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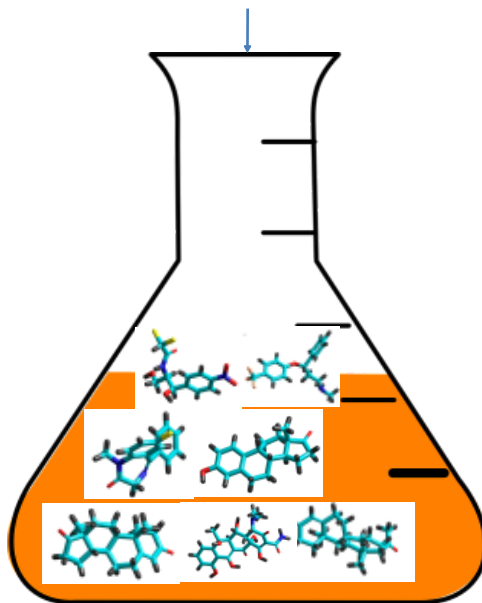
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Title	Bisphenols (A, S, and F) affect the hormonal activity of pharmaceuticals - study of <i>Saccharomyces cerevisiae</i>
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Abstract

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



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

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8 *Abstract:* Pharmaceuticals and analogs of bisphenol A (BPA) are increasingly threatening
9 environmental pollutants. In this study, mixtures of selected pharmaceuticals (diclofenac sodium salt,
10 chloramphenicol, oxytetracycline hydrochloride, fluoxetine hydrochloride, estrone, ketoprofen,
11 progesterone, gemfibrozil and androstenedione) were prepared with BPA and its two analogs
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15 analog. The results obtained were modeled with the concentration addition (CA) and independent
16 action (IA) approaches, the trueness of which was studied with model deviation ratios (MDR). The
17 estrogenic agonistic activity of the drugs studied was most strongly affected by the presence of BPA
18 in solution (twenty-one cases of synergy observed for CA models *versus* twelve cases of antagonism
19 in the case of IA predictions). BPS shows a strong agonistic estrogenic impact on most of the drugs
20 studied at medium and high concentration levels; androgenic agonistic activity was also impaired
21 with elevated concentrations of BPS. Increasing the concentration of BPF in a reaction mixture also
22 increased the number of YES+ synergism incidences (for CA modeling). Estrone, progesterone and
23 androstenedione were mostly affected by the highest BPF concentrations studied in the case of
24 androgenic agonistic research performed.

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

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

29 1. Introduction

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

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

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

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

57 The largest load of pharmaceuticals is introduced into the environment as a result of
58 activities of the pharmaceutical industry, hospital facilities and households (Santos et al. 2010),
59 veterinary and agriculture as well as in animal breeding (Li 2014). Analgetics, anti-inflammatory drugs
60 and anticoagulants are the most frequently detected groups of pharmaceutical residues; ibuprofen,
61 diclofenac and ketoprofen (active ingredients of non-steroid anti-inflammatory drugs) are most
62 frequently found in soil and water samples. The second most frequently detected group of
63 pharmaceutical residues comprises antibiotics and antifungal agents such as oxytetracycline and
64 chloramphenicol, which have been detected in surface waters of Nigeria at concentration levels of
65 460 ng/L and 600 ng/L, respectively (Olatunde et al. 2014). Androgens as well as estrogens such as
66 androstenedione, progesterone and estrone have been detected in surface and wastewaters in the
67 following amounts: >100 ng/L, 66 ng/L, and 36 ng/L, respectively (Kim et al. 2007; Chang et al. 2011).



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

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

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

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



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

109 2. *Materials and methods*

110 2.1. *Chemicals and reagents*

111 The model substances selected for the study, diclofenac sodium salt (diclofenac s.) (CAS no.
112 153907-79-6), chloramphenicol (CAS no. 56-75-7), oxytetracycline hydrochloride (oxytetracycline h.)
113 (CAS no. 2058-46-0), fluoxetine hydrochloride (fluoxetine h.) (CAS no. 56296-78-7), estrone (CAS no.
114 53-16-7), ketoprofen (CAS no. 22071-15-4), progesterone (CAS no. 57-83-0), gemfibrozil (CAS no.
115 25812-30-0), androstenedione (CAS no. 63-05-8), bisphenol A (CAS no. 80-05-7), bisphenol S (CAS no.
116 80-09-1) and bisphenol F (CAS no. 620-92-8), were purchased from Sigma Aldrich (Germany), and
117 they were of analytical grade (>99%). A set of XenoScreen YES/YAS reagents was purchased from
118 Xenometrix AG (Switzerland), namely, a vial with hER α (YES) yeasts (to determine estrogenic activity)
119 and hAR α (YAS) yeasts (to determine androgenic activity) immobilized and dormant on the filtration
120 paper, basal medium, vitamin solution, L-aspartic acid solution, L-threonine solution, CuSO $_4$ solution,
121 17 β -oestradiol (E2, YES+ (agonist) control), 5 α -dihydrotestosterone (DHT, YAS+ (agonist) control), 4-
122 hydroksytamoxyphene (HT, YES- (antagonist) control), and flutamide (FL, YAS- (antagonist) control).
123 DMSO (dimethyl sulphoxide, CAS no. 67-68-5) and CPRG (chlorophenol red- β -D-galactopyranoside)
124 (CAS no. 99792-79-9) dye were purchased from Sigma Aldrich (Germany). Measurements of the
125 OD $_{690}$ cell density (wavelength 690 nm) and of the intensity of the CPRG transformation product
126 OD $_{570}$ (wavelength 570 nm) were performed with a TECAN Infinite M200 spectrophotometer.

