reference sites revealed a background contamination that varies between 10 pg/L (January 2012) and 53 pg/L (January 2013) in site 1 and from no detection to 63 pg/L in site 2.

Discussion and conclusion : Previous studies had reported variable Rs for VEN in fresh water ranging from 0.23 [8] to 0.894 [9]. Our result in salt water was in accordance with [10]. Rs values are also reported for two main metabolites of VEN: ODV and NDV. Rs for ODV was on the same order of magnitude (0.158) while a higher sorption was reported in our study for NDV. Four among the six target compounds have been frequently

detected : VEN, ODV, NODV and NNDDODV that is in accordance with excretion from human after consumption. It has been noted that the relative proportion of metabolites varied between the two sites. The hypotheses examined for the interpretation of the observed differences were the outfall type (submarine or surface) and the size of WWTP. A dispersion of contamination from the outfalls was revealed as can be observed, for example east from site 1 (Figure 2). Target compounds were quantified in reference sites distant of the WWTP outfalls showing the wide distribution of these compounds, suggesting VEN could be proposed as a potential tracer of human stress in urban environment. This study reports for the first time the results on the presence of VEN and its metabolites in sea water near WWTP outfalls. The occurrence of the target compounds revealed an active contamination of the coastal waters in the vicinity of WWTP outfalls and also far from them, as shown by the contamination of the reference sites. This highlights that a focus should be lead on the contribution of WWTP rejects in the marine environment and on contamination by emerging compounds like pharmaceuticals. The calibration experiment allowed us to estimate VEN and metabolites concentration in marine waters on the pg/L levels which are low but continuous.

USE OF PASSIVE SAMPLING (POCIS) FOR AN IMPROVED KNOWLEDGE OF PHARMACEUTICAL PRESENCE IN THE HEADWATER STREAMS: EXAMPLE OF 3 SMALL CATCHMENTS IN THE SOUTH-WEST OF FRANCE.

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Background and objective : During the last decade, attention had been paid to headwater systems due to their environmental interest (e.g. biodiversity, heritage species, quality of downstream waters). However, very little investigations were performed regarding the presence of pharmaceuticals in these rural area with a low population density which is the aim of this work.

Methods and results : Three headwater systems were selected and their main characteristics are displayed in table 1. Facing difficulties in interpreting data from spot water samples, a monitoring by passive sampling has been performed (Polar Organic Chemical Integrative Sampler, POCIS). Eighteen pharmaceuticals (7 antibiotics, 4 antiparasitic agents, 2 antifungals, 2 anti-inflammatory and 1 psychoactive drug) were measured after 15 days of POCIS field deployment. In addition to the quantification of target compounds, the non-target screening of other pharmaceuticals was performed thanks to a Time of Flight mass spectrometer after chromatographic separation (library of c.a. 5000 pharmaceuticals and their metabolites).

Discussion and conclusion : Pharmaceuticals were significantly detected only on the head of the watershed of Aixette and Arnac (Figure 1). Moreover on these 2 catchments, non-target screening showed the presence of sucralose and caffeine. The presence of these molecules seems to be linked to the discharge of treated effluents by a WWTP (wastewater treatment plant). In contrast, the headwaters of La Pude did not displayed a significant content of pharmaceutical residues which can be linked to the lack of a direct WWTP discharge of treated effluents. On the head of watershed of Arnac and Aixette, the content of pharmaceuticals is in the same order of pesticides (Poulier et al, 2014). Although the main micropollutants in the head of watershed with an intensive agriculture remains pesticides (Guibal et al, 2015), pharmaceuticals should also be considered in the local public policy.

SCREENING OF ORGANIC CONTAMINANTS IN SLUDGE AND CATTLE MANURE BY COMBINED USE OF CHROMATOGRAPHIC TECHNIQUES COUPLED WITH HRMS

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Background and objective : One of the technologies to avoid the discharge of cattle manure in the environment and to stabilize the sludge generated in the wastewater treatment plants is the anaerobic digestion (AD), which moreover results in biogas and biofertilizer. However, emerging contaminants (ECs) have been detected in these matrices and have to be deeply analyzed. Organic micropollutants, such as pharmaceutical and personal care products (PPCPs) and endocrine disrupting compounds (EDCs) are included in this category. Little information about ECs in soils is found in literature. [Stasinakis, 2012]. Full spectrum acquisition techniques such as HRMS, offer the possibility of screening a huge number of contaminants, even without reference standards, since the valuable information provided by HRMS allows reliable tentative identifications [Hernández, 2012]. In the last few years, the atmospheric pressure chemical ionization (APCI) source has been successfully implemented in GC-MS instruments, offering attractive features for screening. The availability of this source has allowed the combined use of GC and LC coupled to time-of-flight mass spectrometry (TOF MS). The use of a single TOF platform coupled to both GC and LC opens fascinating perspectives in the environmental field [Hernandez, 2015]. Hybrid quadrupole time-of-flight (QTOF MS), offers additional possibilities for identification or performing additional MS/MS experiments. The aim of this work is to evaluate the potential of QTOF MS coupled to both LC and GC for screening of more than 2.000 compounds in sludge and cattle manure samples. This is an innovative way to deal with the investigation of organic pollutants in complex matrices. As a result of this work, the knowledge of the key pollutants found, will guide the plan to monitor these substances in the project. The final goal will be to improve the chemical quality of the digested sludges, and satisfy both current and future legal requirements.

Methods and results : A hybrid quadrupole-orthogonal acceleration-TOF mass spectrometer (Xevo G2 QTOF, Waters Micromass, Manchester, UK) was interfaced to a Waters Acquity UPLC system (Waters, Milford, MA, USA) or to an Agilent 7890A GC system (Palo Alto, CA, USA), using a single instrument with an orthogonal Z-spray-ESI interface operating in positive and negative ion modes for LC and an APCI source in positive mode for GC. In this work, a LC homemade database has been built containing around 1,600 organic contaminants, including pesticides, pharmaceuticals, drugs, UV-filter agents, colorants, preservatives, and a notable number of degradation products. Regarding GC, the database contained around 560 compounds, including pesticides, PAHs, PCBs, PBDEs, fragrances, antimicrobials, UV filters, PFCs and PCNs. Measurements were made under MSE mode, which consist on a sequential acquisition of accurate-mass spectra at low and high energy in the collision cell. The presence of a chromatographic peak at the expected retention time, together with both the evaluation of the fragment ions and characteristic isotopic ions, allowed the unequivocal confirmation of the identity of the compound detected. It was also compared with the reference standard. When the reference standards were not available, collision induced dissociation fragments and characteristic isotopic ions were evaluated and used for tentative identification, based on structure compatibility and comparison with literature data. After a careful evaluation process, the reference standards were finally acquired and injected to confirm the compound.

Discussion and conclusion : In this work, the results obtained after the screening of the samples are presented. Most of them belonged to the group of pharmaceuticals, although some pesticides among other groups of compounds were also identified. These data are the basis for the next step in the project, since the results of the screening provide the information to select the substances to be monitored, throughout the different stages of the CavO3+DAG-TPAD process. The final purpose is to demonstrate the efficiency of the process in removing hazardous substances from the environment. This work has received funding from European Union's LIFE Programme under grant agreement LIFE14 ENV/ES/000150.

ANALYTICAL METHODS FOR THE DETERMINATION OF RESIDUES OF VETERINARY DRUGS IN ENVIRONMENTAL SAMPLES USING ON-LINE SOLID PHASE EXTRACTION COUPLED TO ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH TANDEM MASS SPECTROMETRY (SPE-UHPLC-MS/MS)

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Background and objective : Veterinary drugs are present in the environment at very low concentrations, in the ng kg⁻¹ and ng L⁻¹ level. Even when using high selective methods as UHPLC-MS/MS, sample preparation procedures are required to extract the analyte from the matrices, to reduce matrix effects and to enhance detectability. The aim of this study was to develop and validate simple methods using on-line SPE process coupled with UHPLC-MS/MS for determining antimicrobials and antiparasitic drug residues in soils and water.

Methods and results : The on-line SPE-UHPLC-MS/MS system used in this work consisted of an autosampler, a column manager, a quaternary and a binary solvent manager pump and a triple quadrupole detector. Two different SPE-columns: XBridge C8 (2.1 x 30 mm, 10 µm) and OASIS HLB (2.1 x 30 mm, 20 µm) were tested using on-line SPE at 30 °C. The analytes were separated in an Acquity UPLC BEH C18 analytical column (50 mm x 2.1 mm, 1.7 µm). Quantitation was performed in the selective reaction monitoring mode. For the method development different SPE sorbents, sample volumes and loading, wash and elution solvents were evaluated to achieve maximum recovery with minimum matrix effect. Five methods were developed and in house validated: (i) sulfonamides in soils, (ii) avermectins and milbeycin in soils, (iii) fluoroquinolones in soils and (iv) in water and (v) anphenicols in water. Before quantitation, the analytes were separated from the soil by solid liquid extraction, using water:acetonitrile (1:1 v/v) for the sulfonamides, acetonitrile for the avermectins and milbemycin and aqueous CaCl₂ 20% w/v, pH 9:methanol (75:25 v/v) for fluoroquinolones. Water samples were only filtered (0.22 µm) and injected directly in the SPE-UHPLC-MS/MS system.

Discussion and conclusion : The combination of the SPE on-line process to UHPLC-MS/MS enabled the establishment of simple and accurate methods for the determination of residues of veterinary drugs in soil and water, with the advantage of lower sample handling, lower costs and analysis time in relation to off-line SPE. All calibration curves presented linearities (r) higher than 0.99. The intra- and inter-precisions were lower than 15%. The limits of quantitation were: 0.5 ng g⁻¹ of sulfonamides, 0.05 ng g⁻¹ of avermectins and moxidectin and 0.1 ng g⁻¹ for fluoroquinolones in soils; 0.1 ng mL⁻¹ for anphenicols and (0.01 to 0.1 ng mL⁻¹) for fluoroquinolones in water. The methods were used to evaluate the concentration of abamectin in soils from an orange plantation and florfenicol in water tanks containing tilapias.

A NEW UHPLC-MS/MS ANALYTICAL METHOD TO ASSESS THE ENVIRONMENTAL AVAILABILITY OF SULFAMETHOXAZOLE AND THREE TRANSFORMATION PRODUCTS IN SOIL AMENDED WITH SOLID MANURE

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Background and objective : The recycling of manure in agriculture may lead to the transfer of antibiotics and their metabolites to cultivated soils. Antibiotics represent a high concern regarding potential impacts on soil microorganisms. The bioavailability of antibiotics to soil microorganisms could be assessed considering the whole system of soil, microorganisms and environmental conditions that could influence both antibiotics availability and/or microbial activity [J. Harmsen et al, 2005]. The objective was to develop a method suitable to measure the environmental availability of sulfamethoxazole (SMX) and three transformation products in soils amended with manure. Soft chemical extractions were therefore developed to target the molecule availability. Given their potentially low available amounts expected, the UHPLC-MS/MS analytical method was optimized to detect and quantify both SMX and derivatives in the extracts. The effect of storing soil samples at -20°C for one month was studied to know if the extraction and analysis could be postponed in the case of a large sample collection. Finally the method was applied to follow the availability of SMX and its transformation products in soil+manure mixtures over 3 months (ageing effect), in function of the initial SMX spiking concentration (dose effect).

Methods and results : Three extracting solutions were selected to extract the available fraction of SMX in manure-amended soils. Throughout the method development and its application, manure was spiked with SMX before its incorporation to the soil at a ratio of 3% in dry mass. The matrix effects and limits of quantification (LOQ) of the UHPLC-MS/MS method were determined for each molecule in each type of extract. The LOQ all ranged between 0.58 and 1.75 µg kg⁻¹ dry mass of soil+manure mixture for SMX and between 0.58 and 2.30 µg kg⁻¹ for the transformation products. Freezing soil samples led to a 20% reduction of the available SMX quantified in the extracts. Consequently, we decided to extract and analyze the available compounds only from freshly-sampled soils. Soil+manure mixtures with increasing SMX doses from a relevant environmental concentration [C1] (0.74 µg g⁻¹ manure dry mass) to [100 x C1], were incubated under controlled aerated conditions. The availability of SMX and transformation products was assessed after 0, 8, 28 and 84 days. The fractions of SMX recovered initially in the mixtures represented 1.9-8.6% of [C1] up to 16.9-63.3% for [100 x C1]. The available fraction of SMX decreased rapidly and reached only 2.2-5.3% after 8 days. After 28 days, SMX was no longer detected for [C1]. For the highest concentration [C1 x 100], only 0.9-2.0% and less than 1% of SMX were available after 28 and 84 days, respectively. Two transformation products were detected during the experiment, reaching a maximum equivalent to around 1% of introduced SMX.

Discussion and conclusion : Soft extractions were performed on fresh samples since freezing was found to decrease the extracted concentrations of SMX. The LOQ obtained for SMX and transformation products allowed quantifying their available fractions in soil+manure samples during a 3-month experiment. In order to assess the availability of SMX in real soil samples, and with regard to the rapid decrease of the antibiotic availability in this experiment, the soft extractions should be performed within the 8 days following the manure application. Moreover, one transformation product was quantified in the extracts just after the incorporation of the manure in the soil; its available fraction decreased as the fraction of the parent molecule. Therefore, SMX could be biodegraded during the days following the amendment [S. Larcher, V. Yargeau, 2011] when its availability is the highest. This might reflect the link between biodegradation and bioavailability. Finally, the assessment of the environmental availability of SMX could be also an indirect method to assess its bioavailability, especially if the measured concentrations are related to impacts of SMX on soil microorganisms.

ANALYSIS OF PHARMACEUTICALS IN SOIL AND ORGANIC WASTE PRODUCTS: AN EXPERIMENTAL DESIGN APPROACH TO STUDY THE FACTORS INFLUENCING THE EXTRACTION STEP

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Background and objective : Pharmaceutical products excreted by treated animals or humans are transferred to manure or wastewater, respectively. In wastewater treatment plants, they can be degraded and/or sorbed to sludge. Therefore pharmaceuticals may be indirectly disseminated into the environment from the recycling of organic waste products in agriculture. In order to evaluate the potential ecotoxicological effects of the pharmaceutical residues in the environment, it is necessary to develop analytical methods for their quantification. Several different extraction conditions are proposed in the literature and most of them have been developed for restricted groups of pharmaceuticals. It is therefore difficult to select the best method to extract pharmaceuticals in the context of multi-residue analysis. Our objective was to determine the influencing factors on the extraction recovery of pharmaceuticals by the experimental design approach, with emphasis on the extraction medium. We selected pharmaceuticals with various physical-chemical properties and representative of different families, to make possible the extension of the conclusions to other compounds belonging to the same families. Two contrasted solid samples were chosen: soil and sewage sludge.

Methods and results : Fourteen pharmaceuticals including eight antibiotics were extracted from solid samples by Ultrasonic-Assisted Extraction (UAE), purified according to an adapted QuEChERS method and analysed by online solid-phase extraction coupled to ultra-high performance liquid chromatography and tandem mass spectrometry (online SPE-UHPLC-MS-MS) [1]. Three successive experimental designs were performed for each matrix. Preliminary experimental designs allowed appreciating the influence of four parameters presumed to be crucial for UAE efficiency: the nature of the organic solvent and its proportion, the nature of the aqueous phase (buffers, modifying agents) and its pH. A response surface design was then performed to better understand the influence on the extraction recovery of the organic solvent proportion in the extractant, the amount of ethylenediaminetetracetic acid (EDTA) added to the buffer and the final pH of the buffer, as well as the interactions between these factors. Our results showed a great influence of interactions between factors for all the fourteen studied pharmaceuticals, responsible for the shapes of response surfaces. Experimental designs were also used to compromise and propose two methods for multi-residue analysis of pharmaceuticals in soil and sewage sludge. The methods were validated with the accuracy profile methodology [2].

Discussion and conclusion : The experimental design approach was a powerful tool to demonstrate that not only parameters of the extraction medium but also interactions between these parameters influence the extraction recovery. The results were explained considering the nature of the solid matrices and the physical-chemical properties of the pharmaceuticals, mainly considering the ionisation state of the pharmaceuticals, their solubility in the extraction medium and their interactions with the solid matrix constituents. Experimental designs also allowed optimising extraction methods that were relevant to analyse environmental samples (see the presentation of Houot et al.). [1] M. Bourdat-Deschamps et al. *J. Chromatogr. A* 1349 (2014) 11–23 [2] S. Ferhi et al. *Anal. Bioanal. Chem.* (2016) submitted.

MYTILUS GALLOPROVINCIALIS (MARINE MUSSEL) AS BIOINDICATOR OF FLUOXETINE CONTAMINATION: BIOCONCENTRATION, METABOLIZATION AND ENVIRONMENTAL OCCURRENCE

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Background and objective : Fluoxetine is among the most prescribed antidepressants nation and worldwide. Within other effects, disruption of invertebrate endocrine systems by increasing the bioavailability of serotonin has been described. We aimed to assess fluoxetine accumulation and metabolization into norfluoxetine in exposed *Mytilus galloprovincialis*, considered an excellent sentinel species for emerging pollutants; and evaluate the use of these marine mussels as fluoxetine bioindicators and as a tool for environmental monitoring in aquatic environments.

Methods and results : Control and exposure treatment (to 75 ng.L⁻¹ of fluoxetine) mussels were removed (n=10) at each set up time (0, 3, 7, and 15 days) from each aquaria for analysis. Moreover, mussels collected from 8 different zones along the Portuguese coast during 6 monitoring campaigns (one year, 2014-2015), totalizing 49 composite samples (each consisting of a pool of 25 mussels) were analyzed. 1g of freeze-dried and grounded mussels was spiked with surrogate standards and extracted with acetonitrile with 0.1% formic acid. Extracts were then loaded into Oasis MCX cartridges and quantified by LC-MSn. Neither compound was detected in un-exposed mussels. Both accumulation and metabolization increased along the exposure period. Fluoxetine detection frequency and mean accumulation, in dry weight (dw), were of 70% and 2.53 ng.g⁻¹ (d3); 80% and 4.43 ng.g⁻¹ (d7); and 100% and 9.31 ng.g⁻¹, respectively. Norfluoxetine frequencies and means were of 10% and 3.06 ng.g⁻¹ (d3); 50% and 2.85 ng.g⁻¹ (d7); and 100% and 11.65 ng.g⁻¹ (d15), respectively. Fluoxetine BCFs and norfluoxetine pseudo-BCFs increased up to 124, and 155, respectively. Fluoxetine kinetic BCF was of 129 (steady state would be reached at d52). Regarding the environmental monitoring data, fluoxetine was found in 40% of the samples in levels (dw) ranging from 2.25 to 9.93 ng.g⁻¹. Norfluoxetine found in 42% of the samples, but in higher levels, ranged between 3.62 and 21.66 ng.g⁻¹.

Discussion and conclusion : Fluoxetine accumulated in mussels is likely metabolized into norfluoxetine with the increase of exposure, suggesting that at this realistic environmental concentration (75 ng.L⁻¹), toxic effects may occur. Moreover, since norfluoxetine is more active than fluoxetine, this metabolization potentiates the biological effects. The presence of fluoxetine in the aquatic environment is commonly associated with the impact of WWTPs, causing growing pressures in heavily populated coastal areas. Discrete seawater sampling may not be adequate to assess such environmental risk. In fact, data on fluoxetine uptake and metabolization by mussels collected along the Portuguese coast proved that these bivalves are good indicators of the presence of this pharmaceutical.

HUMAN EXPOSURE TO CARBAMAZEPINE VIA PRODUCE IRRIGATED WITH TREATED WASTEWATER: A RANDOMIZED CONTROLLED STUDY

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Background and objective : Fresh water scarcity has led to increased use of treated wastewater as an alternative source for crop irrigation. Concerns have been raised regarding pharmaceutical exposure via treated wastewater. Our aim was to assess whether carbamazepine, an anticonvulsant drug highly persistent in wastewater, is present in commercially available treated wastewater-irrigated produce, and whether human exposure to carbamazepine occurs via ingestion of this produce.

Methods and results : We recruited 34 volunteers aged 18-63 years (20 women and 14 men) to a single blind crossover trial. Group I (n=22) was randomized to receive treated wastewater-produce followed by fresh water-irrigated produce; Group II (n=12) received fresh water irrigated-produce followed by supermarket-purchased produce. Each exposure period lasted one week. During the trial, subjects filled food frequency questionnaires and provided urine samples. Carbamazepine and metabolite levels were measured in produce and human urine, following development of a novel method to extract and measure metabolites in urine matrix. We assessed the proportion of individuals with urinary levels above the LOD or LOQ and area under the curve over the study period.

Discussion and conclusion : Treated wastewater-irrigated produce exhibited substantially higher carbamazepine levels than fresh water-irrigated produce. At baseline, urinary carbamazepine was undetectable in 13, between LOD and LOQ in 12, and >LOQ in 9 subjects. Following seven days of consuming treated wastewater irrigated-produce all Group I members exhibited quantifiable levels of carbamazepine, while in Group II the distribution remained unchanged from baseline (between group $P < 0.001$). Area under the curve of carbamazepine excretion was markedly higher in Group I versus II ($P < 0.0001$). Urine levels return to baseline following the exposure periods. This study demonstrates "proof of concept" that human exposure to pharmaceuticals occurs through ingestion of commercially available treated wastewater-irrigated produce, providing data which could guide policy and risk assessments.

DETERMINATION OF ELEVEN THYROID HORMONES AND ASSOCIATED METABOLITES IN PLASMA AND TISSUE: DESCRIPTION OF ANALYTICAL METHOD AND ECOTOXICOLOGICAL CASE STUDIES.

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Background and objective : Thyroid hormones, such as thyroxine (T4) and 3,3',5-triiodothyronine (T3), are vital in numerous physiological processes (e.g. embryonic development, metabolism, cell differentiation and proliferation, cognitive development, and thermogenesis). However, little is known regarding how thyroid hormones affect stress regulation and behavior. Many factors control circulating levels of the bioactive hormone (T3), consequently not only T4 and T3 measurements are vital, but also so-called inactive thyroid hormone metabolites are necessary for a comprehensive description of homeostasis. Circulating thyroid hormones in plasma are typically in low ppt-levels, and can be used as a diagnostic tool during e.g. pregnancy, or for hypothyroidism, hyperthyroidism and endocrine disruption diagnosis. A prerequisite for investigating thyroid hormone disrupting effects is a need for establishing highly sensitive analytical methods. In the present work, we describe an isotopic-dilution LC-MS/MS methodology to determine eleven thyroid hormones and metabolites in 'pico-gram' levels in plasma and tissue from wildlife. The protein-unbound fraction of hormones is largely recognized as the circulating 'bioavailable' fraction. Consequently, free and total thyroid hormone concentrations in blood and plasma are evaluated. Finally, we apply the developed methodology to investigate thyroid hormone levels in individual tadpoles (*Xenopus laevis*) ranging from NF stages 55-61 and in plasma from adult *X. laevis*, both from controlled in-vivo studies, and in wildlife samples (e.g. whale, fish, and amphibian).

Methods and results : Method detection limits are 3.5 pg T4, 1.5 pg T3, 2.9 pg rT3, 1.7 pg 3,3'-T2, 2.3 pg 3,5-T2 and between 0.3-7.5 pg for the remaining six metabolites in 50 µL aliquots of blood sera or plasma. We successfully, applied the novel methodology on blood obtained from amphibian (controlled exposure studies) and free-ranging whales.

Discussion and conclusion : We found not only thyroxine (T4), but also up to six other metabolites are present in tadpole serum. This method will enable researchers to improve the assessment of thyroid homeostasis and endocrine disruption in animals and humans.

IMPACT OF PHARMACEUTICALS DISCHARGES ON THE RECEIVING ENVIRONMENT: RESULTS OF THE LONG-TERM MONITORING PROJECTS SIPIBEL AND IRMISE

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Background and objective : Chemical water pollution is more and more studied and documented. Among these pollutants, drugs are special contaminants, due their very low concentrations in the environment and the potential presence of metabolites. Even though effluents from sewage treatment plants are well known to be one of the major sources for introduction of pharmaceuticals into the aquatic system, contributions of significant sources such as hospitals or agriculture are still unclear. Furthermore, although their behavior in wastewater treatment plants (WWTP) is well documented, many fewer studies are devoted to their fate in the receiving environment.

Methods and results : This work consists in a long-term regular monitoring of fifteen pharmaceutical residues, from treatment plant influents and effluents, including ones from a hospital, to groundwater intended for drinking water production, at the Arve river basin scale. Influent and effluent were sampled from the Bellecombe WWTP, called SIPIBEL, where domestic and hospital wastewaters are treated separately. Target pharmaceuticals were selected according to their consumption at the hospital, as well as their high occurrence and ubiquity in the aquatic environment, and their risk for human health and the environment due to their toxicity or bioaccumulation. This list is composed of a wide variety of substances, including, analgesics, anti-inflammatories, anti-epileptic, steroid, and antibiotics. Presence of diclofenac and sulfamethoxazole metabolites was also studied. Thanks to the use of advanced analytical techniques, limits of quantification below 10 ng/L, consistent with ultra-traces detection, were achieved.

