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Hazard of pharmaceuticals for aquatic environment: Prioritization by structural approaches and prediction of ecotoxicity

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ABSTRACT

Active Pharmaceutical Ingredients (APIs) are recognized as Contaminants of Emerging Concern (CEC) since they are detected in the environment in increasing amount, mainly in aquatic compartment, where they may be hazardous for wildlife. The huge lack of experimental data for a large number of end-points requires tools able to quickly highlight the potentially most hazardous and toxic pharmaceuticals, focusing experiments on the prioritized compounds. *In silico* tools, like QSAR (Quantitative Structure-Activity Relationship) models based on structural molecular descriptors, can predict missing data for toxic end-points necessary to prioritize existing, or even not yet synthesized chemicals for their potential hazard. In the present study, new externally validated QSAR models, specific to predict acute toxicity of APIs in key organisms of the three main aquatic trophic levels, *i.e.* algae, *Daphnia* and two species of fish, were developed using the QSARINS software. These Multiple Linear regressions - Ordinary Least Squares (MLR-OLS) models are based on theoretical molecular descriptors calculated by free PaDEL-Descriptor software and selected by Genetic Algorithm. The models are statistically robust, externally predictive and characterized by a wide structural applicability domain. They were applied to predict acute toxicity for a large set of APIs without experimental data. Then predictions were processed by Principal Component Analysis (PCA) and a trend, driven by the combination of toxicities for all the studied organisms, was highlighted. This trend, named Aquatic Toxicity Index (ATI), allowed the raking of pharmaceuticals according to their potential toxicity upon the whole aquatic environment. Finally a QSAR model for the prediction of this Aquatic Toxicity Index (ATI) was proposed to be applicable in QSARINS for the screening of existing APIs for their potential hazard and the *a priori* chemical design of not environmentally hazardous APIs.

1. Introduction

Pharmaceuticals are essential compounds in the modern society, with undeniable benefits on human health and lifestyle. However, unwanted residues of Active Pharmaceutical Ingredients (APIs) were found in environmental media since 1970th, when the first studies assessing the presence of drugs and their metabolites in domestic wastewaters were published (Aherne et al., 1985; Garrison et al., 1976; Hignite and Azarnoff, 1977; Richardson and Bowron, 1985). In the last decades there was a progressive increase in drug consumption mainly due to irrational use and abuse of pharmaceuticals products (Abraham, 2010; IWW, 2014). Moreover, analytical techniques and methods improved their performances and lowered their limit of detection. These reasons, together with the improper disposal of medicines, lead to enhanced levels of detected and measured medicine residues in the environment all over world. Therefore, pharmaceuticals are now recognized as Contaminants of Emerging Concern (CEC) (Kümmerer, 2009; Taylor and Senac, 2014).

More than 200 APIs, mainly antibiotics, painkillers, vascular drugs and antidepressants, are commonly found in aquatic and terrestrial

Abbreviations: APIs, Active Pharmaceutical Ingredients; AD, applicability domain; ATI, Aquatic Toxicity Index; PBT, Persistence, Bioaccumulation and Toxicity; CAS, Chemical Abstract Service; CCC, concordance correlation coefficient; CEC, Contaminants of Emerging Concern; ECOSAR, Ecological Structure Activity Relationships; E-State, electrotopological state; ERA, Environmental Risk Assessment; EE2, estrogen ethinyl estradiol; EMEA, European Medicines Agency; CSTEE, European Union Commission's Scientific Committee on Toxicity, Ecotoxicity and Environment; GA-VSS, Genetic Algorithm Variable Subset Selection; MLR, Multiple Linear Regression; NCCOS, National Centre for Coastal Ocean Science; NOAA, National Oceanic and Atmospheric Administration; ORe, Ordered by Response; OSt, Ordered by Structure; OLS, Ordinary Least Square; OECD, Organization for Economic Cooperation and Development; PCA, Principal Component Analysis; PCs, Principal Components; QSARINS, QSAR-INSubria; QSAR, Quantitative Structure Activity Relationship; QSTR, Quantitative Structure Toxicity Relationship; Rnd, Random selection; RMSE, Root Mean Squared of Errors; SMILES, Simplified Molecular Input Line Entry System; US-EPA, United States - Environmental Protection Agency; WWTPs, waste water treatment plants.

