ENVIRONMENTAL RISK ASSESSMENT (ERA) OF PHARMACEUTICALS AND PERSONAL CARE PRODUCTS (PPCPs) USING ECOTOXICITY TESTS

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Abstract

A wide range of Pharmaceuticals and Personal Care Products (PPCPs) are present in the environment, and many of their adverse effects are unknown. The environmental risk assessment (ERA) of twenty six PPCPs of high consumption and occurrence in Spain was done in this work. Based on ecotoxicity values obtained by bioluminescence and respirometry assays, the compounds were classified as established the Global Harmonized System of Classification and Labelling of Chemicals (GHS). According to the criteria of the European Medicines Agency (EMEA) the real risk of impact of these compounds in the environment was predicted. Following this methodology and for aquatic environments, clofibric acid, clofibrate and cefaclor can be classified as risk-free. By applying the criteria of the Phase II A of EMEA, three compounds (Ibuprofen, omeprazole and acetaminophen) have resulted with high environmental risk. Nevertheless, applying the Phase II B of EMEA, only the metabolite 1,4-benzoquinone presents high environmental risk. The ERA on WWTPs indicates that ibuprofen, ciprofloxacin, naproxen and acetaminophen show some type of risk (Tier A). Ibuprofen and ciprofloxacin highlight in Tier B with a low risk. In any case, it is necessary to improve acute and chronic ecotoxicity tests and the estimation of predicted environmental concentrations (PECs) of PPCPs to support these studies.

Keywords: Pharmaceuticals and personal care products, ecotoxicity, Environmental Risk Assessment, QSAR.

1. Introduction

The generation and consumption/use of a large amount of synthetic chemicals, specifically pharmaceuticals and personal care products (PPCPs), has led to the detection of these substances with greater frequency and persistence in natural water, wastewater and drinking water systems. This and the lack of knowledge of their negative effects on the environment are the basis of this work which deals with the environmental risk assessment of twenty six PPCPs of high consumption in Spain, based on the occurrence of these compounds in the aquatic environments and the experimental data of their acute ecotoxicity.

Intensive research of PPCPs in the environment began about 15 years ago. Since then it has been published a wide variety of literature, as one of the emerging issues in environmental chemistry [1-7]. More than 80 compounds, pharmaceuticals and metabolites were detected in aquatic environments specifically in wastewater, surface water, drinking water, and agricultural soils, in recent researches conducted in Austria, Brazil, Canada, Croatia, England, Germany, Greece, Italy, Spain, Switzerland, the Netherlands and the U.S.A. [3]. Income, occurrence and fate of these substances in aquatic environments have been and continue to be of great interest [8].

Many effects and negative impacts that can cause PPCPs in the environment are still unknown. Numerous studies confirm the persistence of these compounds in aquatic media, sediment, sludge and soil and their bioaccumulation and toxicity to wildlife. Depending on their physicochemical properties, most of these substances become part of the municipal wastewater after they had been consumed, metabolized and excreted by living organisms. In many cases, wastewater treatment plants (WWTPs) are unable to efficiently remove these compounds and are adsorbed by the sludge of primary and secondary treatments, or they remain in the treated wastewater, and finally, they are distributed in surface waters and groundwaters, sediments and/or tissues of exposed wildlife. The health disorders most frequently associated with the exposure to these chemicals, both in

animals of different species and humans, mainly include the development of resistance to pathogens [9] hormone-dependent diseases, immune system disorders, and even increased incidence of different types of cancer [10].

An alternative to estimate the fate and the negative effects of these substances in the environment is applying the methodology of "quantitative structure-activity relationship" (QSAR) that takes into account the physicochemical properties and molecular structure to estimate their biodegradability, biological ecotoxicity, mutagenicity and carcinogenicity, among other adverse effects. However, the combination of these predictive models with experimental tests of ecotoxicity, generally of low complexity and cost, provide a first scientific approach to rapidly identify compounds that require immediate attention for their potential adverse effects on the environment.