Discussion and conclusion : Ten substances were always quantified in wastewaters. Influent analysis showed significantly higher concentrations in the hospital wastewaters of four pharmaceutical substances: an anti-inflammatory, ketoprofen, and 3 antibiotics ciprofloxacin, sulfamethoxazole, and vancomycin. But, in terms of mass flows, the hospital contribution appeared relatively low compared to domestic discharges, except for ciprofloxacin and vancomycin. Removal efficiencies were higher than 50%, except for two compounds, diclofenac and carbamazepine. However, variability was observed according to the season, and higher removal efficiency was obtained for the hospital wastewater, probably due to different hydraulic retention times. Eight molecules, quantified in WWTP influent and effluent at $\mu\text{g/L}$ levels, were also detected in the receiving environment, which is the Arve River, in concentrations between the ng/L and 100 ng/L for acetaminophen. Three substances, among the eight, were also quantified in groundwater, particularly sulfamethoxazole, at the order of 2 ng/L . This substance is very resistant to water treatments, including those made for drinking water production and therefore may be a good anthropogenic indicator.

PHARMACEUTICALS IN WATERS OF THE PEARL RIVER CATCHMENT, CHINA

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Background and objective : Pharmaceuticals in the environment have attracted increasing concerns due to their potential impact on ecological systems. Occurrence, stereoisomeric (enantiomeric) compositions, behavior and fate of a variety of pharmaceuticals including antibacterials, antiviral, antifungals, non-steroid anti-inflammatory drugs, lipid regulators, β -blockers, antiepileptic drug carbamazepine and its metabolite, and X-ray contrast agent iopromide were investigated in municipal wastewater, river water, and groundwater in the Pearl River catchment, China.

Methods and results : The pharmaceuticals were widely present in the wastewater, whereas their fate varied depending on the compound. In the untreated wastewater, fluoroquinolones were generally the most abundant with a maximal concentration up to 6415 ng L⁻¹ observed for norfloxacin. A median percentage of 67% of sulfonamides and 86% of macrolides remained in the final effluent after treatment in sewage treatment plants (STPs) whereas approximately half of the fluoroquinolones finally ended up and persisted in sludge. In the river water, on the other hand, sulfonamides, trimethoprim, and macrolides were widely present whereas fluoroquinolones were only occasionally, probably due to their strong tendency of partition to sediment and/or photodegradability. Dehydroerythromycin and sulfamethoxazole were frequently detected in the groundwater. Azole antifungals had a maximum level of 1834 ng L⁻¹ (miconazole) in the wastewater. Fluconazole passed through the STP treatment and largely remained in the final effluent. Ketoconazole was more readily biotransformed with a transformation rate of 72±14%. Clotrimazole, econazole, and miconazole were more likely to be adsorbed onto and persisted in sewage sludge as shown by the mass balance and enantiomeric compositions. The azole antifungals were widely present in the rivers, generally non-racemic. Acyclovir was the only antiviral detected in the wastewater and only partly eliminated in the STPs during biodegradation. Acyclovir was also the only antiviral quantitatively detected in the Pearl River and its tributaries, with a maximum concentration up to 113 ng L⁻¹. Concentrations of the pharmaceuticals in the raw wastewater were mostly at ng L⁻¹ levels and were moderately to greatly removed/transformed during treatment in the STPs except carbamazepine which kept almost unchanged. Generally, biodegradation was the governing process for elimination of the investigated pharmaceuticals. The pharmaceuticals were widely detected in the river water.

Discussion and conclusion : Seasonal variations of the pharmaceuticals distribution in the wastewater are mainly ascribed to different consumption and the seasonal difference was smoothed out after treatment in the STPs. Dilution effect by precipitation plays an important role in seasonal distribution of the pharmaceuticals in the river water. The pharmaceuticals in the groundwater did not show significant seasonal differences. Overall, the pharmaceuticals in the river water and groundwater posed low to high ecological risks and may be of serious concern.

STUDY ON HUMAN AND VETERINARY ANTIBIOTICS IN RAW AND TREATED WATER FROM A MAJOR FRENCH RIVER BASIN

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Background and objective : During the latest years, the interest on the presence of micropollutants in water resources, and especially in drinking water, has been growing up, particularly due to the increase of media pressure. Pharmaceuticals represent one of the main targets regarding the public awareness and among them, veterinary compounds are largely used in high amounts worldwide. According to ANSES (French National Agency for the Sanitary Safety of Food, the Environment and Labour), pigs and cattle are the largest consumers of veterinary medicines. Moreover, in the human society, the consumption of pharmaceuticals appears in constant increase. The main objectives of this work were to determine the presence of veterinary medicines in the Seine-Normandy basin, where the livestock breeding is widely developed, and to evaluate their behaviour along drinking water treatment plants (DWTP). In France, cattle breeding represents 85 % of livestock breeding and is particularly present in the Basin of Seine-Normandy where 17% of the national cattle livestock is produced. Normandy is placing in the 3rd rank of national level with more than 1,9 million of cattle breed. Moreover, this area occupies also the 3rd rank concerning pig breeding in France. Then, in order to evaluate the efficiency of DWTP in this area, the risk of *Cryptosporidium* was also taken into account to select the points of interest. Indeed, the presence of *Cryptosporidium* was regularly observed in one of the main DWTP studied in this work, showing the impact and the importance of breeding in this specific agriculture area. Although this study was primarily focused on veterinary drugs, some of the targeted compounds are used both in human and veterinary medicine.

Methods and results : Raw and treated waters were sampled along six different DWTP. Two were studied upstream Paris and four downstream. Different treatments are employed in the DWTP: ozonation, UV, granular activated carbon, chlorination... Note that two DWTP are flown into ground water. In parallel, water was collected in four sites along the Seine River and in eight rivers in Normandy. The 18 sites sampled at two seasons, represent a total of 64 samples. After filtration on glass fibre filter (0.47 µm), two types of solid phase extractions (SPE) and liquid chromatography analysis coupled with tandem mass spectrometry detection (LC-MS/MS) were performed in order to quantify veterinary medicines. 17 antibiotics (sulfonamides, quinolones and fluoroquinolones, tetracyclines and macrolides), 2 pesticides (potential tracer for farming activity) and iopromide (iodinated contrast used as human activity tracer) were extracted on Oasis HLB cartridges and eluted with acetonitrile at pH 4 in a first extraction step and 14 antibiotics (beta-lactames, anthelmintics, coccidiostats, antiparasitics) at pH 7 on Oasis HLB cartridges with water/methanol (10/90, v/v) + 2% formic acid. Depending on the physico-chemical properties of compounds, LC-MS/MS analyses were performed in positive and negative ionisation mode for both analytical methodologies. Note that quality controls were performed in order to validate the results. For example, extraction and injection blanks, spiked water control or internal standards for quantification were used. First of all, a large panel of antibiotics are detected in the studied rivers with highest concentrations from 100 to 500 ng/L where livestock breeding is the most important. Fluoroquinolones and clorsulon (antiparasitic compound) are major and ubiquitous compounds in this farming area but the origin of these compounds are not necessarily from veterinary usage and could also represent a human contamination. Sulfonamides and tetracyclines are quantified around 45 – 60 ng/L at various sampling sites. Then, in ground water, some antibiotics, such as oxolinic acid, marbofloxacin sulfonamides and some pesticides, are quantified in the range of 4 - 39 ng/L, depending on the seasonality of sampling. As in the rivers, the simultaneous presence of veterinary and human antibiotics demonstrates the impact of livestock breeding and farming but also human activities. Therefore, concerning the efficiency of various treatments of DWTP, results of “Amont 2” site show the benefits of clarification using a carbon filter (1st stage of filtration) instead of a conventional sand filter for fluoroquinolones and other antibiotics. Then, UV treatment seems to eliminate remaining compounds except danofloxacin. These behaviours are also reported for the 2nd sampling campaign and marbofloxacin seems to show the same trend as danofloxacin. Regarding other DWTP, both molecules are ubiquitous. Finally,

ozonation coupled with granular activated carbon or chlorination have no impact on the elimination of antibiotics in water. On the contrary, the results about a settling-filtration-GAC filtration combination and a flotation-GAC filtration combined techniques appeared contradictory and depended on the compound of interest.

Discussion and conclusion : The detection of a large panel of antibiotics in different types of waters (raw, treated, groundwater...) show the importance and the ubiquity of these compounds in the environment. The various sources of these molecules (human and/or veterinary) and the detected concentration in natural waters could be a major risk for the environment and indirectly for human health, particularly with the development of antibiotic resistance. The health risk evaluation (following ANSES recommendations, 2013) shows that remaining antibiotics in treated waters do not represent a significant risk for human consumption.

OCCURRENCE AND DETERMINATION OF PHARMACEUTICALS AND ILLICIT DRUGS IN WASTEWATER SAMPLES IN NORTH-EASTERN TUNISIA. ESTIMATING COMMUNITY DRUG ABUSE BY WASTEWATER ANALYSIS

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Background and objective : Background: Pharmaceutically active substances (PhACs) and drugs of abuse (DAs) are two classes of new so-called “emerging” contaminants that have raised great concern in the last years. Numerous studies revealed their presence in the effluent wastewaters. This is mainly due to the fact that some compounds are not efficiently removed during wastewater treatment processes, being able to reach the aquatic environment. Recently, some investigations have shown that the same methodology can be used to measure also drugs of abuse in wastewater (UNODC 2011; EMCDDA 2015). Most methods to estimate drug abuse in Tunisia and in the world are based on surveys, crime statistics and drug seizures by law enforcement. But much illegal drug use happens off the radar. To better approximate usage, scientists have been turning to wastewater. In Europe, a number of studies have been done to see how well wastewater treatment plants are removing illicit drugs from sludge before treated water is released into the environment. But until now, no study in Tunisia had looked at this, likely leading to underestimates of abuse. objectives: The aim of this work was to survey the exposure of Tunisian STP effluents to both licit and illicit drugs. The novelty of this research work is to investigate for the first time in Tunisia the occurrence and removal efficiency of these two major groups of emerging contaminants in Tunisian STP effluents. This research work presents the first effort for revealing data of the concentration of illicit drugs in WWTP influents, therefore, estimate and monitor drug consumption in the population in real time, helping social scientists and authorities to combat drug abuse in an African country, which presents a different case study compared to other mainland countries.

Methods and results : Methods: Composite 24-h wastewater samples used in this study were collected from seven different Wastewater Treatment Plants (WWTPs) located in north-eastern Tunisia. The samples were pretreated via off-line solid phase extraction followed by UPLC-MS/MS. Target residues are; Pharmaceuticals: Paracetamol, Caffeine, Propranolol, Atenolol, Carbamazepine, Erythromycin, Clarithromycin, Ofloxacin, Ciprofloxacin, Sulfamethoxazole, Ampicillin, Trimethoprim. Illicit drugs: AMP, MDMA, METH, COC, BE The analysis was performed with an ACQUITY TQD UPLC-MS/MS system (From Waters Corporation). A triple quadrupole mass spectrometer coupled with electrospray ionization (ESI) source was used for sample analysis. Data acquisition was performed with MassLynx™ software. Cocaine consumption was backcalculated from the measured daily loads of the drug target residues DTR using the model suggested by Zuccato et al. (2008). Results: Out of 17 compound analyzed, 14 of them were detected, mainly in effluent wastewaters. In both matrices, antibiotics and β -blockers were the most detected groups. This suggests that these compound show noticeable resistance against biological treatment in WWTPs. The estimated concentrations of antibiotics in effluents ranged from ca. 35 ng/L to 1.2 μ g/L. Quantification ranges were found to be 25-450 ng/L for cocaine and its metabolite in influent samples. Cocaine consumption was back-calculated and the city linked to WWTP1 (NE1) presents the highest consumption of cocaine 600mg/day/1000 inhabitants.

Discussion and conclusion : Discussion: - Concerning pharmaceuticals, Only, ampicillin was not detected. Probably this compound is metabolized during the treatment. - The highest concentration corresponds to caffeine in the influent samples. This is mainly due to the consumption of caffeine, especially to the intake of the coffee as drink. - Antibiotics and β -blockers were the major groups of compounds detected in the effluents, a fact suggesting that they are bio-resistant. Their concentrations in effluents predominantly ranged from 100 ng /L to 1 μ g/L. - The most important pathways for removal of PhACs during wastewater treatment are biotransformation/biodegradation and abiotic removal by adsorption to the sludge. - The unexplained variations of the concentration observed for antibiotics during the time, are due to the deconjugation processes which are occurred during the treatment with activated sludge. - The increased concentration of Antibiotics and β -blockers in almost all effluents samples examined as compared with that found in influents samples is attributed to the fact that metabolites and conjugated forms which are also present in the influent samples, may degrade and retransform into the parent compounds (Göbel et al. 2004). - The presence of the five compounds was

confirmed across all the WWTP influent samples. However, only cocaine was detected in the most of influents samples. - A fluctuation of the concentrations is observed through all the IWW's which is attributed to the different usage of illicit drugs in Tunisia and the consumption among regions. - The highest consumption of cocaine was found in the NE1 region which correspond to the population plugged to WWTP1 (600.000 habitants). Conclusion: Most existing conventional treatment processes applied are not designed to completely remove different classes of xenobiotics organic compounds including active pharmaceutical ingredients. The presence of licit and illicit drugs in wastewater samples from several WWTPs in north-eastern Tunisia has been demonstrated, the difference between the concentration of the pharmaceuticals in the influent of the seven WWTPs, probably is attributed to the variations of the consumption among regions in Tunisia and the volume of wastewater inflow of WWTPs which is in accordance to the large number of population which is plugged to each sewage treatment plant, respectively. The results of illicit drugs in influents wastewater samples were useful to estimate and monitor the initial load of the illicit drugs in influent samples and thereafter, the real-time estimation of the consumption, helping social scientists and authorities to combat drug abuse.

DETERMINATION OF CARBAMAZEPINE AND METABOLITES IN WASTE WATER USING HIGH RESOLUTION LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY (LC-MS).

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Background and objective : The presence of pharmaceuticals and personal care products in waste water effluent and aquatic systems is ubiquitous and well-studied. Pharmaceuticals will enter the waste water system via human excretion of the unchanged parent drug or by improper disposal of unused medicines. Excretion rates of the parent drug are compound dependent and metabolites and transformation products should also be considered. Although much data is now available on the removal of pharmaceuticals in waste water treatment plants (WWTPs) and their subsequent discharge to the environment, relatively few studies have looked at the removal of metabolites and transformation products and their potential environmental toxicity. As part of the noPILLS project, based on usage data and environmental risk, the widely used antiepileptic carbamazepine (CBZ) was selected for an in-depth study. There are several CBZ metabolites, including trans-10,11-dihydro-10,11-dihydroxy carbamazepine (trans-CBZdiOH), which should be excreted in concentrations greater than CBZ, and 10,11-epoxy-10,11-dihydrocarbamazepine (CBZEP), which is reported as being toxic in the environment. High resolution liquid chromatography-mass spectrometry (LC-MS) was used to identify metabolites in waste water and to calculate removal rates in a WWTP.

Methods and results : Flow composite samples of influent and effluent were collected every 2 hours over a 46 hour period from a WWTP utilising activated sludge treatment. Hospital waste water was also sampled directly (weekly composite samples). Samples were sequentially filtered and prepared using solid phase extraction (Strata X, Phenomenex). Analysis was carried out using a Thermo Scientific Orbitrap Q-Exactive mass spectrometer linked to a Dionex Ultimate 3000 binary HPLC system. Standards of carbamazepine, cis and trans-CBZdiOH, oxcarbazepine (OxCBZ), eslicarbazepine acetate (Elsi-CBZ), 10-monohydroxy carbamazepine (10-CBZmonohydroxy) and CBZEP were used to characterise the chromatographic retention times and mass spectral fragmentation patterns of these compounds and associated degradation products. Deuterated carbamazepine was used as internal standard. Concentrations of CBZ and metabolites in WWTP influent were highly variable throughout the 46 hour period. The mean concentrations of CBZ, trans-CBZdiOH and CBZEP were 0.46 ng/ml, 0.49 ng/ml and 0.06 ng/ml respectively. Effluent measurements showed that these compounds are poorly removed in the WWTP and the effluent concentration of CBZ was higher than the influent. This can be explained by the breakdown of the CBZ-glucuronide during waste water treatment. Hospital waste water CBZ concentrations were extremely high (maximum 5832 ng/l) in some samples but did not give the corresponding expected metabolite ratio. This might indicate that some CBZ has been improperly disposed of directly to the waste system.

Discussion and conclusion : Analysis of influent and effluent samples shows that CBZ and its main metabolites are poorly removed during waste water treatment and therefore they have the potential to reach the aquatic environment. Effluent concentrations of CBZ are above the PNEC value of 0.42 ug/L. Measurement of only the parent compound may not provide enough information about the overall environmental impact. However, more ecotoxicological data for the metabolites is required to make this evaluation. Calculation of the parent compound to metabolite ratio might provide a method of detecting if pharmaceuticals have been improperly disposed of in the waste water system.

EVALUATION OF THE IMPACT OF HOSPITAL EFFLUENTS ON THE ANTIBIOTIC RESISTANCE DISSEMINATION IN THE ENVIRONMENT

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Background and objective : The spread of multiresistant bacteria (MRB) to antibiotics is a major public health issue. Some anthropogenic effluents from antibiotic-consuming activities such as clinical are suspected to contribute to the MRB dissemination into the environment. Resistant integrons (RIs) are bacterial genetic elements capable of acquiring and expressing antibiotic resistance genes. These genetic elements mainly found in Gram-negative bacteria are widely involved in the antibiotic resistance issue in clinical and veterinary settings. Indeed, several studies have underlined the class 1 RI as a global genetic marker of the dissemination of MRB. One part of the objectives of the Pills and noPills project was firstly, to assess, by monitoring class 1 RI, the care facilities effluents contribution to the environmental spread of MRB and secondly, to evaluate the efficiency of advanced processes to minimise this dissemination.

Methods and results : Twenty hours representative samples of effluents from (i) 7 European hospital centres (HCs), (ii) 2 geriatric units (GUs), (iii) 3 urban areas (UAs) unaffected by significant clinical activities and natural waters (rivers and ponds) were sampled. In the same way industrial or pilot wastewater treatment plants (WWTP) using advanced treatment (membrane, UV, Ozone..) for the treatment of hospital effluents were selected in European countries. Influent and effluents were sampled and concentrated using cellulose ester filters (0.45 µm pore size) until saturation of the filter was achieved. Each water or effluent was concentrated onto 0.45µm-pore size filter, and total DNA of bacteria retained on filter was extracted. Total genomic DNA was extracted in triplicate, using the PowerWater™ DNA isolation kit (MoBio Laboratories Inc.). Class 1, 2 and 3 MI were quantified using a multiplex qPCR and normalized to the 16S RNA-encoding DNA gene copy number so as to calculate the relative abundance of RIs. In all anthropogenic effluents, concentrations of class 1 RIs ranged from 3.0×10^9 to 6.4×10^{11} copies of R.L-1 regardless of their origin (HCs, GUs or UAs). In natural waters, concentrations of class 1 RIs were lower and ranged from less than 6.6×10^4 to 3.0×10^7 copies of R.L-1. These results underlined that, independently of the origins of their activity, anthropogenic effluents studied could be involved in class 1 RIs dissemination in the environment. The relative abundances of class 1 RIs in bacterial communities from effluents of HCs were highest compared to other samples, with values comprised between 0.38 and 2.22 (median = 1.68), while relative abundances of effluents from the GUs and UAs were comprised between 0.06 and 0.25 (median = 0.09). In all influents of each WWTP, quantities of class 1 MI were the most important (1010-1011 copies.L-1) with more class 3 MI (109-1010) than class 2 (108-109). All the processes reduced the concentrations of MI and bacteria by 2 to 5 log (2 for a classical WWTP and 5 for the ultrafiltration membrane). The addition of another advanced treatment step (such as ozonation) was only of benefit when the first step was not efficient.

Discussion and conclusion : The conclusion of the noPills project was that effluent produced by care centers may contain a resistant bacteria load and the relative abundance of resistant bacteria in a hospital effluent was higher than in an urban effluent. Otherwise, it was demonstrated that the quantification of integrons and relative abundance could be a method to evaluate an overall resistance. It was also concluded that the fight against antibiotic resistance requires a range of approach (standardization of methods, definition of indicators, methodology for risk assessment, the evaluation of gene transfers in anthropic systems, and that fundamental research on resistant bacteria and gene transfer is recommended.

SPATIAL DISTRIBUTION OF PHARMACEUTICAL POLLUTION WITHIN A RIVER CATCHMENT - RESULTS FROM THE NOPILLS PROJECT

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Background and objective : Pharmaceutical concentrations in surface waters depend on the pollutant load as discharged from multiple point sources (STW effluent), environmental degradation and / or sequestration during transport and the available dilution provided by the river and its tributaries. Presented are the results of a sampling campaign to 1) compare river loads and concentrations upstream, 400m and 10km downstream from a trickling filter plant, and 2) establish mass balance before and after a confluence of a tributary and the main river.

Methods and results : Daily grab samples were taken at 5 locations within the catchment for 4 consecutive days in June 2013. Concentrations for Atenolol, Ibuprofen, Bezafibrate, Carbamazepine, Lidocaine and Clarithromycin were determined with a Thermo Scientific Q Exactive orbitrap LCMSMS.

Discussion and conclusion : The mean concentration of Atenolol and Clarithromycin was a factor 5 lower at the downstream location. The mean values for Carbamazepine, Ibuprofen and Bezafibrate were only slightly (30%) reduced downstream. Unexpectedly, Lidocaine was found in considerable higher concentrations at the downstream location. Although no other STW inputs are present along the stretch, there are 18 authorised private sewage discharge points (e.g. septic tanks) in the tributary's catchment. For Atenolol the lowest concentration was found downstream from the confluence. It is likely that degradation between the upstream and the downstream sampling points played a role as no other dilution takes place. As concentrations for Bezafibrate, Carbamazepine, and Clarithromycin in the two contributing streams are similar, it is no surprise that the concentrations downstream from the confluence are also similar. For Lidocaine and Ibuprofen, concentrations in the tributary are higher than upstream, resulting – as expected - in an increase of concentrations downstream from the confluence. Although mass balance calculations have not yet been possible pending the processing of flow data, the results appear to be broadly consistent with expectations. Conclusions Pharmaceutical pollutants can be transported over a long range. Some compounds, such as Atenolol, appear to be degraded or sequestered considerably along a 10km stretch of river, but Carbamazepine and Ibuprofen are not, although the influence of private sewage discharges must be investigated further. Despite multiple larger STW discharging into the main river, it is the smaller tributary, which receives effluent only from one relatively small STW, that shows the highest levels of pharmaceutical pollution due to low available dilution. The high levels of Lidocaine apparently not issuing from any STW remind us that other sources of pharmaceutical pollution should not be ignored.

INVESTIGATION OF PHARMACEUTICALS IN THE BORDEAUX URBAN WATERS: A MULTISOURCE ASSESSMENT

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Background and objective : The global contamination of surface waters by pharmaceuticals is widely recognized. This contamination can be linked to human consumption, as wastewater treatment plants (WWTP) are not able to remove the totality of pharmaceuticals present in wastewater effluents. If the improvement of WWTP efficiency is one possible solution, the reduction of contamination at sources is another one and it is then necessary to characterize the various sources of pharmaceuticals. In the framework of the project RESEAU/REGARD, the aim of this study was to perform a multisource assessment of pharmaceuticals in the Bordeaux urban waters.

Methods and results : Time proportional 24 h composite water samples were collected at 6 points of a small Garonne river tributary (south-west of France), the Jalle of Blanquefort, between July 2013 and August 2015 (6 campaigns). Flow proportional 24 h composite water samples from influent and effluent of a WWTP, discharging into the Jalle of Blanquefort were collected). The upstream of the sewage network was also studied, with the collection of samples from industrial, hospital and domestic areas. In parallel, samples were collected in rainfall outlets. A list of 43 pharmaceuticals was investigated using a multi-residues protocol using solid-phase extraction and liquid phase chromatography.

Discussion and conclusion : Amongst the most emblematic molecules quantified in the Jalle of Blanquefort are diclofenac, gabapentin and hydroxy-ibuprofen which were quantified in median concentrations of 108, 262 and 166 ng.L⁻¹. These molecules were also present in WWTP influent and still in the WWTP effluent at concentrations above 800 ng/L as they were not entirely removed by the WWTP biological treatments. Paracetamol, the most abundant pharmaceutical in waste waters but which is however well removed when biological treatments are applied, was quantified only in 32 % of samples in concentrations ranging from 14 to 216 ng/L. Pharmaceuticals were also found in samples collected in industrial and hospital sewage network. For example, paracetamol concentrations were comprised between 36,303 and 622,771 ng/L for industrial samples, and between 23,871 and 1,747,929 ng/L for hospital samples, which is in accordance with the widespread use of this molecule. Unexpectedly, pharmaceuticals were also found in samples collected in rainfall outlets. For example, paracetamol concentrations ranged from the non detection to 67,974 ng/L. As this molecule is well removed by WWTP treatments, this highlights the presence of untreated wastewater. Finally, the results of this multisource assessment of pharmaceuticals showed that pharmaceuticals are ubiquitous molecules. Further studies are ongoing in order to investigate hospital and pluvial specificities.

IMPLEMENTATION OF COMPLEMENTARY MONITORING STRATEGIES TO SUSTAIN THE NEEDS FOR A BETTER EVALUATION OF DRINKING WATER TREATMENT EFFICIENCY: CASE STUDY ON PSYCHOTROPIC DRUGS AND METABOLITES IN THE PARISIAN AREA

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Background and objective : Through the last decades, ubiquitous contamination of water, all through its cycle, by pharmaceuticals residues has been demonstrated. More recently, the scope of attention has been widening to psychotropic drugs.

