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Dynamic and differential expression of the gonadal aromatase during the process of sexual differentiation in a novel transgenic *cyp19a1a*-GFP zebrafish line

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Environmental protection in a multi-stressed world: challenges for science, industry and regulators

BARCELONA²⁵
SETAC Europe 2015 

ABSTRACT BOOK



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directly compared to trigger values for water, sediment and soil to identify persistent compounds. However, for many substances there are no data from simulation studies available. Thus, a screening assessment for persistence is performed under REACH that is mainly based on ready biodegradability tests according to OECD 301 and extrapolation of these data to derive half-lives for water as well as soil and sediment by multiplication with default factors. In addition to experimental mineralization half-lives, alternative approaches described in literature and software packages were used in the present study to determine half-lives for the 15 test compounds in water, sediment and soil. By means of these data, a screening assessment for persistence was performed by comparing the half-lives to the persistence criteria under REACH.

MO444

Sorption and degradation of pharmaceuticals in soil and biosolids amended soil

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Pharmaceuticals are emerging contaminants which have drawn concerns about possible harmful effects in the environment or the potential entry of drugs into the human food chain. Most studies on the environmental fate of pharmaceuticals have focused on their behaviours during wastewater treatment processes, the sludge and in environmental waters. However, there is still a significant lack of knowledge of their transport and fate in agricultural soils. Sewage sludge from wastewater treatment plants (WWTPs) is commonly used as soil amendment because it contains nutrients and organic matter which can improve the soil quality. Since pharmaceuticals are routinely found in biosolids from WWTPs, once land applied they are potentially mobile and available for plant uptake and bioaccumulation. The fate of pharmaceuticals in soils is mostly determined by sorption and degradation processes. This group of contaminants consist of a wide range of compounds. Their sorption behaviours vary from compound to compound and are difficult to predict because is often controlled by interactions with specific functional groups and pH-dependent speciation. Moreover, land application of biosolids can alter the chemical and physical properties of the soil, with an increase in soil-water retention and organic matter content which can consequently affects the interaction with chemicals. In this study, laboratory experiments were performed to investigate the sorption and degradation of three anti-inflammatory (naproxen, ibuprofen and diclofenac) and five antidepressant drugs (citalopran, fluoxetine, norfluoxetine, paroxetine and sertraline), with different physicochemical characteristics, in an agricultural soil. The effect of biosolid amendment on sorption and degradation was also examined. As expected, antidepressants adsorbed very strongly to the soil with and without addition of biosolids. Adsorption tests using a batch equilibrium method showed that adsorption of all tested pharmaceuticals could be well described with Freundlich equation. Competitive sorption was observed when the pharmaceuticals coexisted. This cosolute effect was no observed for anti-inflammatory drugs in absence of antidepressants. Biosolids amendment increased the sorptive strength for all the compound studied and, in general, had also an effect on degradation under the conditions of this study.

MO445

Bioremediation of Hydrocarbons with Microorganisms

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Bioremediation or the use of microorganisms for environmental cleanup is usually the preferred and major route of PAH removal from contaminated environments because it is cost effectiveness and complete cleanup (Pothuluri and Cerniglia, 1994). Several isolated microorganisms have been successfully utilized in major hazardous waste cleanup processes such as, in industrial process streams and effluents (Levinson *et al.*, 1994 and Al-Wasify and Hamed, 2014). Unfortunately, most of these studies were carried out in Western countries, to a limited extent in South America and Asia (Prantera *et al.*, 2002). In Qatar, there is limited information on microbial degradation of polycyclic aromatic hydrocarbons (Al-Thani *et al.*, 2009). In this work, we reported the isolation and characterization of some bacterial and yeast species from soils and industrial wastewater (IWW) collected from a native plant experiment conducted at Environmental Studies Center (ESC) of Qatar University. The native plant experiment project had two phases. Sand samples were collected in sterile containers after mixing the soil-sand culture and before and after irrigating with IWW. Samples were collected every 15 days according to the project plan. Water samples were collected from the first and second IWW deliveries. The same procedure was repeated for the second phase of the project. After using the serial dilution method for the microbial total count, preparation of several media such as (nutrient agar, Pseudomonas Agar Base, Cetrimide Agar, Trypticase Soy Agar, yeast extract agar & Czapek-Dox Agar) was done to promote the bacterial and yeast growth. After isolation and purification of bacterial species, identification was done using **API 20NE & API Coryne** as an identification system from **bioMérieux** then, confirmation was done using **VITEK 2 Systems Version: 04.02 MIC Interpretation Guideline** to some selected strains. Our results showed that, twenty nine species belonged to twenty genera were isolated from the IWW and Soil samples. Isolation and screening cultures on mineral salts medium and enrichment medium were done. The effect of different diesel concentration on the growth of these species was studied. **API 20 AUX** as a

yeast identification system from bioMérieux was used to identify the most common yeast isolates which show thirteen species which were belonged to eight different genera. The most common were *Candida spp.*, *Trichosporon mucoides* and *Cryptococcus spp.*

