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Abstract

Pharmaceuticals and analogs of bisphenol A (BPA) are increasingly threatening environmental pollutants. In this study, mixtures of selected pharmaceuticals (diclofenac sodium salt, chloramphenicol, oxytetracycline hydrochloride, fluoxetine hydrochloride, estrone, ketoprofen, progesterone, gemfibrozil and androstenedione) were prepared with BPA and its two analogs (namely, bisphenols F and S) at such ratios to reflect environmentally detectable levels. Then, the mixture solutions were studied with a XenoScreen YES/YAS assay to determine the variations in the initial hormonal response of each pharmaceutical compound due to the presence of a bisphenol analog. The results obtained were modeled with the concentration addition (CA) and independent action (IA) approaches, the trueness of which was studied with model deviation ratios (MDR). The estrogenic agonistic activity of the drugs studied was most strongly affected by the presence of BPA in solution (twenty-one cases of synergy observed for CA models versus twelve cases of antagonism in the case of IA predictions). BPS shows a strong agonistic estrogenic impact on most of the drugs studied at medium and high concentration levels; androgenic agonistic activity was also impaired with elevated concentrations of BPS. Increasing the concentration of BPF in a reaction mixture also increased the number of YES+ synergism incidences (for CA modeling). Estrone, progesterone and androstenedione were mostly affected by the highest BPF concentrations studied in the case of androgenic agonistic research performed.

Keywords	bisphenol A, bisphenol A analogues, pharmaceuticals, endocrine disruptors of mixtures, environmental pollution
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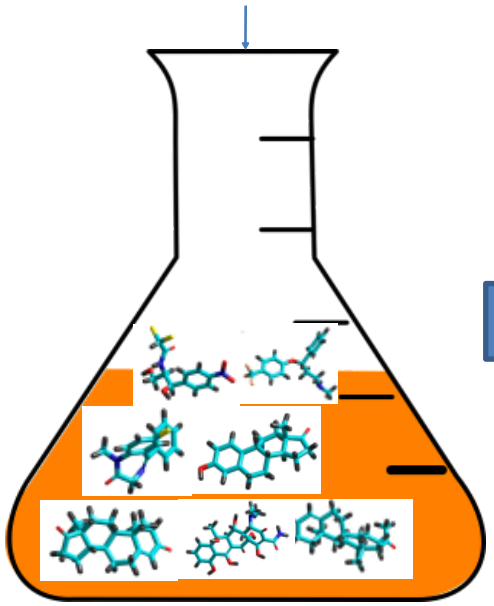
Pharmaceuticals + bisphenols (A, S, F)



XenoScreen
YES/YAS

Mixtures
toxicity
models

Bisphenols (A, S, and F) affect the basic hormonal activity determined for pharmaceuticals – study of *Saccharomyces cerevisiae*

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Abstract: Pharmaceuticals and analogs of bisphenol A (BPA) are increasingly threatening environmental pollutants. In this study, mixtures of selected pharmaceuticals (diclofenac sodium salt, chloramphenicol, oxytetracycline hydrochloride, fluoxetine hydrochloride, estrone, ketoprofen, progesterone, gemfibrozil and androstenedione) were prepared with BPA and its two analogs (namely, bisphenols F and S) at such ratios to reflect environmentally detectable levels. Then, the mixture solutions were studied with a XenoScreen YES/YAS assay to determine the variations in the initial hormonal response of each pharmaceutical compound due to the presence of a bisphenol analog. The results obtained were modeled with the concentration addition (CA) and independent action (IA) approaches, the trueness of which was studied with model deviation ratios (MDR). The estrogenic agonistic activity of the drugs studied was most strongly affected by the presence of BPA in solution (twenty-one cases of synergy observed for CA models *versus* twelve cases of antagonism in the case of IA predictions). BPS shows a strong agonistic estrogenic impact on most of the drugs studied at medium and high concentration levels; androgenic agonistic activity was also impaired with elevated concentrations of BPS. Increasing the concentration of BPF in a reaction mixture also increased the number of YES+ synergism incidences (for CA modeling). Estrone, progesterone and androstenedione were mostly affected by the highest BPF concentrations studied in the case of androgenic agonistic research performed.

Keywords: bisphenol A, bisphenol A analogs, pharmaceuticals, endocrine disruptors of mixtures, environmental pollution

Capsule: Analogs of BPA affect endocrine potential of numerous pharmaceuticals; the extent and magnitude of disruption is compound and concentration dependent.

1. *Introduction*

Comprehensive studies on the environmental fate and impact of pharmaceuticals (and their residues) as well as plasticizers on living organisms are a relatively new research topic. Unfortunately, many studies and literature reports in this area concern only the instrumental quantification of the

presence of a given xenobiotics (and in best case scenario - possible transformation products). Certainly, this type of information is very useful, but may be insufficient to determine the impact of given pharmaceuticals on living organisms existing in a particular environment. Most importantly, residues of pharmaceuticals occur in the environment in mixtures with other pollutants, and the degree of their toxicity may vary depending on the influence of environmental factors (such as pH, salinity, and ion co-presence (Wieczerzak et al. 2018).

Bisphenols (BPs) are quite widely used and have been detected in significant amounts in drinking water, foods (especially pre-packaged foods) and beverages, as well as in environmental samples (Eladak et al. 2015; Danzl et al. 2009). In 2015, Corrales et al. have reviewed over 500 publications dealing with the prevalence of BPA, noting that the average amount of BPA detected in wastewater treatment plants (WWTP) effluents worldwide ranged from 5 up to 370 µg/L, and surface waters samples may contain even 56 μgL^{-1} of BPA; however, salty waters of the Baltic Sea may contain up to 67.7 ng/L according to Staniszewska et al. (2014). Lower levels of BPA were reported in potable tap water samples levels, up to 1.3 µg/L (France) (Colin et al. 2014). The amount of BPA polluting sludges and biosolids has been reported to vary from 10 to > 100 000 µg/kg in industrial areas (dry weight) (Meesters and Schroder 2002).

The literature is not rich in data on BPA analogs; the analysis of wastewater from WWTPs in Slovakia revealed that only BPF and BPS (but not other BPs) were detected in the WWTP influents (in amounts of 36.7 ng/L and 40.6 ng/L, respectively) (Česen et al. 2018). Another study detected concentrations of BPF as high as 2850 ng/L in the Tamagawa River in Japan (Yamazaki et al. 2015). Liao and Kannan in 2013 studied a variety of food samples from the US and determined that the highest amount of BPF (1130 ng/g) was present in samples of mustard (dressing) and ginger; additionally, the studies confirmed that canned foods contained higher concentrations of bisphenols than foods stored in glass, paper or even plastic containers (Liao and Kannan 2013).

The largest load of pharmaceuticals is introduced into the environment as a result of activities of the pharmaceutical industry, hospital facilities and households (Santos et al. 2010), veterinary and agriculture as well as in animal breeding (Li 2014). Analgetics, anti-inflammatory drugs and anticoagulants are the most frequently detected groups of pharmaceutical residues; ibuprofen, diclofenac and ketoprofen (active ingredients of non-steroid anti-inflammatory drugs) are most frequently found in soil and water samples. The second most frequently detected group of pharmaceutical residues comprises antibiotics and antifungal agents such as oxytetracycline and chloramphenicol, which have been detected in surface waters of Nigeria at concentration levels of 460 ng/L and 600 ng/L, respectively (Olatunde et al. 2014). Androgens as well as estrogens such as androstenedione, progesterone and estrone have been detected in surface and wastewaters in the following amounts: >100 ng/L, 66 ng/L, and 36 ng/L, respectively (Kim et al. 2007; Chang et al. 2011).

In environmental samples, residues of anti-epileptic and antidepressant drugs have also been found; among them, fluoxetine is one of the most frequently detected, and its average surface water concentrations ranges globally from 0.012 to 1.4 μg/L (Weinberger and Klaper 2014). Studies have shown that lipid regulators (such as gemfibrozil) are also present in the environment at concentration of 70.27 ng/L e.g. in the Llobregat River (Osorio et al. 2016).