Methods and results : According to this recent interest and to French consumption specificity, this work presents the survey of 68 psychotropic compounds including 29 metabolites and glucuronides. More specifically, a broad range of benzodiazepines, hypnotic drugs, antidepressants, stimulants, opiates and opioids, anticonvulsants, anti-dementia drugs as well as anthropic tracers were investigated. To ensure the reliability of the data, a particular attention has been paid to Quality Control/Quality Assurance from the method development, to its performances validation and finally implementation. Moreover, two complementary sampling approaches were simultaneously implemented on two drinking water treatment plants located nearby Paris; raw and treated water were investigated. The first sampling approach was based on grab sampling and the second on passive sampling using polar organic compound integrative samplers (POCIS). Those last enable to concentrate in situ trace pollutants and yields in time-weighted averaged concentrations

Discussion and conclusion : Main results, lessons from the implemented strategy, its advantages and limits will be presented and discussed.

INVESTIGATION OF PHARMACEUTICALS IN AQUATIC ENVIRONMENTS ON A NATIONAL SCALE IN FRANCE AND FRENCH OVERSEAS DEPARTMENTS

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Background and objective : A screening study of emerging contaminants was carried out in 2012 in surface waters in both metropolitan France and overseas departments (Martinique, Guadeloupe, Reunion, Mayotte and French Guiana) as part of the National Action Plan against pollution of the aquatic environment, which implies the regular update of the lists of substances to be included in monitoring programs. The study on 182 emerging compounds (100 molecules measured in surface waters, 134 in sediments and 191 in groundwater in overseas only) was performed at 200 sites (rivers and lakes in metropolitan France and overseas territories).

Methods and results : Among the substances selected, more than 40 pharmaceuticals were analysed, with a total of 400 data collected for each substance for the surface waters (3 sampling campaigns) and 150 data for the sediments (1 sampling campaign).

Discussion and conclusion : Overall, out of the 22 pharmaceuticals monitored in surface water, 16 were analysed above the LOQ at least once in rivers during the three sampling campaigns in metropolitan France. Highest concentrations were measured for anxiolytics (oxazepam, lorazepam and diazepam). For the sediment matrix, out of the 28 measured pharmaceuticals, 15 were quantified in rivers or in lakes in metropolitan France. The highest concentrations were measured for amiodarone, diosgenin and econazole (Max. Conc. 18 ng/g). It was the first time that Water Agencies acquired data on these compounds in sediments. For many compounds such as carbamazepine (frequency of quantification of 71.0%), oxazepam (60.3%) or ofloxacin (23.7%), it was the first time that they were measured at large spatial scale (115 sites) and with such a number of samples (n=400) in France. For some pharmaceuticals, the improvement of analytical methods (order of LOQ of ng/L, significantly lower as compared to previous studies carried out by water agencies) helped to have a better frequency of quantification. As an example, the frequency of quantification of ketoprofen increased up to 51.5% (instead of 9.3%) and sulfamethoxazole up to 38.0% (instead of 1.4%). Almost all substances were quantified at reference sites in the water matrix, but for sulfamethoxazole, ketoprofen and oxazepam concentrations were near the LoQ values. For sediments, urban and agricultural sites were overall more contaminated as compared to reference sites. Diosgenin was highly measured in agricultural and reference sites but not in urban sites. Nicotinamide was observed in both agricultural and urban sites but never quantified in reference sites. In overseas territories, a short list of pharmaceuticals was monitored in both groundwater and surface water to identify a possible link between the two water categories. These combined studies allowed to identify which water bodies is more contaminated (ex. ketoprofen, even if always detected in both water categories, is more an issue for surface water as compared to groundwater). In marine compartments, two pharmaceuticals were quantified at high concentrations in POCIS (carbamazepine and ketoprofen) in a large number of sites in Metropolitan France. Miconazole, amiodarone and diosgenin displayed a frequency of quantification of about 30% in sediments. In conclusion, this screening study allowed the collection of about 15000 records for pharmaceuticals in French aquatic environments. Further to this screening campaign, 11 pharmaceuticals substances (8 for water and 3 for sediment) have been included in the national Watch List for future monitoring in the French surface aquatic environment (2016–2021) (as a result of a prioritisation exercise based on frequency of quantification, frequency and degree of PNEC exceedance and hazardous properties). This study also contributed to increase the knowledge about emerging pharmaceuticals in French marine waters.

IMPACT OF AGRICULTURAL PRACTICES ON VETERINARY PHARMACEUTICAL OCCURRENCE IN SUPERFICIAL WATERS

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Background and objective : Diffuse contamination of surface water by veterinary pharmaceuticals (VP) has been poorly studied in intensive breeding context. Recycling animal waste on soil is supported to fertilize crops and reduce utilization of mineral fertilizer but may have some environmental impact by recycling pollutants on soils and transferring pollutants from soils to rivers. Most of pharmaceuticals are consumed indifferently by human and animal, especially antibiotics. This study aims to quantify veterinary pharmaceuticals (VP) in superficial water in two agricultural catchments. One is dedicated to the production of drinkable water. Agriculture but also waste water treatment plant may contribute to the contamination of water and it is difficult to distinguish animal versus human source of contamination.

Methods and results : Grab and storm events water sampling were carried out from March 2013 to December 2014 on the 4,9 km² Kervidy-Naizin headwater catchment located in Brittany (western France) belonging to the AgrHyS environmental research observatories and part of Network in Drainage Basins. Water quality is impacted by intensive breeding (swine, cattle) with mean nitrate concentration of about 80 mg/l. Grab sampling were performed on different sub catchments from 2 to 80 km² located in Ille et Vilaine, impacted both by agricultural diffuse pollution and a sewage treatment plant. Priorization of molecules was established by a veterinary survey. On Kervidy-Naizin, Thrimethoprim, Oxytetracyclin and Enrofloxacin are frequently quantified (from 57 to 42%). Mean and max concentrations respectively (12 to 80 ng/l) and (20 to 230 ng/l) are identical in flood and non-flood events. The frequency of quantification of Eprinomectin, a cattle veterinary antimicrobial, is 31% and mean concentration is high (400 ng/l). On the Ille et Vilaine catchment, human pharmaceuticals are frequently quantified Diclofénac (77%), et Carbamazepin (55%). But human/veterinary Flunixin (69 %), Lincomycin (56%) and Fluméquin (55%) or specifically veterinary ,Sulfaméthazin (67%), are also quantified. Cumulated concentrations ranged from 1 to 3178 ng/L on Kervidy Naizin and 8 to 2500 ng/L on Ille et Vilaine site. Maximum VP concentrations are associated to the transfer of one or two molecules, mostly antimicrobial. High temporal variability of the nature of the molecules and the concentration were observed during the seven storm events.

Discussion and conclusion : Specifically veterinary pharmaceutical were quantified in both sites and particularly in headwater catchments above the plant. The impact of agricultural practices on water contamination by VP was confirmed. Presence of human/veterinary molecules suggested that diffuse agricultural pollution may really contribute to the presence of pharmaceutical in water but it is difficult to identify the main sources.

MONITORING ENDOCRINE DISRUPTING COMPOUNDS AND ESTROGENIC, ANDROGENIC AND THYROIDAL ACTIVITY IN TAP WATER FROM CENTRAL SPAIN.

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Background and objective : Endocrine disruptors (EDs) are defined as exogenous substances or mixtures that alter the function(s) of the endocrine system, thereby causing adverse health effects on an intact organism, its progeny, or (sub)populations, even when present at very low concentrations (ng/L). EDs include natural substances such as reproductive hormones (e.g. estrogens, androgens, progestogens), thyroid hormones and corticosteroids, as well as a wide range of chemicals, including synthetic hormones, polycyclic aromatic hydrocarbons, dioxins, plastics, pharmaceuticals, and pesticides. Given their potential threat to human and wildlife reproduction, there has been a great deal of interest in their identification and characterization. Most studies concerning endocrine disruptors have focused on the interference of these chemicals with the estrogen and androgen signaling pathways. More recently, however, the thyroid axis has also been recognized as a target of EDs. The thyroid hormones (THs) triiodothyronine (T3) and thyroxine (T4) play a crucial role in maintaining homeostasis in vertebrates, notably in the control of development, growth, energy provision, reproduction and behavior. When assessing the potential (anti)hormonal activity of a given compound, mixture, or environmental sample, in vitro assays have proven to be a quick and reliable tool for biological and environmental screening. In particular, reporter-gene assays with high sensitivity, reproducibility and low cost are particularly appropriate for the detection of endocrine activity. In this context, the main objectives of the present study were: i) to monitor the occurrence of 30 EDs and related compounds in the drinking water from different supply regions (different water supply sources) in the Madrid Region (Central Spain); ii) to evaluate the anti-androgen, anti-estrogen, and anti-thyroid activity of drinking water from the supply regions studied.

Methods and results : points representative of three drinking water supply areas in the Madrid Region (MR). Samples were collected over 7 days in the month of November in 1-L glass bottles and were treated with sodium thiosulfate (60 mg/L) to remove any residual chlorine that may interfere with subsequent test results. Water samples were collected over a period of 8 hours and were stored cold until analysis. All samples were analysed as described in detail previously [1] and in the previous study of the presence of EDs in surface water reported by Esteban et al. [2] Cell lines stably transfected with hormone receptors and the luciferase reporter gene were used to evaluate the presence of substances that may be able to activate or block estrogen, androgen, and thyroid hormone receptors. A total of 11 of the 30 substances analysed were detected, with Methylparaben, Nonylphenol, Tolytriazol, Tris (2-cloro ethyl) phosphate being detected in all samples. Sampling point DW1 presented the highest overall concentrations 1203 ng/L. The substances found in the highest concentrations were the flame retardant Tris (2-cloro ethyl)phosphate (DW1; 404 ng/L), followed by nonylphenol (DW4; 356 ng/L). Flame retardants and alkylphenols were the families of compounds detected in the highest concentrations. The in vitro assays performed did not show any (anti) androgenic, (anti) estrogenic, or (anti) thyroid activity for any samples, except for one sample from point DW2, which exhibited (anti) thyroidal activity.

Discussion and conclusion : The water purification processes used in the DWTPs in the Madrid Region appear to be unable to remove the chemicals analysed, and the drinking water in this region contains also contain derivatives of the plastics found in the pipes used to supply treated water to consumers. As such, the presence of these substances, all of which have hormonal activity, in drinking water must be monitored regularly, and their possible risk for human health at the concentrations found normally must be evaluated, especially in sensitive groups. References 1. Gorga, M.; Petrovic, M.; Barcelo, D. 2013. Multi-residue analytical method for the determination of endocrine disruptors and related compounds in river and waste water using dual column liquid chromatography switching system coupled to mass spectrometry . *Journal of Chromatography A*. 1295:57-66. 2. Esteban, S ; Gorga, M; Gonzalez-Alonso, S ; Barcelo, D; Valcárcel, Y. 2014 Analysis and occurrence of endocrine-disrupting compounds and estrogenic activity in the surface waters of Central Spain *Science of the*

Total Environment. 466: 939-951. Acknowledgments The authors would like to thank the Spanish Ministry of Economy and Competitiveness for financial support via the Carlos III Health Institute and the program "Proyectos de Investigación en Salud 2014-2016" FIS (PI14/00516), the financial support of the Generalitat de Catalunya (Consolidated Research Groups "2014 SGR 418 - Water and Soil Quality Unit" and 2014 SGR 291 - ICRA), and the Departamento de Medio Ambiente, of the Instituto de Investigación Agraria y Alimentaria INIA, for its help with this study. Merck is acknowledged for the gift of LC columns.

RESIDUES OF SELECTED ANTIBIOTICS IN NEAR BOTTOM WATERS COLLECTED FROM THE SOUTHERN BALTIC SEA - CONCENTRATIONS AND RISK ASSESSMENT

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Background and objective : Antibiotics enter the environment as a consequence of veterinary and human uses. Continuous discharge of these bioactive compounds to the environment and prolonged exposure of biota even to low concentrations may induce biological effects in non-target organisms from different trophic chain levels, potentially disrupting ecosystem processes. Seas can be seen as the final sink of the most persistent antibiotic residues, however the availability of data on pharmaceuticals concentrations and ecotoxicological consequences in marine or estuarine water is still very limited. Natural features of the Baltic Sea like water residence time of around 30 years, its shallowness and particularly large catchment area make it susceptible to the accumulation of hazardous substances. This could be particularly true in case of antibiotics due to the fact that the Baltic Sea catchment area is home to about 85 millions habitants and various branches of drugs industry, intensive farming and animals husbandry. Our previous results revealed the presence of selected antibiotics in the sediments from southern Baltic Sea. This study presents the results concerning the concentrations of the same antibiotics in the near-bottom waters and preliminary conclusions on environmental consequences.

Methods and results : 21 samples of near-bottom water were taken from the southern Baltic along the polish coast. Samples were collected during r/v "Oceania" cruises in 2011-2012 period. Samples were analyzed for determination of 12 antibiotic residues from group of sulfonamides, quinolones and trimethoprim. The procedures for the analysis of target compounds applied tandem SPE technique for isolation, enrichment and clean-up and liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) with electrospray ionization for final quantitative and qualitative analysis. Of the 12 target antibiotics 11 were detected in water samples. Antibiotics residues were present in 52% of the analysed samples and sulfamethoxazole and trimethoprim were the most frequently detected compounds. In general concentrations ranged from <MDL to 311 ng L⁻¹ for sulfonamides and from <MDL to 279 ng L⁻¹ for trimethoprim. The measured values were also used for sediment/water pseudo-partitioning coefficients calculations.

Discussion and conclusion : With the obtained environmental concentrations, a risk assessment for 8 antibiotics has been carried out, based on available acute and chronic toxicity data in target organisms. The results shows that sulfamethoxazole concentrations measured in near bottom waters in the Gulf of Gdańsk can affect the ecosystem. A relatively low risk for other antibiotics measured in the collected samples was predicted.

EXPOSURE ASSESSMENT IN A COASTAL ZONE RECEIVING TREATED WASTEWATERS TO THE ANTIDEPRESSANT DRUG VENLAFAXINE AND ITS METABOLITES

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Background and objective : Pharmaceuticals reached the marine environment mostly after therapeutic use through WTPs, as parent compounds and/or metabolites. The occurrence of numerous pharmaceuticals has been described in marine waters and occasionally in marine organisms but few studies reported data on a substance and its relevant metabolites. In the present work, the presence of venlafaxine and its metabolites, O-desmethylvenlafaxine (ODV), N-desmethylvenlafaxine and N,O-didesmethylvenlafaxine, was reported in a wastewater treatment plant effluent and in the receiving marine environment. The aim of the study was to assess their occurrence by predicting and measuring their concentrations in seawater and in marine mussels.

Methods and results : Concentrations of VLF and its metabolites were measured in the different compartments i.e. in water with passive samplers and in caged mussels. In seawater, predicted environmental concentrations (PEC_{sw}) of VLF and its main human metabolites were estimated taking into account the sold amounts of VLF, its human metabolism and the diffusion and dilution of the compounds in the coastal zone using an adapted hydrodynamic model (MARS 3D) developed by Ifremer. Predicted concentrations in mussels were estimated using seawater concentrations and BCF obtained with linear QSAR models described in the literature for aquatic organisms. Estimated concentrations in biota were then compared to the concentrations in mussels detected at the studied site. In seawater, the studied compounds were detected with concentrations ranging from under the limit of detection to 0.89ng/L for ODV. These measured concentrations were in the range of the predicted concentrations. In organisms, concentrations estimated with MEC_{sw} were in good agreement with measured concentrations in caged mussels thus appearing more adequate for estimating accumulation in mussels than PEC_{sw} which produced overestimation.

Discussion and conclusion : The proposed models to calculate the PEC in seawater and in mussels are suitable for obtaining relevant information on likely levels in a coastal area receiving WTPs effluents. However, molecules were considered conservative in the simulation and a possible metabolism was not taken into account in mussels, leading to uncertainties. Further studies on degradation, sorption in the receiving marine environment and on metabolism of these compounds in mussels should be performed, in order to improve estimations. The results obtained in the present study highlighted that organisms living in coastal environments are exposed for long-term to very low concentrations of pharmaceuticals and its metabolites. Then, it could be suggested that possible and subtle effects deserved further research as they cannot be excluded. Adequate methods able to highlight the effects in the conditions encountered on the coast remain to be developed to study the consequences of those exposures.

IMPACT OF WASTE WATER TREATMENT PLANT ON ANTIBIOTIC RESISTANCE GENES DISSEMINATION A CASE STUDY: AMOXICILLIN

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Background and objective : Domestic wastewaters are potential new water source in the context of water scarcity and climate changes but they are increasingly contaminated with toxic organic pollutants. Among these pollutants, pharmaceutical micropollutants got concerns since the late 1990s, as these compounds are designed to have some biological effects on living organisms and may have potential adverse effects on human health or aquatic organisms even at very low concentrations. Also, important concerns have been raised regarding the continuous discharge of antibiotics and anti-microbial products to aquatic environment which may facilitate the development or proliferation of resistant strains of bacteria. Indeed, the emergence and spread of antibiotic resistant bacteria have been classified by the World Health Organization as one of the three biggest threats to public health in the 21st century. Moreover, chronic toxicity effects have been reported in many places for aquatic organisms exposed to pharmaceutical micropollutants at trace concentrations. So far, there is evidence of occurrence of 160 different drugs in the effluents of domestic and urban waste water treatment plants (WWTP).

Methods and results : Most of the worldwide WWTP includes a treatment step by biological treatment called conventional activated sludge process. Therefore, organic and potentially toxic micropollutants could be oxidized or biosorbed on sludge within this bioreactor where specific concentration of biomass (measured as mixed liquor suspended solids (MLSS)) is maintained along with sufficient dissolved oxygen concentration to allow possible biodegradation of such pollutants. The presence of hardly biodegradable substances in wastewater stream, such as antibiotics is also considered as biological inhibitors and should deteriorates biological treatment systems by reducing microbial activity. Nevertheless, bacteria can acquire antibiotic resistance genes and overcomes the presence of antibiotics. The aims of the study are first to assess respirometric method to quantify antibiotic (Amoxicillin) toxicity, then to measure the amoxicillin toxicity on several sludge samples and finally to try to explain antibiotics and antibiotic resistance genes fate in WWTP. These samples have been chosen in order to cover different WWTP operating conditions (activated sludge age which equals to solid retention time) and treatment scheme (Membrane bioreactor (MBR) versus conventional activated sludge (CAS)). In this work, experimental inhibition evaluation and definition of Amoxicilline effect on activated sludge were undertaken using continuous aeration respirometric techniques. This is based on monitoring oxygen uptake rate of samples containing the inhibitor and others free of Amoxicilline. The inhibition level was evaluated using the effective concentration index (EC index). Amoxicilline concentrations of 0.5 mg/l to 50 mg/l were used. Inhibition starts to appear at a concentration of less than 3 mg/l and attain 14.71 % at a concentration of 11.5 mg/l. Furthermore, EC index attained 20 % for a concentration of 14.08 mg/l and decreases thereafter.

Discussion and conclusion : Biological inhibitions start to appear at different concentration depending of sludge origin. MBR sludges were able to oxidize more the amoxicillin in comparison to CAS, and EC index were measured at lower values in membrane biological systems. Viruses are probably the main responsible of passing resistance genes to bacteria. As the membrane cut off is able to retain some virus in MBR, they may inject the resistance genes into any new activated sludge bacteria. Thus, MBR biomass has the ability to acquire "free" DNA thanks to the WWTP scheme where sludge is perfectly mixed and viruses partially retained.

PHARMACEUTICALS IN SOIL LEACHATES AFTER RAW AND TREATED SLUDGE SPREADING: IMPACT OF SLUDGE TREATMENTS

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Background and objective : Sewage sludge contains a huge diversity of organic contaminants including pharmaceuticals (1). These compounds may interact with the organo-mineral environment prevailing in such complex matrix through various sorption/complexation mechanisms. These interactions could be modified by the treatment applied to sludge and this reactivity modification could thus influence the contaminants fate after sludge disposal onto soil. In order to study the impact of sludge treatments on the fate of pharmaceuticals after sludge spreading on soil, we have conducted lab-scale leaching experiments with 4 types of sludge sampled on an industrial sludge treatment line including anaerobic digestion, drying and composting.

Methods and results : Batch and column assays (based on EU standards XP CEN ISO/TS 21268-1, XP CEN ISO/TS 21268-2, XP CEN/TS 14997 et XP CEN ISO/TS 21268-3) and lysimeters experiments were conducted to assess contaminant release from 4 sludge: a thickened sludge (S), the same sludge after anaerobic digestion (DS), then after drying (DDS) and finally composted with green wastes (C-DDS). The release was also assessed from soil-sludge mixtures. Carbamazepin (CBZ), ciprofloxacin (CIP), sulfamethoxazole (SMX) and ibuprofen (IBP) were quantified in the raw and treated samples and in the leachates of the assays. CIP and IBP transfer from a non-contaminated soil was also assessed by means of laboratory column experiments. CIP was present at the highest concentration in the 4 sludge samples, the three other compounds were present at concentration around and below 100 µg/kgDM. During all the leaching experiments, only CIP, CBZ and IBP were detected. Experiments carried out on sludge showed that different sludge treatments can lead to different contaminant releases. Indeed, the dried anaerobic digested sludge and the composted sludge led to a better retention of CIP and CBZ as shown during batch and column experiments. Sludge thickening and composting led to a greater retention of IBP as shown in batch experiments. However, the sludge exhibited the same behavior in column experiments concerning IBP. In addition, CIP was neither detected in batch nor in column experiments carried out with soil-sludge mixtures whereas IBP and CBZ were either quantified or detected. In batch experiments IBP was quantified in leachates coming from soil-DS mixture and only detected in leachates of other mixtures. Under the same batch conditions, CBZ was quantified in each leachate with a lower level in the soil-C-DDS. In the column experiments, IBP was quantified in all soil-sludge mixtures as well as CBZ. In addition, column experiments with the non-contaminated soil showed that IBP can migrate in soil. But, a fraction of IBP seemed irreversibly sorbed to the soil. On the contrary, CIP showed no migration in laboratory column experiments. In lysimeter vessel under unsaturated conditions, CIP was not detected in leachates. IBP was quantified in only one leachate coming from the lysimeter filled with the soil-DS mixture and was also detected in some other leachates. CBZ was quantified in all leachates, the lowest concentration was found for the soil-compost mixture. These results confirmed what was already observed during batch and column experiments: CIP is highly retained in soils whereas IBP and CBZ can be mobilized. These results are also in accordance with results obtained on field-experiment where CBZ and IBP were detected at low frequency in the soil-leachates (2).

Discussion and conclusion : According to the concentrations found in the raw and treated sludge, it seems that ibuprofen is preferentially released compared to carbamazepin and ciprofloxacin. Ciprofloxacin is highly retained in the soil. Ibuprofen release is nevertheless moderate. The releases of ciprofloxacin and carbamazepin are lower is the case of compost supply.

REPEATED APPLICATIONS OF ORGANIC WASTE PRODUCTS IN FIELD CONDITIONS HARDLY IMPACT PHARMACEUTICAL CONCENTRATIONS IN SOIL AND SOIL LEACHATES

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Background and objective : Pharmaceutical products excreted by treated animals or humans are transferred to manure or wastewater, respectively. In wastewater treatment plants, pharmaceuticals can be degraded and/or removed from water through adsorption to sludge. The recycling of the organic waste products (sludge, compost, slurry, manure...) in agriculture is largely encouraged nowadays for their fertilising properties, but it may indirectly contribute to the dissemination of pharmaceuticals in the environment (soils, surface and ground waters). Pharmaceuticals are considered as emerging pollutants due to their potential activity against non-target organisms. The objective was to determine the concentration levels of pharmaceuticals in different real environmental matrices: (i) organic waste products (OWP) such as sewage sludge, different composts, pig slurry and poultry manure, (ii) soils that received the OWP for a decade of years, (iii) and soil leachates. Measurements were done in 3 sites (QualiAgro, PRO'spective and La Mare) from the network of long-term experimental fields called "SOERE-PRO". It has been created to study the effects of repeated applications of different OWP under different soil and climate conditions and realistic rates of OWP application.

Methods and results : Organic waste products were sampled on the experimental fields on the day of their application. Soils were sampled before the application of the OWP (QualiAgro and PRO'spective sites). It corresponded to two years after the seventh and eighth application in PRO'spective and QualiAgro sites, respectively. Soil was also sampled one month and one year after the new application (QualiAgro). Soil leachates were collected with lysimeters installed at 45 and 100 cm depth. The pharmaceutical concentration levels in solid and liquid matrices were determined with analytical methods developed in our laboratory and based on ultrasound assisted extraction for solid matrices and online SPE-UHPLC-MS-MS. Urban OWP contained pharmaceutical compounds at different concentration levels, from few $\mu\text{g}/\text{kg}$ dry weight (e.g. 37 and 7.6 $\mu\text{g}/\text{kg}$ carbamazepin in sludge and composted sludge) up to hundreds and thousands $\mu\text{g}/\text{kg}$ dry weight (e.g. 4411 and 347 $\mu\text{g}/\text{kg}$ ofloxacin in sludge and composted sludge). Animal OWP also contained variable concentrations of pharmaceuticals, e.g. 9 $\mu\text{g}/\text{kg}$ and 196 mg/kg dry weight of sulfamethazine and doxycycline in liquid pig slurry. The soils amended with OWP contained only few pharmaceuticals with concentrations below 10 $\mu\text{g}/\text{kg}$ dry weight. Their concentration increased from 1.6 to 12 times after OWP application, depending on the compounds. In leachates, the detection frequency of pharmaceuticals was below 11% and the quantification frequency below 1%, without differences between treatments.