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compartments in concentration ranging from few nanograms/liter to thousand micrograms/liter (Hughes et al., 2013). Many APIs are not completely degraded by the ordinary microbiological treatments in waste water treatment plants (WWTPs) and can be discharged in the aquatic environment (Han et al., 2006; Joss et al., 2005), leading to an ubiquitous and continuous contamination (Daughton and Ternes, 1999; Fernandez et al., 2010). It is widely recognized that the main route of entry in the environment for pharmaceuticals are WWTPs that collect urban waste water containing APIs and their metabolites, excreted with faeces and urines, and also drugs improperly disposed (Nebot et al., 2015; Papageorgiou et al., 2016). Other important sources of pharmaceuticals are hospitals and factories, whose effluents are loaded with very high concentration of APIs (Cardoso et al., 2014; Oliveira et al., 2015; Orias and Perrodin, 2013; Santos et al., 2013).

Pharmaceuticals are biologically active substances, specifically designed to interact with living organisms. They can cause biological effects even at low concentration. When released in the environment, in particular in the aquatic media, their biological activity may adversely affect wildlife and in general have negative impact on the ecosystem health. A lot of acute and chronic effects have been assessed, due to exposure of non-target organisms to environmental concentrations of pharmaceutical residues (Baldigo et al., 2015; Bartikova et al., 2016; Brausch et al., 2012; Damasceno de Oliveira et al., 2016; Godoy et al., 2015; Lumaret et al., 2012; Overturf et al., 2015). Some pharmaceuticals could pose a risk for aquatic plants and algae, similarly to some compounds used as herbicides (Guo et al., 2015). For example, two fluoroquinolone antibiotics, enrofloxacin and ciprofloxacin, frequently detected in wastewaters and in surface waters, were assessed to be toxic to algae (Ebert et al., 2011). Some antidepressant drugs affect invertebrates at concentrations commonly found in the environment (Fong and Ford, 2014). The steroid estrogen ethinyl estradiol (EE2), used in the contraceptive "pill", causes the feminization of male fish in river and water bodies, even at concentrations of few nanograms/liter (Nash et al., 2004; Sumpter et al., 2006). Also the anti-inflammatory drug diclofenac seems to be cause of concern for aquatic organisms (Eades and Waring, 2010; Triebskorn et al., 2004).

The standard regulatory characterization of the hazard of chemicals for the aquatic compartment is performed by standard ecotoxicity assays on selected aquatic organisms of different trophic levels. Different species of fish, crustaceans and algae are used in standard tests following the respective OECD guidelines. Such tests are required also in the guidelines for environmental risk assessment (ERA) of pharmaceutical substances, which was requested by the European Medicines Agency (EMEA) in the pre-approval phase for the marketing authorisation of new medicinal products (EMEA, 2006). The EMEA guidelines are limited to the new substances even if some authors suggested to enlarge the ERA procedure also for the "existing pharmaceuticals", on the market before 2006 (Ågerstrand et al., 2015).

Anyway, such risk assessment issue requires a high amount of experimental data with consequent high costs, time consuming and animal lives sacrificed for *in vivo* testing. Unfortunately these kind of experimental data are very limited. When data are not available, Quantitative Structure Activity Relationship (QSAR) methodologies represent a valid alternative to estimate the potential hazard of substances, inherent in their chemical structure. QSAR can find the structural features that are related to a specific end-point of biological activity, models this relationship from the experimentally available data, and exploits the established relationship for predicting missing data. The integration of the few experimentally available data with validated QSAR predictions is also useful for ranking the studied chemicals according to their potential toxicity: in this way a prioritization of the most hazardous compounds can be achieved (Cassani and Gramatica, 2015; Gramatica, 2013; Gramatica et al., 2016a, 2016b, 2015; Mendoza et al., 2015).