Environmental contamination still seems to be underestimated by policy makers and entrepreneurs. Scientists continue to learn how to better quantitatively and qualitatively determine the concentrations of pollutants in complex mixtures to assess acute and chronic exposure of different organs/tissues/organisms to single compounds belonging to different groups and to predict their impact on ecosystems. Tanaka et al. (2018) confirmed that a time-weighted average concentration exposure to pyriproxyfen during a period of sensitivity affects the sex ratio, causing approximately equivalent population-level effects as reproductive inhibition regardless of the exposure scenario. Wan et al. (2018) concluded that one in-unit increase in urinary BPS was correlated with a 0.72-day increase in pregnancy duration, and in case of fetal sex each in-unit increase in maternal urinary BPS was associated with increased gestational age; on the other hand, in this study no associations of BPS with birth weight or length were found. Chen et al. (2018) confirmed the presence of seven bisphenols and TCS in 283 urine samples collected from children from South China aged between 3 and 11 years and noticed that age, but not gender, was negatively associated with urinary levels of BPA and BPS.

Synergism and antagonism are two basic phenomena that may describe effect of one substance/factor combination (Wieczerzak et al. 2015 and 2016). Among several mathematical models enabling prediction if any of the two phenomena occurred the concentration addition (CA) and independent action (IA) are most commonly used. Briefly the CA model is used to study if analytes in a mixture exhibit a similar mode of action. The concept is that the similarly acting substances act jointly in an additive manner when present together in mixture. The IA model should be used to test toxicants of dissimilar mode of action when present in a mixture – assumption is done that they act independently. This model is rather a form of a statistical approach to predict if one of multiple events could occur.

In this study, BPS and BPF were selected as plausible mixture components (next to BPA) due to fact that manufacturers are gradually replacing BPA with these compounds to comply with restrictions and regulations on BPA (Chen et al. 2016, IRIS 1988, Rochester 2013). BPS is also used as an anticorrosive agent in epoxy glues or as a reagent in polymer reactions; the presence of BPS has also been confirmed in canned foodstuffs. BPS is less prone to environmental degradation than BPA and BPF and has been found in over 80% of the urine samples studied. BPF, on the other hand, is used in manufacturing to increase the durability and thickness of the materials produced (not to

mention its usage in liners, lacquers, adhesives, plastics, coating of drink containers and food cans and dental sealant). BPF was confirmed to cause genotoxicity when introduced to Hep G2 cells, and its endocrine potential is also well confirmed. Due to the confirmed endocrine potential of numerous pharmaceuticals (Wieczerzak et al. 2016) and BPA analogs (Owczarek et al. 2018a and 2018b), it is important to study the behavior of these common environmental pollutants when present in mixtures, which was the goal of this study.

2. *Materials and methods*

2.1. Chemicals and reagents

The model substances selected for the study, diclofenac sodium salt (diclofenac s.) (CAS no. 153907-79-6), chloramphenicol (CAS no. 56-75-7), oxytetracycline hydrochloride (oxytetracycline h.) (CAS no. 2058-46-0), fluoxetine hydrochloride (fluoxetine h.) (CAS no. 56296-78-7), estrone (CAS no. 53-16-7), ketoprofen (CAS no. 22071-15-4), progesterone (CAS no. 57-83-0), gemfibrozil (CAS no. 25812-30-0), androstenedione (CAS no. 63-05-8), bisphenol A (CAS no. 80-05-7), bisphenol S (CAS no. 80-09-1) and bisphenol F (CAS no. 620-92-8), were purchased from Sigma Aldrich (Germany), and they were of analytical grade (>99%). A set of XenoScreen YES/YAS reagents was purchased from Xenometrix AG (Switzerland), namely, a vial with hERα (YES) yeasts (to determine estrogenic activity) and hARα (YAS) yeasts (to determine androgenic activity) immobilized and dormant on the filtration paper, basal medium, vitamin solution, L-aspartic acid solution, L-threonine solution, $CuSO_4$ solution, 17β-oestradiol (E2, YES+ (agonist) control), 5α-dihydrotestosterone (DHT, YAS+ (agonist) control), 4-hydroksytamoxyphene (HT, YES- (antagonist) control), and flutamide (FL, YAS- (antagonist) control). DMSO (dimethyl sulphoxide, CAS no. 67-68-5) and CPRG (chlorophenol red-β-D-galactopyranoside) (CAS no. 99792-79-9) dye were purchased from Sigma Aldrich (Germany). Measurements of the OD_{690} cell density (wavelength 690 nm) and of the intensity of the CPRG transformation product OD_{570} (wavelength 570 nm) were performed with a TECAN Infinite M200 spectrophotometer.

2.2. XenoScreen YES/YAS methodology

To investigate the endocrine potential of the solutions, a slightly modified protocol for XenoScreen YES/YAS was utilized. *Saccharomyces cerevisiae* yeast cells were cultivated from the filter papers in a growth medium (basic medium with a vitamin solution and a solution of L-threonine, L-aspartic acid and copper (II) sulfate (VI)). Then, 5 mL of the growth medium was transferred to labeled culture bottles with caps with a gas permeable filter. Afterwards, the yeast disks were sterilely transferred and placed on an orbital shaker set at 32 °C and 100 rpm for 48 h. Then, 100 µL of DMSO was added to each control vial containing the standards: E2 (17β-oestradiol control of YES agonist), DHT (5α-dihydrotestosterone control of YAS agonist), HT (4-hydroxytamoxifen control of

YES antagonist), and FL (flutamide control of YAS antagonist). Test plates were prepared in such a way that the controls were in duplicate in eight serial dilutions:

- YES agonist plate E2 (min. concentration $1 \cdot 10^{-11}$ M, max. concentration $1 \cdot 10^{-8}$ M),
- YES antagonist plate HT (min. concentration $1 \cdot 10^{-8}$ M, max. concentration $1 \cdot 10^{-5}$ M; additionally, in the entire plate, E2 was present at a constant concentration of $1 \cdot 10^{-9}$ M),
- YAS agonist plate DHT (min. concentration $1 \cdot 10^{-9}$ M, max. concentration $1 \cdot 10^{-6}$ M),
- YAS antagonist plate FL (min. concentration 1×10^{-7} M, max. concentration $1 \cdot 10^{-4}$ M; additionally, in the entire plate, DHT was present at a constant concentration of $3 \cdot 10^{-8}$ M).

The addition of E2 or DHT at the same concentration as the entire YES or YAS antagonist plate, respectively, was intended to help examine (confirm/deny) the andro- and estrogenic antagonistic activity of the samples. A substance with antagonist properties competes with the E2 or DHT present on the plate and binds to the receptor without inducing the expression of β-galactosidase. Without the enzyme, substrate staining does not occur. However, if the test sample does not contain antagonistic substances, then the E2 and DHT present in the wells bind with the receptor, expressing β-galactosidase; thus, the staining of the substrate occurs.

Twenty microliters of adjusted drug sample and sixty µL of 6 mM CRPG dye were added to each assay well. Pharmaceuticals were mixed in three concentration ratios in such a way as to detect a broad range of possible interactions. All of the studies on the mixtures were performed in triplicate; furthermore, controls were made for pure substances in duplicate, and YES and YAS suspensions of yeast cultures (100 µL; yeast cell density >0.3 OD_{690}) were added into the agonist and antagonist YES and YAS plates, respectively. Assay plates were sealed with semipermeable membranes and placed in the zipper bag moistened with watered gauze on an orbital shaker for 48 h at 32 °C and 100 rpm. After 48 h of incubation, the cell density determined by OD was read at a wavelength of 690 nm, and the color intensity at a wavelength of 570 nm was determined. Afterwards, the activity of β-galactosidase was calculated as the ratio of $[(OD_{570}-OD_{690})/OD_{690}]$. All experiments were run and measured in triplicate.

To determine whether the addition of selected bisphenol analogs to the pharmaceutical solutions would affect the endocrine potential, preconcentrated solutions of pharmaceuticals were prepared. The study of the effects of BPA analogues on the toxicity of the pharmaceuticals was conducted at three concentration levels (listed for each substance in Table 1).

2.3. Calculation of MDR values

In order to determine whether the presence of one compound in a binary mixture with another substance would affect the endocrine potential against *Saccharomyces cerevisiae*, the mixtures were prepared such that the compounds were present in appropriate ratios to reflect, respectively, the C1 concentration of first substance with the C1, C2 or C3 concentration of the second substance;

subsequently the C2 concentration of the first substance with the C1, C2 or C3 concentration of second substance, *etc.* C2 represents the concentration of analyte's solutions calculated from the XenoScreen YES/YAS test, and it is summarized in Table 1. C1 represents the concentrations of substance reduced to 50% of C2, and respectively C3 is the concentration elevated to 150% of C2 – the same scheme was applied to each pharmaceutical studied.