This research focuses on the environmental risk assessment of twenty six PPCPs which are highly consumed in Spain, using acute ecotoxicity experimental data and the estimation of the occurrence of these compounds in aquatic environments and WWTPs.

2. Materials and methods

Figure 1 shows a scheme of the methodology used in the research presented in this paper. The PPCPs investigated were selected based on their physicochemical properties (water solubility, solid/water and octanol/water partition coefficients), their high consumption and their occurrence in aquatic environments in Spain [11].

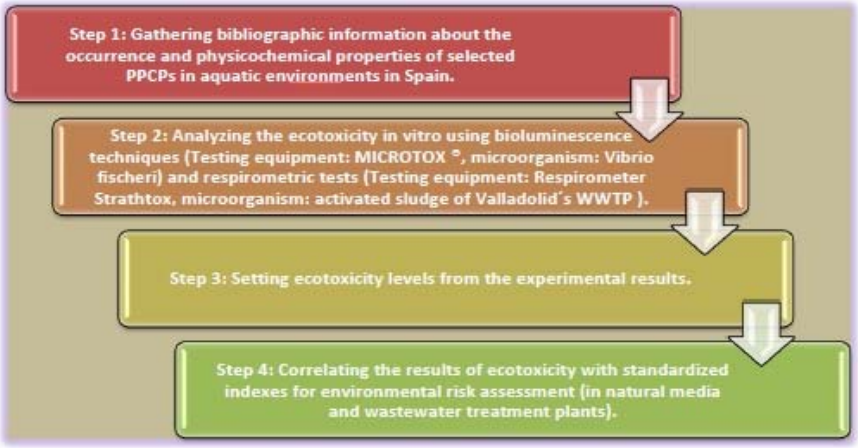

Figure 1. Stages involved in this research

2.1. Chemicals, test organisms and media

Twenty six PPCPs were selected for this study: acetaminophen, 1,4-benzoquinone (acetaminophen metabolite), ibuprofen, ibuprofen sodium salt, diclofenac sodium salt, naproxen, naproxen sodium salt, acetylsalicylic acid, salicylic acid, amoxicillin, sulfamethoxazole, cefaclor, ciprofloxacin, ciprofloxacin hydrochloride monohydrate, clarithromycin, erythromycin, levofloxacin, norfloxacin, omeprazole, clofibrate, clofibric acid, methylparaben, ethylparaben, propylparaben, p-hydroxybenzoic acid, triclosan. All these compounds were of analytical grade, purity ≥ 99.5% and were purchased from Sigma-Aldrich Chemicals (Madrid, Spain).

The Microtox® acute ecotoxicity test was performed with the marine bioluminescent bacteria *Vibrio fisheri*, as the test organism. The bacteria were purchased from Instrumentación Analítica S.A. (Spain) as a freeze-dried form and were stored at -20 to -25 ºC to preserve microbial activity. All the PPCPs solutions were prepared with Milli-Q® water. Two solutions of NaCl (2% and 22% w/w) were used as a salt medium and osmotic adjustment.

The respirometry test was performed with: i) aerobic sludge taken from the secondary treatment of the Valladolid´s WWTP, ii) synthetic wastewater purchased from Strathkelvin Instruments (Lanarkshire, Scotland) and iii) distilled water.

2.2. Ecotoxicity tests

The acute effects on bioluminescence of the *Vibro fisheri* bacteria were carried out using a Microtox® Model 500 ecotoxicity analyzer according to the manufacturer`s instructions (Azur Environmental, Newark, Delaware, USA) and ISO 11348-3:2007 protocol [12]. In the Microtox® test, the inhibition of light emission was measured in relative units of luminescence. The data were used to calculate the EC_{50}, which is the mean sample concentration that causes a 50% reduction in bacteria bioluminescence [13]. Each test was carried out in

duplicate at five different concentrations which were obtained by serial dilution from the prepared stock solution. A reference toxicant, zinc sulfate ($ZnSO_4 \cdot 7H_2O$) was used as positive control. The positive control was performed concurrently with the sample as a quality control test. Temperature, pH, solubility, turbidity and color were adjusted or measured if they were necessary. The dose-response curves were obtained for each PPCP at 5 and 15 minutes. The acute ecotoxicity endpoint was determined as the EC_{50} at both times with 95% confidence limits using a linear regression model.