Discussion and conclusion : Even though pharmaceutical concentrations in OWP were sometimes high, they were low in soils after decades of OWP applications. Pharmaceuticals were hardly detected in soil leachates. Research is currently carried out to know if these low concentration levels could have some ecotoxicological impact. See also the presentations of D. Patureau et al. and Bourdat-Deschamps et al.

USE OF VETERINARY PHARMACEUTICALS IN A STUD FARM: OPTIMIZATION OF THE ANALYTICAL PROCEDURE AND PRESENCE IN HORSE MANURE

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Background and objective : In France, horse breeding represents more than a million livestock. Horses can receive antibiotic, anti-inflammatory or anti-parasitic treatments. These pharmaceutical substances are then partly excreted with urine and feces and, consequently, can reach the soil and water compartments. The present study, undertaken with the financial support of the ADEME (French Environment and Energy Management Agency) and IFCE (French Institute for Horses and Horse-Riding), aimed at evaluating the fate of veterinary substances in the context of horse breeding and, in particular, the production of compost from horse manure.

Methods and results : The analytical procedure had to be optimized and validated for the quantification of 11 veterinary substances in the complex manure matrix. Manure samples were extracted using an Accelerated Solvent Extractor (ASE) and the obtained extracts were purified by Solid Phase Extraction (SPE, with Oasis HLB sorbent) and analysed by Ultra High Performance Liquid Chromatography with Time of Flight mass spectrometry (UHPLC-Q-ToF). An experimental design has been applied for the optimization of ASE parameters (temperature, solvent composition and pH, number and duration of extraction cycles). With a spiked manure (2 µg/g), recoveries of the veterinary substances were greater than 80% for antiparasitic (except pyrantel), ranged from 52% to 74% for antibiotic (except dihydrostreptomycin) and from 59% to 81% for anti-inflammatory. The proposed analytical procedure has been applied to horse manures collected in a stud farm (Chamberet, France). The concerned animals were 18 male foals receiving an anti-parasitic (ivermectine), antibiotic (penicillin G and dihydrostreptomycin) and anti-inflammatory (dexamethasone) treatment during the period of castration. 3 substances were identified and quantified in manure at a concentration level of 8,2 µg/g for ivermectin, 21 to 31 µg/g for penicillin G and 0.4 to 1 µg/g for dihydrostreptomycin.

Discussion and conclusion : Ivermectine, which was found 3 weeks after the treatment at a concentration level close to the theoretical one, is mainly excreted with feces and has a strong affinity with organic matter. Partial degradation of this substance can be suspected since a lower concentration was measured 6 weeks after the treatment. No degradation was noted for penicillin G 6 weeks after horse treatment, in accordance with its urinary excretion in the active form and its higher persistence. Dexamethasone (48-hour half-life) was not detected in horse manure. Composting tests did not evidence an effect of pharmaceutical residues on the stabilization and transformation processes of organic matter; temperature increase probably accelerating their degradation. This work will be completed by a toxicity evaluation.

IMPACT OF CYTOTOXIC DRUG COMPOUNDING ON CONTAMINATION OF OCCUPATIONAL ENVIRONMENT

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Background and objective : Nowadays, cancers are one of the first causes of mortality in the world and antineoplastic drugs are commonly used to treat cancerous patients. Treatments are individually adapted by physicians and specifically prepared by pharmacists in compounding unit in hospital pharmacy. The aim of this study was to evaluate the residual cytotoxic contamination in the chemotherapy compounding process.

Methods and results : Seven French hospital pharmaceutical units preparing antineoplastic drugs have been investigated. Samples were collected according to a standardized protocol on workplace and cytotoxic vials surface in pharmacy units and analyzed by graphite furnace atomic absorption after pre-concentration by cloud point extraction (limit of detection and quantification of 2 and 6 ng/sample). A total of 37% contaminated samples (n=517) were identified: 33% on external vial surfaces (n=111, contamination from 2 to 290 ng), 29% on chemotherapy packaging (n=41, from 2 to 78 ng) and 38% on workplace surfaces (n=365, from 2 to 20000 ng detected on cytotoxic raw material storage box) with 6% inside the working area (n=55), 71% of detectable contamination inside collective protective equipment used for drug compounding (n=169) , 18% in the storage area (n=89), 4 % in the control area (n=25) and 1% in adjacent area (n=27).

Discussion and conclusion : Although cytotoxic drugs definitely contribute to reduce tumor growth, cytotoxic drugs may affect normal cells. Due to inherent toxicity, cytotoxic drugs are potentially dangerous for exposed healthcare workers. Regarding results, exposure may occur during all steps involved with cytotoxic drugs from reception of raw materials, cytotoxic compounding to patient administration. Prevent exposure with appropriate personal protective equipments is a major hospital preoccupation.

ESTIMATING THE CONCENTRATION OF PHARMACEUTICAL RESIDUES IN HOSPITAL WASTEWATER

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Background and objective : Among emerging pollutants, pharmaceuticals are of major concern with regard to environment management and public health. In recent years, due to advances in the field of analytical sciences, pharmaceuticals have been detected in wastewaters, surface water, ground water and even in drinking water. Among the different contributors hospitals have been pointed out as a source of pharmaceuticals discharged in sewage treatment-plant. Recent studies having assessed the contribution of hospital towards the pharmaceutical load in municipal wastewater seem to show that the impact of hospitals remains limited. In spite of these results, increasing attention is given by hospitals to the environmental impact of their effluents. Despite the availability of high-sensitive analytical methods, analysis and monitoring of pharmaceuticals remains a challenge. Due to the wide number of pharmacologically active substances available, combined to the challenging sampling and analytical method, an alternative tool is needed in order to estimate the pharmaceuticals discharged. The purpose of this work was to provide a reliable method in order to assess pharmaceuticals release from hospital wastewater. In this way, we first performed calculation of the predicted concentrations of several pharmaceuticals in the effluents of two french hospital from consumption data. Then, in order to assess the reliability of the method, we compare the predicted concentrations to measured concentrations in the hospital effluents.

Methods and results : This study has been conducted in a university hospital and in private hospital located in different French area. We have first selected a list of the more relevant molecules according to consumptions data and available analytical methods. Then we have fully described in details their pharmacokinetics to finally develop a method in order to provide predicted concentrations of the selected molecules in wastewater. In a second step several sites throughout the hospitals have been selected in which several sampling campaigns were performed. Fifty molecules have been identified and then quantified covering twelve pharmacotherapeutic area. Only three molecules were not detected in any wastewater samples. Fifteen molecules were found in both hospitals. The pharmaceuticals concentrations obtained were within the range of micrograms per liter to hundred micrograms per liter; only concentrations of iodinated contrast media were in the range of milligrams per liter. Our model showed a high level of concordance (> 75 %) between predicted and measured concentrations.

Discussion and conclusion : Comparison of measured concentrations to predicted ones provided a good fit for most of the pharmaceuticals. The proposed method easily allows predicting the effluent concentrations of all the drugs used in a hospital, without having to perform expensive analytical measurements.

HOMOLOGY MODELING, MOLECULAR DYNAMICS AND DOCKING TO PREDICT INTERNAL EXPOSURE OF FRESHWATER FISH TO IONISABLE ORGANIC CHEMICALS

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Background and objective : Upon release in the environment, anthropogenic chemicals like pharmaceuticals, herbicides and pesticides can be taken up directly and indirectly by plants, animals and humans. As these emerging pollutants are mostly ionizable and/or polar, most of them end up in aquatic matrices. In environmental risk assessment, there is a need to predict the internal concentrations of these compounds in fish.

Methods and results : Computational methods including homology modeling, quantum chemistry, molecular dynamics and docking were used to quantify the binding to relevant carrier proteins. Fish were selected based on genetic variability and data availability, 3D models of relevant carrier proteins and chemical pollutants were generated, and docking was performed to predict affinities between the two. The method gave average binding for fish plasma protein with low-micromolar to nanomolar affinities, comparable to the average human plasma protein binding. However, differences in relative binding of individual compounds in fish plasma were found when compared to humans.

Discussion and conclusion : Results obtained are valuable, for example for establishing species-specific environmental concentration limits. The in silico method created also reduces the need for laborious experimentation, potentially increasing the awareness of risks posed by anthropogenic chemicals at low cost.

FATE OF PHARMACEUTICALS IN SURFACE WATERS; A CASE STUDY OF THE RIVER DOMMEL, THE NETHERLANDS

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Background and objective : Nowadays pharmaceuticals are measured frequently in surface water monitoring schemes. These substances reach surface waters via waste water treatment plants and are distributed throughout the receiving water system. Their distribution in water and sediment depends on various parameters, e.g., substance characteristics, degradation and sorption kinetics. The parameters, however, are usually not known hampering predictions on their environmental fate. In this project a micropollutant fate model is used and applied in the DUFLOW 1D modelling tool. The model was parameterized to simulate the distribution of diclofenac, metoprolol, carbamazepine and sulfamethoxazole (SMX) in the river Dommel, The Netherlands. The simulations were validated using available monitoring data.

Methods and results : A deterministic fate model was parameterized to study the environmental fate dynamics of diclofenac, metoprolol, carbamazepine and SMX in the river Dommel, The Netherlands. Parameterization was performed using tailored laboratory experiments and surface water and sediment monitoring data. In the laboratory set-up the impact of temperature, photolysis and microbial activity on compound degradation was tested. The data on surface water concentrations was obtained from regional surface water quality surveys, while sediment data was obtained by sampling and analysing sediment from different locations in the Dommel system. In the lab study, diclofenac degraded fastest (DT50=2.8d), followed by SMX (DT50=8.3d), metoprolol (DT50=10.6d) and carbamazepine (DT50=22.2d). Photolysis, increased temperature, and microbial activity all enhanced compound degradation. During the modelling it became apparent that besides degradation, sorption processes were an important factor determining the fate and transport of the compounds in the Dommel water system. Although modelled dynamics of carbamazepine and metoprolol fitted the measured concentrations well, this was not the case for diclofenac. Since SMX was not included in the monitoring surveys, the modelled dynamics could not be validated against measured concentrations in water and sediment.

Discussion and conclusion : The DUFLOW model predicted the dynamics of metoprolol and carbamazepine well but did not so for diclofenac, which was the most degradable compound tested. A sensitivity analysis on the different model parameters, i.e., degradation, resuspension, sedimentation rate and partitioning coefficients, revealed that model sensitivity was high for degradation rates of the compounds. Since the laboratory study showed that degradation was driven for a large extent by photolysis, this indicates that good knowledge of this process and its confounding factors (e.g. DOC, DIC, algae Chl-a) is key to adequately model the fate dynamics of fast degradable pharmaceuticals in water systems. For slower degrading compounds the current model already provides adequate fits with measured pharmaceutical levels.

PRELIMINARY FATE ASSESSMENT OF ANTIDIABETIC PHARMACEUTICALS IN THE ENVIRONMENT

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Background and objective : In recent years there has been growing interest on the prevalence and fate of pharmaceuticals in the natural environment. Pharmaceuticals may undergo incomplete, or even resist degradation at all in traditional waste water treatment plants (WWTPs), meaning they will enter receiving waters, soils and sediments. Hypoglycaemic pharmaceuticals are often taken in high doses and are excreted unchanged or only partially metabolised. These drugs and their transformation products may retain their pharmacological activity, and thus, being potentially harmful to the wild life.

Methods and results : Within this work we tested primary and ultimate biodegradability of selected antidiabetic pharmaceuticals in wastewater. To determine further fate we performed studies on interactions of these drugs in selected soils. OECD biodegradation tests were used to assess sorption and biodegradation potential. In wastewater complete primary degradation was observed for glibenclamide and glimepiride whereas gliclazide was shown to be resistant to biodegradation. We confirmed dead-end product –guanylurea – from metformin. In soil environment we observed strong sorption and thus lower biodegradation of sulfonylurea derivatives. Metformin exhibit much higher mobility and greater degradation potential.

Discussion and conclusion : Strong sorption adversely affects biodegradation and transformation. Binding of sulfonylurea derivatives to soils was higher than, for instance, sulfonylurea herbicides and exhibited much lower biodegradation potential. During the time of the experiment, transformation processes were observed mainly under aerobic conditions. All but one of tested compounds were completely biologically transformed within two weeks in wastewater biodegradation experiments. The influx of antidiabetic pharmaceuticals into the environment is and will remain significant. Our studies allow a preliminary assessment of environmental persistency of a very important group of pharmaceuticals and show need for implementing monitoring programs.

RETENTION MECHANISM OF SULFAMETHOXAZOLE IN A SILTY-LOAM SOIL.

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Background and objective : Sulfamethoxazole (SMX) is a relatively weakly sorbing sulfonamide type antibiotics. It is commonly found in soil, ground and surface waters. Predicting its dissemination in these reservoirs requires the parameters and sorption mechanisms on suspended, dissolved and stationary mineral and organic phases to be known. Its sorption and transfer properties were investigated on an agricultural silty-loam soil (INRA Paris, Feucherolles site) dependent on pH, soil organic matter content and trace elemental (especially Cu and Zn) concentration.

Methods and results : The isotherm sorption experiments performed at variable SMX total concentration ($0.33 \leq [\text{SMX}]_{\text{tot}} \leq 1000 \mu\text{mol/gdry-soil}$) were linear at the soil pH of 6.1 (10 % of $[\text{SMX}]_{\text{tot}}$ sorbed at the 1/10 solid/liquid ratio). Variable pH sorption experiments showed a sorption increase at $\text{pH} < 6$. Contaminating the soil with Cu(II) and Zn(II) ions increased the SMX sorption. The data curve analysis evidenced that the strongly sorbed Cu and Zn ions act as bond strength enhancing SMX bridging surface agents. The following non-electrostatic model fitted conveniently the Cu and Zn related SMX sorption enhancement effect (\equiv symbolizes the surface attachment of the Q_x site): $\equiv\text{Q}_x\text{-2} + \text{Smx-} + \text{Zn}^{+2} \rightleftharpoons \equiv\text{Q}_x\text{SmxZn-}$; $\text{LogK} = 7.4$ $\equiv\text{Q}_x\text{-2} + \text{Smx-} + \text{Cu}^{+2} \rightleftharpoons \equiv\text{Q}_x\text{SmxCu-}$; $\text{LogK} = 9.3$ The model is based on separate sorption experiments determining the sorption strength of Cu and Zn on that soil $\equiv\text{Q}_x\text{-2} + \text{Cu}^{+2} \rightleftharpoons \equiv\text{Q}_x\text{Cu}$; $\text{LogK} = 5.4$ $\equiv\text{Q}_x\text{-2} + \text{Zn}^{+2} \rightleftharpoons \equiv\text{Q}_x\text{Zn}$; $\text{LogK} = 4$ and its acid-base properties modeled as 4pK reaction system ($\equiv\text{Q}_x$ represents one of the four $\equiv\text{Q}_a, \equiv\text{Q}_b, \equiv\text{Q}_c, \equiv\text{Q}_d$ sites). The total surface concentration of weakly acidic SMX complexing sites was determined to be 0.11 mmol/gdry_soil.

Discussion and conclusion : Organic matter is strongly involved in SMX sorption. Separate sorption of SMX on humic acids revealed a similar sorption strength but at a 43 times higher site density compared to the whole soil. This suggests that more than 50 % of SMX fixes to the 1 % of organic matter present in that soil. Sorption of SMX on metal-free humic acids finally revealed that sorption persists at only slightly decreased sorption strength on all investigated pH ranges even in absence of metals. This suggests that other mechanism than metal-bridging and hydrophobic interactions are involved in the fixation of SMX on soils. The model obtained from batch experiments successfully predicted the SMX eluate concentrations obtained from prepared soil transfer experiments. This suggests that sorption constants obtained from batches may be used for predicting the retention of SMX in natural soils.

CO-OCCURRENCE OF VETERINARY ANTIBIOTICS AND FECAL SOURCE MARKERS IN BRITTANY SUPERFICIAL WATERS: AN EXPERIMENTAL STUDY.

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Background and objective : After absorption by human or animals, pharmaceuticals compounds are excreted either in their free, conjugated or metabolized form in soil and water. The dissemination of those compounds through water resources can lead to antibiotic resistance and could have health effects on living organisms. Numerous studies attest the contamination of various aquatic environments by pharmaceutical compounds, including water resources. The two major contaminations sources are : i) the localized release by industrial and municipal wastewater treatment plants ; ii) non-point dispersion through soil amendment using animal manure or release from animal pastures or by individual septic tanks (Sarmah et al. 2006 ; Ruhoya et Daughton, 2008). Prerequisite of any assessment of the health risks induced by these emerging contaminants requires identification of the contamination sources, close quantification of the input to the water environmental compartment, while developing knowledge on the chemical speciation and fate of the residues once released in the environment. Some residues found in waters may have a mixed origin (human and animal), such as some hormones or antibiotics that can be used indifferently (Kemper, 2008). Identification of the source relies then on co-tracers specific to humans or animals (Murata et al., 2011). This is particularly important in area of intensive livestock activities such as Brittany, region in the North-West part of France, with mixed watershed. We propose to evaluate by experimentation in laboratory the co-persistence of veterinary pharmaceuticals and fecal markers (fecal stanols) in water from agricultural watershed. Fecal stanols associated with usual bacterial indicators of fecal contamination are microbial source tracking markers used to distinguish among human, bovine and porcine fecal contamination in water (Jardé et al., 2007 ; Gourmelon et al., 2010 ; Jeanneau et al., 2011 ; Derrien et al., 2012).

Methods and results : Experimental study has been carried out at the laboratory scale to evaluate the persistence of veterinary antibiotics and fecal indicators and markers (E.coli and fecal stanols) during 21 days. Microcosms of freshwater have been inoculated with pig slurry contaminated by sulfadiazine, sulfamethazine and oxytetracycline. The microcosms were maintained under aerobic conditions with constant mixing and at constant temperature ($20^{\circ}\text{C} \pm 1^{\circ}\text{C}$). These experiments were conducted in darkness to avoid heterogeneous lighting due to the turbidity of the system. Antibiotics concentration, E.coli and fecal stanols were quantified in microcosms at the starting day and on days 1, 2, 3, 8, 15 and 21.

Discussion and conclusion : Decay rates and the length of time to obtain a reduction of 50% of the initial inoculum ($T_{1/2}$) were calculated for E.coli, fecal stanols and antibiotics in the microcosms inoculated with pig slurry. $T_{1/2}$ was lower for E.coli (1.2 days) than for fecal stanols (between 3.4 to 4.6 days depending on the stanol) and antibiotics (between 2.2 days for oxytetracycline to 7 days for sulfamides). Among antibiotics, the persistence of oxytetracycline is similar to that of fecal stanols and lower than persistence of sulfamides. These differences might be linked to different sorption properties of tetracycline and sulfamides. Fecal stanols seem to co-occur with tetracycline in water samples and might be useful to track sources of pharmaceuticals contamination at the watershed scale.

EVALUATION OF SIMPLETREAT 4.0: PHARMACEUTICAL DEGRADATION IN WASTEWATER TREATMENT PLANTS IN PROBABILISTIC MODEL SIMULATIONS

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Background and objective : Due to the increased use of human pharmaceuticals, emission of those substances into the environment have become a major concern. After consumption, pharmaceuticals and some of their metabolites ends up in wastewater and wastewater treatment plants (WWTPs). Because many compounds are incompletely transformed in WWTPs, they are discharged into the environment via effluent water and sludge, which forms a potential risk for humans and ecosystems. Therefore, understanding and predicting the fate of pharmaceuticals through WWTPs is important. SimpleTreat is a simple box model used to estimate the emission of a chemical from a WWTP to the environment. The model has become accepted as an evaluation tool for generic exposure assessment of substances emitted via sewage treatment plants in the European Union. Recently, the model was adapted to ionized substances and provided with new equations for a higher variability in operation parameters.

Methods and results : In this study, it is tested whether elimination of pharmaceuticals is well predicted by the improved SimpleTreat 4.0. Field data obtained from literature of 44 pharmaceuticals, which are measured in 52 different in activated sludge WWTPs, were used. The modelled concentrations of SimpleTreat 4.0 were compared to observed effluent concentrations as derived from reported influent concentrations. The model predicts well when applied to experimental data of pharmaceuticals, using the specific WWTP parameters as well as SimpleTreat default parameters.

Discussion and conclusion : Differences in detection techniques and sampling methods can cause variability in data, which can especially influence the measured effluent concentrations. In a second step, we focus on a probabilistic parameterization, reflecting actual conditions of conventional activated sludge WWTP, combined with statistical uncertainty in physicochemical properties of pharmaceuticals. With these probabilistic Monte Carlo simulations we provide insight into the validity of results as well as in the sensitivity of model parameters to ultimately come to more realistic model estimations.

SORPTION AND DISSIPATION OF ANTIMICROBIALS AND ANTIPARASITIC VETERINARY DRUGS ON CHARACTERISTIC SOILS FROM THE STATE OF SÃO PAULO, BRAZIL

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Background and objective : Veterinary drugs may be considered diffuse source of pollutants affecting ecosystems. Brazil is one of the largest animal producers in the world. This large-scale production directly affects the domestic veterinary drug market which reached approximately US\$ 4.4 billion in 2014, with 22% and 17% due to antiparasitic and antibacterial drugs, respectively. The aim of this work was to obtain a first dataset on sorption and dissipation of antimicrobials and antiparasitic veterinary drugs in soils from the São Paulo state.

Methods and results : Sorption/desorption studies were conducted with 18 drugs from seven different families: sulfonamides, fluoroquinolones, tetracyclines, anphenicols, benzimidazoles, milbemycin and avermectins on seven soils belonging to the following textural classes: sandy, sandy-clay and clay. The experiments were performed according to the OECD 106 Guide. Dissipation was evaluated in two soils according to OECD 307 Guideline. The Freundlich sorption coefficients (in $\mu\text{g l}^{-1/n} (\text{cm}^3)^{1/n} \text{g}^{-1}$) were in the range of: sulfonamides (0.45 to 19), fluoroquinolones (72 – 2410), thiabendazole (9 to 58), florfenicol (0.03 to 0.48), oxitetracycline (3.2 to 16), moxidectin (105 to 424) and avermectins (14 to 184). The Freundlich desorption coefficients (in $\mu\text{g l}^{-1/n} (\text{cm}^3)^{1/n} \text{g}^{-1}$) were in the range of: sulfonamides (0.2 to 37), fluoroquinolones (110 – 3293), thiabendazole (10 to 73), florfenicol (2.6 to 3.7), oxitetracycline (16 to 62), moxidectin (145 to 565) and avermectins (24 to 236). The DT50 of abamectin and ivermectin were in the range of 1-4 days for abamectin and 11-15 for ivermectin.

Discussion and conclusion : The composition of the studied soils favors a strong sorption of fluoroquinolones, which are in their cationic form in the soil pH (4 to 6). The main interactions were cation exchange, cation bridging and surface complexation. On the contrary, sulfonamides presented a low sorption potential and are likely mobile. The sorption is related to the lipophilicity of the sulfonamides and sorption is enhanced in soils with a higher organic matter content. Regarding florfenicol, its affinity to soil particles is negligible indicating that this molecule is highly mobile and would easily reach surface and ground waters. All the assessed antiparasitic drugs interacts more strongly with soils containing a higher organic matter content, and the results indicate that hydrophobic interactions as well as ion exchange with clay minerals play an important role in the sorption onto soils. Also, it was verified that the aerobic microbial degradation must have been the primary mechanism responsible for the dissipation of abamectin in soils.

THE INFLUENCE OF UNCERTAINTY AND LOCATION-SPECIFIC CONDITIONS ON THE ENVIRONMENTAL PRIORITISATION OF HUMAN PHARMACEUTICALS IN EUROPE

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Background and objective : The selection of priority APIs can benefit from a spatially explicit approach, since an API might exceed the threshold of environmental concern in one location, while staying below that same threshold in another. However, such a spatially explicit approach is relatively data intensive and subject to parameter uncertainty due to limited data. We investigated the added value of a spatially explicit EU-wide modelling approach for the local prioritisation of legacy APIs based on their risks for the aquatic environment. We did this for a set of nine antibiotics, with and without the consideration of uncertainty in input parameters.

Methods and results : We adapted a previously developed spatially explicit prioritisation tool for human APIs in Europe. It estimates regionalised concentrations in the aquatic environment throughout 100*100 km² grid cells in Europe, and compares them with API-specific species sensitivity distributions (SSDs) to derive the local potentially affected fraction of species (PAF). These PAFs were then compared with different threshold PAFs upon which the selection as priority API could be based. Based on an extensive literature search, deterministic input parameters for each of the antibiotics were replaced with distributions reflecting their uncertainty. This uncertainty was then propagated into regionalised PAFs via Monte Carlo simulations. By using the 95th percentile of these PAF distributions as a basis for prioritisation of the APIs, rather than the most likely (median) PAF, it was investigated whether a local approach in priority setting of APIs was still worthwhile when considering uncertainty. The added value of inclusion of spatially explicit information was expressed as the selection reduction factor (SRF): the extent to which the amount of priority APIs can be reduced compared with a non-spatial EU-wide approach. Figure 1 illustrates the advantage of following a location-specific approach in the prioritisation of APIs: in 94% of the grid cells in Europe, no APIs exceed either of the thresholds. This added value remains when accounting for uncertainty in parameter settings. In 96% of the grid cells, the location-specific approach still enables a reduction of the selection of at least 50%, compared with a EU-wide prioritisation.