The toxicological effect of a mixture of pharmaceuticals with BPA and its analogues on *S. cerevisiae* cells was mathematically assessed with both the Concentration Addition - CA and Independent Action - IA models using equations 1 and 2, respectively (Kudłak et al. 2016, Wieczerzak et al. 2015):

$$EC_{X_{Mix}} = \left(\sum_{i=1}^{n} \frac{P_i}{EC_{x_i}} \right)^{-1} \quad \text{(eq. 1.)}$$

where:

- ECx_{mix} is the x_{mix} effect caused by the total concentration of the mixture of studied chemicals (components) (Expected value);
- p_i indicates the fraction of component *i* in the mixture, calculated on the basis of the concentration of component *i* in the mixture;
- *n* indicates the number of components in the mixture;
- and ECx_i indicates the x_i effect caused by component *i* at a given studied concentration in the mixture.

$$E(C_{mix}) = 1 - \prod_{i=1}^{n} (1 - E(c_1)) \quad \text{(eq. 2.)}$$

where:

- EC_{mix} is the overall effect expressed as a fraction of the maximal possible effect of a mixture of chemical *i* (expected value);
- c_i indicates the concentration of component *i* in the mixture;
- *n* indicates the number of components in the mixture;
- $E(c_i)$ indicates the effect of component *i*, applied separately.

To verify the difference between the predicted and observed effect, the Model Deviation Ratio (MDR) approach was applied, defined as shown in equation 3 (Wieczerzak *et al.* 2016; Kudłak *et al.* 2016):

$$MDR = \frac{Expected}{Observed} \qquad \text{(eq. 3.)}$$

where:

- *Expected* is the effective toxicity (raw values of genotoxicity calculated according to eq. 1. or 2.) of the mixture predicted by the CA or IA model, and

- *Observed* is the effective toxicity (raw values of genotoxicity calculated) for the mixture obtained during the toxicity studies.

MDR values lower than 0.50 are indicators of synergic behavior of substances present in mixture while values >2.00 justify the statement of antagonistic action of analytes studied; MDR values within 0.50–0.71 and 1.40–2.00 mean, respectively, under- and overestimation of calculated models.

3. Results and discussion

3.1. Results of hormonal activity studies of selected analytes

Based on previous experience and in relation to published data, the proper concentrations (3 constant levels for each analyte) were selected for the studies (as presented in Table 1) to reflect the environmental concentration levels of the analytes. Then, mixtures of BPA analogs with respective pharmaceuticals were prepared, and their endocrine potential was studied as described in subchapter 2.3.

Table 1.

3.2. Results for BPA impact on the hormonal activity of pharmaceuticals

BPA is a well-known to bind both nuclear estrogen receptors (ERα and ERβ) and strongly binds to estrogen related receptors γ (ERR-γ). In other studies it has been shown that BPA also acts as a weak antagonist of androgen receptors (AR) (Stossi et al. 2014). This type of multiple mechanisms of interaction on receptors contributes to the observation of many different effects, both estrogenic and androgenic.

The MDR values presenting a variation of modeled values from measured values in the case of studies of BPA impact on the endocrine potential of selected drugs are given in Table 2 below.

Table 2.

3.2.1. YES+

The estrogenic agonistic activity of the drugs studied seems to be most strongly affected by the presence of BPA in solution. In twenty one cases (mixtures with diclofenac, chloramphenicol, oxytetracycline and fluoxetine) synergy was observed for the CA models, whereas antagonism was

observed only for progesterone and gemfibrozil in the case of twelve IA predictions (please refer to Table C in the electronic supplementary material for details on the percentile values for the MDRs of the experiments on BPA YES+).

Antagonism was observed for IA model for mixtures of gemfibrozil (at all concentrations levels) with BPA (only for the lowest concentration - C1). Cases of antagonism were observed also for all mixtures of BPA and progesterone. Although the tests performed do not answer the question what is happening at the cellular level, so reference data were needed to better interpret the test results. The literature is a bit skimpy regarding the impact of BPA on gemfibrozil, but it can contain data on the effect of BPA, in clinical trials, on the development of progesterone resistance, which may explain the strong antagonism observed in these mixtures (Aldad et al. 2011).

In twenty one cases (mixtures with diclofenac, chloramphenicol, oxytetracycline and fluoxetine) synergy was observed for the CA models with the strongest synergism shown for BPA and oxytetracycline h. pair (please refer to Table C in the electronic supplementary material for details on the percentile values for the MDRs of the experiments on BPA YES+). In these cases the metabolic pathway of these substances may be responsible, because the enzymes from the P450 group (CYP1A, CYP2A, etc.) are involved in the detoxification of all these substances, the occurrence of two substances that involve the same detoxification enzymes may burden the system leading to greater toxicity (endocrine disruption) (Hanioka et al. 2000; DrugBank 2018a, 2018b, 2018c,). Similar results for agonist interactions were obtained for the combination of these drugs with BPS and BPF. However, this should be verified in further studies.

3.2.2. YES-

Estrogenic antagonistic activity was not affected by the presence of BPA in a synergic manner; no such cases are observed. Seventeen cases of antagonistic behavior were confirmed for mixtures with chloramphenicol, fluoxetine, progesterone and gemfibrozil with IA model (please refer to Table D in the electronic supplementary material for details on the percentile values for the MDRs of the experiments on BPA YES-). The most antagonistically potent to estrogen receptors was the mixture of BPA and fluoxetine (at all concentrations studied); however, the observed phenomenon had a weak character and was strengthened with increasing concentration of both substances (please refer to Table D in the electronic supplementary material for details).

3.2.3. YAS+

BPA at the lowest concentration level studied (C1) was adequately modeled with the CA approach (one case of synergism for androstenedione can be noticed, the number of observed cases of synergy increases with increasing concentration of BPA in the mixture) while IA shows several cases of antagonism (for diclofenac, chloramphenicol, oxytetracycline, fluoxetine and gemfibrozil). At higher concentration levels synergism was observable for mixtures with estrone, progesterone and

androstenedione (with CA approach) showing the importance of BPA in cases of co-presence with these hormonal chemicals. In the case of androgenic agonistic effects, all observed cases of synergy were in mixtures of BPA with hormonal substances. The IA models showed twenty cases of antagonistic action (as presented in table 2) (please refer also to Table E in the electronic supplementary material for details on the percentile values for the MDRs of the experiments on BPA YAS+).

3.2.4. YAS-

In the case of androgenic antagonistic studies, no cases of synergism were observed for either the IA or CA models. Interestingly, antagonistic behavior was observed only for the highest concentration level of fluoxetine (four cases) (please refer to Table F in the electronic supplementary material for details on the percentile values for the MDRs of the experiments on BPA YAS-).

3.3. Results of BPS impact on the hormonal activity of pharmaceuticals

BPS (just like BPA) is able to easily bind to nuclear receptors (the data shows that this bond is weaker than of BPA and estradiol); there also some studies that show androgenic activity of BPS (Rochester and Bolden 2015). The results obtained for BPS and BPF (described below) are similar to what could indicate a similar mechanism of action in mixtures. The MDR values presenting a variation of modeled values from measured ones in the case of studies of BPS impact on the endocrine potential of selected drugs are given in table A (placed in electronic supplementary material).

3.3.1. YES+

BPS showed strong agonistic estrogenic impact on most of the drugs studied at a medium (C2) and high (C3) concentration of BPS. Forty cases of synergism (with diclofenac, chloramphenicol, oxytetracycline, fluoxetine, ketoprofen, progesterone and gemfibrozil) and nine cases of underestimation were observed for the CA models. The strongest synergistic effect was observed for gemfibrozil at the level C2 and BPS at the highest concentration (C3). With the increase in BPS concentration, one can observe a "transition" from antagonistic interactions to synergistic properties for progesterone, androstenedione, chloramphenicol and gemfibrozil couples, which would mean that the higher concentration of BPS in these mixtures affect dominantly estrogen receptors, which may be related to the metabolic pathway of these substances. At the lowest concentration level of BPS studied almost no impact on other analytes was found with the possible exception of androstenedione where antagonistic behavior was observed for both models used; with increasing concentration of BPS there was a tendency for underestimating the impact of BPS and even exhibiting synergic action (please refer to Table G in the electronic supplementary material for details on the percentile values for the MDRs of the experiments on BPS YES+).