The respiration inhibition test (immediate) was used in this study to measure the ecotoxicity of each PPCP on the activated sludge taken from the secondary treatment of Valladolid´s WWTP and was accomplished in the Strathtox Unit SI500 from Strathkelvin Instruments (Lanarkshire, Scotland) and according to its procedure manual and the EPA 712-C-014 OCSPP 850.3300 method [14]. The respiration inhibition test calculates EC_{50}, EC_{20} and EC_{10} values, i.e. the concentration of wastewater causing 50%, 20% and 10% inhibition of the respiration rate. The activated sludge was kept fully aerated during the test and the Mixed Liquor Suspended Solids concentration (MLSS) was kept between 2 and 4 g/L. The PPCPs solutions were added directly to the respirometer tubes and they were mixed with distilled water to obtained five different dilutions for a total volume of 10 mL. Then, the synthetic wastewater and the activated sludge were added to measure the respiration rate (based on oxygen concentration decrease over the time). The respiration inhibition tests were evaluated in triplicated for each PPCP solution and for each concentration. The EC_{50} was calculated with a 95% confidence limit using a linear regression model, as in the Microtox® test.

2.3. Ecotoxicity levels

Based on ecotoxicity values obtained by bioluminescence and respirometry assays, the compounds were classified as is established by the Global Harmonized System of Classification and Labelling of Chemicals (GHS) [15]:

i) highly toxic: $EC_{50} \leq 1$ mg/L;

ii) toxic: 1 mg/L < $EC_{50} \leq 10$ mg/L;

iii) harmful to aquatic organisms: 10 mg/L < $EC_{50} \leq 100$ mg/L.

Some regulatory systems include a fourth category (nontoxic) for those compounds having an EC_{50} > 100 mg/L.

2.4. Environmental risk assessment (ERA) for PPCPs carried out considering the framework of the European Agency for the Evaluation of Medicinal Products (EMEA)

Figure 2 shows a summary of the schematic procedure to perform ERA of medicinal products for human use following the EMEA guidelines. The assessment of the potential risks of this kind of compounds to the environment is a step-wise, phased procedure, consisting of two phases.

Figure 2. Diagram of EMEA guidelines for ERAs of PPCPs

In phase I, the estimation was based only on the substance characteristics, irrespective of its route of administration, pharmaceutical form, metabolism and excretion. If the Predicted Environmental Concentration (PEC) value is below 0.01 µg/L, and no other environmental concerns are apparent, it is assumed that the medicinal product is unlikely to represent a risk for the environment following its prescribed usage in patients. If the PEC value is equal to or above 0.01 µg/L, then an environmental fate and effect analysis Phase II should be performed. In some cases, the action limit may not be applicable. Nevertheless, some drug substances may affect the reproduction of vertebrate or lower animals at concentrations lower than 0.01 µg/L. In these cases, these substances should enter Phase II and a tailored risk assessment strategy should be followed that addresses its specific mechanism of action [16].

Moreover, following the guidelines of the EMEA an evaluation of persistence, bioaccumulation and ecotoxicity (PBT) according to EU TGD [17] was done for such selected PPCPs with an octanol-water partition coefficient greater than 4.5. The PBT indexes were evaluated using the models implemented in the EPI Suite™ interface [18].

In the second phase (Phase II), information about the fate and effects in the environment was obtained and assessed. Phase II is divided in two parts, Tier A and B [16]. In this study, PEC values were taken from a recent research [11] for the both parts, II A (simple PEC: excluding for calculations metabolization in humans and removal in WWTPs) and II B (refined PEC: considering metabolization in humans and removal in WWTPs). Predicted no effect concentrations (PNECs) for aquatic environments were obtained as the ratio of the lower value of Microtox® EC_{50} and the standard assessment factor recommended by the EMEA (1000) for these ecotoxicity tests. PNECs for WWTPs were calculated from respirometry tests and the standard assessment factor recommended by the EMEA (100).