Discussion and conclusion : Inclusion of spatially explicit information might be highly relevant for a meaningful and efficient local prioritisation of APIs. Indeed, it enables national and regional regulators to decrease the risk of misguided allocation of resources to APIs of no local environmental concern. Conversely, it allows to focus risk management options to where they may be expected to be most effective.

SORPTION OF PHARMACEUTICALS IN SOIL SYSTEM

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Background and objective : After use, active pharmaceutical ingredients (APIs) are excreted with urine and feces into domestic wastewater systems. This ultimately poses a risk to terrestrial environment via application of wastewater treatment by-products containing API residues. It is essential to assess the fate, mobility, and bioavailability of pharmaceuticals in soil to fully understand the risks these chemicals pose to terrestrial dwelling organisms. In the present study, in order to explore the relationships between the properties of neutral pharmaceuticals and sorption kinetics. A system of regression model has been derived which is based on a reliable set of experimental log Koc values and the octanol–water partition coefficient (log Kow). The resulting regression was tested and its performance compared to the existing models, including the regressions developed by Sabljic et al., (1995), Schwarzenbach R P., (2005), Karickhoff, S. W. (1981) and Franco's (2008) which were applied for hydrophobic chemicals. The second regression was developed by using the deviations between the measured log Koc and the Log Koc estimated with these existing models.

Methods and results : Data on the soil-water partition coefficient (Kd), soil properties, and physical-chemical properties of APIs were gathered from the scientific literature. Chemicals were classified as acidic, basic, neutral or zwitterionic based on the soil pH reported in the literature and the chemical pKa. Log Kow as the only model parameter used to develop linear regression models were recommended in the Technical Guidance Document (TGD). The first regressions related measured log Koc values of neutral pharmaceuticals to Log Kow which used the least-squares method with Microsoft Excel Add-In Solver. In order to test the goodness of fit of existing models, the second regressions were built between experimental log Koc values and the calculated log Koc values using Sabljic et al., (1995), Schwarzenbach R P., (2005) Karickhoff, S. W. (1981) and Franco's (2008) models, respectively. For unionised chemicals, poor regression relationship between measured sorption coefficients collected from the literature and log Kow ($r^2= 0.2897$). In order to reduce the interference of soil texture, another model based on sand samples from the whole neutral group was built. The correlation improved significantly ($r^2= 0.8747$). Sabljic et al., (1995) model provide a better prediction for hydrophobic chemicals. But his model is likely to underestimate sorption by 4 orders of magnitude for the compounds with low log Kow values range from -1 to 1 from my database. Similar results were found in Karickhoff's and Franco's model. However, Schwarzenbach R P., (2005) (1981) model provided a poor prediction which need to be further examined.

Discussion and conclusion : For the neutral pharmaceuticals with low log Kow values, their log Koc data are systematically underestimated by the existing models. This result indicates that these chemical groups establish specific bindings with appropriate soil constituents which is demonstrated by the larger soil sorption coefficients than can be expected from their octanol-water partition coefficients. Sorption has been measured with many different soils having widely varying characteristics, resulting in diverse values for the soil sorption coefficient even when that parameter is normalized to organic carbon content as Koc. In order to reduce the interference of soil texture, further sorption experiments for the wide range of pharmaceuticals in same soil condition are necessary.

QSAR STUDIES ON THE POTENTIAL ENVIRONMENTAL HAZARD OF PHARMACEUTICALS

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Background and objective : Active Pharmaceutical Ingredients (API) have become ubiquitarily present in the environment, for this reason in 2006 the European Medicines Evaluation Agency (EMA) published guidelines for the environmental risk assessment of human pharmaceuticals. Every environmental risk assessment (ERA) requires large amounts of data for each chemical but, unfortunately, information on PBT behaviour and ecotoxicological data are available only for a little percentage of API in literature and databases.

Methods and results : We propose the characterization of the intrinsic hazard potential of pharmaceuticals by QSARs development and application. Some pharmaceuticals have been prioritized as potential PBTs by both Insubria PBT Index in QSARINS and US-EPA PBT Profiler in consensus approach. Then, from literature, we collected and carefully curated datasets for ecotoxicity data of species at different trophic levels (algae, Daphnia, fish) in order to consider a simplified aquatic ecosystem. For each species we developed ad hoc QSAR acute toxicity models, based on PaDEL molecular descriptors using OLS method and Genetic Algorithm for Variable Subset Selection in QSARINS software. All models are robust ($R^2 > 0.75$, $Q^2_{loo} > 0.70$) and externally validated ($CCC > 0.85$) on different splitting schemes.

Discussion and conclusion : The structural applicability domain (AD) of the proposed models to pharmaceuticals without experimental data has been verified and demonstrated to be very high with the 74% of chemicals inside the AD for all the toxicity models. Moreover, reliable predictions from different models applied on a set of more than 1000 pharmaceuticals were combined by Principal Component Analysis (PCA) to find an ecotoxicity trend representative of the pharmaceuticals' toxicity in the whole aquatic ecosystem. This trend, called overall aquatic toxicity index (ATI), has been then modelled by molecular descriptors to obtain a QSAR model useful to highlight, directly from the chemical structure, the pharmaceuticals potentially most hazardous for the environment. This index and the predictions obtained by it could be used to refine procedures of input prevention and control at consumer level as well as a priori in the rational design of environmentally safer pharmaceuticals. Finally, we propose also interspecies correlation models to extrapolate information from one simpler trophic level (such as Daphnia) to another more complex (Fish), reducing the use of animal tests.

PRAGMATIC PHARMACEUTICAL PRIORITISATION: USING ACCUMULATION AND ELIMINATION AS KEY STEPS IN PRIORITISING DRUGS FOR ENVIRONMENTAL RISK

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Background and objective : In order to prioritise the risk of pharmaceuticals in the aquatic environment it is critically important to understand exactly how much of any particular compound is able to enter a fish. The potential for uptake, elimination and net bioaccumulation are therefore key factors. We present a simple model for Pragmatic Pharmaceutical Prioritisation that undertakes progressive steps to assess the utility of the fish plasma model to assess the risk of key compounds. The fish plasma model predicts pharmacological effect only when plasma concentrations of a drug are similar to that of therapeutic effect in humans. However to date this has proven to be a simplistic calculation based on physiochemical properties and it is clear that further refinement is required.

Methods and results : Among the first steps towards this improved model for pharmaceuticals (typically ionized; low logP) we need to accurately predict the uptake of a compound across the gill. We believe in silico models are still some way off understanding the processes involved for these compounds; so have developed the in vitro reconstruction using primary culture and experimental use of rainbow trout gill epithelia organoid which replicates the 'live' gill to demonstrate uptake and elimination by both passive, and most importantly, active processes.

Discussion and conclusion : Once inside a fish, the availability of a pharmaceutical is principally driven by the pharmacokinetics of that compound. The metabolism and elimination, tissue distribution, protein binding and availability to susceptible targets are all key features of how simplistic bioaccumulation can be tuned to predict plasma concentrations and therefore accurately model risk in fish.

PRIORITIZATION OF PHARMACEUTICALS IN THE AQUATIC ENVIRONMENT FOR REGIONS WITH LIMITED USAGE INFORMATION

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Background and objective : As we only have data on the environmental impact of a small proportion of the 1500 active pharmaceutical ingredients (APIs) in use, it is important to establish approaches for rapidly identifying APIs in the environment that really matter. A number of researchers have reported various approaches for prioritization of APIs in the environment. Most of these require annual pharmaceutical usage data for the region of interest. It is therefore difficult to apply the approach in countries, like Kazakhstan, which have limited data on API usage. The aim of the study was therefore to develop a new ranking method for pharmaceuticals in surface water in regions with limited data and to illustrate the approach using Kazakhstan as a case study.

Methods and results : Initially, data were collected on the number of products and active ingredients for different therapeutic classes in use in Kazakhstan and on the typical doses used in humans. These data were then used alongside simple exposure modelling approaches to develop an exposure index for the active ingredients (about 250 APIs) in surface waters in Kazakhstan. The exposure values were then compared to ecotoxicological indices to identify APIs of highest risk. Using the approach, amoxicillin, clarithromycin, azithromycin, sulfamethoxazole and ketoconazole were identified as of most concern.

Discussion and conclusion : Our study presents a novel approach for prioritising pharmaceuticals in the environment for regions with limited data on API usage. While the approach has only been applied to Kazakhstan, we believe it has much wider applicability for prioritising pharmaceuticals in countries where data on product usage is limited. A major assumption of the approach is that number of products containing an API provides an indication of the usage of an active ingredient. To test this assumption and the proposed approach, we recommend that future research will focus on developing analytical methods for these compounds and on monitoring concentration in surface waters in Kazakhstan.

WHAT HAPPENS TO THE PHARMACEUTICAL MOLECULES DURING THE SLUDGE TREATMENT PROCESSES?

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Background and objective : As landspreading is one of the main routes for sludge disposal in many countries after wastewater treatment, the behaviour of micropollutants, as pharmaceuticals, during sludge treatment before land application has to be monitored. The physicochemical properties of pharmaceutical compounds, such as hydrophobicity or sorption capacity could lead to sorption onto sludge without biodegradation by transfers between the liquid phase to the solid phase. (Carballa et al., 2008). Before landspreading, sewage sludge follows treatment processes, as thickening, dewatering, drying, sanitation, and stabilization. In France, the main processes for sludge stabilization are liming, anaerobic digestion and composting. The objectives of the work were to follow the behaviour of pharmaceuticals during sludge treatment, and especially stabilisation, and to assess a potential risk when returning to the ground.

Methods and results : Removal and phase repartition of 11 pharmaceuticals compounds was investigated and compared according to the kind of sludge stabilization (chemical or biological). Pharmaceutical compounds studied were: carbamazepine (CBZ), ciprofloxacin (CIP), sulfamethoxazole (SMX), salicylic acid (SAL), ibuprofen (IBU), paracetamol (PAR), diclofenac (DIC), ketoprofen (KTP), econazole (ECZ), atenolol (ATN) and propranolol (PRP). They were extracted and analyzed with the technique described by Peysson and Vulliet (2013). Their phase distribution was determined in the particulate fraction (and in the soluble fractions of sludge and empirical K_d values have been determined. Sludge was sampled from a wastewater treatment plant (WWTP) in Bellecombe (France) designed to treat separately hospital wastewater and domestic wastewater with conventional activated sludge systems and have undergone a stabilization treatment (liming, anaerobic digestion). The obtained value of desorption potential were correlated and/or their partition coefficients as biochemical composition, fonctionnal groups or pH. This work demonstrated that the pH, phosphoric fonctionnal groups in the soluble phase, or the amine/hydroxyl groups in the particulate fraction have the main impacts on the sorption behavior. Furthermore, it was showed that the desorption (and accordingly their availability or mobility during return to the ground) is different depending on the applied treatments of stabilization. For exemple, ibuprofen, salicylic acid, diclofenac and paracetamol were found in soil column leaching.

Discussion and conclusion : The sorption of pharmaceutical compounds onto the biological sludge during the wastewater treatment and the soluble/particulate distribution during subsequent treatment of the sludge will be influenced by the interactions related to chemical functions present in the sludge. a statistical model was proposed to represent these interactions. Various interactions exist between pharmaceutical compounds and sludge as hydrophobic or electrostatic interactions but also the negative charges of microorganisms, which act as cation echangeurs (such as for carbamazepine or atenolol). A consequence of the mobility of molecules in the ground is the emergence of an environmental risk. A risk quotient (RQs) were calculated for the different sludge sample and a predictive value was calculated in case of land-spreading. The results showed that risk quotient were very low and if some environmental risks can exist for some compounds in limed sludge, the dilution achieve during landspreading led to a complete reduction of environmental risk for the eleven pharmaceutical compounds investigated.

EFFICIENT REMOVAL OF PHARMACEUTICALS BY A SURFACE WATER TREATMENT PLANT DELIVERING DRINKING WATER TO THE CITY OF BRUSSELS

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Background and objective : The drinking water company VIVAQUA, delivering water to more than two million people in Belgium, especially to the city of Brussels and its surrounding, is using the river Meuse as a resource for 30% of its total production. Monitoring of the raw river water shows the presence of several pharmaceuticals at low concentrations. With its surface water treatment plant using a multi-barrier design with ozone and activated carbon, the pharmaceuticals found in the raw water can be removed, to produce drinking water without pharmaceuticals. The objective is to show that the exposure of the population to pharmaceuticals present in surface water is greatly reduced by a treatment plant designed to remove other micropollutants as well.

Methods and results : Monitoring of several pharmaceuticals and some of their metabolites, both by target analysis and target screening will show the evolution of several pharmaceuticals in the river and across the treatment plant. The monitored pharmaceuticals and their metabolites are efficiently removed mainly by the biological activated carbon filtration. The monitoring of a reaction product of carbamazepine, the 9-acridine carboxylic acid, is also followed, but has not been found so far.

Discussion and conclusion : By selecting the most likely pharmaceuticals, from the sold amounts on the Belgian market, from the commonly pharmaceuticals found in other surface waters and with the help of target screening, the monitoring of the river Meuse at the location of VIVAQUA's surface water treatment plant shows some pharmaceuticals and human metabolites to be present. These micropollutants are easily removed by the treatment plant, designed to remove other micropollutants, by using several treatment steps of which biological activated carbon filtration and ozonation. Therefore, the drinking water delivered to the city of Brussels doesn't expose the consumer to a cocktail of pharmaceuticals. Moreover, drinking water is not really an exposure issue when a conventional surface water treatment is applied, using activated carbon and ozone. The sum of all commonly monitored pharmaceuticals is also made available to public consultation on the internet site, to insure a transparent communication and reassure the consumer.

ESTROGENICITY AND TOXICITY OF ENVIRONMENTALLY RELEVANT MIXTURES OF ENDOCRINE ACTIVE COMPOUNDS

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Background and objective : The presence of endocrine active compounds (EACs) in wastewater, surface water, groundwater and even drinking water has become a major public concern worldwide. Exposure to a mixture of EACs has been predicted to result in additive or possibly synergistic effects, which is difficult to detect by chemical analysis. Bioanalysis, such as using *in vitro* or *in vivo* methods, has the advantage of measuring the mixture effects of EACs, but this has not been well studied using environmentally relevant mixtures of EACs. In a previous study, we evaluated the estrogenic effects of 11 EACs of high environmental concern using the *in vitro* yeast estrogen screen (YES) method. The full mixture of all these chemicals at an environmentally relevant ratio also showed weak anti-estrogenic activity. Further, EE2 did not have a prominent contribution to the estrogenic activity of the mixture. The objective of the current study was to evaluate the *in vivo* response to exposure to eleven endocrine active compounds (EACs) commonly found in surface water globally.

Methods and results : We evaluated the mixture toxicity of three naturally occurring estrogens (17 β -estradiol–E2, estrone–E1 and estriol–E3), one synthetic estrogen (17 α -ethinyl estradiol–EE2), one phytoestrogen (genistein–GEN), three phenolic xenoestrogens (bisphenol A–BPA, nonyl phenol–NP, octyl phenol–OP) and three phthalates (dibutyl phthalate–DBP, butyl benzyl phthalate–BBP and bis(2-ethylhexyl) phthalate–DEHP) on the model aquatic organisms, zebrafish. This is the same set of EACs we used in the *in vitro* YES study. We followed the OECD 236 guideline and the U.S. Environmental Protection Agency (EPA) method EPA/600/4-91/002. The zebrafish embryos were exposed to 6 dilutions of the EAC mixture, varying from 0.1 to 100 times the environmental concentrations. A parallel set of experiments with the same chemicals will be conducted in the transgenic *cyp19a1b*-GFP zebrafish embryo assay (EASZY) to gain information on mixture effects *in vivo*. The transgenic zebrafish embryos will be exposed to 6 dilutions of the EAC mixture in the same range of concentrations. A comparison of the two methods will be presented at the meeting, after analysis is completed.

Discussion and conclusion : For exposure experiments, EDCs mixtures at environmentally-relevant concentration level were used. Based on our preliminary analysis, we conclude that a holistic evaluation of the toxicity and estrogenic activity is necessary to evaluate the risk of exposure to a mixture of EACs in the environment.

ASSESSMENT OF THE SUITABILITY AND PERFORMANCE OF IN VITRO BIOASSAY TOOLS FOR ANALYZING MIXTURE TOXICITY AND ECOTOXICITY EFFECTS OF ANDROGENIC, THYROID, GLUCOCORTICOID AND PROGESTERONE HORMONAL ACTIVITY IN ENVIRONMENTAL AND DRINKING WATER

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Background and objective : If much of endocrine disruption research from environmental exposure has been carried out on the estrogenic axis assessment, recent works have confirmed that environmental micropollutants may also affect other primary endocrine/hormonal pathways, including androgen, progestagen, glucocorticoid, retinoid, thyroid and mineral corticoid activity. Those pathways also play a crucial role in the maintenance of homeostasis, sexual development, metabolism, growth and behavior. These observations call for urgent improvement of the tools available to assess endocrine activity in water cycle quality assessment and monitoring, to better assess potential endocrine effects in wildlife and human health. While chemical analyses alone often provide very little information on the biological effects and do not take into account interactions among individual chemicals in mixtures, in vitro bioassays are finding increasing added value as screening tools by addressing EDC characteristics.

Methods and results : This project, funded by the Global Water Research Coalition (GWRC), had four main aims: (i) review available in vitro methods for a selection of hormonal endpoints from the literature and identify a suite of in vitro bioassays suitable for laboratory screening in one or more water matrices (drinking water, surface water, treated wastewater and recycled water), (ii) develop and validate methods to extract and analyze a variety of endocrine-active compounds from water, (iii) benchmark different in vitro and in vivo assays addressing different thyroid mode of disruptive activity; and (iv) apply the newly validated battery of assays to measure a panel of endocrine pathways activity in three different water matrices (treated wastewater, surface and drinking) from six participating countries. The purpose of the presentation is to inform a broader scientific audience about the aim of this collaborative project including preliminary findings and follow-up activities.

Discussion and conclusion : Results so far suggest that i. As for an extensive literature review conducted to identify available in vitro bioassays for non-estrogenic endocrine activity based on a large set of parameters [specific to their application to water such as sensitivity, selectivity, reproducibility, comprehensiveness (i.e. agreement with in vivo effects), and robustness, but also measures specific to the assay that affect its applicability in the water sector, such as timeliness, ease-of use maturity, availability, throughput, and cost.], mammalian reporter gene assays and cell proliferation assays are the most sensitive methods, but significant sample enrichment will be necessary to detect non-estrogenic endocrine activity in water samples based on current (often incomplete) knowledge. ii. Solid Phase Extraction (SPE) with a StrataX cartridge at pH 2 was the most efficient method to extract a variety of endocrine active compounds from different types of water samples, although extraction with C18 and Oasis HLB cartridges likewise were very efficient iii. Thyroid receptor reporter gene assays do not detect significant thyroid activity in environmental waters as for the inter-assay comparison that included ten of the most promising in vitro bioassays (TH biosynthesis, TH transport, TR mediated action) using 9 model compounds and 1 full mix in four water samples (drinking, surface, ground and treated waste water), one spiked and one non-spiked, to determine the likelihood of matrix effects (using the extraction method identified in step ii above). The extracts have been analysed by both chemical analysis (list of 51 substances' including hormones and pharmaceuticals) and in the bioassay battery, as well as in two in vivo assays tested in parallel. The performance of each assay with the model compounds, the field samples have been compared among in vitro methods but also against the in vivo response to determine if the in vitro bioassays are representative of potential adverse effects that could impact normal physiology and applicable for future evaluation of water and wastewater treatment technologies. The data analysis is currently underway and data will be presented at the conference. iv. Finally, a battery of 6 bioassays and various chemicals were applied to 24 environmental samples (treated wastewater, surface, drinking and ultrapure water) collected from 6 countries

(The Netherlands, Germany, Spain, France, Australia and South Africa) to determine androgenic, progestagenic, glucocorticoid, retinoid, mineralcorticoid and thyroid activity. Participating laboratories include Veolia, CIRSEE and TZW for chemical analysis and Griffith University, Univ Pretoria, BDS, UFZ-Helmholtz Leipzig and IRCM Montpellier for bioassay analyses. Bioassay endpoints include: estrogen (ER-GeneBLazer), androgen (AR-GeneBLazer, MDA-kb2), progestagen (PR-GeneBLazer, PR-CALUX), glucocorticoid (GR-GeneBLazer, GR-CALUX), RXR (RXR-CALUX, HG5LN-RXR), PPAR (HG5LN-PPARg), mineralcorticoid (HG5LN-MR) and thyroid activity (GH3-Luc). Chemical analysis include the initial screen developed at TZW for 51 target compounds including hormones and pharmaceuticals), but also various analytes by silylation-GC/MS and LC/MS at Suez-CIRSEE and a LC/MS screening on 112 compounds by Veolia-VERI. Data analysis for the 4th aim is currently underway and will be presented at the conference.

ASSESSMENT of SYNERGISM and ANTAGONISM in BINARY MIXTURES of PHARMACEUTICALS and PHENOLS TOWARDS a NON-TARGET ORGANISM *Chlorella vulgaris*

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Background and objective : Organisms in the aquatic environment are exposed to a variety of substances of numerous chemical classes. The unintentional co-occurrence of pharmaceuticals and other contaminants of emerging concern may pose risk to non-target organisms. The chemicals legislation, spearheaded by REACH and CLP, aims to ensure a high level of protection in human health and the environment, but it is rarely based on the assessment of combination effects of chemicals. In this study, individual and binary mixture toxicity experiments of selected pharmaceuticals (ibuprofen and ciprofloxacin) and chlorophenols (2,4-dichlorophenol (2,4-DCP) and 3-chlorophenol (3-CP)) have been performed with freshwater algae *Chlorella vulgaris*.

Methods and results : All experiments have been carried out according to the 96-h algal growth inhibition test OECD No. 201. Binary mixture tests were conducted using proportions of the respective IC50s in terms of toxic unit (TU). The mixture concentration-response curve was compared to predicted effects based on both the concentration addition (CA) and the independent action (IA) model as suggested in regulatory risk assessment. Additionally, the Combination Index (CI)- isobologram equation method was used to assess toxicological interactions of the binary mixtures.

Discussion and conclusion : All substances individually tested had a significant effect on *C. vulgaris* population density and revealed IC50 values < 100 mg.L-1 after exposure period of 96-h. The toxic ranking of these four compounds to *C. vulgaris* was 2,4-DCP > ciprofloxacin > 3-CP > ibuprofen. Generally, it can be concluded from this study that toxic mixture effects of all tested chemicals to *C. vulgaris* are higher than the individual effect of each mixture component. The CA model is appropriate to estimate mixture toxicity, while the IA model tends to underestimate the joint toxic effect. The CI-isobologram equation method elicited synergism at low effect levels for the majority of tested combinations and revealed that interactions between the toxicants can change with effect levels. The IC50 values of the tested mixtures predominately lead to additive effects. The CI method can be applied in ecotoxicology to define interactions of potential toxicants in mixtures towards non-target organisms and may be especially useful for risk assessment strategies.

PRIORITISATION OF ACTIVE PHARMACEUTICAL INGREDIENTS MIXTURE IN THE AQUATIC ENVIRONMENT

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Background and objective : As living conditions become better, the need to live healthier, happier and longer has increased the use of pharmaceutical and personal care products. After use, there is the potential for these chemicals and their metabolites to be released into the aquatic environment, where non target organisms will be exposed to a complex mixture of active pharmaceutical ingredients (APIs) and the substances. Once in the environment, APIs may interact in either an additive, synergistic or antagonistic manner. Therefore to fully understand the impact of pharmaceuticals in the environment, it is important to understand these interactions. As there are over 1500 APIs in use, it would be impossible to explore the mixture interactions of all compounds so it would be valuable to develop prioritisation approaches that identify API that are most likely to interact. In this study, we present an approach for doing this, using information on likely environmental exposure and on drug – drug interaction that are known to occur in humans.

Methods and results : A screening and prioritisation exercise was carried out to determine which APIs present the greatest risk to aquatic organisms in the environment. Specifically, for the APIs with the highest usage in UK, a risk characterisation ratio (RCR) was calculated that incorporated information on the predicted exposure concentration (PEC), predicted fish steady state plasma concentrations (FSSPC) and maximum human therapeutic concentration (HTPC). In total, 27 APIs were identified as having fish plasma concentrations > 0.1 times the level needed to elicit a human therapeutic effect. For these APIs, information on drug-drug interactions in humans was extracted and used to identify APIs combinations that could interact to cause side effect in the environment (i.e if RCRs for two APIs that interact in humans were both > 0.1 then this combination was considered likely to show a similar interaction in the environment). Results from the screening and prioritisation exercise indicate that Amlodipine, Felodipine, Amiodarone, Simvastatin, Citalopram and Phenothiazine have a RCR > 0.1 and severe drug-drug interactions have been reported in humans. For these APIs there is the potential for the drug-drug interactions observed in humans such as hypotension, myopathy and cardiac arrhythmias to be observed in aquatic organisms.

Discussion and conclusion : The study has developed a pragmatic approach for identifying APIs that could interact in a negative way in the environment. Using the approach, seven APIs have been identified for further study. Future investigations will focus on the effect of these APIs in mixtures using environmentally relevant concentrations and measurements will be made on the heart rate, movement, agitation and survival of *Daphnia magna*. As organisms are exposed to a multitude of chemicals in the environment, including APIs, it is important to consider how exposure to mixture influences organism toxicity. More research is required to comprehensively explore mixture toxicity in non-target organism in order to provide a comprehensive assessment of the risk of pharmaceuticals in the environment.