3.3.2. YES-

The impact of BPS on the estrogenic antagonistic properties of selected pharmaceuticals was adequately modeled with both the CA and IA approaches. IA showed twenty-four cases of antagonistic activity (mostly at the lowest concentration level of BPS) for mixtures with diclofenac, chloramphenicol, progesterone, gemfibrozil and the lowest concentration levels of oxytetracycline and fluoxetine (with the highest MDR values for 2.74 for BPS (C1) and gemfibrozil (C3)); (refer to Tables A and G in the electronic supplementary material for details on the results and on the percentile values for the MDRs of the experiments on BPS YES-).

3.3.3. YAS+

Synergism and underestimation were observed in forty five cases of CA modeling while the IA models showed five cases of antagonistic behavior of BPS on drugs when androgenic agonistic behavior is concerned. BPS had a synergic impact on estrone, progesterone and androstenedione, confirming the environmental threats posed by this substance (please refer to Table I in the electronic supplementary material for details on the percentile values for the MDRs of the experiments o BPS YAS+).

3.3.4. YAS-

The androgenic antagonistic impact of BPS was noted in only four cases of IA modeling (for fluoxetine and estrone). In other cases both models adequately forecast the behavior of BPS-drug mixtures (MDR values close to 1.00) (please refer to Table J in the electronic supplementary material for details on the percentile values for the MDRs of the experiments on BPS YAS-).

3.4. Results of BPF impact on the hormonal activity of pharmaceuticals

BPF (similarly to its analogs) shows estrogenic and antiandrogenic properties ((Rochester and Bolden 2015). The results obtained were similar to the BPF, both agonistically and antagonistically to estrogen and androgenic receptors, suggesting similarity in MOD. The MDR values presenting a variation of the modeled values from measured values in the case of studies of BPS impact on the endocrine potential of selected drugs are given in table B (in electronic supplementary material).

3.4.1. YES+

The strongest synergistic effect was observed for gemfibrozil at the level C3 and BPF at the highest concentration (C3). As in the case of BPS, with increase of BPF concentration, a "transition" from antagonistic interactions to synergistic properties can be observed for progesterone, androstenedione, chloramphenicol and gemfibrozil pairs. At the lowest concentration level studied BPF did not show any synergic impact on the estrogenic agonistic activity of the drugs selected. Such behavior (for CA modeling) was observable in numerous cases with increasing concentration levels of BPF. Only the activity of estrone was not greatly impacted by the presence of BPF. No antagonistic

behavior of BPF with the CA modeling approach was observed, and seven cases of antagonism were detected with IA modeling (for progesterone, androstenedione and chloramphenicol) (please refer to Table I in the electronic supplementary material for details on the percentile values for the MDRs of the experiments on BPF YES+).

3.4.2. YES-

For the estrogenic antagonistic impact of BPF only one case of synergism (with ketoprofen) was observed while sixteen results confirm underestimation by CA modeling. Antagonistic behavior is more likely and was observed in fourteen cases, and forty-four overestimations were noted as a result of IA modeling (refer to table B for details) (please refer also to Table L in the electronic supplementary material for details on the percentile values for the MDRs of the experiments on BPF YES-).

3.4.3.YAS+

Androgenic agonistic endpoints were in most cases not affected by the presence of mixtures of the lowest BPF concentration with the drugs studied. Several exceptions include ten synergic mixtures of BPF with estrone, progesterone and androstenedione and two antagonistic mixtures with diclofenac and chloramphenicol (in IA studies); at higher concentration levels BPF showed a synergic impact on the drugs studied. Thirty nine cases of underestimation were present in the CA studies, and twenty-six cases of overestimation were found for IA modeling (please refer to Table M in the electronic supplementary material for details on the percentile values for the MDRs of the experiments on BPF YAS+).

3.4.4.YAS-

Androgenic antagonistic activity seemed to be only slightly impacted by mixtures of BPF and the drugs studied. Such synergic impact was seen for six mixtures with chloramphenicol while five antagonistic incidences were observed for diclofenac and fluoxetine (for IA modeling) (please refer to Table N in the electronic supplementary material for details on the percentile values for the MDRs of the experiments on BPF YAS-).

4. Conclusions

As stated in this research, the variations in endocrine potential are to a great extent concentration dependent. YES+ and YAS+ activity in most cases was affected in a synergic manner while YES- and YAS- were affected in an antagonistic manner in most of experiments performed. As can be easily noticed, the magnitudes of interactions were dependent on the chemicals (BPA, BPS or BPF) admixed in solution as well as their concentration levels.

Certainly, the work does not give the ultimate answer on possible interactions of BPA analogues and pharmaceuticals but constitutes one of next steps and guidelines proving the necessity of taking into account phenomena of antagonism or synergism when studying target-oriented processes occurring at levels of ecosystems. Future research should focus on studying higher order mixtures of pollutants of persistent character belonging to other chemical groups (e.g., derivatives of bisphenol A, diglycidyl ether, other pharmaceuticals, pesticides, etc.) as well as their degradation products and on wider sets of bioassays with varying biotest endpoints. BPS and BPF are unfortunately considered to be safe replacements of BPA; therefore, their amount in the environment starts increasing and for this reason it is important to monitor them in terms of their properties also in mixtures – what always happens in case of environmental samples (especially due to the fact that the literature data is less rich in information about BPS, BPS than in BPA). In this way the information coming from instrumental studies will be broadened with newly developed, accurate mathematical models describing the environmental threat posed by mixtures of pollutants.

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515

Table 1. Concentration values of analytes (of bisphenol analogues and pharmaceuticals) selected and used during research

	C1	C2	C3
	μM		
BPA	0.548	1.368	2.190
BPS	0.274	0.684	1.095
BPF	0.274	0.684	1.095
Diclofenac s.	5.750	14.375	23.000
Chloramphenicol	8.704	21.759	34.816
Oxytetracycline h.	5.750	14.375	23.000
Fluoxetine h.	8.299	20.748	33.197
Estrone	8.531	21.329	34.127
Ketoprofen	6.865	17.162	27.460
Progesterone	9.516	23.791	38.065
Gemfibrozil	3.736	9.339	14.943
Androstenedione	6.530	16.326	26.121

516

Table 2. MDR values variations depending on BPA concentration change for solutions of selected pharmaceuticals studied (red - synergism, blue - antagonism, green - overestimation, yellow - underestimation, for values of particular concentrations C1, C2 and C3 of all analytes please refer to Table 1.)

