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Modeling of pharmaceuticals mixtures toxicity with deviation ratio and best-fit functions models

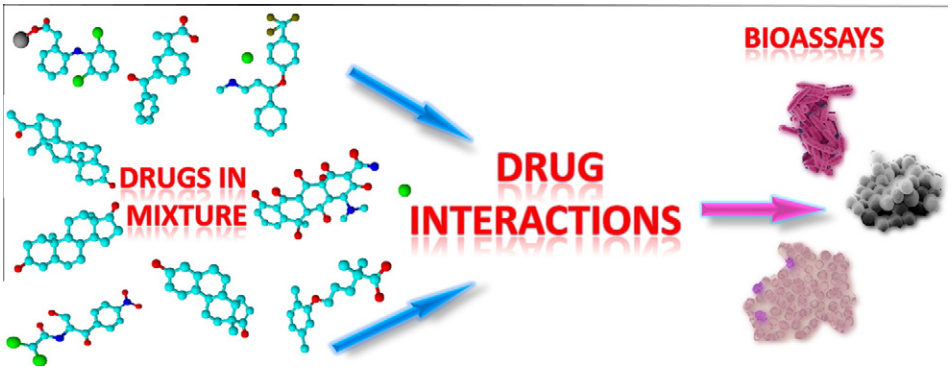
Monika Wieczerzak [a], Błażej Kudłak [a,*], Galina Yotova [b], Miroslava Nedyalkova [c], Stefan Tsakovski [b], Vasil Simeonov [b], Jacek Namieśnik [a]

[a] Department of Analytical Chemistry, Faculty of Chemistry, Gdańsk University of Technology, 11/12 Narutowicza, 80-233 Gdańsk, Poland
[b] Chair of Analytical Chemistry, Faculty of Chemistry and Pharmacy, University of Sofia "St. Kl. Okhridski", 1, J. Bourchier Blvd., 1164 Sofia, Bulgaria
[c] Chair of General and Inorganic Chemistry, Faculty of Chemistry and Pharmacy, University of Sofia "St. Kl. Okhridski", 1, J. Bourchier Blvd., 1164 Sofia, Bulgaria

HIGHLIGHTS

- Drug synergism or antagonism is *carte blanche* in modern environmental science.
- Quantitative assessment of drug mixtures toxicological parameters is given.
- CI, IA and SI by model deviation ratio (MDR) studies were carried out.
- Best-fit function modeling was also applied as an assessment option.
- Independent action was stated for most of drug mixtures studied with bioassays.

GRAPHICAL ABSTRACT



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ABSTRACT

The present study deals with assessment of ecotoxicological parameters of 9 drugs (diclofenac (sodium salt), oxytetracycline hydrochloride, fluoxetine hydrochloride, chloramphenicol, ketoprofen, progesterone, estrone, androstenedione and gemfibrozil), present in the environmental compartments at specific concentration levels, and their mutual combinations by couples against Microtox® and XenoScreen YES/YAS® bioassays. As the quantitative assessment of ecotoxicity of drug mixtures is an complex and sophisticated topic in the present study we have used two major approaches to gain specific information on the mutual impact of two separate drugs present in a mixture. The first approach is well documented in many toxicological studies and follows the procedure for assessing three types of models, namely concentration addition (CA), independent action (IA) and simple interaction (SI) by calculation of a model deviation ratio (MDR) for each one of the experiments carried out. The second approach used was based on the assumption that the mutual impact in each mixture of two drugs could be described by a best-fit model function with calculation of weight (regression coefficient or other model parameter) for each of the participants in the mixture or by correlation analysis. It was shown that the sign and the absolute value of the weight or the correlation coefficient could be a reliable measure for the impact of either drug A on drug B or, vice versa, of B on A. Results of studies justify the statement, that both of the approaches show

Abbreviations: CA, concentration addition; MDR, independent action (IA) and simple interaction (SI) by calculation of a model deviation ratio; YES+, YES agonist; YES−, YES antagonist; YAS+, YAS agonist; YAS−, YAS antagonist.

* Corresponding author.
E-mail address: monwiecz@student.pg.gda.pl (B. Kudłak).

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similar assessment of the mode of mutual interaction of the drugs studied. It was found that most of the drug mixtures exhibit independent action and quite few of the mixtures show synergic or dependent action.

1. Introduction

Numerous biologically active compounds are produced by humans and are present in the environment. Main sources of drugs' residues in the environment are considered to be products of pharmaceutical and veterinary industry, hospital facilities and agriculture, minor sources include municipal wastewaters and poorly sealed medical waste landfills. Despite the constantly increasing knowledge on environmental fate of pharmaceuticals we still do not fully understand all the processes that can occur between drug residues in the environment and their effects on living organisms (Kudłak et al., 2011; Halling-Sørensen et al., 1998). The risk of environmental exposure to residues of pharmaceuticals becomes greater due to the fact that they are biologically active substances, and often are not subjected to proper biodegradation in sewage treatment plants (Fatta-Kassinos et al., 2011). The literature provides some information in order to establish a uniform definition of drug interactions, namely: synergism, antagonism and additivity or to predict risk assessment of chemical mixtures (U.S. EPA, 1986; Wieczerzak et al., 2015; Backhaus and Faust, 2012; Vasquez et al., 2014; Watanabe et al., 2016). Within the environmental research on fate of pharmaceuticals it should not be forgotten that the residues of pharmaceuticals are present in the ecosystem in a mixture with other drugs and various stressors. Although numerous treatment methodologies involve the use of drug mixtures to achieve adequate therapeutic effect, this type of drugs co-presence is undesirable for the environment. As presented in Supplementary Table 1 studies on mutual impact (as well as in mechanistic response of drug in living body) of drugs when present in complex matrices were conducted mostly for health studies and in case of higher animals what does not reflect processes that occur in different environmental compartments. Environmental ecotoxicological studies in this area are still scarce and are conducted by few scientific centres (Watanabe et al., 2016, Altenburger et al., 2004; Backhaus, 2014, Silva E, Rajapakse N, Kortenkamp, 2002, Escher and Hermens, 2002, Dubiella-Jackowska et al., 2010).