USE OF HAZARD ASSESSMENT TOOLS OF INCREASING LEVEL OF COMPLEXITY FOR 2 PHARMACEUTICALS: BIOLOGICAL AND ECOLOGICAL RELEVANCE

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Background and objective : Use of pharmaceuticals and their subsequent indirect emissions as unchanged parent, metabolized drugs or degradation products in aquatic ecosystems implies to increase the scientific knowledge on their likely ecotoxicological impacts and the improvement of the tools to assess them. This is the goal of the DOREMIPHARM project, which aims at developing robust hazard assessment tools for pharmaceutical substances. As these substances are biologically active and might be persistent, toxic effects on organisms are suspected at low concentrations. The project involves different partners and their corresponding skills in order to implement a large number of tools allowing the testing of various scales of experimentation (in vitro, ex vivo, in vivo and mesocosms study) and different level of biological responses. In order to assess whether the current regulatory requirements were sufficient to assess the ecotoxicity of pharmaceuticals, conventional and non conventional endpoints (from a regulatory viewpoint) were tested.

Methods and results : The aim of this work is to give an overview of the possible levels of assessment that may be taken on board for environmental hazard assessment of pharmaceuticals, focusing on the work done on diclofenac sodium and carbamazepine. Traditional and regulatory Predicted No Effect Concentrations (PNECs) based on the so-called “conventional” endpoints such as mortality and sublethal endpoints (e.g. growth, reproduction, development) were calculated. These values are derived from standardised laboratory tests on 3 trophic levels: primary producers (algae), primary consumer (invertebrates) and secondary consumers (fish). They were used to compare how the inclusion of less conventional endpoints may drive differently the hazard assessment. These endpoints depict different complexity scales as regards space (laboratory versus mesocosm data) and as regards biological organisation (community, population, individuals, cellular, histological findings, biochemical responses, including immunotoxicity and endocrine activity). It is shown that the concentrations at which effects occur cover several orders of magnitude.

Discussion and conclusion : Applying this methodology, the so-called early-warning endpoints are compared to effects observed at community or populational level. Then, different PNECs are derived and presented : “traditional PNECs” covers conventional endpoints for at least three trophic levels (algae, crustaceans and fish), while “non conventional PNECs” may take account of the mesocosm study data obtained during the project but also of biomarkers response (e.g. biomarkers of effects such as immunotoxicological or respiratory burst effects). the ecological relevance of various endpoint is discussed.

APPLICATION OF RISK ASSESSMENT TOOLS FOR A COMPARATIVE CHARACTERISATION OF WASTEWATER POLLUTED BY PHARMACEUTICALS FROM DIFFERENT SOURCES

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Background and objective : Hospital wastewater contains high concentrations of micropollutants like pharmaceuticals, disinfectants, pathogens and multi-resistant bacteria (Kümmerer, 2009; Ort et al., 2010; Verlicchi et al., 2010). To assess the contribution of hospital to the emission of selected pharmaceuticals a well-defined urban catchment area with a medium sized hospital was characterized by an adapted sampling and measurement campaign of hospital wastewater and urban wastewater. In addition to measured concentrations mass balances of selected antibiotics and cytostatics the potential relevance of the wastewater from different sources was evaluated by adopting risk assessment tools.

Methods and results : To assess the ecotoxicity potential of hospital wastewater a risk quotient (RQ) was evaluated according Escher et al. (2011). RQ was defined as measured concentration (MC) divided by the predicted no-effect concentration (PNEC). This approach allows for an evaluation of the risk stemming from pharmaceuticals and the contribution of hospitals in comparison to urban wastewater. In the present study this approach was applied to a catchment area of a medium sized German hospital (560 beds) discharging into a sewer which collects the urban wastewater of 76,000 inhabitants living upstream (Fig. 1). The bed per 1000 inhabitants ratio of 7.4 of the investigated catchment area is in good agreement with other hospital wastewater studies (Ort et al., 2010). Flow proportional composite samples were taken and analysed for 124 pharmaceuticals, hormones, contrast media and disinfectants. This study focuses on the results of 26 different antibiotics, 5 antibiotic metabolites and 12 different cytostatics. Figure 1. Schematic drawing of the sampling points. Significant differences were observed for the concentrations and mass fluxes depending on the considered compound, the sampling point and the sampling day. For some compounds a pronounced weekly variation was observed. Consumption data of selected antibiotics and substance-specific excretion rates were used to calculate predicted environmental concentrations (PEC) and to validate the measured concentrations (MC). The assessment of the measured loads was conducted according to a classification system developed by Ort et al. (2010). In the second part of this study risk quotients (RQ) were calculated using both measured data as well as literature data (PNEC of selected compounds). Additionally a QSAR approach according to Escher et al. (2011) was applied as an option for the calculation of PNEC values based on physico-chemical properties. Finally, the calculated RQ and its relevance were critically reviewed and compared with related studies (Weißbrodt et al., 2009; Escher et al., 2010; Verlicchi et al., 2012). This final assessment was based on a comparison between the mass load ranking of the selected compounds and their risk level. Fig. 2 gives a summary of this assessment. The data representation in Fig. 2 was oriented on the RQ classification originally proposed by Hernando et al. (2006) and allows a direct comparison with the results from Verlicchi et al. (2012). Figure 2. Comparison of risk quotients for selected antibiotics and cytostatics in the hospital and urban wastewater.

Discussion and conclusion : The results of this study support the current discussion on the relevance of source control measures to reduce the impact of pharmaceutical compounds in surface water bodies. Given the lack of assessment and monitoring values for pharmaceutical compounds in the existing regulatory framework the adopted approach revealed that there are no simple answers. Nevertheless, the approach provides operators of wastewater infrastructure with additional aspects for the discussion of management options at source or end-of-pipe. The present study was part of the European INTERREG IV B project PILLS (Pharmaceutical Input and Elimination from Local Sources) (www.pills-project.eu).

PRIORITIZATION APPROACH FOR ANTIBIOTICS IN HOSPITAL EFFLUENTS BASED ON THEIR PREDICTED NO EFFECT CONCENTRATION FOR RESISTANCE SELECTION. A PORTUGUESE CASE-STUDY.

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Background and objective : The use of some antibiotics is now restricted mainly to the hospital centers. Therefore hospital wastewater (HWW) effluents may result in significant sources of antibiotics, providing favorable conditions for the dissemination of resistant bacteria (Peak, et al., 2007). The main objective of this work was to prioritize hospital antibiotics based on its microbial risk quotients (HQs).

Methods and results : Predicted environmental concentrations (PEC) and the predicted no effects concentrations for resistance selection (PNEC) were calculated to obtain HQs for HWW and waste water treatment plan (WWTP). The PECHWW values were obtained by dividing the total used amount (considering the excretion factor), with the volume of wastewater (assuming the amount of consumed water in the 46 hospitals analyzed during 2014) (ACSS, 2014). PNEC values corresponded to the size-adjusted lowest minimal inhibition concentration (Bengtsson-Palme and Larsson, 2016). In order to predict concentrations in wastewater treatment plants (WWTP) a dilution factor of 100 has been applied (Kümmerer and Henninger, 2003). The 2014 annual crude amount of antibiotics used was about 11.7 tons. It was markedly larger for penicillins (66%), followed by cephalosporins (16%), carbapenems (7%) and quinolones (2.4%). Piperacillin, ceftriaxone, meropenem, and vancomycin were identified as hospital-specific compounds. The estimated 5 most hazardous antibiotics ($HQ \geq 500$) were: ceftriaxone, meropenem, piperacillin, amoxicillin and ciprofloxacin.

Discussion and conclusion : Antibiotics may exert antimicrobial effects at exposures levels below those required to produce ecotoxicological effects and should be complied as a significant end-point. This approach, can support the hospital management board to acquire knowledge about its effluents contamination, identifying those antibiotics of particular concern.

ANALYSIS OF THE LIFE CYCLE IMPACT ASSESSMENT OF PHARMACEUTICAL PRODUCT INVENTORIES

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Background and objective : INTRODUCTION The assessment is done by means of LCA, according to the ISO 14040 series by using SimaPro.6 LCA Software using the Eco Indicator 95 methodology. Which is a "damage oriented" impact assessment method with clearly detailed steps such as fate, exposure, effect and damage analysis. A report containing a complete description of the methodology, as well an overview of all damage (characterization) factors can be downloaded from the web site link. [1] [2] The objective of this study is to: Identify the potential environmental impact associated with the production of drug mycocide250 (pomade) in this factory using the SimaPro.6 software. Evaluate the potential impact related to a mixture of and using the experience plan by SimaPro.6. Establish relation between potential impact and the compound of Mycocide drug.

Methods and results : MATERIALS AND METHODS Different pomade drugs are produced in this factory, but in the case study; MYCOCIDE is selected for evaluation of potential impacts. These choose is based on the most quantity produced or commercialized in year. The inventory data collected was calculated to quantify the inputs from the environment and outputs to the environment for every 250kg produced of mycocide, this inventory is naming life cycle inventory LCI. The life cycle impacts assessment LCIA was conducted using the SimaPro.6 soft ware version 6.0; Eco Indicator 95 and 99 methodologies are selected to conduct the LCIA. Table N°1 : Life Cycle Inventory of pomade factory. Input Unit Dematerialized water 54 m³ / J Electricity 933 M WH / J Natural gas 562 N m³ / J Out put unit Chemical oxygen demand COD 406 mg d'O / l Biological oxygen demand DBO 163 Mater in suspension MES 13 mg / l Volatile mater MVS 2 mg / l Waste carton 42 Kg / J System boundary and functional unit: The system boundary was set to include only the production process of this drug selected (mycocide) as shown in figure 1. The society and co treatment consequence is not taking consideration in this case. The data collected has been adapted to database of the software used. The functional unit is 250 kg of mycocide produced by preparation. RESULTS Potential impacts of mycocide pomade Table N°1 show the LCIA of 250 kg of mycocide produced in this factory. Figure 2 shows the single score of the LCIA of the production of 1 kg of mycocide drug which shows that there are only 3 potential impacts as acidification, winter smog and heavy metals expressed by mPts unit and figure 3 shows the whitening of the LCIA for the same production, which detect the same potential impacts. All impacts detected have origins of the traces of principal's actives maters in the compound of this pomade, such as acidity due to pH of pomade, the purity of his compound, presence of minerals and heavy metals of each element.

Potential impacts of the elements composition of mycocide: Three (3) elements are chosen in this case with experience plan as NYSTATINE, NEOMYCINE SULFATE, and TRIAMCONOLONE ACETONIDE. The concentration of actives elements is gives in the table 3. 1st mixture Composition elements (%) Nystatine (NYST) 1 2.268 1 2.268 Triamcinolone Acetonide (TRIAM) 0.048 0.048 0.096 0.096 2nd mixture Composition elements (%) Nystatine (NYST) 1 2.268 1 2.268 Néomycine Sulfate (NEO) 0.206 0.206 0.412 0.412 Table.2: The mixture of actives elements using the experience plan

Variables Number of tray X 1 X 2 1 - 1 -1 2 +1 -1 3 -1 +1 4 +1 +1 As: X1: is the fist variable. X2: is the second variable. -1: is the low or minimum concentration. +1: is the height or maximum concentration. In this step for each mixture, potentials impacts was determinate by the same software; SimaPro.6 using the EcoIndicator 99 methodology. The LCIA of two mixtures are shown in the figures below. For each mixture, five potentials impacts; fossil fuels, respiratory inorganic, climate change, Ecotoxicity and acidification / Eutrophisation, all these impacts are related to: - Height consumption of natural gaz and electricity. - Using height quantity of raw material and actives maters witch have residues in the liquid waste. - Using organics and inorganic, acid and basic product in the process production. Comparison potentials impacts between mixture is resumed in table 4 Table 4: Comparison potentials impacts. Impacts Category Impacts (Pts) Nyst Min Triam min Nyst max Triam min Nyst Min Triam max Nyst Max Triam max Nyst Min Neom min Nyst Max Neom min Nyst Min Neom max Nyst Max Neom max Carcinogen 1.13E5 5.13E4 1.08E5 5.03E4 428 189 428 4.44E4 Respiratory organic 6.8E3 3.08E3 6.5E3 3.01E3 25.6 11.3 25.6 2.66E3 Respiratory inorganic 2.39E6 1.08E3 2.29E6

1.06E6 9.03E3 3.98E3 9.03E3 9.36E5 Climate change 8.18E5 3.7E5 7.83E5 3.63E5 3.09E3 1.36E3
 3.09E3 3.2E5 Radiation 2.27E3 1.03E3 2.17E3 1.01E3 8.57 3.78 8.57 888 Ozone layer 3.2E3 1.45E3
 3.06E3 1.42E3 12.1 5.33 12.1 1.25E3 Ecotoxicity 4.46E5 2.02E5 4.26E5 1.98E5 1.68E3 741 1.68E3
 1.74E5 Acidification/ Eutrophisation 3.64E5 1.65E5 3.48E5 1.61E5 1.37E3 606 1.37E3 1.42E5 Land use
 6.09E4 2.75E4 5.82E4 2.7E4 230 101 230 2.38E4 Minerals 3.98E4 1.8E4 3.18E4 1.77E4 150 66.3 150
 1.56E4 Fossil fuels 5.71E6 2.58E6 5.46E6 2.53E6 2.16E4 9.5E3 2.15E4 2.23E6 .

Discussion and conclusion : CONCLUSION All the while environmental management was categorized more for image purposes. However in recent developments of shift towards wanting a more green earth, environmental demands are becoming marketing tools. It is becoming a determining factor for use products.

DOES THE PRESENCE OF CAFFEINE IN THE MARINE ENVIRONMENT REPRESENT AN ENVIRONMENTAL RISK?

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Background and objective : Caffeine is widely consumed as a stimulant by the general population (also it is used in hospitals as a drug). In Europe, the adult population consumes an average of 200 mg caffeine, mainly in tea and coffee. Indeed, coffee is the most widely consumed drink in Spain, with the average consumption of one or two cups per person per day representing an average intake of 91.32 mg of caffeine (EUFIC, 2007). The ability of caffeine to enhance the state of alert and long-term attentiveness is well known, and its primary role as a central nervous system stimulant arises due to its action as an adenosine antagonist. Caffeine, and its main metabolite paraxanthine, are considered to be indicators of anthropogenic contamination given their widespread use by the population, their ubiquity in the environment, their chemical stability and their relationship to other contaminants associated with human activities. As such, caffeine has been included in the group of emerging contaminants and may be subject to regulation in the future in light of research into its negative effects on the environment. The presence of caffeine in river water has been widely documented, reaching concentrations of up to 34 µg/L (Paxéus et al., 1996); 22 µg/L (Gagné et al., 2006) or 13 µg/L in Spain (Valcárcel et al.). In the latter study, Valcárcel et al. obtained an HQ value of more than 10 for the caffeine concentrations found in the rivers of central Spain (Madrid Region), thereby representing a high environmental risk. However, its presence in the marine environment has not been as widely studied, and, in Spain in particular, there are no studies of the concentration of caffeine present in this medium and very few studies of its toxic potential once it reaches this environment.

Methods and results : The regions studied were the coastal inlets (rías) of Muros and Noia, Arousa and Pontevedra (NW Spain), which are located on the west coast of Galicia close to A Coruña and Pontevedra. These three inlets form part of a larger grouping known in Spain as the Rías Bajas (or rías baixas) in reference to their geographical location. Galicia has a population of around 2.7 million, around 35% of which lives close to the western seaboard and is therefore in direct contact with the inlets sampled (INE). To conduct this study, a series of specific points located in the aforementioned inlets was sampled in the second and third weeks of July 2015. Pre-established sampling times were not used as they were of no specific interest for this study. The number and location of the points selected in each inlet were based on the local population and nearby fish and shellfish production. The system used was a simple, one-off sampling in which a volume of 0.5 litres was collected in opaque PET bottles previously washed with distilled water and stored cold after use until being frozen for subsequent transport to the IDAEA-CSIC group for analysis. The risk assessment was based on the concentrations obtained (MECs) and the predictive toxicity values (PNECs). In addition, the risk was assessed on the basis of persistence, bioaccumulation and toxicity (PBT). The results obtained for caffeine show maximum and average concentrations of 289 and 16 ng/L for Pontevedra, where the lowest concentrations were measured, 18,493 µg/L and 10.6 n/L for Muros/Noia and 10.4 and 857 ng/L for Arousa. Overall, this means a considerably high occurrence and environmental impact in a region that is highly dependent on fish and shellfish production.

Discussion and conclusion : The purification processes applied at the WWTPs in the coastal regions of NW Spain, specifically around the Rías Bajas, which are internationally renowned as one of the regions with the greatest production of fish and shellfish, do not remove the majority of the caffeine and paraxanthine that reaches them in wastewater from the main urban centres. As such, the ecotoxicological risk of the concentrations obtained in seawater in this region must be assessed to determine whether caffeine represents an environmental risk, especially given the importance of the large number of species for human consumption found in this region.

Acknowledgments

The authors would like to thank the Spanish Ministry of Economy and Competitiveness for financial support via the Carlos III Health Institute and the Program “Projects on Health Research” 2014-2016 FIS (PI14/00516) and the European Regional Development Funds (ERDF), as well as the financial support of the European Commission through the project SOLUTIONS (contract 603437), and the Generalitat de Catalunya (Consolidated Research Groups “2014SGR418-Water and Soil Quality Unit” and 2014SGR291-ICRA65). Merck is acknowledged for the gift of LC columns.

ECOTOXICOLOGICAL RISK ASSESSMENT AND PRIORITIZATION OF PHARMACEUTICALS IN IBERIAN RIVERS

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Background and objective : 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 generally at low concentrations. 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 threat. . For the purpose creating a more comprehensive picture of the environmental risk of the pharmaceuticals is necessary.

Methods and results : 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 2011 with different river flow and meteorological conditions. The ecotoxicological risk was estimated by toxic unit approach (TU) for three standard test species (algae, invertebrates and fish). For the prioritization purpose ranking index (RI) was developed which is based on the frequency of compounds TU being in the certain importance 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.

Discussion and conclusion : 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 increment of risk of micropollutants which is evident already.

SPATIAL PATTERNS OF ENVIRONMENTAL RISK FROM PHARMACEUTICALS: THE INFLUENCE OF HOSPITALS

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Background and objective : Pharmaceuticals are used ubiquitously in hospitals as well as in the community. Following excretion, residues enter the sewer and can enter the aquatic environment. Whilst overall, the amount used in the community is much greater than that used in hospitals, variation in the relative contributions varies by drug, by hospital type and by geographical setting. This paper investigates the spatial variation in risk for selected drugs, some used predominantly in hospitals and some associated with community use.

Methods and results : Using datasets on hospital and community consumption of selected pharmaceuticals, typical consumption values per bed and per head of population were determined for a range of different types of hospitals and for domestic wastewater, for selected compounds. These were combined with location and size data on hospitals of different types and of wastewater treatment plants, subsequently with flow and catchment data from a network of river gauging stations, and with published data on compound ecotoxicity, enabling the calculation of spatially differentiated environmental risk quotients for each of the selected compounds, visualised as risk maps.

Discussion and conclusion : Both the influence of dilution and the influence of the presence of point sources are clearly visible on the maps. Pharmaceuticals predominantly used in hospitals were likely to be concentrated in 'hotspots' in surfacewaters where discharges containing hospital effluents are released into the environment. Locally, risk quotients for hospital drugs reached values much higher than risk quotients based on national consumption datasets and default dilutions. It is therefore recommended that an additional Assessment Factor is used in environmental risk assessments for drugs predominantly used in hospitals.

POST-APPROVAL ENVIRONMENTAL MANAGEMENT OF HUMAN MEDICINAL PRODUCTS: AN EXTENDED ENVIRONMENTAL RISK ASSESSMENT (EERA) FRAMEWORK.

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Background and objective : The Association of European Self-Medication Industry (AESGP), European Federation of Pharmaceutical Industry Associations (Efpia) and the European Generics Association (EGA) are currently looking to implement a holistic approach to the environmental management of human medicines products called EcoPharmacoStewardship (EPS). Within this EPS initiative, AESGP, Efpia and EGA propose to extend the regulatory ERA to formally capture post-approval commitments, periodic environmental updates, and where required, risk refinement.

Methods and results : The eERA framework has been developed for APIs marketed post-2006 and addresses environmental risk based on total substance use and not product specific use. Should the ERA outcome change as a result of these periodic eERA activities, environmental risk refinement and management options would be discussed with stakeholders and appropriate follow-up measures agreed by all interested parties. The eERA framework has three distinct phases and allows the effective management of environmental risk of medicinal products as they lose patent protection through advocating the increased transparency and accessibility of ERA data. The benefits the eERA framework brings to the environmental assessment of human medicinal products includes: (i) formalising post-launch commitments for addressing environment risk without impacting patient access to medicines, (ii) a risk assessment based on the total predicted environmental concentration (PEC) arising from all human medicinal products containing the same API, and (iii) on-going assessment of the relevance and reliability of published data to ensure the ERA is up to date.

Discussion and conclusion : eERA offers a possible mechanism where industry can agree with the EMA and other stakeholders (including national competent authorities (NCAs)) on proportionate risk management measures where any significant environmental risks identified post-patient use would trigger appropriate further work to refine the ERA. Conversely, where post-authorisation surveillance does not indicate any significant risk for an API then no further action is needed until the next periodic review of the ERA based on total PEC. This presentation will describe the eERA process, its benefits, and discuss some of the challenges posed in its implementation.

DRUG RESIDUES (DR) OCCURRENCE IN TOULOUSE CITY SEWAGE, IN WWTP EFFLUENT AND AFTER ARRIVAL INTO THE GARONNE RIVER: ECOTOXICOLOGICAL RISKS CHARACTERIZATION (2011-2013 STUDY).

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Background and objective : DR occurrence in aquatic environments, especially coming from Waste Water Treatment Plants (WWTP) effluents, is documented. Ecotoxicological studies showed in vitro impact on aquatic organisms. However, there is a lack of evidence about ecotoxicological risks. The aim of this study, performed from 2011 to 2013, was to assess DR occurrence in Toulouse city sewage and downstream effluents release in the Garonne River.

Methods and results : Monthly samplings were performed on WWTP effluents in 2012 and 2013 during flu epidemic peaks, according to Sentinelles website data. Among 52 antibiotics sought from January to March 2012, at most 26 were detected. The 3 most concentrated were Ciprofloxacin, Spiramycin and Clarithromycin. Therefore, only these molecules were sought in March 2012 in the Garonne, and then, during 2013 campaigns (January, February, April) in WWTP effluents and the Garonne. Analyses were performed by physical and toxicological chemistry of the environment laboratory (Bordeaux). For each considered substance, following information were studied: - Measured Environmental Concentration (MEC) in the Garonne; - Garonne Predicted Environmental Concentration (PEC), obtained from WWTP effluent concentrations; - Predicted No Effect Concentration (PNEC), obtained from scientific literature; - PEC/PNEC and MEC/PNEC ratios, underlining an environmental risk when they are greater than 1. In most cases, Spiramycin and Ciprofloxacin PEC/PNEC ratios were greater than 1 in 2012 and lower than 1 in 2013. Clarithromycin PEC/PNEC ratios were systematically greater than 1 (except in April 2013). In March 2012, Ciprofloxacin and Spiramycin MEC values were greater than PNEC, underlining a potential ecotoxicological risk. In 2013, Ciprofloxacin MEC did not allow to determine if there was an environmental risk. In 2012 and 2013 campaigns, Clarithromycin showed MEC values greater than PNEC, demonstrating an environmental risk. In 2013 campaigns, Clarithromycin MEC were systematically greater than PEC, contrary to Spiramycin MEC.

Discussion and conclusion : This study highlighted potential ecotoxicological risks in the Garonne. 2013 PEC were lower than 2012 ones, demonstrating drugs arrival in the river decreased. Previous studies highlighted flu epidemic peaks are linked to higher antibiotic consumption. However, number of flu cases was greater in 2013 than 2012 (26,000 against 9,000 in Midi-Pyrénées), thus, ecotoxicological risks temporal variations seems not related to flu peaks. Other parameters (WWTP degradation capacity, physicochemical drugs properties) should explain environmental risks variations for the 3 antibiotics considered. This study demonstrated that PEC were greater than MEC in most cases. A complementary study is underway to determine a correction factor to estimate DR MEC from PEC values.

DRUG RESIDUES IN AN URBAN RIVER: DEVELOPMENT OF A REFERENCE SYSTEM FOR ECOTOXICOLOGICAL RISKS MANAGEMENT BASED ON THEIR TEMPORAL VARIATIONS.

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Background and objective : Drug residues (DR) occurrence in Waste Water Treatment Plants (WWTP) effluents and aquatic environments is known. A study, performed from 2011 to 2013, aimed to assess the DR occurrence in Toulouse city sewage and in the receiver aquatic environment (the Garonne River). Several DR were identified in 2012 in WWTP effluents, then, analyses focused on the 3 first in terms of concentration: Ciprofloxacin, Spiramycin and Clarithromycin. The risks associated to their presence in the Garonne were highlighted. However, the samplings effort did not permit to explain temporal variations of ecotoxicological risks. Moreover, it was not possible to demonstrate a relationship between the Measured Environmental Concentration (MEC) and the Predicted Environmental Concentration (PEC). The aim of the present study is to develop a database incorporating the temporal variations of ecotoxicological risks.