		YES+						YES-						YAS+						YAS-					
		MDR						MDR						MDR						MDR					
		BPA C1		BPA C2		BPA C3		BPA C1		BPA C2		BPA C3		BPA C1		BPA C2		BPA C3		BPA C1		BPA C2		BPA C3	
		CA	IA	CA	IA	CA	IA	CA	IA	CA	IA	CA	IA	CA	IA	CA	IA	CA	IA	CA	IA	CA	IA	CA	IA
Diclofenac	C1	0.76	1.49	0.44	1.12	0.33	0.97	0.92	1.64	0.88	1.63	0.83	1.51	1.09	2.22	0.72	1.51	0.68	1.36	1.07	1.93	0.91	1.60	0.95	1.66
	C2	0.78	1.51	0.50	1.30	0.32	1.02	0.90	1.52	0.88	1.57	0.92	1.59	1.05	1.85	0.96	1.75	0.87	1.57	0.87	1.52	0.90	1.53	0.90	1.50
	C3	0.92	1.81	0.53	1.45	0.36	1.23	0.92	1.56	0.93	1.65	0.98	1.70	1.06	1.82	0.91	1.63	0.89	1.58	0.91	1.57	0.92	1.55	0.94	1.53
Chloramphe-nicol	C1	0.57	1.42	0.37	1.28	0.24	1.00	1.02	1.82	1.03	1.92	0.93	1.70	1.15	2.38	0.84	1.78	0.83	1.69	0.99	1.80	0.93	1.65	0.85	1.47
	C2	1.03	1.98	0.55	1.43	0.30	0.97	0.98	1.72	0.91	1.68	0.92	1.64	1.19	2.27	1.01	1.99	0.82	1.57	0.92	1.66	0.99	1.74	0.94	1.59
	C3	0.73	1.68	0.38	1.26	0.23	0.97	1.12	1.90	1.13	2.00	0.99	1.69	0.98	2.03	0.82	1.76	0.77	1.59	1.18	1.88	1.18	1.86	1.07	1.65
Oxytetracy-cline h.	C1	0.56	1.06	0.39	0.93	0.27	0.74	1.08	1.79	0.98	1.72	0.99	1.75	1.51	3.38	1.03	2.33	1.00	2.14	0.99	1.68	0.96	1.62	0.89	1.51
	C2	0.78	1.40	0.37	0.87	0.22	0.62	0.78	1.31	0.91	1.61	0.95	1.63	1.31	2.63	1.03	2.14	1.10	2.21	0.90	1.50	0.95	1.56	0.86	1.39
	C3	0.63	1.17	0.38	0.95	0.24	0.73	1.10	1.73	1.11	1.83	1.10	1.78	1.15	2.30	0.84	1.75	0.97	1.95	0.97	1.59	0.94	1.51	0.91	1.43
Fluoxetine h.	C1	0.66	1.35	0.43	1.18	0.29	0.95	1.14	2.03	1.09	2.01	1.11	2.01	0.74	1.86	0.66	1.67	0.62	1.47	0.86	1.84	0.76	1.54	0.68	1.31
	C2	0.65	1.48	0.41	1.35	0.27	1.12	1.34	2.36	1.22	2.24	1.26	2.23	1.08	2.56	0.99	2.45	0.79	1.85	0.85	2.30	0.75	1.95	0.76	1.81
	C3	0.77	1.74	0.42	1.35	0.36	1.48	1.20	2.11	1.33	2.45	1.27	2.24	1.13	2.41	0.86	1.91	1.17	2.51	1.19	2.19	1.35	2.42	2.00	3.43
Estrone	C1	0.94	1.45	0.79	1.33	0.81	1.46	0.99	1.69	0.88	1.54	0.86	1.59	0.62	0.97	0.45	0.97	0.35	0.96	0.86	1.70	0.81	1.52	0.80	1.47
	C2	0.88	1.30	0.88	1.38	0.77	1.28	1.08	1.80	0.98	1.62	0.90	1.57	0.74	0.96	0.60	0.96	0.50	0.94	0.91	1.73	0.89	1.59	0.84	1.46
	C3	0.96	1.38	0.96	1.45	0.87	1.40	1.07	1.75	1.02	1.65	0.94	1.59	0.81	1.00	0.67	0.95	0.60	0.96	0.93	1.77	0.80	1.44	0.88	1.50
Ketoprofen	C1	0.78	1.52	0.78	1.57	0.67	1.40	0.98	1.95	0.91	1.76	0.89	1.79	0.66	1.50	0.68	1.49	0.68	1.43	0.72	1.45	0.87	1.66	0.62	1.16
	C2	0.80	1.51	0.74	1.46	0.68	1.43	0.95	1.90	0.89	1.73	0.93	1.90	0.70	1.64	0.70	1.60	0.75	1.66	0.75	1.67	0.76	1.57	0.89	1.75
	C3	0.67	1.22	0.70	1.34	0.69	1.40	1.00	1.87	0.95	1.74	0.97	1.86	0.73	1.63	0.72	1.60	0.62	1.32	0.83	1.77	0.85	1.68	0.81	1.53
Progesterone	C1	1.06	3.82	0.80	2.92	0.64	2.50	1.09	2.11	1.00	1.89	0.94	1.85	0.61	0.95	0.43	0.93	0.34	0.94	0.82	1.48	0.78	1.36	0.71	1.24
	C2	1.07	4.02	0.86	3.39	0.71	3.06	1.04	2.03	0.97	1.85	0.95	1.91	0.66	0.85	0.55	0.89	0.44	0.85	0.80	1.27	0.85	1.31	0.73	1.12
	C3	1.05	3.81	0.82	3.16	0.74	3.11	1.03	1.99	0.95	1.78	1.05	2.08	0.76	0.93	0.59	0.83	0.53	0.87	0.81	1.27	0.77	1.17	0.76	1.13
Gemfibrozil	C1	1.19	2.41	0.84	1.74	0.81	1.70	1.10	2.24	0.91	1.81	0.84	1.70	1.30	2.93	0.90	1.93	0.87	1.80	0.89	1.73	0.91	1.71	0.95	1.78
	C2	0.99	2.14	0.85	1.89	0.79	1.87	0.98	2.11	0.88	1.81	0.85	1.83	1.24	2.35	1.23	2.32	1.16	2.19	0.87	1.70	0.95	1.77	1.00	1.80
	C3	0.94	2.22	0.81	1.98	0.72	1.89	0.96	2.12	0.81	1.71	0.72	1.58	0.94	2.11	0.85	1.87	0.87	1.84	0.88	1.74	0.95	1.77	1.00	1.80
Androstenedi one	C1	1.10	1.99	0.85	1.62	0.77	1.53	1.12	2.08	0.97	1.81	0.97	1.87	0.39	0.77	0.29	0.94	0.19	0.85	0.87	1.36	0.77	1.26	0.65	1.16
	C2	0.97	1.56	1.00	1.70	0.84	1.52	0.99	1.70	0.97	1.66	0.90	1.62	0.63	0.97	0.42	0.96	0.29	0.90	0.86	1.20	0.81	1.18	0.70	1.07
	C3	1.08	1.87	0.89	1.61	0.99	1.90	0.92	1.72	0.87	1.58	0.96	1.84	0.66	0.88	0.55	0.94	0.43	0.92	0.95	1.39	0.92	1.33	0.80	1.17

Table 2. MDR values variations depending on BPA concentration change for solutions of selected pharmaceuticals studied (legend for printed B&W version: MDR >2.0 exhibits antagonism, MDR < 0.5 shows synergism, MDR values within 0.50–0.71 and 1.40–2.00 values mean, respectively, under- and overestimation of presented models, for values of particular concentrations C1, C2 and C3 of all analytes please refer to Table 1.)