The clarification of the toxic impact of different chemicals is a difficult, complex and, often, disputable problem. This holds true for assessing the effect of a single compound (e.g. a certain drug) and the task is much more complex in assessing the toxicity of drug mixtures. The traditional experimentation in studies of this type relies on the use of the responses of laboratory animals (usually rats) to the administration of drug combinations (Jakovljevic et al., 2009, Gan, 2010). In cases of this type the interaction between the administered drugs is considered to be “independent”, “dependent” or “synergetic” if the drug impact is neutral, negative or additive with respect to the influence on the general toxicity.

The problem is becoming even more complicated if the drugs mixture impact is regarded with respect to the ecotoxicity response of environmental compartments to the administration of drugs as wastes to environmental phases like surface waters, soils or sediments. On one hand the environmental systems are very different when compared to animal ones (including human), so it becomes a problem to interpret the mechanism of possible drug interactions offered for biota and to apply it the environmental samples. Different ecotoxicological tests (for acute ecotoxicity, chronic ecotoxicity, endocrine potential or DNA disruptors) require specific experimental design and assessment of results obtained, on the other hand. Therefore, the organization and performance of model experiments using different ecotoxicity test could be of significant importance in detection of any possible kind of interaction between drugs in waste water samples (independent, dependent or synergetic). The model experiment output should be considered as response of a black box system where the input is the concentration of each drug in a mixture and the output – the ecotoxicity measure for a certain type of bioassay (EC50, mortality, inhibition of luminescence etc.). Using the best fit function approach or MDR method as modeling procedures an adequate modeling of the mutual impact of the drugs in a mixture is possible although without exact description of the possible interaction mechanism.

It is the aim of the present study to assess by model experiments in a semi-quantitative way the combined ecotoxicological and endocrine impact of two drugs in a mixture at levels stated in environmental samples and to determine the possible independent, dependent and synergetic behavior of the separate drugs.

2. Materials and methods

In the present work, the influence of mixtures of 9 pharmaceuticals against 2 organisms from different trophic levels was assessed. The selected organisms were: bacterium *Vibrio fischeri* (Microtox®) and genetically modified yeast (XenoScreen YES/YAS®).

Vibrio fischeri is a G(−) bacteria found in salt and brackish waters. Any change in the bioluminescence of bacterial suspension after a period of incubation with the test sample is the basis of the Microtox® calculations used in this study (Marugán et al., 2012). In the XenoScreen YES/YAS® test genetically modified yeasts are used which, due to genetically introduced the androgenic (YAS) and estrogenic (YES) receptors, are sensitive to presence of substances with hormonal properties. The test allows the assessment of the agonistic and the antagonistic properties of chemicals present in the sample. Stained by β-galactosidase growth medium with CPRG is measured using a spectrophotometer.

Tested pharmaceuticals, namely: diclofenac (sodium salt), oxytetracycline hydrochloride, fluoxetine hydrochloride, chloramphenicol, ketoprofen, progesterone, estrone, androstenedione and gemfibrozil, are widely used in various therapeutic treatments. Their presence in the environment at different concentration levels has been confirmed in numerous studies (Vulliet et al., 2011; Kasprzyk-Hordern et al., 2008; Kim et al., 2007; Lin and Tsai, 2009). In Table 1 information on concentration levels of select group of pharmaceuticals in the environment is summarized. The data indicates that there is a risk of adverse effects of those compounds' presence. The compounds selected for studies are those prescribed in the highest quantitates and representing chemicals of different mode of action to human beings and for this reason determining toxicity of such mixtures is environmentally relevant.

2.1. Microtox® bioassay protocol

The Microtox® test acute reagent (lyophilized *Vibrio fischeri*), osmotic adjustment solution (OAS, 22% solution of sodium chloride), reconstitution solution (RS), and diluent (2% solution of sodium chloride) were purchased from Modern Waters (USA). The study was conducted using Microtox® Analyzer M500 model. Apparatus is equipped with 30 incubation wells as well as reagent (bacterial suspensions) and read wells. Temperatures are assigned to the corresponding type of performed test (in this case acute toxicity test) and the internally maintained at 5.5 ± 1 °C for reagent well and 15 ± 0.5 °C for both the incubator part and the read well.

2.2. XenoScreen YES/YAS® bioassay protocol

A set of XenoScreen YES/YAS® was purchased in Xenometrix AG (Switzerland), namely: hERα yeasts (to determine estrogenic activity)