Methods and results : This study is based on the risk definition: a relationship between the adverse impacts of DR (hazard) on aquatic organisms and the contamination level in the river (exposition). Predicted No Effect Concentration (PNEC), MEC and PEC data are gathered in a database developed with DataGrip software (JetBrains Company). Compared to the 2011-2013 study, list of searched molecules is increased. These molecules were selected by compiling: - DR existing in the European Water Framework Directive vigilance list; - DR pointed out by 2011-2013 study and by other national projects about residues in WWTP effluents and aquatic environments in France. To assess the environmental exposition, a monthly sampling is performed since May 2015 until May 2017 in WWTP, and every two months since June 2015 until May 2016 in the Garonne. The physical and toxical chemistry of the environment laboratory in Bordeaux will perform DR analyses, and results will be incorporated in the database. PNEC values (which characterize hazard) are necessary to risk assessment. These data coming from scientific literature are integrated in the database. Finally, for each DR, the following parameters are studied: - MEC, obtained from measurement in the Garonne; - Garonne PEC, obtained from WWTP effluents concentration; - PNEC; - PEC/PNEC and MEC/PNEC ratios, which indicate an environmental risk when they are greater than 1.

Discussion and conclusion : This study will try to find a correction factor to apply to the PEC in order to estimate the MEC. This database will characterize the temporal variation of environmental risks related to DR. Identification of pollution peaks will facilitate the management of these risks.

HARMONISED ENVIRONMENTAL INFORMATION OF PHARMACEUTICAL SUBSTANCES – THE ESSENTIAL BASE FOR RISK ASSESSMENT AND RISK MANAGEMENT

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Background and objective : Even more than 10 years after implementing the environmental risk assessment (ERA) into the EU pharmaceutical legislation there is still a lack of data on fate and effects of active pharmaceutical substances. The reason for that is quite simple: A review program of ‘legacy’ products, which were approved before the ERA requirement was set, was never envisaged. However, even for newly approved pharmaceuticals sometimes no full data sets essential for an ERA are available.

Methods and results : In the pre-market phase, an ERA is required for all new applications of human and veterinary medicinal products. This often leads to duplication of studies and assessments and hence, to a waste of resources. This may lead to diverging assessments and even to different risk mitigation measures for medicinal products intended for the same indication. This situation is far from being satisfactory and could be solved with a paradigm shift from product-based toward a substance-based ERA. First of all a register of harmonised environmental information of active pharmaceutical substances (HEIPS) should be established. The aim of such a system is to generate a comprehensive set of valid fate and effects data. Therefore, existing data are compiled and gaps are filled. The compiled data set undergoes an evaluation process to agree on the information to be used for ERAs of medicinal products on EU level. Once established all applications for marketing authorisation of a medicinal product will use the agreed information from the monograph of the respective active substance to perform the ERA of the product. This will make the current authorisation procedure much more effective and enable a harmonised environmental risk assessment which is the essential base for any kind of risk management.

Discussion and conclusion : The poster will present results from a research project funded by the German UBA. This project investigated how such a register could be established and what implication the concerned stakeholders need to expect. In conclusion, only up-to-date ERAs, which are based on harmonised environmental information of pharmaceutical substances and regular adjustments of this information to the scientific and technical progress, will be able to ensure the environmental safety of medicinal products in use. Additionally, the HEIPS system could also provide data to be used for risk management in other regulatory frameworks, e.g. the EU water policy. This holds true for human as well as for veterinary medicinal products.

A FEASIBLE CONCEPT TO PRIORITISE VETERINARY PHARMACEUTICAL SUBSTANCES

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Background and objective : The poster presents a new and feasible prioritisation concept for pharmaceutically active substances in veterinary medicines. Since 2005 an environmental risk assessment (ERA) is required for all new veterinary pharmaceuticals within the marketing authorisation procedure. For those pharmaceutically active substances which came on the market before 2005 no or insufficient environmental data are available. In order to fill this data gap a substance-based review system, a so-called “monograph system”, is under discussion at European level. At present about 500 different veterinary active substances are on the European market. Only a limited number of these are environmentally relevant and should be considered for an in-depth environmental risk assessment. Therefore, it is considered necessary to prioritise these substances. The goal of our work was to establish few and relatively simple criteria for a rough classification.

Methods and results : Our prioritisation concept aims at identifying the substances with the highest potential environmental risk and/or the highest potential presence in the environment. We propose a simple three-step approach: The prioritisation starts with a “pre-filter” step where those substances can be identified which are considered environmentally relevant according to the EMA/VICH Guideline for the environmental risk assessment of veterinary pharmaceuticals. In a second step, the prioritisation is refined using publicly available specific product information e.g. dose, application. Finally the substances are classified into four priority categories (very high, high, moderate, low) considering substance specific data on consumption and exposure, and information on fate incl. monitoring data and effects if available.

Discussion and conclusion : Prioritisation was done exemplarily with data available to the German Environment Agency (Umweltbundesamt). It showed that most of the 84 substances considered for an in-depth environmental risk assessment are expected to be in class II (high priority) and class III (moderate priority). Several parasiticides and antibiotics turned out to have the highest priority (class I). The suggested approach is considered to be applicable to other EU countries since all data necessary for prioritisation should be available to other national regulatory authorities as well. In conclusion, the number of prioritised substances for inclusion in a substance based review system seems to be manageable.

PRESENTATION OF FRENCH SCIENTIFIC INTEREST GROUP « PHARMACEUTICALS IN THE ENVIRONMENT »

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Background and objective : 20 institutions are participating in this scientific interest group (universities*, CNRS , INRA, IFREMER, IRSTEA, INERIS, MEDD, ONEMA). * Albi University, Bordeaux University, Limoge University, Lorraine University, Lyon University, Montpellier University, Nice University, Nîmes University, Paris-Sud University, Poitiers University, Reims University, Toulouse University (INP, INSA). GIS aims to promote interdisciplinary researches and exchanges with public and private decision-makers to: - Contribute to the assessment of health and environmental risks associated with pharmaceutical residues in the environment, - Understand and describe the mechanisms of effects and impact of pharmaceutical residues in the environment, - Help to reduce the risk by contributing to the development of misuse prevention guidance and improved treatments for reducing the pharmaceutical concentrations in the environment.

Methods and results : To meet these objectives, the GIS will take action along four main areas of research: - Axe 1 : Link between the uses and the presence of pharmaceutical residues in the environment; - Axe 2 : Mechanisms of action of pharmaceutical residues on non-target organisms that are exposed to environmental concentrations and development of diagnostic tools; - Axe 3 : Perception of general question of pharmaceutical in the environment and acceptability of risk management measures; - Axe 4 : technological and socio-economic approaches to reduce the presence of pharmaceutical residues in the environment.

Discussion and conclusion : The scientific interest group « Pharmaceuticals in the environment » is a grouping of multidisciplinary public researchers having a common goal of reducing the impact of drugs in the environment.

COMMUNICATING ENVIRONMENTAL RISKS OF VETERINARY MEDICINAL PRODUCTS IN BENEFIT-RISK ASSESSMENT

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Background and objective : As part of the marketing authorization process, veterinary medicinal products (VMPs) require consideration of how environmental risks weigh up against the wider benefits of the product: a risk:benefit assessment. However, environmental risks are not directly comparable to therapeutic benefits (i.e., efficacy). Approaches are therefore needed to allow environmental risks to be weighed up against the benefits.

Methods and results : We have developed a methodology for communicating and comparing the environmental risks and benefits of VMPs to stakeholders, regulators, and risk managers in a summarized format. Criteria were developed to categorize compounds into risk and benefits levels. We applied this methodology to three case-study compounds (ivermectin, tylosin and diclofenac) for which there are concerns about impacts on the environment. Using summaries of product characteristics, literature sources, and regulatory guidelines, the risks of the compounds were characterized and used in the methodology to classify the compounds. Due to data limitations the classification of benefits were not tested on the case-study compounds.

Discussion and conclusion : We found comparisons of risks and benefits were highly limited by differences in data sets. To support transparent decision-making future research should aim to create standardized data sets and consider methodologies for implementing benefit-risk assessments. Future implementation of the categorization approaches has potential to be a valuable approach to supporting the benefit-risk assessments required for VMPs.

RESULTS OF THE EU PROJECT NOPILLS – RISK PERCEPTION AND COMMUNITY ENGAGEMENT REGARDING PHARMACEUTICALS IN THE AQUATIC ENVIRONMENT (THE ‘DÜLMEN CASE STUDY’)

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Background and objective : A large case study was conducted in the local town of Dülmen, focusing on capturing risk perception, attitudes and behaviours and on implementing and evaluating intervention in the form of awareness campaigns. This included public awareness campaigns over one and a half years and involved not only members of the public but also medical and pharmaceutical professionals and various other stakeholders.

Methods and results : A case study in Dülmen consisted of the following: • Two-stage survey to assess risk perception and attitudes and behaviour patterns for medicine use and disposal before and after public awareness campaigns. • Survey to assess attitudes and behaviour patterns for medicine prescription and dispensing. • Public awareness campaigns in the city. • Information and education campaigns for medical and pharmaceutical professionals. About 400 households were interviewed before and after a public awareness campaign. To assess attitudes and behaviour patterns of 36 medical and pharmaceutical professionals a written survey was conducted. The awareness-raising campaigns included: discussions with medical and pharmaceutical professionals to share information about the issue of medicines in water; continuing education seminar for medical and pharmaceutical professionals; education projects in schools; a running event and various other events to encourage direct public involvement.

Discussion and conclusion : The effect of the awareness campaigns in the Dülmen case study was evaluated empirically by means of a ‘before-and-after’ comparison that shows: • 70% of questioned medical professionals rated the pollution of waters by pharmaceutical residues as a strong ecological hazard. 50% of them estimate the risk to human health as relevant. • 71% of the interviewees took notice of at least one of the printed information materials. • 20% of them actively participated in at least one of the campaigns. • Knowledge about medicines residues in waters increased by 19% and about proper disposal ways by 20%. • 34% of the respondents stated they had changed their own habit regarding the disposal of leftover medicines. • 16% of the interviewees claimed to have changed their own behaviour regarding medicines consumption. The Dülmen case study indicate a clear sense that members of the public, in particular have a considered view on the (over)use of medication. There is a consistent message that they would wish to have more information on appropriate use and disposal. There is a more general view on the lack of information about appropriate disposal mechanisms. And yet there is a great desire by members of the public in particular to ‘do the right thing’.

SEVEN MEANS OF REDUCING PHARMACEUTICAL RESIDUES IN WATER-MEASURES PROPOSED BY ACTORS IN THE PHARCEUTICAL LIFE CYCLE

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Background and objective : As part of European project NoPills, a sociological study on perceptions of the problem of drug residues in water was conducted by International Office for Water and University of Limoges. The study centered in particular on current practices in every stage of the medicine cycle and on the actual or symbolic link between various actors with on one hand, the medicine and on the other hand the environment, by assuming that the perception of the medicinal residues in the water is connected to the social, cultural and symbolic representation of the medicine as well as to the practices that ensue from it. Poster objectives are to show the results of the survey : take into account societal issues and measures proposed by actors involved in the life cycle of medicine to reduce pharmaceuticals residues at source -

Methods and results : The study concerns perceptions of water issues and cultural, social or imaginary representations relating to medicinal residues and their presence in waters for the actors involved in the life cycle of medicine. Assuming that the entire chain of actors plays a role in the problem of medicinal residues in waters and that any 'mean of action' will relate to perceptions of actors in each category. The typology of social population used for this study is as follows: • producers: pharmaceutical companies, pharmaceutical sales representatives / medical visitors • Distributing: wholesalers-distributors, • Dispensers: pharmacists, • prescribing doctor : doctors, specialists, • Administrators : nurses, midwives, • Regulators: Onema, Regional Health Agency (ARS), Regional health insurance, mutual insurance company, • Professionals of the water, • the Actors : "recycling", • Users: patients, consumers, • Throwers of alert: associations, Non-Governmental Organisations (NGO) • Scientific community The survey was structured by a qualitative investigation in the form of semi-directive conversation with 21 individuals representing the pharmaceuticals life cycle, a quantitative survey (questionnaires for practitioners, dispensers and inhabitants in Limoges city) and 3 workshops followed with a debate. The results of the survey show that the majority of respondents across all categories consider that pharmaceuticals have a strong impact on flora and fauna. Seven means of action to reduce pollution by drugs were identified and discussed: training and research, improved water treatment, prescribing and consumption practices, environmental awareness and education, communication, but also legislation. Measures to follow have been proposed by the actors.

Discussion and conclusion : In conclusion, different measures to follow were proposed by actors involved in the life cycle of pharmaceuticals. The poster exposes measures and mean of action, that are results of the sociological study.

STRATEGICAL IRMISE PROJECT STUDY: WHICH LEVERS AND WHICH SCENARIOS TO REDUCE PHARMACEUTICALS RESIDUES IN WATER CYCLE (BELLECOMBE'S PILOTE SITE – SIPIBEL)?

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Background and objective : As researches progress and means of detection enhance, it has become obvious that there are, before and after treatment, residues of pharmaceuticals products in waste water, therefore in environment and in water supply. This strategic study has set the main issues on that cross border territory at stake. In a context of high level population dynamics, the drugs individual consumption is still increasing. A part of ingested substances (varying according to the molecule) can be found in water circle, by renal excretion. Some purification processes are able to remove residues of medicinal products in water, but with significant economics and environmental costs.

Methods and results : The investigation phase, based on the perception of more than 200 people, shows that measures of rationalization of drugs consumption, as well as limitation of unused medicines, are considered as complementary to waste water treatment. In this context, regulation can be a significant support. The involvement of health stakeholders is considered as essential, especially because they are highly believed when they are bearing awareness raising messages.

Discussion and conclusion : Several levers can thus be used to improve the situation. Health professionals, as prescribers, but also in their professional practices, are both priority targets for training activities and essential drivers for awareness raising initiatives. They must get reliable scientific information. They must be able to take their own position on the issue, for their professional action to make sense and contribute to reducing residues of pharmaceuticals products in water. Water professional should be able to support them on every topic. But the measures will be effective, only if the issue of medicinal products in water is considered in a wider framework, including for example quality of water in general, or improvement of environmental and economical practices in public health, and if the positions of the two countries are considered as complementary.

COMPLEMENTARITY BETWEEN CHEMICAL ANALYSIS AND SOCIOLOGICAL APPROACHES IN THE STUDY OF PHARMACEUTICALS IN HOSPITAL

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Background and objective : In order to study the contribution of an important hospital centre to the pharmaceutical pollution at the scale of Bordeaux Metropolis, with the objective to reduce it, the Bordeaux University Teaching Hospital (BUTH), the University of Bordeaux, the LyRE and Irstea have launched in 2013 a research program called RESEAU/REGARD. The objectives of this program were to estimate the consumption of pharmaceuticals at the BUTH, to know their release in the sewage network, to understand how the problematic of the environmental pollution by pharmaceutical residues is taking into account by the hospital staff (manager, doctors, nurses, technical services ...) and to analyze the hospital actor measures to “green” their professional practices.

Methods and results : To achieve these objectives, chemical and sociological complementary studies were conducted in parallel. - The chemical study based on a 2 step strategy: 1) the analysis of the hospital pharmaceutical consumptions through the analysis of the hospital pharmacy distributions; 2) the collection and analysis of 30 wastewater samples collected at the outlet of different hospital services or buildings and in the sewage network before and after hospital effluents. - The sociological study based on 2 strategies: 1) 41 semi-directive interviews on medical practices and patient relationships; 2) A lexicological study of two BUTHs' internal newsletters in order to analyse the institutional discourse of the BUTH on that problematic.

Discussion and conclusion : The analysis of the hospital pharmacy distributions has allowed to list the most distributed pharmaceuticals and thus, the probably most consumed ones at the BUTH. In view of those consumptions, 78 pharmaceuticals from various therapeutic classes were selected and searched in water samples. Paracetamol, which is the second most prescribed pharmaceutical with 1500 kg distributed in 2014, was also the most quantified compound in the water samples with concentrations ranging from 30 to 1,748 µg/L. The first results of the sociological study has highlighted three categories of actors: - Those for whom the greening possibilities of the professional core are limited, - Those who proceed to a partial greening of their professional core, - Those who operate a complete greening of their medical activities. The affiliation to each actor's category can be explained by three factors: 1) The proximity of the profession to the patient and to the health stake management. 2) The organizational transformations of the hospital embody by the outpatient care services and by the implementation of the healthcare circuit. 3) The occupational health risk knowledge which leads to realise a causality link between the health consequences of medicinal and their impacts on environment.

MANAGING THE IMPACTS OF PHARMACEUTICAL WASTE ON THE ENVIRONMENT

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Background and objective :Unused or expired medicines from hospital and household waste can ultimately end up in landfills. There is therefore the potential for active pharmaceutical ingredients, from a range of medicinal products, to be present in landfill leachate. Landfill leachate is often released into the sewage system, and therefore pharmaceutical residues, which are not removed during the wastewater treatment stages, will be present in the resulting effluent and subsequently released into the aquatic environment. In order to understand the impacts of pharmaceuticals present in landfill systems, it is important to identify typical waste disposal habits of medicinal products.

Methods and results : A critical review of pharmaceutical waste management practices and the potential environmental impacts has been carried out to quantify the waste emission pathways of major-use pharmaceuticals and determine the potential environmental impacts associated with these. A survey of household and hospitals has been performed to establish current disposal practices. Future work will involve the analysis of drugs in leachate samples and life cycle assessment of the pharmaceutical waste management approaches. Preliminary results from a survey carried out to understand household pharmaceutical waste management in the York, UK catchment showed that a higher proportion of over-the-counter (OTC) medicines are disposed of compared to prescription medicines. Between 20 and 24% of households return their unused medicines to the pharmacy whilst the majority of people surveyed dispose of their OTC medicines in the trash, which would ultimately end up in landfill.

Discussion and conclusion : To date, research assessing the impact of pharmaceuticals in landfills and landfill leachate is particularly scarce yet presents a potential threat to the environment. This work begins to understand the typical disposal habits of households with regards to pharmaceutical waste and provides a basis for estimating the proportion of pharmaceuticals reaching the landfill. As disposal of pharmaceutical waste to landfill is a potential route of entry for pharmaceuticals to the environment it is important that this exposure route is comprehensively evaluated and explored in the context of environmental risk assessment. Understanding typical pharmaceutical waste disposal habits is the first step in improving public awareness and government policy to ensure best practice of pharmaceutical waste management is carried out.

ENVIRONMENTAL DIAGNOSIS METHODOLOGY FOR HEALTH ESTABLISHMENTS THAT HAVE REJECTED PHARMACEUTICAL COMPOUNDS IN THE ENVIRONMENT.

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Background and objective : This work aims to develop a reproducible diagnosis methodology, applied to healthcare establishments, in order to assess and reduce pharmaceutical residues release in the environment. The method will be developed from various healthcare establishments. Then, it can be transposed to other establishments and even proposed to other applications (cities, watershed,...)

Methods and results : The method will consider (1) data from hospital: activities, patient, pharmaceutical consumption, water consumption; (2) data from the pharmaceuticals consumed: excretion rate, physico-chemical, pharmacological and toxicity properties and (3) data from the urban network: wastewater flow, wastewater treatment plant (WWTP) process, network structure... By integrating these data in a calculation model, the aim of the method will be to propose indexes representing the potential risk of the hospital releases for the network, the WWTP and the receptor environment. Real measurement will be performed to verify the calculated prediction data. This project will be carried out by the University of Nîmes in partnership with healthcare establishments and community health volunteers in PACA region. This work is part of the PNSE3 in several actions: to improve knowledge of emerging contaminants in water (actions 32 and 33), to act for a better water quality (actions 53 and 54), to make environmental health data accessible and available and, to better identify emergences (Action 46).

Discussion and conclusion : The methodology and indexes would assess the potential risk for the environment of the pharmaceutical residues released by healthcare establishment and to propose, if necessary the required corrective actions. The project will associated different actors concerned by the life cycle of the pharmaceutical product, including the medical, water manager and risk assessor sectors. Such indexes will bring very informative data for the management of hospital wastewater.

BELLECOMBE'S PILOT SITE (SIPIBEL) ON IMPACTS OF HOSPITAL EFFLUENTS IN AN URBAN SEWAGE TREATMENT PLANT

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Background and objective : Bellecombe's site –Sipibel– was created in 2010 to study the characterisation, treatability and impacts of hospital effluents in an urban sewage treatment plant. It focuses on pharmaceuticals as well as disinfectant and detergent products used in hospitals. This pilot site is composed of one hospital, which opened in February 2012, a treatment plant, with 2 distinct treatment lines allowing to isolate hospital effluents, and receiving waters, the Arve river. It mobilises local organisations involved in water and hospital management, industrials and scientists to define and organise this observatory and research programs.

Methods and results : SIPIBEL is a research and observation site, which includes: • A field observatory, which aims to monitor effluents and their impacts on receiving waters, • Research actions, developed in association with the field observatory – These actions are part of the SIPIBEL research programme, divided into 4 themes: o Theme 1: knowledge and modelling of urban and hospital flows o Theme 2: treatment process of micropollutants in wastewaters o Theme 3: ecotoxicological, ecological and health risks o Theme 4: sociology • Coordination and enhancement of the project, with: a new website, scientific publications, conferences and press conferences. Bellecombe's pilote site is the support of two programs: • IRMISE is a Franco-Swiss Interreg project focusing on the impact of Micropollutant (and pharmaceutical) Discharges from wastewater treatment plants downstream of the Arve catchment and on the Genevese Aquifer, based on the Bellecombe site – SIPIBEL. This project integrated SIPIBEL in a larger and cross-border framework and extended the micropollutants and their impact issues in the whole water cycle. Carried out between 2013 and 2015, it included a strategic study that put into perspective the other project actions, created dialogue between local water and health stakeholders and aimed to build water management and micropollutant flow scenarios. • The RILACT project (Risks and Measures related to pharmaceutical, detergent and biocide discharges in hospital and urban effluents) started in November 2014 in response to the "Innovation and change in practices: micropollutants in urban water" national call for projects. It is complementary to the device already set up in order to meet the three following main objectives: -better understand discharge sources, their metabolism and degradation processes in hospital and urban sewage networks; -characterize sanitary and environmental risks related to these effluents; - identify levers by involving the whole chain of responsibility.

Discussion and conclusion : The project dynamics and hospital opening deadlines allowed a very quick and effective establishment of the observatory, even though some adjustments are always necessary with regards to the difficult detection of micro-pollutant traces. After four years of observation, notably for 15 pharmaceuticals, microbiological and ecotoxicological indicators, 350 samples and 41 000 analysis results have been collected and put in a databank. Results confirm observed tendencies found in other French and European research programs and provides new knowledges about pharmaceutical compounds emissions and hospital effluents impacts. These conclusions allowed a local strategy for hospital effluent treatment management, in terms of separating or not the effluents and pollution reduction at source. The "Micropollutants" national call for projects permitted to develop a French observatory network to support the national strategy for the control of micro-pollutant discharges, influents or potential treatments, especially for pharmaceuticals.

REDUCING EMISSIONS OF PHARMACEUTICALS TO SURFACE WATERS BY MEASURES AT SOURCE – A CASE STUDY IN THE FRAMEWORK OF THE EUROPEAN NOPILLS PROJECT

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Background and objective : An important aim of the noPILLS project was to assess the feasibility and the efficiency of source segregation measures on hospital level. A separate collection and disposal of pharmaceutical residues at source can be efficient in view of the elimination of specific molecules and of the local context. Iodinated x-ray contrast media (ICM) are an example of a substance group which could be easily collected and disposed separately after administration. ICM are predominantly excreted via urine within 24h after administration. Accordingly, the separate collection and disposal of urine can be a measure to reduce ICM emissions to sewer systems and surface waters. In this context the objectives of a case study for a separate collection of the iodinated x-ray contrast medium iobitridol in the Centre Hospitalier Emile Mayrisch (CHEM) in Esch-sur-Alzette (Luxembourg) were to 1. Evaluate real and theoretical potentials to reduce emissions to water systems 2. Assess the willingness of hospital staff and patients to support the measure at source

Methods and results : Within a time period of three consecutive two week periods detailed data of patients getting an iobitridol injection were collected by the radiology staff (dose, ambulant/stationary patient etc.) In parallel substance flows of iobitridol were monitored in the hospital sewer as well as in the inflow and effluent of the downstream sewage treatment plant. During the second period of two weeks five urine bags, information on campaign and a questionnaire were handed out to patients willing to participate. A comparison of expected and detected iobitridol loads on catchment level indicates a reduction of iobitridol emissions to the sewer system of 17% during the campaign. The theoretical potential of load reduction involving ambulant patients into a separation campaign would be about 65%. Further, the results of the patients' survey show the willingness of ambulant patients to participate in this kind of measure at source.

Discussion and conclusion : The results indicate a significant potential of emission reduction by segregation measures at source on hospital level. An important element of the measures is an efficient involvement of medical staff. The study reveals a lack of knowledge of medical staff in view of the fate of pharmaceuticals in the environment and particularly in water systems. Additionally, a separate collection of specific substances offers the possibility of a substance recovery.

IMPROVING RISK MITIGATION OF VETERINARY MEDICAL PRODUCTS

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Background and objective :The levels of environmental risk from veterinary medicinal products (VMPs) can be reduced through application of appropriate risk mitigation measures (RMMs). The RMMs are applied during the authorization process by regulators and authorization applicants. Knowledge of RMMs, their practicality and application is unevenly available to stakeholders, regulators and managers. We aim to gain insights into the perceptions and practicality of RMMs.

Methods and results : We investigated VMP user (i.e., veterinarians and farmers) perspectives and attitudes towards RMMs via key-informant interviews. Interviews followed a semi-structured approach and a questionnaire was developed to guide the discussions.