MO446

Effects of HP-β-CD concentrations and repeated exposures to diesel on biodegradation of benzo[a]pyrene in soil

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Aim: It is well-known that low aqueous solubility and poor microbial catabolic activity limit benzo[a]pyrene biodegradation in soil. Used as a solubility-enhancement agent, the effect of increasing hydroxypropyl-β-cyclodextrin (HP-β-CD) concentrations (0, 12.5, 25 and 50 mM) on benzo[a]pyrene mineralisation was assessed in soil. The effect of repeated exposures over 150 d to diesel oil (1x500, 1x5000, 2x250, 2x2500, 5x100 and 5x1000 mg_{oil-c} kg⁻¹ soil) on the development of benzo[a]pyrene catabolic activity was also assessed. *Materials and Methods:* Sequential aqueous, HP-β-CD and alkaline extractions were used to quantify benzo[a]pyrene bioavailability and partitioning in soil. Standard radiorespirometric assays were used to measure catabolic activity and mineralisation of ¹⁴C-benzo[a]pyrene in soil. *Results:* Indigenous catabolic activity towards benzo[a]pyrene was low in control soil (mineralisation extent < 1%). However, pre-exposure to diesel oil significantly enhanced (11–55times) catabolic activity; this being greatest in soils repeatedly exposed to lower diesel concentrations. Overall, whilst the presence of 50 mM HP-β-CD significantly increased (26–67times) benzo[a]pyrene bioavailability in soil, it failed to enhance benzo[a]pyrene mineralisation; however, lower HP-β-CD concentrations appeared to have some impact on mineralisation. *Conclusion:* Increasing HP-β-CD concentration significantly increased the apparent aqueous dissolution of benzo[a]pyrene from soil matrices, but failed to further enhance ¹⁴C-benzo[a]pyrene mineralisation in diesel-contaminated soil. These findings are important in assessing the fate of benzo[a]pyrene and designing bespoke remediation strategies for soils chronically exposed to petroleum-derived oils.

MO447

Humic acids of Patagonian soils. Isolation and kinetic study of the photochemical action in the natural degradation of environmental pollutants

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Humic substances, among which humic acids (HAs) constitute a relevant fraction, are natural mixtures of organic compounds, abundant in terrestrial and aquatic ecosystems [1][2] In some sense, the same can be said about riboflavin (Rf, vitamin B₂) which is also present in waters of rivers, lakes and seas. This contribution presents a systematic kinetic and mechanistic study on the photosensitizing properties of the mixture HA plus Rf, in order to determine potential synergistic or inhibitory effects of the photosensitizers. As compounds models for Ar-OH contaminants, phenol (PhOH) and 3-hydroxypyridine (3-OHP) were employed. The HA used was isolated from a forest soil near El Chalten, Santa Cruz, Argentina. The fraction was obtained by sequential dissolution using hydrochloric acid, sodium hydroxide and hydrofluoric acid and purified by dialysis in cellulose tube Sigma D-7884.[3] A solution of 50µg/ml of HA plus 0,04 mM Rf was used as sensitizer. As an overall result, it was determine that the visible-light-mediated photooxidation of 3-OHP at pH 7, sensitized by low Rf and HA concentrations, and standardized by comparison to the photooxidation rate of the known oxidizable target FFA, is an efficient process. It should be mention that the experiments of oxygen uptake for 3-OHP and FFA were made under identical experimental conditions, including the concentration of the oxidizable substrates. On the other hand, the degradation of PhOH only operates in the alkaline pH range. A moderate decrease in the rate of oxygen consumption by 3-OHP upon Rf +HA sensitization was observed as compared to the simple addition of the oxygen uptake rates for 3-OHP upon individual Rf and HA sensitization. It is attributed to a catalytic decomposition of O₂•- by HA, which competes with 3-OHP by the oxidative species. <br clear="all" /> [1] J.I. Edges, J.M. Oades, Comparative organic geochemistry's of soils and marine sediments, Org. Geochem. 27 (1997) 319–361. [2] M.C. Scapini, V.H. Conzonno, V.T. Balzaretti, A. Fernández Cirelli, Comparison of marine and river water humic substances in a Patagonian environment (Argentina), Aquat. Sci. 72 (2010) 1–12. [3] Mac Carthy, P., R.L. Malcom, A proposal to establish a reference collection of humic materials for interlaboratory comparisons, Geoderma, 16, pp 179-181 (1976).