In the Phase II A and II B the PEC:PNEC ratio was calculated to predict: (i) if the compound needs more attention, (ii) if other tests have to be done to demostrate its adverse effects on environmental or otherwise (iii) if it is not harmful.

Other more restrictive ranking of environmental risk impact [19] establishes the risk quotient, RQ (PEC:PNEC): (i) High toxicity: RQ>1, (ii) medium toxicity: 0.1<RQ<1 and (iii) low toxicity: 0.01<RQ<0.1.

These two classifications were used to rank the risk for the PPCPs under study.

3. Results and discussion

Figure 3 shows the ecotoxicity values (EC_{50}) obtained by bioluminescence and respirometry assays and the ecotoxicity levels according to the GHS classification.

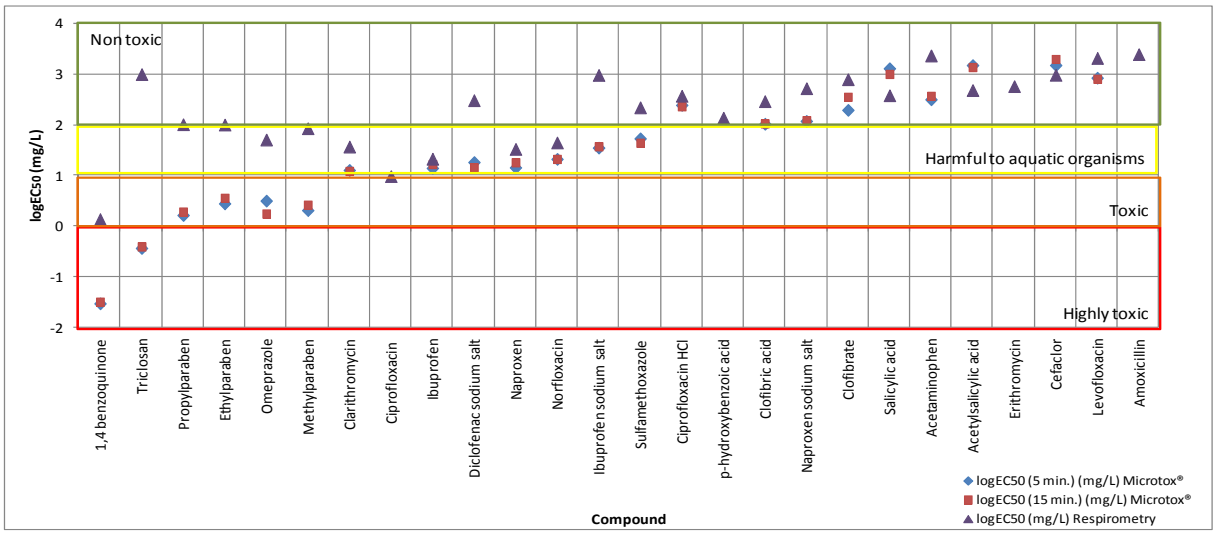

Figure 3. Ecotoxicity tests results with GHS classification

These ecotoxicity values show that *Vibrio fischeri* is more sensitive than biomass microorganisms from secondary treatment WWTPs because these microorganisms are adapted to receive large amount of toxic compounds from urban and industrial sewage. Despite this, 53.8% of PPCPs under study are harmful to aquatic organisms in at least two ecotoxicity tests according to the GHS ecotoxicity classification which gives preliminary evidence about negative affectation of these compounds on the environment.