Pharmaceuticals were studied for their potential Persistent Bioaccumulative (PB) behaviour by Howard and Muir (Howard and Muir, 2011) applying QSAR models. Recently a large set of more than 1200 pharmaceuticals was screened and prioritized for overall Persistence, Bioaccumulation and Toxicity (PBT) potential, in our lab, by comparing the predictions from the Insubria PBT Index and the US-EPA PBT Profiler (Sangion and Gramatica, 2016). A priority list of 35 pharmaceuticals, which were predicted as potential PBTs by consensus of both applied modelling methods, was proposed. These compounds were highlighted as potentially hazardous for their cumulative behaviour as persistent, bioaccumulative and toxic chemicals, but not specifically for their toxicity on some definite endpoints. Sanderson, in an interesting paper, stated that QSAR models can be useful to prioritize pharmaceuticals according to their acute toxicity, even if much future developments were needed to address the regulatory purposes (Sanderson, 2012). Also the European Union Commission's Scientific Committee on Toxicity, Ecotoxicity and Environment (CSTEE) had recommended the use of QSAR models for screening purposes of pharmaceutical ingredients (European Commission (EC), 2001).

So far, the main used tool to predict ecotoxicity of APIs by QSAR is ECOSAR (US EPA, 2012), a widely applied online modelling tool, based on the octanol-water partition coefficient (LogKow). Even thought a lot of studies used these models to fill the data gap (Fent et al., 2006; Mendoza et al., 2015; Ortiz de García et al., 2013; Sanderson et al., 2003; Sanderson and Thomsen, 2009, 2007), Madden et al. stated that the applicability domain of the ECOSAR program to predict pharmaceuticals effects should be carefully evaluated, because the models were developed using small industrial chemicals as training sets (Madden et al., 2009). We agree with this observation. In fact, such models are based on very small sets of molecules, mainly of simple chemical structure with only a single functional group, while pharmaceuticals are complex chemicals often with a plurality of functional groups.

Recently some authors tried to develop QSAR models for pharmaceuticals developing statistically validated QSAR models which are based on theoretical molecular descriptors (Jiang et al., 2010; Roy and Ghosh, 2010; Tugcu et al., 2012). However, also these models were developed on very small sets of molecules and, most importantly, are not available for all the required trophic levels, a necessary condition for a comprehensive assessment of the potential hazard for the aquatic compartment. Recently, Singh et al. tried to overcome this problem, developing QSTR (Quantitative Structure Toxicity Relationship) models to predict the ecotoxicity of pharmaceuticals in multiple test species (Singh et al., 2015).

The aim of the present work is to propose an alternative framework, based on externally validated QSAR models, specifically developed on pharmaceuticals, to prioritize the potentially most hazardous compounds according to their intrinsic aquatic toxicity by proposing a cumulative Aquatic Toxicity Index (ATI). To reach this global aim, the present study was based on various steps: a) the development of QSAR models of ecotoxicity on three trophic levels of aquatic organisms, specifically for APIs; b) the comparison of our model results with the predictions obtained by ECOSAR (US EPA, 2012); c) the application of our *ad hoc* QSAR models to a large set of more than 1200 APIs, collected in our previous study (Sangion and Gramatica, 2016); d) the ranking of the studied compounds, combining the ecotoxicity data on different aquatic organisms by Principal Component Analysis (PCA), to highlight and prioritize the most hazardous pharmaceuticals; e) the proposal of a final QSAR model of the cumulative aquatic toxicity trend, obtained by PCA and defined as an Aquatic Toxicity Index (ATI). All the proposed QSAR models will be applicable by using the QSARINS-Chem module in the software QSARINS (Gramatica et al., 2014, 2013).

Such comprehensive framework would allow prioritizing the existing APIs according to their cumulative aquatic toxicity potential, just on the basis of their molecular structure. Moreover it could be applied, *a priori*, in the preliminary assessment of not yet synthetized and commercialized pharmaceuticals in the hazard evaluation phase of the ERA procedure.

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