Engineering in vivo fish models for advancing environmental hazard and risk assessment (P)

MO448

Dynamic and differential expression of the gonadal aromatase during the process of sexual differentiation in a novel transgenic cyp19a1a-GFP zebrafish line

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In most gonochoristic fish species, aromatase, the enzyme responsible for the synthesis of estrogens, has been shown to play a critical role in the process of sexual differentiation (SD). In zebrafish, that undergoes a juvenile intersex stage, there is still a lack of knowledge regarding the precise localization and pattern of expression of gonadal aromatase (Cyp19a1a) impeding the formal characterization of the role of *cyp19a1a* in this process. To fulfill this gap, the spatio-temporal expression of *cyp19a1a* was analyzed in a novel transgenic zebrafish model expressing Green Fluorescent Protein (GFP) under the control of the zebrafish *cyp19a1a* gene promoter. First, we showed a perfect co-expression of GFP and endogenous Cyp19a1a protein in adult gonads that was localized in the cytoplasm of oögonia, young oocytes and peri-follicular cells of the ovary and in the cytoplasm of Leydig and germ cells in the testis. Then, the spatio-temporal expression pattern of *cyp19a1a* was studied during SD using GFP fluorescence imaging in gonads at 20, 30, 35 and 40 day post-fertilisation (dpf). Our data showed that GFP was expressed in all undifferentiated gonads of 20 dpf-old zebrafish. At later stages of development, GFP expression increased in early differentiated female at 30 and 35 dpf to reach high GFP intensity in well-differentiated ovaries at 40 dpf. This pattern of gonadal GFP expression markedly differed from that of males which consistently displayed low GFP expression as compared to female whatever their stage of development, resulting in a clear dimorphic expression between males and females. Interestingly, fish that undergoes ovary-to-testis transition (35 and 40 dpf) presented an intermediary GFP level as compared to those measured in males and females. Our results suggest that (1) *cyp19a1a* is expressed early during gonadal development before the histological process of SD, (2) the down-regulation of *cyp19a1a* expression is critical for the testicular differentiation, (3) although *cyp19a1a* expression exhibit a clear dimorphic expression in gonads during SD, its expression persists whatever the sex suggesting that estradiol synthesis is important for gonadal development in both male and female. Monitoring the expression of the GFP in control and exposed-fish will help to identify the interest of this model to study the mechanisms of action of endocrine disrupting chemicals on critical physiological processes such as SD.

MO449

Application of transgenic zebrafish to enable adverse outcome pathway analysis for endocrine disrupting mechanisms

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Around 800 chemicals are known, or suspected, to interact with hormone receptors, and/or to affect hormone metabolism, with the theoretical potential to affect physiological function, if exposures exceed thresholds for activation of molecular initiating events (MIEs). However, only a small fraction of these chemicals have been investigated in tests capable of identifying overt endocrine disrupting adverse effects in intact organisms or their progeny or (sub)populations (Weybridge, 1996, WHO/UNEP, 2012). Whilst *in silico* and *in vitro* tools may provide some indication of the potential endocrine activity of chemicals, reliable *in vivo* systems are required to assess for potential adverse effects, ideally in a high through-put/high content manner. Transgenic animal models (e.g. zebrafish) offer systems whereby MIEs that trigger adverse outcome pathways (AOPs) can be identified and quantified by fluorescent protein reporters linked to specific receptors or enzymes. Spatial resolution of receptor or enzyme responses at the organ and tissue levels potentially enables targeted assessment of downstream molecular events and subsequent pathological, physiological and functional responses. Temporal resolution is also possible in individual fish, enabling the identification of latent responses in organs and tissues. Quantification of MIEs and other key events and their calibration against adverse outcomes are crucial next steps for moving the application of AOPs from basic hazard assessment to intelligent risk assessment. Using the 'ERE-Casper' zebrafish as an illustrative model, we present a scheme for the application of transgenic zebrafish in chemical hazard and risk assessment and, at a more mechanistic level, show how they can serve to enable adverse outcome pathway analysis. The ERE-Casper zebrafish possesses a green fluorescent protein (GFP) reporter activated by estrogen response elements (ERE) and amplified via a two plasmid system, making it highly sensitive for detecting environmentally realistic exposure concentrations of selected estrogens (Lee et al., 2012). Furthermore, this model lacks skin pigmentation, making detection of the GFP response possible in both larval and juvenile life-stages and allowing for direct comparisons against adverse effects on development and function of targeted organs for these life-stages.

MO450

A newly *cyp11c1*-GFP transgenic zebrafish model to study the expression and the perturbation of corticosteroidogenesis by endocrine active substances at early developmental stages.