Based on the ecotoxicity of nonsteroidal antiinflammatories drugs and acetylsalicylic acid data evaluated with algae and *Daphia magna* [22]; can be indicated that diclofenac, ibuprofen and naproxen were more toxic for *Vibrio fischeri* (this study) than for algae and *Daphia magna,* but acetylsalicylic acid showed the opposite behavior. Acute ecotoxicity for levofloxacin in algae and crustaceans were higher than in *Vibrio fischeri* [21], for norfloxacin, in rotifers and algae, were similar to than in *Vibrio fischeri*. In other studies [23-24] *Vibrio fischeri* acute ecotoxicities of sulfamethoxazole were EC_{50}=78.1 mg/L (15 minutes) and EC_{50}=23.3 mg/L (30 minutes). In crustaceans were between 15 y 35 mg/L [24]. Triclosan was reported as "very toxic" according to acute ecotoxicity in different species and exposure times [25]. Parabens could cause adverse effects on environment [26] which confirms the results on *Vibrio fischeri* and respirometry of this study. Ecotoxicity of clofibric acid has been evaluated using three different aquatic microorganisms [9], one of them the *Vibrio fischeri* bacteria, although we have found an EC_{50} 2.8 times greater than other study [9], the risk classification for this PPCP is similar in both studies.

Discrepant values of EC_{50} can be attributed to the complexity of the biological tests, changes in the organisms' sensibility and interlaboratory differences. Thus, risk categorization is a useful method to establish a reasonable range to classify the ecotoxicity values and their adverse effects, and to interrelate results from different researches.

According to the EMEA guidelines (Phase I) the PEC values were verified for the PPCPs investigated in this study (the PPCPs most abundant in aquatic environments in Spain). In a recent study for occurrence of PPCPs [11] it was observed that clofibric acid and clofibrate (blood lipid regulators) and cefaclor (antibiotic) had a PEC <10 ng/L, and therefore can be classified as risk-free. Aditionally, octanol-water partition coefficient was investigated, and only the octanol-water partition coefficient for triclosan was higher than 4.5, therefore, a PBT analysis was done for this compound by EPI Suite™ interface [14]. The results displayed that the triclosan is persistent and toxic but not bioaccumulative. Despite this, a recent research [25] indicates that there are contradictory studies about of the bioaccumulation potential of the triclosan. Therefore, it should be performed bioaccumulation tests for the triclosan in the specific geographic area under study.

When the phase II of the EMEA guidelines is applied and the RQ ratio calculated without consider pharmacokinetics in humans and removal in WWTPs, then only acetaminophen, ibuprofen and omeprazole have a RQ>1. Therefore, these compounds have to be evaluated in Tier B. Nevertheless, if a second more restrictive classification [19] is applied, then the 63.3% of the compounds investigated have some kind of risk, see Figure 4.

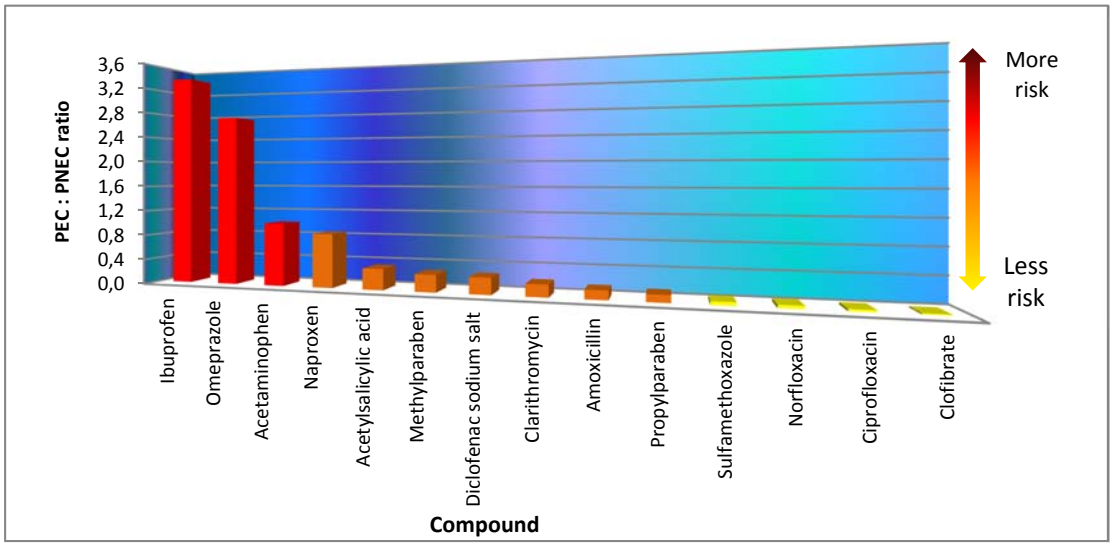

Figure 4. ERA of the PPCPs under study with more environmental risk.