		YES+						YES-						YAS+						YAS-					
		MDR						MDR						MDR						MDR					
		BPA C1		BPA C2		BPA C3		BPA C1		BPA C2		BPA C3		BPA C1		BPA C2		BPA C3		BPA C1		BPA C2		BPA C3	
		CA	IA	CA	IA	CA	IA	CA	IA	CA	IA	CA	IA	CA	IA	CA	IA	CA	IA	CA	IA	CA	IA	CA	IA
Diclofenac	C1	0.76	1.49	0.44	1.12	0.33	0.97	0.92	1.64	0.88	1.63	0.83	1.51	1.09	2.22	0.72	1.51	0.68	1.36	1.07	1.93	0.91	1.60	0.95	1.66
	C2	0.78	1.51	0.50	1.30	0.32	1.02	0.90	1.52	0.88	1.57	0.92	1.59	1.05	1.85	0.96	1.75	0.87	1.57	0.87	1.52	0.90	1.53	0.90	1.50
	C3	0.92	1.81	0.53	1.45	0.36	1.23	0.92	1.56	0.93	1.65	0.98	1.70	1.06	1.82	0.91	1.63	0.89	1.58	0.91	1.57	0.92	1.55	0.94	1.53
Chloramphe-nicol	C1	0.57	1.42	0.37	1.28	0.24	1.00	1.02	1.82	1.03	1.92	0.93	1.70	1.15	2.38	0.84	1.78	0.83	1.69	0.99	1.80	0.93	1.65	0.85	1.47
	C2	1.03	1.98	0.55	1.43	0.30	0.97	0.98	1.72	0.91	1.68	0.92	1.64	1.19	2.27	1.01	1.99	0.82	1.57	0.92	1.66	0.99	1.74	0.94	1.59
	C3	0.73	1.68	0.38	1.26	0.23	0.97	1.12	1.90	1.13	2.00	0.99	1.69	0.98	2.03	0.82	1.76	0.77	1.59	1.18	1.88	1.18	1.86	1.07	1.65
Oxytetracy-cline h.	C1	0.56	1.06	0.39	0.93	0.27	0.74	1.08	1.79	0.98	1.72	0.99	1.75	1.51	3.38	1.03	2.33	1.00	2.14	0.99	1.68	0.96	1.62	0.89	1.51
	C2	0.78	1.40	0.37	0.87	0.22	0.62	0.78	1.31	0.91	1.61	0.95	1.63	1.31	2.63	1.03	2.14	1.10	2.21	0.90	1.50	0.95	1.56	0.86	1.39
	C3	0.63	1.17	0.38	0.95	0.24	0.73	1.10	1.73	1.11	1.83	1.10	1.78	1.15	2.30	0.84	1.75	0.97	1.95	0.97	1.59	0.94	1.51	0.91	1.43
Fluoxetine h.	C1	0.66	1.35	0.43	1.18	0.29	0.95	1.14	2.03	1.09	2.01	1.11	2.01	0.74	1.86	0.66	1.67	0.62	1.47	0.86	1.84	0.76	1.54	0.68	1.31
	C2	0.65	1.48	0.41	1.35	0.27	1.12	1.34	2.36	1.22	2.24	1.26	2.23	1.08	2.56	0.99	2.45	0.79	1.85	0.85	2.30	0.75	1.95	0.76	1.81
	C3	0.77	1.74	0.42	1.35	0.36	1.48	1.20	2.11	1.33	2.45	1.27	2.24	1.13	2.41	0.86	1.91	1.17	2.51	1.19	2.19	1.35	2.42	2.00	3.43
Estrone	C1	0.94	1.45	0.79	1.33	0.81	1.46	0.99	1.69	0.88	1.54	0.86	1.59	0.62	0.97	0.45	0.97	0.35	0.96	0.86	1.70	0.81	1.52	0.80	1.47
	C2	0.88	1.30	0.88	1.38	0.77	1.28	1.08	1.80	0.98	1.62	0.90	1.57	0.74	0.96	0.60	0.96	0.50	0.94	0.91	1.73	0.89	1.59	0.84	1.46
	C3	0.96	1.38	0.96	1.45	0.87	1.40	1.07	1.75	1.02	1.65	0.94	1.59	0.81	1.00	0.67	0.95	0.60	0.96	0.93	1.77	0.80	1.44	0.88	1.50
Ketoprofen	C1	0.78	1.52	0.78	1.57	0.67	1.40	0.98	1.95	0.91	1.76	0.89	1.79	0.66	1.50	0.68	1.49	0.68	1.43	0.72	1.45	0.87	1.66	0.62	1.16
	C2	0.80	1.51	0.74	1.46	0.68	1.43	0.95	1.90	0.89	1.73	0.93	1.90	0.70	1.64	0.70	1.60	0.75	1.66	0.75	1.67	0.76	1.57	0.89	1.75
	C3	0.67	1.22	0.70	1.34	0.69	1.40	1.00	1.87	0.95	1.74	0.97	1.86	0.73	1.63	0.72	1.60	0.62	1.32	0.83	1.77	0.85	1.68	0.81	1.53
Progesterone	C1	1.06	3.82	0.80	2.92	0.64	2.50	1.09	2.11	1.00	1.89	0.94	1.85	0.61	0.95	0.43	0.93	0.34	0.94	0.82	1.48	0.78	1.36	0.71	1.24
	C2	1.07	4.02	0.86	3.39	0.71	3.06	1.04	2.03	0.97	1.85	0.95	1.91	0.66	0.85	0.55	0.89	0.44	0.85	0.80	1.27	0.85	1.31	0.73	1.12
	C3	1.05	3.81	0.82	3.16	0.74	3.11	1.03	1.99	0.95	1.78	1.05	2.08	0.76	0.93	0.59	0.83	0.53	0.87	0.81	1.27	0.77	1.17	0.76	1.13
Gemfibrozil	C1	1.19	2.41	0.84	1.74	0.81	1.70	1.10	2.24	0.91	1.81	0.84	1.70	1.30	2.93	0.90	1.93	0.87	1.80	0.89	1.73	0.91	1.71	0.95	1.78
	C2	0.99	2.14	0.85	1.89	0.79	1.87	0.98	2.11	0.88	1.81	0.85	1.83	1.24	2.35	1.23	2.32	1.16	2.19	0.87	1.70	0.95	1.77	1.00	1.80
	C3	0.94	2.22	0.81	1.98	0.72	1.89	0.96	2.12	0.81	1.71	0.72	1.58	0.94	2.11	0.85	1.87	0.87	1.84	0.88	1.74	0.95	1.77	1.00	1.80
Androstenedi one	C1	1.10	1.99	0.85	1.62	0.77	1.53	1.12	2.08	0.97	1.81	0.97	1.87	0.39	0.77	0.29	0.94	0.19	0.85	0.87	1.36	0.77	1.26	0.65	1.16
	C2	0.97	1.56	1.00	1.70	0.84	1.52	0.99	1.70	0.97	1.66	0.90	1.62	0.63	0.97	0.42	0.96	0.29	0.90	0.86	1.20	0.81	1.18	0.70	1.07
	C3	1.08	1.87	0.89	1.61	0.99	1.90	0.92	1.72	0.87	1.58	0.96	1.84	0.66	0.88	0.55	0.94	0.43	0.92	0.95	1.39	0.92	1.33	0.80	1.17

Bisphenols (A, S, and F) affect the basic hormonal activity determined for pharmaceuticals – study of *Saccharomyces cerevisiae*

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Table A. MDR values variations depending on BPS concentration change for solutions of selected pharmaceuticals studied (red - synergism, blue - antagonism, green - overestimation, yellow – underestimation, for values of particular concentrations C1, C2 and C3 of all analytes please refer to Table 1.)