127

128 2.2. *XenoScreen YES/YAS methodology*

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

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

139 - YES agonist plate E2 (min. concentration $1 \cdot 10^{-11}$ M, max. concentration $1 \cdot 10^{-8}$ M),

140 - YES antagonist plate HT (min. concentration $1 \cdot 10^{-8}$ M, max. concentration $1 \cdot 10^{-5}$ M; additionally, in
141 the entire plate, E2 was present at a constant concentration of $1 \cdot 10^{-9}$ M),

142 - YAS agonist plate DHT (min. concentration $1 \cdot 10^{-9}$ M, max. concentration $1 \cdot 10^{-6}$ M),

143 - YAS antagonist plate FL (min. concentration $1 \cdot 10^{-7}$ M, max. concentration $1 \cdot 10^{-4}$ M; additionally, in
144 the entire plate, DHT was present at a constant concentration of $3 \cdot 10^{-8}$ M).

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

152 Twenty microliters of adjusted drug sample and sixty μ L of 6 mM CRPG dye were added to
153 each assay well. Pharmaceuticals were mixed in three concentration ratios in such a way as to detect
154 a broad range of possible interactions. All of the studies on the mixtures were performed in
155 triplicate; furthermore, controls were made for pure substances in duplicate, and YES and YAS
156 suspensions of yeast cultures (100 μ L; yeast cell density >0.3 OD₆₉₀) were added into the agonist and
157 antagonist YES and YAS plates, respectively. Assay plates were sealed with semipermeable
158 membranes and placed in the zipper bag moistened with watered gauze on an orbital shaker for 48 h
159 at 32 °C and 100 rpm. After 48 h of incubation, the cell density determined by OD was read at a
160 wavelength of 690 nm, and the color intensity at a wavelength of 570 nm was determined.
161 Afterwards, the activity of β -galactosidase was calculated as the ratio of $[(OD_{570}-OD_{690})/OD_{690}]$. All
162 experiments were run and measured in triplicate.

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

167 2.3. Calculation of MDR values

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



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

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

181

182
$$EC_{X_{Mix}} = \left(\sum_{i=1}^n \frac{P_i}{EC_{x_i}} \right)^{-1} \quad (\text{eq. 1.})$$

183 where:

184 - $EC_{X_{mix}}$ is the x_{mix} effect caused by the total concentration of the mixture of studied chemicals
185 (components) (Expected value);

186 - p_i indicates the fraction of component i in the mixture, calculated on the basis of the
187 concentration of component i in the mixture;

188 - n indicates the number of components in the mixture;

189 - and EC_{x_i} indicates the x_i effect caused by component i at a given studied concentration in
190 the mixture.

191

192
$$E(C_{mix}) = 1 - \prod_{i=1}^n (1 - E(c_i)) \quad (\text{eq. 2.})$$

193 where:

194 - EC_{mix} is the overall effect expressed as a fraction of the maximal possible effect of a mixture
195 of chemical i (expected value);

196 - c_i indicates the concentration of component i in the mixture;

197 - n indicates the number of components in the mixture;

198 - $E(c_i)$ indicates the effect of component i , applied separately.

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

202

203
$$MDR = \frac{Expected}{Observed} \quad (\text{eq. 3.})$$

204 where:

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

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

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

213 3. *Results and discussion*

214 3.1. *Results of hormonal activity studies of selected analytes*

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

220 **Table 1.**

221

222 3.2. *Results for BPA impact on the hormonal activity of pharmaceuticals*

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

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

230

231 **Table 2.**

232

233 3.2.1. *YES+*

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

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

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

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

257 3.2.2. YES-

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

266 3.2.3. YAS+

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



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

278 3.2.4. YAS-

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

283

284 3.3. Results of BPS impact on the hormonal activity of pharmaceuticals

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

291 3.3.1. YES+

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

306 3.3.2. YES-

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

314 3.3.3. YAS+

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

321 3.3.4. YAS-

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

326

327 3.4. Results of BPF impact on the hormonal activity of pharmaceuticals

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

333

334 3.4.1. YES+

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



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

346 3.4.2. YES-

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

353 3.4.3. YAS+

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

363 3.4.4. YAS-

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

369 4. Conclusions

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

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

388

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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

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
Chloramphenicol	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
Oxytetracycline 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
Androstenedione	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
Chloramphenicol	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
Oxytetracycline 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
Androstenedione	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+ MDR						YES- MDR						YAS+ MDR						YAS- 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
Chloramphenicol	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
Oxytetracycline 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
Progesterone	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
Androstenedione	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



Electronic supplementary material

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-MDR						YAS+						YAS-					
		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
Chloramphenicol	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
Oxytetracycline 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
Androstenedione	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	Antagonism	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	Antagonism	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	Antagonism	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	Antagonism	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	Antagonism	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	Antagonism	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	Antagonism	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	Antagonism	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	Antagonism	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	Antagonism	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	Antagonism	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