Discussion and conclusion : We found the major factors influencing the practicality of RMMs were the attributes of individual farms and the knowledge base of individual farmers. We highlight and discuss the importance of information communication for the practical and consistent application of RMMs. Increased understanding of the application of RMMs will decrease uncertainty in risk assessments and provide a foundation for the development and application of additional RMMs.

IMPROVEMENT OF MICROPOLLUTANTS TREATMENT AND NUTRIENTS RECOVERY BY SOURCE SEPARATION OF URINE

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Background and objective : Due to low efficiency of conventional treatments in waste water treatment plants (WWTPs), a high diversity of common pharmaceutical compounds (Phacs) is found in natural environments that induce a suspected ecotoxicological effect. This lack of treatment efficiency is partially due to the mixture of micropollutants with numerous other substances and to their immense dilution. Based on a demonstration platform, the SMS project (Separation of Micropollutants at Source) proposes a change in practice with urine separation at source because it represents a small volume (<1% of waste water) and contains an important part of the measured ecotoxicity (65%). The challenge of the project is to reduce both the discharges to the environment and their ecotoxicological impact by limitation of the micropollutants degree of mixing and dilution.

Methods and results : The project is supported by 2 territorial collectivities: the SIVOM de la Saudrune (31) and the Portet-sur-Garonne city (31). It includes four companies (POLYMEM/Ozoval/JP Coste/Adict), offering their technologies and knowledge, and three scientific research laboratories (LGC/LISBP/Ecolab) bringing their expertise in engineering processes and environmental assessments. Chosen technologies and actions include: - Urine-separating toilets (JP Coste) - The urine treatment chain: precipitation of N, P and COD degradation (Laboratories), the later facilitate the catalytic ozonation of micropollutants (Ozoval, laboratories). - The sewage sector with a membrane bioreactor (Polymem, laboratories), coupled with a double digestion of sludge (anaerobic/thermophilic aerobic), targeting degradation of organic matter and micropollutants. - An integrative method of ecotoxicological assessment (Laboratories). - A social acceptance assessment (Adict).

Discussion and conclusion : The analysis of micropollutants and their impact on environment represent a strong issue. Therefore, in order to assess the ecotoxicological impact of effluents, the project promotes a multi-criteria analysis strategy integrating physicochemical approaches on targeted pollutants and an ecotoxicological approach analyzing comprehensive and integrated effects. The effectiveness of treatment technologies proposed have already been proven in laboratory. The demonstration platform allows quantification of the performances of integrated technologies that will give more technical and economic indicators. The Sivom de la Saudrune and the city of Portet-sur-Garonne wish to anticipate regulations on micropollutants to protect natural environment, and to implement innovative solutions, especially in the field of recovery and valorization in WWTP. The SMS project is a structuring and driver model for the community because it is a starting point for solutions that could be implemented in future projects.

REMPAR – REseau MicroPolluants du Bassin d'ARcachon

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Background and objective : The Arcachon Bay is a mesotidal shallow lagoon located on the French Atlantic coast. It is a major core for oyster farming and recreational activities. In recent years, several “ecological crises” have affected the Bay’s ecosystem (such as death rates in oyster juveniles and decline in eelgrass *zostera* beds), and therefore raised the question of the impact of micropollutants (MP) on its ecosystem. As a consequence, a monitoring network on micropollutants was born from the initiative of local elected authorities and stakeholders to establish an active monitoring of MP on the Bay and to reduce their footprint on the Bay. This network called REMPAR is an ambitious project aiming at i) tracking sources of several micropollutants, including pharmaceuticals, ii) assess their impact, iii) appraise efficacy and propose appropriate treatments at the local scale and iv) adjust our day-today behavior concerning the water resource.

Methods and results : Within REMPAR, an integrated approach on pharmaceutical residues is implemented: Mapping sources in wastewater: the occurrence of about 50 pharmaceuticals is assessed in raw and treated urban, industrial and hospital wastewater. A specific focus will be made on the behavior and degradation of several pharmaceuticals in raw hospital wastewater. Propose appropriate treatments: a study aims at assessing the effectiveness and the techno-economic interest of a pilot scale membrane bioreactor (MBR) for the treatment of Arcachon’s hospital wastewater. Assessing ecotoxicity: an in situ laboratory has been set up to assess the toxicity of the hospital effluents and also to assess the efficiency of the MBR to reduce such toxicity. Cell based screening tests and in vivo bioassays on aquatic species focusing on biochemical and genetic markers are implemented within this laboratory. Understanding and adjusting our behaviour: a study of practices and social perception to pharmaceutical compounds is initiated to identify levers for their source reduction. This study is divided in three steps: i) semi-structured interviews with health professionals, ii) focus groups with residents and stakeholders iii) questionnaires carried out with residents and tourists.

Discussion and conclusion : Beyond providing a comprehensive monitoring of micropollutants on the Arcachon Bay, REMPAR is intended to create links between people so that everybody feels implied in the preservation of water. Therefore, communication and awareness raising actions will be set up at the hospital, in retail pharmacies and several public places, focusing on pharmaceuticals residues, micropollutants and treatment and management of the water resources.

HYDRODYNAMICS AND MASS TRANSFER IN A STIRRED TANK REACTOR: APPLICATION TO THE DEGRADATION OF PHARMACEUTICAL POLLUTANTS IN WASTEWATER

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Background and objective : Aeration of a liquid phase by gas is the basis of many chemical oxidation, absorption and biological treatment of urban wastewater processes. To optimize the performance of oxygen transfer, it is essential to consider the hydrodynamic phase that determines the exchange surface, the thermodynamic properties of phases that control the exchange and the transfer coefficient material between the gas bubbles and the associated liquid.

Methods and results : In this study, the experimental gas (air)-liquid (water) reactor consists essentially of a flat bottom tank (DT=23.6cm) designed according to the standard configuration of HOLLAND with four radial baffles, placed on the wall of the tank to avoid the formation of vortex, and a stirrer. The gas, supplied by a blower, is injected in the reactor via a single nozzle sparger ($d_p=6\text{mm}$). Six configurations for the stirrer have been tested for the hydrodynamic study: a simple configuration called single-stage (Rushton turbine and turbine with six inclined blades) and a two-stage configuration (combination of two Rushton turbines, combination of two turbines with six inclined blades, combination of a Rushton turbine above and a six inclined blades below and finally a combination of a six inclined blades above and a Rushton turbine below). For these configurations, the gas hold-up was measured at room temperature and atmospheric pressure while two process parameters varied: the superficial gas velocity (from 0 to $2.8 \times 10^{-3} \text{ m.s}^{-1}$) and the stirrer rotational speed (from 0 to 450 rpm). The three hydrodynamic regimes (short circuit, mixing and flooding) were observed for both the single-stage configuration and the two-stage one. The results show that the gas hold-up gets a maximum value for the mixed two-stage combination (six inclined blades turbine above and Rushton turbine below). This optimal combination was retained for hydrodynamics and mass transfer study under various conditions of superficial gas velocity (from 0 to $2.8 \times 10^{-3} \text{ m.s}^{-1}$) and stirrer rotational speed (from 0 to 450 rpm). The residence time distribution (RTD) was conducted using the well-known tracer method. The experiments show that the stirred reactor is perfectly mixed. The photographic method was employed for the bubble size distribution and the gassing out method was applied for the determination of the volumetric mass transfer coefficient. The influence of the superficial gas velocity and stirrer rotational speed on oxygen mass transfer was related to gas hold-up and bubble size. Oxygen transfer increases with increasing gas hold-up and decreasing with bubble size. Wastewater treatment experiments were then carried out in order to evaluate the removal of pharmaceutical pollutants (antibiotics) by the lab scale reactor.

Discussion and conclusion : The first results are very interesting and the reactor designed seems to be a promising process for the degradation of pharmaceutical pollutants present in wastewaters.

DICLOFENAC REMOVAL FROM WASTEWATER BY ADSORPTION ONTO POPULUS SP. CHAR

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Background and objective : The European Union has set a 20% target for the overall share of energy from renewable sources (RES) by 2020 (Directive 2009/28/EC). Among them, biomass - and particularly woody biomass from energy crops - has many advantages and may play an important role in displacing fossil fuels. In this sense, thermo-chemical processes, such as pyrolysis, are used for the production of energy (Sait et al., 2012). However, a solid by-product is generated from the thermo-chemical processing of woody biomass, which, in the case of pyrolysis is called char. Utilization and/or valorization of that solid by-product should be pursued under a Circular Economy basis, as encouraged by the European Commission. In this context, the objective of this work was to assess the utilization of the char obtained from the pyrolysis of a *Populus* sp. energy crop for the adsorptive removal of pharmaceuticals from wastewater. Pharmaceuticals represent an especially worrying class among emerging contaminants (ECs) since they were designed to cause a physiological response (Santos et al., 2010; Pal et al., 2014). Among them, diclofenac was included by the Directive 2013/39/EU in the first watch list of substances to be monitored in all member states to support future reviews of the priority substances within the Water Framework Directive (2000/60/EC) (WFD).

Methods and results : Using char from poplar pyrolysis as adsorbent, kinetic and equilibrium adsorption experiments were carried out under batch operation both from ultrapure water and from the secondary effluent of a sewage treatment plant (STP). Also, and for comparison purposes, a commercial activated carbon offered by Chemviron Calgon, was used under identical experimental conditions. Fittings of the obtained results to kinetic and equilibrium models were determined and made evident differences between the char and the commercial activated carbon, which displayed a more efficient performance in the adsorption of diclofenac. However, for both the adsorbents considered, it was proved that the adsorption from the two aqueous matrixes considered was quite similar.

Discussion and conclusion : These results are especially relevant and open new perspectives on to the utilization of char from the pyrolysis of biomass from energy crops in the tertiary treatment of sewage, namely for the removal of ECs.

TESTING AND IMPLEMENTING AN ADVANCED WASTEWATER TREATMENT TECHNOLOGY TO REMOVE PHARMACEUTICAL MICRO-POLLUTANTS FROM MANUFACTURING EFFLUENTS: COMBINATION OF CHEMICAL AND BIOLOGICAL ANALYSIS AS PILLARS OF THE TECHNOLOGY PERFORMANCE MONITORING.

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Background and objective : Losses of APIs from manufacturing are considered as a minor source of pharmaceuticals to the environment compared to the use of medicines by patients. However manufacturing sites may constitute point source discharges of APIs which raise the question of a potential impact to the environment. In 2008, abnormalities were reported in gudgeons in the Dore river, France. Environmental studies conducted by INERIS revealed complex endocrine disrupting effects with vitellogenin induction, intersex and sex-ratio alteration in gudgeons sampled from sites of the river located downstream from both an API production site's WWTP and an urban WWTP. A link with an exposure to some pollutants was suggested by the analytical detection of xenobiotics and the measurement of steroid-like activities downstream of the industrial discharge.

Methods and results : With respect to this information, an action plan was set up, with two main axes: o Reducing the discharge of micropollutants into the river: a BAT based on activated charcoal capture has been selected, tested and implemented in addition to source reduction measures o Implementing a specific monitoring program in the WWTP effluents and the river upstream and downstream of the discharge point: targeted chemical analysis and bioassays have been conducted on a monthly base

Discussion and conclusion : The focus will be made on the different actions implemented and specifically on the pilot study to define an effective wastewater treatment technology capable of reducing biologically active micro pollutants.

OXIDATIVE TRANSFORMATION IN THE ENVIRONMENT OF A QUINOLONE SORBED ON NANO-MAGNETITE VIA Fe^{2+}/O_2 MEDIATED REACTIONS

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Background and objective : Organic pollution has become a critical issue worldwide due to the increasing input and persistence of organic compounds in the environment. Various minerals, which are ubiquitous in natural waters, sediments and soils, are able to adsorb organic pollutants on their surfaces and enhance their degradation via oxidative and reductive transformation processes [1]. Recently, increasing attention has been paid to heterogeneous Fenton-like reactions using iron-bearing minerals as catalyst. Iron nano-minerals have attracted attention because of their nanometric-size particles, large surface area, high sorption ability and reactivity. They have been proven to efficiently degrade several organic contaminants sorbed to their surfaces at circumneutral pH. In the present study, we explored the oxidative capacity of a mixed iron oxide, nano-magnetite, as catalyst for heterogeneous Fenton-like reactions mediated by oxygen, for the removal from waters of a recalcitrant quinolone antibacterial agent, nalidixic acid [2].

Methods and results : Nano-magnetite (Fe_3O_4) having 10-12 nm particle size and 93 m^2/g surface area was synthesized by aqueous coprecipitation of Fe^{2+} and Fe^{3+} ions in a glovebox under N_2 atmosphere. The sorption of nalidixic acid onto nano-magnetite was performed under anoxic conditions for 3 days at pH 6.5. After two to six periods of oxygenation (15 min each), the quinolone and its by-products, as well as the solid and solvated iron oxides were characterized and quantified. Results showed, under anoxic conditions, an efficient sorption of nalidixic acid at the surface of magnetite (97-99%). This step was followed by a rapid and efficient degradation of nalidixic acid when exposed to air (60% after 30 min), while negligible degradation was observed in the absence of oxygen or of iron oxides. Five by-products issuing from the oxidative degradation of the antibacterial agent were identified by liquid chromatography-mass spectrometry (UHPLC-MS/MS), and degradation pathways were proposed. X-ray powder diffraction and Fe K-edge X-ray absorption spectroscopy were used to investigate mineralogical and iron redox changes upon oxidative degradation of the contaminant.

Discussion and conclusion : The nalidixic acid degradation was accompanied by a significant and systematic oxidation of the solid phase under oxic conditions. This suggested that the iron oxide enhanced the degradation of the contaminant: magnetite oxidation into maghemite led to the degradation of nalidixic acid under acidic to weakly alkaline pH conditions. The quenching of the degradation reaction in the presence of ethanol provided evidence that hydroxyl radicals ($HO\bullet$) were involved in the oxidative degradation of nalidixic acid. A degradation mechanism was thus proposed. This study points out the promising potentialities of mixed valence iron oxides for the treatment of soils and wastewater contaminated by organic pollutants. 1. T. Borch, R. Kretzschmar, A. Kappler, P. Van Cappellen, M. Ginder-Vogel, A. Voegelin, K. Campbell, Environ. Sci. Technol. 44:15-23 (2010) 2. S.G. Ardo, S. Nélieu, G. Ona-Nguema, G. Delarue, J. Brest, E. Pironin, G. Morin, Environ. Sci. Technol. 49:4506-4514 (2015).

ECOTOXICOLOGICAL EVALUATION TO FOLLOW A 3 YEARS IN SITU-EXPERIMENT OF A SUBMERGED MBR FOR ONCOLOGICAL HOSPITAL EFFLUENT TREATMENT.

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Background and objective :In the hospitals and pharmaceutical industry effluents, as well as in wastewater-treatment plants and in wastewaters, several pollutants with various effects were able to be identified, genotoxics (hydrocarbons), endocrine disruptors (personal care products) or plastics (Bisphenol A) The antitumor molecules, although in lesser quantity represent an increasing danger. Considerable contents of these metabolites can be found in rivers. In this context, their removal seems to be a security measure. MBR appears to be good candidates: based on previous lab experiments the aims of the PANACEA project, are split into 3 interdependent objectives: 1) the evaluation of the occurrence of molecules, used in the treatments of cancers, in the effluents of the corresponding services; 2) The measure and quantification of the toxic effects, (geno / cytotoxic and endocrine disruptors); 3) the development of an hybrid process constituted by a combination of biological and physical-chemical treatments (coupling of a membrane bioreactor with adsorption / oxidation processes or nanofiltration).

Methods and results : After presentation of the sampling protocol, the obtaining of the physico-chemical parameters and the quantification of 125 pharmaceutical molecules, allowed "to describe" the variability of the hospital effluent. From the processing point of view, the MBR was operated with sludge residence time (SRT) equal to 40 days; the study consisted in estimating the effect of the hydraulic residence time (HRT), the other parameters staying equal. Two experimental campaigns were conducted for HRT = 24 and 48 hours. From a hydraulic point of view the filtration performances are satisfactory with the chosen operating conditions (no backflushing, sequenced filtration). The treatment performances (macroscopic quality parameters) remain corresponding to standards of discharge.

Discussion and conclusion : The 125 molecules quantification revealed that the MBR removals are very variable, from a total elimination to a fictive "production", as if un-conjugations would occur. On the same samples, series of tests of ecotoxicity and endocrine disruptors measurements were applied. They highlight a removal of the global ecotoxicity, oestrogenic, androgenic activities and glucocorticoïde. More over these global effect analyses coupled with chemical quantifications allowed to appreciate at which level pharmaceuticals molecules are implied in the environmental impact of such effluent, and in which extend MBR may help to reduce it.

ANTIBIOTIC RESISTANCE GENES AS NEW CONTAMINANTS OF EMERGING CONCERN: INVESTIGATING THEIR FATE DURING ADVANCED WASTEWATER TREATMENT

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Background and objective : Background Urban wastewater treatment plants have been recently highlighted as important factors in the dissemination of Antibiotic Resistance (AR) due to the continuous exposure of pathogenic and environmental bacteria to sub-lethal concentrations of antibiotics through treated wastewater, contributing to the prevalence of AR. Currently, there is very limited published research available on the integration of alternative wastewater treatments aiming to manage the presence of AR determinants in wastewater matrices. Objectives Consequently, the fate of total DNA content and contaminants of emerging concern, namely total DNA, opportunistic bacteria and various ARG was investigated during an integrated Membrane BioReactor (MBR)-solar Fenton oxidation process, at a pilot scale.

Methods and results : Methods The pilot-scale MBR has a treatment capacity of 10 m³ day⁻¹. The solar Fenton experiments were conducted in a compound parabolic collector (CPC) pilot plant. The amount of the DNA was measured using the NanoDrop ND-1000 instrument. The q-PCR assessment was carried out using the Bio-Rad CFX 96 Touch™ Real-Time PCR Detection System. Results and discussion The total DNA concentration reduction after the integrated MBR-solar Fenton treatment was 97%, with a final DNA concentration of 0.16 mg 100 mL⁻¹ at the end of solar Fenton treatment of the MBR effluent. The presence of the Enterococcus-specific and Pseudomonas-specific taxon marker in the MBR effluent shows the challenge faced for their removal by biological processes. Sul1 was abundant in all MBR treatment steps while ampC concentration seems to be highest in the MLSS, despite its absence in the incoming primary influent into the MBR reactor, suggesting the accumulation of beta-lactam resistant bacteria inside the MBR reactor. Enterococci were the most prevalent parameter during the solar Fenton treatment, with limited removal during oxidation. Pseudomonas bacteria were also detected in a similar manner to enterococci. Along the examined ARGs, ampC was detected in the initial sample but was not detected after 180 minutes of solar Fenton treatment. Sul1 and ermB ARG were not effectively treated after 180 minutes of treatment with no change in concentrations compared to initial solar Fenton concentrations (1.56 and 1.53 log₁₀ Cell Equivalents per 100 ng⁻¹ DNA, respectively).

Discussion and conclusion : Conclusion Despite a 97% reduction in the total DNA found after the solar Fenton treatment, the taxon-specific markers of Pseudomonas and Enterococci, and the ARG sul1 and ermB were still prevalent in the examined samples. Additionally, it was demonstrated in this study for the first time, that the solar Fenton oxidation process has the potential to reduce the abundance of certain ARG i.e. ampC. It was further shown that enterococci- and Pseudomonas- specific markers may require a different approach of removal.

REMOVAL OF A PERSISTENT ORGANIC POLLUTANT BY SOLAR PHOTOCATALYSIS

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Background and objective : Many pharmaceutically active compounds (PhACs) behave as persistent organic micro-pollutants, as evidenced by their continuous input and accumulation in the environment the accumulation of PhACs poses a threat to the quality of water resources. Advanced technologies such as photocatalysis have been considered as an effective remediation tool for pharmaceutical compounds. In this context, photochemical treatments offer an alternative. They use sunlight radiation as an energy source capable of initiating chemical reactions that attack and destroy the pollutants. Solar photocatalysis has gradually become an alternative technology for water pollution control by proving its effectiveness through the mineralization in aqueous medium, organic molecules toxic to humans and the environment. It is a combination of a catalyst semiconductor is titanium dioxide TiO_2 with an ultraviolet light source is the sun. It has the added advantage of not introducing additives in the medium to be treated. In the present paper, we present the development of a new system employing TiO_2 in a tubular photocatalytic reactor for the degradation of the Spiramycin, as pharmaceutical pollutant model, under solar irradiation.

Methods and results : Solar photocatalytic experiments were carried out in the photoreactor developed for photocatalytic application installed at the Solar Equipment Development Unit (UDES) on the north of Algeria (latitude $36^\circ.39'$; longitude $2^\circ.42'$ at sea level), using natural sunlight radiation. Solar radiation was measured by global radiometer (KIPP&ZONZN, CMP11) mounted on a platform horizontal tilted as the same as the reactor. The degradation of Spiramycin (SPM) was investigated in an aqueous suspension of TiO_2 (PC 500) using solar energy in tubular reactor. The objective of the study was to assess the influence of various parameters such as initial spiramycin concentration, PC500 catalyst concentration and pH on the photocatalytic degradation rate of Spiramycin (SPM) under UV sunlight radiation.

Discussion and conclusion : The optimum rate of photodegradation (99.3%) was obtained with a flow rate and a catalyst coating equal to 1.74 L/mn and 0.75 g/L respectively. Adjusting the pH favors the photocatalytic reaction and the best performance was obtained for acid pH (3-5) adjusted. Thus, the (UVsun/ TiO_2 PC500) photocatalysis process is very efficient and can be suggested for the degradation of spiramycin in aqueous solution.

ADSORPTION OF OXYTETRACYCLIN ON ORANGE PEELS

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Background and objective : It is impossible to imagine our society without effective modern drugs. They help us to prevent and treat disease. Nowadays, large quantities of various pharmaceutical active substances are manufactured to protect humans and animals. Following the improvement of medical care, longer life expectancy and progressive industrialisation of agriculture, the amounts of drugs consumed have increased. However, in many cases, these products are not fully absorbed and metabolised by patients and are partially excreted. So, traces of these products join the water cycle. With significant advances in chemical analysis technology, it is now possible to measure many pharmaceutical residues in water at extremely low concentrations, often several times lower than those that were possible to measure a few years ago. Indeed, studies have highlighted the presence of more than 80 pharmaceutical products in water. We do not know exactly what effects these residues have on the aquatic environment for example on microorganisms. Which implications these drugs have on the food chain, on the biodiversity and on the entire ecosystem, is a frequently cited issue. At first glance, it would seem reasonable to completely avoid the rejection of pharmaceutical residues in order to protect our water system. The abandonment of production and medication is not a realistic or desirable scenario. Another option is to take technical measures to treat contaminated water. In order to achieve the removal of a pharmaceutical micro pollutant (oxytetracyclin) from the aquatic environment, its adsorption was conducted on orange peels, a food waste largely abundant in Algeria. Our study has three main objectives: reduce pollution levels and in the same time manage and valorise an agricultural waste.

Methods and results : Adsorption of oxytetracyclin on grinded orange peels (particle size of 100 μm) was studied in batch reactor. This study investigated the influence of various parameters on the oxytetracyclin removal efficiency. These include contact time, initial concentration of oxytetracyclin, mass and particle size of orange peel powder and pH of the medium.

Discussion and conclusion : Results show that for a solid/liquid ratio of 8 g/L, the removal efficiencies of oxytetracyclin are of 100% and 94 % for antibiotic concentrations of 5 and 10 ppm, respectively. The adsorption kinetics are well described by the pseudo second-order model. The adsorption isotherms obtained are in accordance with the Langmuir model. In addition, the adsorbed amounts decrease with increasing particle size and the adsorption of oxytetracyclin is favoured in the acidic reaction medium, which is the natural pH of orange peels in solution.

PHARMACEUTICALS REMOVAL FROM WASTEWATER BY THE MICROALGAE SCENEDESMUS OBLIQUUS

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Background and objective : The microalgae capability for the removal of nutrients from wastewater has been widely studied (Prandini et al., 2016; Arbib et al., 2013) , showing high removal rates and proposing the production of microalgae coupled to wastewater treatment. However, due to the recent concern of the scientific community about the risks of emerging contaminants and especially of pharmaceuticals, their removal from water by microalgae has become a new objective of research (de Wilt et al., 2016; Wang et al., 2016; Hom-Díaz et al., 2015; Matamoros et al., 2015; Gattullo et al., 2012). The aim of this work was to prove the feasibility of the microalgae *Scenedesmus obliquus* to remove diclofenac, salicylic acid and paracetamol from water.

Methods and results : The assays were performed in photobioreactors under controlled conditions and were operated in batch mode until the end of the exponential growth phase and then in semicontinuous mode, at a daily dilution rate of 30%, until the growth parameters remained constant. The obtained results showed that the growth of the culture was increased under the presence of diclofenac and salicylic acid and it was not modified under paracetamol. On the other hand, the removal of pharmaceuticals showed efficiencies higher than 98% for diclofenac, 93% for salicylic acid and 40% for paracetamol at the end of the batch culture; and higher than 79%, 99% and 9% for diclofenac, salicylic acid and paracetamol, respectively, at the steady state of the semicontinuous culture.

Discussion and conclusion : The obtained results proved that *Scenedesmus obliquus* may be applied for the removal of these pharmaceuticals, with removal efficiencies nearly complete in the case of diclofenac and salicylic acid. The high removal rates here determined point to the possible application of microalgae cultures as bioremediation systems.

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