		YES+						YES-						YAS+						YAS-					
		MDR						MDR						MDR						MDR					
		BPS C1		BPS C2		BPS C3		BPS C1		BPS C2		BPS C3		BPS C1		BPS C2		BPS C3		BPS C1		BPS C2		BPS C3	
		CA	IA	CA	IA	CA	IA	CA	IA	CA	IA	CA	IA	CA	IA	CA	IA	CA	IA	CA	IA	CA	IA	CA	IA
Diclofenac	C1	0.69	1.46	0.49	1.40	0.22	0.95	1.17	2.43	0.91	2.14	0.67	1.48	1.21	2.22	0.63	1.28	0.63	1.27	0.90	1.45	0.91	1.48	0.78	1.27
	C2	0.77	1.52	0.49	1.35	0.28	1.19	0.97	2.06	0.89	2.20	0.56	1.32	0.74	1.29	0.70	1.36	0.56	1.08	0.97	1.51	0.91	1.45	0.85	1.36
	C3	0.73	1.49	0.51	1.46	0.29	1.31	1.29	2.57	0.97	2.25	0.67	1.47	0.78	1.39	0.73	1.43	0.69	1.34	0.90	1.41	0.88	1.41	0.87	1.39
Chloramphe-nicol	C1	0.80	1.69	0.46	1.35	0.23	1.05	1.06	2.37	0.94	2.42	0.52	1.26	1.05	1.93	0.82	1.68	0.69	1.37	0.97	1.55	0.96	1.56	0.81	1.31
	C2	0.93	1.83	0.60	1.64	0.25	1.10	1.06	2.13	0.85	1.98	0.63	1.40	1.02	1.77	0.94	1.82	0.76	1.44	1.05	1.63	0.97	1.55	0.90	1.43
	C3	0.84	1.70	0.49	1.40	0.24	1.09	1.17	2.29	0.94	2.12	0.66	1.43	1.04	1.87	0.96	1.92	0.67	1.31	0.93	1.46	0.93	1.49	0.90	1.44
Oxytetracy-cline h.	C1	1.04	2.02	0.49	1.27	0.21	0.82	1.08	2.09	0.74	1.61	0.66	1.38	0.88	1.68	0.67	1.42	0.68	1.39	0.93	1.50	0.87	1.42	0.92	1.50
	C2	0.94	1.86	0.46	1.26	0.29	1.25	1.08	1.93	0.74	1.49	0.75	1.46	0.90	1.59	0.79	1.55	0.76	1.47	0.96	1.51	0.99	1.58	0.93	1.48
	C3	0.84	1.55	0.38	0.97	0.26	1.01	0.89	1.64	0.62	1.30	0.61	1.23	0.99	1.66	0.77	1.44	0.79	1.45	0.97	1.51	0.97	1.54	0.88	1.40
Fluoxetine h.	C1	0.80	1.59	0.53	1.45	0.27	1.16	0.99	2.05	0.63	1.49	0.63	1.41	0.84	1.63	0.75	1.61	0.69	1.47	0.88	1.60	0.80	1.47	0.82	1.49
	C2	0.82	1.67	0.47	1.34	0.31	1.41	0.82	1.75	0.60	1.49	0.59	1.40	0.74	1.71	0.74	1.94	0.77	1.97	0.84	2.04	0.74	1.84	0.77	1.89
	C3	0.79	1.67	0.47	1.43	0.26	1.28	0.83	1.66	0.56	1.29	0.73	1.62	0.96	2.16	1.01	2.60	0.98	2.45	1.05	2.57	1.19	3.02	1.33	3.35
Estrone	C1	0.82	1.20	0.76	1.12	0.82	1.26	1.02	1.42	0.99	1.53	0.98	1.54	0.69	0.93	0.42	0.68	0.37	0.75	0.90	1.58	0.74	1.33	0.72	1.21
	C2	1.00	1.22	0.83	1.08	0.94	1.30	1.09	1.44	1.00	1.47	1.13	1.66	0.76	0.92	0.52	0.68	0.49	0.74	0.90	1.64	0.65	1.25	0.79	1.38
	C3	1.02	1.19	0.89	1.12	0.94	1.29	0.87	1.68	0.64	1.52	0.79	1.79	0.85	0.98	0.54	0.67	0.56	0.76	0.84	1.27	0.72	1.12	0.85	1.26
Ketoprofen	C1	0.67	1.12	0.30	0.87	0.25	0.94	0.81	1.54	0.57	1.31	0.67	1.46	0.95	1.84	0.95	1.84	0.69	1.36	0.82	1.34	0.81	1.35	0.69	1.08
	C2	0.91	1.43	0.31	0.83	0.28	0.99	0.78	1.60	0.59	1.49	0.76	1.86	0.89	1.81	0.89	1.81	0.68	1.40	0.83	1.44	0.59	1.06	0.70	1.18
	C3	0.83	1.14	0.39	0.82	0.48	1.29	0.86	1.58	0.66	1.48	0.74	1.61	0.98	1.93	0.98	1.93	0.69	1.38	0.74	1.31	0.63	1.17	0.78	1.32
Progesteron e	C1	0.86	2.78	0.30	2.33	0.18	1.96	1.00	2.48	0.64	1.99	0.65	1.90	0.56	0.81	0.41	0.73	0.30	0.74	0.77	1.50	0.69	1.40	0.67	1.24
	C2	0.65	2.04	0.32	2.40	0.17	1.87	0.93	2.15	0.76	2.22	0.72	2.02	0.72	0.89	0.56	0.79	0.52	0.92	0.78	1.41	0.65	1.22	0.72	1.24
	C3	0.67	1.96	0.31	2.16	0.22	2.18	0.91	2.11	0.72	2.14	0.70	1.97	0.73	0.84	0.68	0.86	0.62	0.92	0.81	1.45	0.74	1.39	0.83	1.44
Gemfibrozil	C1	1.24	2.17	0.39	1.14	0.17	0.65	1.30	2.47	0.77	1.72	0.90	1.90	1.69	3.27	0.56	1.21	0.66	1.31	0.85	1.37	0.82	1.34	0.82	1.27
	C2	1.79	2.83	0.42	1.11	0.23	0.80	1.10	2.26	0.69	1.73	0.81	1.92	0.89	1.82	0.55	1.26	0.58	1.18	0.74	1.29	0.72	1.29	0.77	1.28
	C3	1.24	1.98	0.26	0.71	0.34	1.23	1.48	2.74	0.87	1.95	1.10	2.36	0.87	1.72	0.49	1.11	0.69	1.38	0.83	1.45	0.69	1.26	0.79	1.34
Androstene-dione	C1	1.60	2.23	0.76	1.38	0.32	0.68	0.94	1.77	0.63	1.42	0.71	1.52	0.49	0.94	0.34	0.88	0.22	0.87	0.94	1.68	0.89	1.63	0.88	1.48
	C2	3.22	4.00	1.15	1.58	0.74	1.10	0.83	1.39	0.72	1.43	0.70	1.34	0.67	0.95	0.53	0.93	0.39	0.97	0.84	1.43	0.86	1.53	0.81	1.34
	C3	2.91	3.48	0.58	0.84	0.68	1.12	1.18	2.15	0.64	1.42	0.89	1.90	0.70	0.88	0.62	0.91	0.46	0.89	0.96	1.60	0.92	1.60	0.88	1.43

Table B. MDR values variations depending on BPF concentration change for solutions of selected pharmaceuticals studied (red - synergism, blue - antagonism, green - overestimation, yellow - underestimation, for values of particular concentrations C1, C2 and C3 of all analytes please refer to Table 1.)

		YES+						YES-						YAS+						YAS-					
		MDR						MDR						MDR						MDR					
		BPF C1		BPF C2		BPF C3		BPF C1		BPF C2		BPF C3		BPF C1		BPF C2		BPF C3		BPF C1		BPF C2		BPF C3	
		CA	IA	CA	IA	CA	IA	CA	IA	CA	IA	CA	IA	CA	IA	CA	IA	CA	IA	CA	IA	CA	IA	CA	IA
Diclofenac	C1	0.77	1.98	0.33	1.39	0.27	1.15	1.02	2.24	0.69	1.57	0.66	1.52	1.26	2.57	0.73	1.45	0.65	1.32	1.31	2.55	1.14	2.12	1.05	1.94
	C2	0.65	1.77	0.29	1.34	0.26	1.27	0.91	1.96	0.74	1.65	0.73	1.71	0.84	1.69	0.71	1.39	0.56	1.13	0.99	1.96	0.97	1.81	0.95	1.75
	C3	0.70	1.91	0.31	1.49	0.28	1.38	0.95	2.07	0.78	1.76	0.74	1.75	0.97	1.63	0.81	1.35	0.76	1.30	1.01	1.88	0.91	1.59	0.91	1.59
Chloramphe-nicol	C1	0.77	1.94	0.38	1.57	0.29	1.25	1.31	2.53	0.88	1.76	0.82	1.70	1.17	2.48	0.68	1.40	0.61	1.27	0.86	1.61	0.75	1.32	0.74	1.27
	C2	0.76	1.91	0.28	1.18	0.29	1.31	1.13	2.18	0.91	1.82	0.90	1.88	0.74	1.59	0.63	1.31	0.49	1.06	0.47	1.26	0.47	1.15	0.46	1.08
	C3	0.84	2.03	0.42	1.69	0.31	1.34	1.28	2.38	1.04	2.01	0.98	1.99	0.76	1.42	0.64	1.17	0.60	1.13	0.43	1.19	0.42	1.05	0.42	1.04
Oxytetracy-cline h.	C1	0.77	1.72	0.30	1.04	0.27	0.96	0.93	1.84	0.73	1.49	0.66	1.39	0.96	1.81	0.85	1.57	0.73	1.40	0.97	1.54	0.94	1.44	0.81	1.25
	C2	0.72	1.60	0.29	1.05	0.25	0.93	0.92	1.68	0.86	1.63	0.74	1.46	0.92	1.65	0.88	1.55	0.69	1.25	0.98	1.49	0.99	1.44	0.96	1.39
	C3	0.79	1.70	0.32	1.11	0.30	1.09	1.18	2.10	0.76	1.40	0.82	1.57	1.00	1.69	0.93	1.55	0.85	1.46	1.04	1.58	0.99	1.44	0.91	1.32
Fluoxetine h.	C1	0.92	1.93	0.37	1.22	0.31	1.05	1.04	2.00	0.83	1.66	0.77	1.59	0.71	1.50	0.74	1.52	0.69	1.44	0.82	1.53	0.82	1.44	0.79	1.36
	C2	0.97	1.98	0.39	1.27	0.37	1.26	0.91	1.76	0.90	1.79	0.83	1.71	0.82	1.77	0.84	1.75	0.81	1.75	0.85	2.26	0.81	1.97	0.89	2.10
	C3	0.80	1.71	0.35	1.22	0.32	1.14	0.94	1.75	0.87	1.69	0.77	1.56	0.83	1.55	0.89	1.62	0.95	1.78	0.68	1.87	0.79	1.97	0.97	2.36
Estrone	C1	0.78	1.15	0.75	1.15	0.68	1.17	1.10	1.50	0.78	1.15	0.80	1.27	0.62	0.89	0.43	0.85	0.36	0.82	0.87	1.55	0.84	1.53	0.80	1.40
	C2	0.96	1.18	0.94	1.24	0.77	1.08	1.00	1.33	0.82	1.10	0.81	1.19	0.77	0.93	0.59	0.84	0.55	0.86	1.02	1.85	0.92	1.72	0.90	1.61
	C3	1.02	1.19	0.90	1.14	0.77	1.01	1.17	1.49	1.00	1.29	1.02	1.42	0.68	0.79	0.66	0.85	0.60	0.83	0.92	1.77	0.79	1.55	0.78	1.48
Ketoprofen	C1	0.60	0.92	0.43	0.91	0.35	0.75	0.95	1.49	0.67	1.07	0.69	1.22	0.74	1.17	0.70	1.16	0.68	1.18	0.80	1.28	0.82	1.34	0.79	1.26
	C2	0.93	1.46	0.52	1.19	0.49	1.13	0.69	1.33	0.47	0.88	0.58	1.28	0.71	1.36	0.62	1.18	0.56	1.13	0.85	1.41	0.77	1.31	0.74	1.23
	C3	0.66	0.94	0.54	1.07	0.44	0.89	0.89	1.50	0.68	1.11	0.64	1.22	0.63	1.11	0.63	1.11	0.76	1.43	0.79	1.27	0.80	1.31	0.82	1.31
Progesterone	C1	0.91	2.41	0.42	2.00	0.35	1.66	1.13	2.39	0.74	1.49	0.65	1.55	0.58	0.84	0.40	0.80	0.35	0.82	0.78	1.60	0.63	1.32	0.70	1.39
	C2	0.83	2.21	0.39	1.92	0.35	1.73	1.07	2.13	0.82	1.57	0.75	1.72	0.68	0.82	0.55	0.80	0.50	0.81	0.89	1.55	0.87	1.56	0.87	1.51
	C3	0.83	2.12	0.39	1.87	0.32	1.54	0.92	1.78	0.76	1.41	0.80	1.79	0.74	0.86	0.59	0.78	0.65	0.92	0.89	1.59	0.77	1.41	0.91	1.62
Gemfibrozil	C1	0.90	2.18	0.26	1.14	0.20	0.89	1.13	2.21	0.60	1.14	0.62	1.32	0.89	1.69	0.64	1.23	0.48	0.96	0.75	1.22	0.77	1.28	0.73	1.19
	C2	0.69	1.88	0.27	1.38	0.22	1.16	0.90	2.19	0.55	1.26	0.54	1.49	0.71	1.47	0.61	1.26	0.53	1.15	0.86	1.42	0.80	1.36	0.79	1.30
	C3	0.59	1.55	0.27	1.33	0.22	1.08	0.94	2.05	0.66	1.36	0.70	1.76	0.72	1.43	0.62	1.22	0.65	1.37	0.75	1.24	0.76	1.30	0.92	1.53
Androstene-dione	C1	1.49	2.27	0.68	1.42	0.50	1.04	1.33	2.20	0.80	1.33	0.75	1.39	0.57	1.06	0.34	1.02	0.27	0.96	0.88	1.41	0.87	1.42	0.83	1.34
	C2	0.85	1.23	0.44	0.88	0.37	0.75	1.08	1.53	0.85	1.23	0.78	1.24	0.67	0.91	0.49	0.91	0.40	0.86	0.97	1.50	0.92	1.46	0.85	1.32
	C3	1.56	2.21	0.70	1.36	0.67	1.33	1.06	1.73	0.81	1.30	0.83	1.53	0.75	0.93	0.60	0.92	0.53	0.93	0.97	1.52	0.96	1.54	1.00	1.56