If the tier B (with refined data) -phase II- of the EMEA methodology is applied, then only 1,4-benzoquinone is found as hazardous. . Nevertheless, using the more restrictive classification [19] aforementioned with refines data, besides the 1,4-benzoquinone (high risk), omeprazole and triclosan have medium risk and clarithromycin, ethylparaben, methylparaben have a low risk.

Other studies on aquatic environments have reported the RQ ratio according to the EMEA guidelines for different PPCPs and metabolites [19, 27-31]. In Switzerland [28] was analyzed the environmental risk of five pharmaceutical compounds in three WWTPs, they predicted the PECs values (refined or not) and measured the concentrations of the substances in the effluent of the WWTPs and the PNEC values were calculated from acute or chronic values of standard species or with ECOSAR™ predictions. In the Ebro River Basin (Spain) [31] RQ ratios were estimated for effluent of WWTPs located in the principal cities. The RQs values were calculated with the detected MECs and the estimated PNECs from fish, *Daphnia* and algae acute ecotoxicity values. Other study [19] discusses the potential of environmental impact of WWTP effluent, surface water and sediments, using acute ecotoxicity values for fish, *Daphnia* and algae. Their results show a wide divergence between the RQs due to, mainly, the assumptions made, the different ways to calculate PECs and the different available values of ecotoxicity. These issues highlight the need to further refine and improve the ERA methodologies and to estimate the acute and chronic ecotoxicities of these compounds.

The ERA of WWTPs (Tier A) indicates that ibuprofen, ciprofloxacin, naproxen and acetaminophen show some type of risk in these facilities. However, ibuprofen and ciprofloxacin highlight a low risk when Tier B is applied. These calculations were done with inhibition respirometry tests using WWTPs biomass. There is no evidence of respirometry ecotoxicity values on biomass according the EMEA guideline, at least in Spain.

Inhibition of respiration on biomass assays has a high dependence on laboratory conditions: temperature, equipment calibration, agitation, and especially the biomass, which is particular to each WWTP. Despite this, ERA in WWTPs allows predict the behavior of these compounds in these facilities and implement actions to improve their performance and prevent that PPCPs reached to the aquatic environment. Moreover, the classification obtained could be used as starting point for a more detailed analysis, including the occurrence, fate and effects in the long term of these compounds in WWTPs, with particular emphasis on secondary treatment.

Conclusions

The experimental results of ecotoxicity show that 53.8% of PPCPs under study are at least harmful to aquatic organisms according to the GHS classification based on two different ecotoxicity tests, which gives preliminary evidence about negative affectation of these compounds in the environment.

ERAs of PPCPs in aquatic environments and WWTPs were done following the EMEA guidelines to predict the real risk of impact of these compounds in these compartments. Using this methodology, can be concluded that clofibric acid, clofibrate and cefaclor can be classified as risk-free. Triclosan is persistent and toxic but not bioaccumulative. Applying Phase II A on aquatic environments, three compounds (Ibuprofen, Omeprazole and Acetaminophen) have resulted with high environmental risk; but in WWTPs, ibuprofen, ciprofloxacin, naproxen and acetaminophen show some type of risk. When PECs are refined (Phase II B), only the metabolite 1,4-benzoquinone presents high risk in aquatic environments and ibuprofen and ciprofloxacin highlight low risk in WWTPs.

This study underlines the need for further refining and improving the ERA methodologies for PPCPs disposed on the environment and for estimating acute and chronic ecotoxicity for PPCPs as a way to accurately predict their behaviors in the environment.

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