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The 11 β -hydroxylase (11 β H) is the enzyme responsible for the biosynthesis of cortisol, the main corticosteroid in fish, which is implicated in the regulation of

multiple physiological processes including energy metabolism, hydromineral balance, reproduction and the immune system. In fish, the gene coding for 11 β H, namely *cyp11c1*, is expressed early in interrenal cells during the development participating to the emergence of the corticosteroid axis. To further investigate the expression *cyp11c1* and its potential deregulation by stressors and pharmaceutical ligands, we established a transgenic zebrafish line that expresses Green Fluorescent Protein (GFP) under the control of the zebrafish *cyp11c1* promoter. Using *in vivo* fluorescence imaging techniques on whole larvae, we showed a weak expression of the reporter gene from 4 to 7 days post-fertilisation (dpf) in an anatomical region where interrenal cells are known to be present. Tissue sections and immunostaining with specific 11 β H antibodies revealed that GFP is co-localized with the endogenous *cyp11c1* protein in interrenal cells. This well agrees with the expression and localization of other steroidogenic interrenal marker genes such as *star* and *cyp11a1* in wild-type zebrafish at this stage of development (To et al. 2007). To further characterize the *cyp11c1*-GFP zebrafish model, GFP was quantified in 6-dpf old zebrafish after osmotic stress or exposure to glucocorticoids receptor (GR) agonists (Dexamethasone, DEX) and antagonists (Mifepristone, MIF). Exposure to salt water led to a rapid induction of GFP, showing that *cyp11c1* responds to an osmotic shock. Exposure to MIF (1 μ M from 5 to 6 dpf) decreases GFP intensity, suggesting that GR is involved in the transcriptional regulation of *cyp11c1* during development. In DEX-exposed larvae (10 μ M, 5-6dpf), GFP intensity was inhibited as compared to controls, this effect being abolished when zebrafish larvae were co-exposed with MIF. These data agree with the known negative feedback of DEX on pituitary expression of Pro-opiomelanocortin (POMC), leading in turn to a down-regulation of genes involved in corticosteroidogenesis (Sun et al., 2010). Altogether, these data suggest that the newly developed transgenic *cyp11c1*-GFP zebrafish is an interesting model to study the expression of 11 β -hydroxylase gene in interrenal cells and its perturbation by endocrine active compounds acting on the Hypothalamic-Pituitary-Interrenal axis, which is critical for cortisol homeostasis.

Ecotoxicology of amphibians and reptiles. Resolving uncertainties about the impact of pollutants on individuals, populations and communities (P)

TU001

Pesticide risk assessment needed for amphibians and reptiles?

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Many amphibians and reptile species are globally in decline and it is hypothesized that one of the causes for their decline is exposure to xenobiotic chemicals and complex interactions with other environmental stressors [1] [2] [3] [4]. Many amphibian and reptile species occur in agricultural landscapes and can be exposed to pesticides in the aquatic and the terrestrial environment. Exposure to pesticides could potentially lead to severe impacts on amphibians [5]. The EU regulation EU 1107/2009 requires for approval of plant protection products no unacceptable effects on the environment, by considering particularly their impacts on non-target species including on the ongoing behaviour of those species and their impacts on biodiversity and the ecosystem. The data requirements for active substances (Regulation EU 283 / 2013) and formulations (Regulation EU 284 / 2013) ask for information on effects on terrestrial wildlife including amphibians and reptiles as well as for consideration of endocrine mediated effects. Information is available on the toxicity of aquatic life stages of amphibians which enabled a comparison of acute toxicity endpoints with other aquatic organisms. It is concluded in the aquatic guidance document [8] that the acute risk to aquatic life stages of amphibians is covered by the standard first tier risk assessment for fish. It would need to be investigated further whether this covers also the risk to amphibians which develop in shallow non-permanent water bodies since exposure may be different than in the currently assessed FOCUS scenarios (i.e. permanent water bodies with a depth of 30 cm). Dermal exposure to pesticides is likely to be a major route of exposure for amphibians. In particular movements through treated fields could present a worst case exposure situation. EFSA has recently published a guidance document on non-dietary human exposure covering in-field and edge-of-field assessments, some elements could serve as a basis for future developments to address the dermal and inhalation exposure of other terrestrial vertebrates. In order to draw conclusions on what is covered by the current risk assessments more information on exposure estimates for shallow non-permanent water bodies, toxicity data (dermal and oral), feeding patterns and potential coverage of the risk by human health non-dietary risk assessments is needed.

TU002

Challenges facing terrestrial amphibian risk assessment

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Risk assessment processes for agricultural chemicals are generally well developed for protecting aquatic species; however, there is currently no risk assessment