Table C. Percentile values for model deviation ratios (MDR) and numbers of cases for each group of CA and IA experiments of BPA YES+ toxicity studies.

	80%	90%	95%	99%	Synergism	Under-estimation	Over-estimation	Antagonism	**Total**
CA	0.89	0.99	1.06	1.11	21	14	0	0	81
IA	1.89	2.50	3.16	3.86	0	1	34	12	81

Table D. Percentile values for model deviation ratios (MDR) and numbers of cases for each group of CA and IA experiments of BPA YES- toxicity studies.

	80%	90%	95%	99%	Synergism	Under-estimation	Over-estimation	Antago-nism	**Total**
CA	1.09	1.12	1.22	1.34	0	0	0	0	81
IA	2.00	2.11	2.24	2.38	0	0	63	17	81

Table E. Percentile values for model deviation ratios (MDR) and numbers of cases for each group of CA and IA experiments of BPA YAS+ toxicity studies.

	80%	90%	95%	99%	Synergism	Under-estimation	Over-estimation	Antago-nism	**Total**
CA	1.03	1.15	1.23	1.35	12	21	1	0	81
IA	2.14	2.35	2.51	3.02	0	0	32	20	81

Table F. Percentile values for model deviation ratios (MDR) and numbers of cases for each group of CA and IA experiments of BPA YAS- toxicity studies.

	80%	90%	95%	99%	Synergism	Under-estimation	Over-estimation	Antago-nism	**Total**
CA	0.95	1.00	1.18	1.48	0	4	1	0	81
IA	1.77	1.84	1.95	2.62	0	0	57	4	81

Table G. Percentile values for model deviation ratios (MDR) and numbers of cases for each group of CA and IA experiments of BPS toxicity studies.

	80%	90%	95%	99%	Synergism	Under-estimation	Over-estimation	Antago-nism	**Total**
CA	0.86	1.02	1.24	2.97	40	9	2	2	81
IA	1.86	2.17	2.40	3.59	0	3	22	12	81

Table H. Percentile values for model deviation ratios (MDR) and numbers of cases for each group of CA and IA experiments of BPS YES- toxicity studies.

	80%	90%	95%	99%	Synergism	Under-estimation	Over-estimation	Antago-nism	**Total**
CA	1.00	1.10	1.17	1.34	0	27	1	0	81
IA	2.14	2.29	2.43	2.61	0	0	46	24	81

Table I. Percentile values for model deviation ratios (MDR) and numbers of cases for each group of CA and IA experiments of BPS YAS+ toxicity studies.

	80%	90%	95%	99%	Synergism	Under-estimation	Over-estimation	Antago-nism	**Total**
CA	0.88	0.98	1.02	1.30	11	34	1	0	81
IA	1.72	1.93	2.16	2.73	0	3	31	5	81

Table J. Percentile values for model deviation ratios (MDR) and numbers of cases for each group of CA and IA experiments of BPS YAS- toxicity studies.

	80%	90%	95%	99%	Synergism	Under-estimation	Over-estimation	Antago-nism	**Total**
CA	0.93	0.97	0.99	1.22	0	9	0	0	81
IA	1.55	1.63	1.89	3.08	0	0	43	4	81

Table K. Percentile values for model deviation ratios (MDR) and numbers of cases for each group of CA and IA experiments of BPF YES+ toxicity studies.

	80%	90%	95%	99%	Synergism	Under-estimation	Over-estimation	Antago-nism	**Total**
CA	0.79	0.91	0.96	1.50	43	12	2	0	81
IA	1.87	1.98	2.18	2.30	0	0	23	8	81

Table L. Percentile values for model deviation ratios (MDR) and numbers of cases for each group of CA and IA experiments of BPF YES- toxicity studies.

	80%	90%	95%	99%	Synergism	Under-estimation	Over-estimation	Antago-nism	**Total**
CA	1.00	1.10	1.17	1.31	1	16	0	0	81
IA	1.88	2.13	2.21	2.41	0	0	44	14	81

Table M. Percentile values for model deviation ratios (MDR) and numbers of cases for each group of CA and IA experiments of BPF YAS+ toxicity studies.

	80%	90%	95%	99%	Synergism	Under-estimation	Over-estimation	Antago-nism	**Total**
CA	0.82	0.89	0.96	1.19	10	39	0	0	81
IA	1.55	1.69	1.77	2.49	0	0	26	2	81

Table N. Percentile values for model deviation ratios (MDR) and numbers of cases for each group of CA and IA experiments of BPF YAS- toxicity studies.

	80%	90%	95%	99%	Synergism	Under-estimation	Over-estimation	Antago-nism	**Total**
CA	0.96	0.99	1.02	1.18	6	3	0	0	81
IA	1.62	1.94	2.10	2.40	0	0	45	5	81