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1 **Estrogenic and anti-estrogenic activity of 23 commercial** 2 **textile dyes**

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16 **Abstract**

17 The presence of dyes in wastewater effluent of textile industry is well documented. In
18 contrast, the endocrine disrupting effects of these dyes and wastewater effluent have been
19 poorly investigated. Herein, we studied twenty-three commercial dyes, usually used in the
20 textile industry, and extracts of blue jean textile wastewater samples were evaluated for their
21 agonistic and antagonistic estrogen activity. Total estrogenic and anti-estrogenic activities
22 were measured using the Yeast Estrogen Screen bioassay (YES) that evaluates estrogen
23 receptor binding-dependent transcriptional and translational activities. The estrogenic
24 potencies of the dyes and wastewater samples were evaluated by dose-response curves and
25 compared to the dose-response curve of 17 β -estradiol (E2), the reference compound. The
26 dose-dependent anti-estrogenic activities of the dyes and wastewater samples were
27 normalized to the known antagonistic effect of 4-hydroxytamoxifen (4-OHT) on the induction
28 of the lac Z reporter gene by E2. About half azo textile dyes have anti-estrogenic activity with
29 the most active being Blue HFRL. Most azo dyes however have no or weak estrogenic
30 activity. E2/dye or E2/waste water ER competitive binding assays show activity of Blue
31

32 HFRL, benzopurpurine 4B, Everzol Navy Blue FBN, direct red 89 BNL 200% and waste
33 water samples indicating a mechanism of action common to E2. Our results indicate that
34 several textile dyes are potential endocrine disrupting agents. The presence of some of these
35 dyes in textile industry wastewater may thus impact the aquatic ecosystem.

36

37 *Keywords:* textile dyes, estrogenic activity, anti-estrogenic activity, industrial textile effluent.

38

39 **1. Introduction**

40

41 Dyes are widely used in most industries such as those manufacturing papers, plastics, food,
42 cosmetics, textiles or leathers. These dyes are useful to colour the final products. Dyes are
43 classified depending on their colors, their chemical structures and/or their origin (natural or
44 synthetic). Natural dyes most frequently originate from plants (such as riboflavin or β -
45 carotene). Determination of the chemical structures of natural dyes and the accomplishment of
46 their synthesis allow the gradual replacement of these natural dyes by their synthetic
47 counterparts. Approximately 10.000 commercial dyes are used in the coloring industry. More
48 than 10% of dyestuff used during the coloring processes does not bind to the fibers and
49 therefore these excess dyes are released into the environment producing serious
50 environmental pollution (Pearce et al., 2003; Rajeswari et al., 2011). The presence of these
51 dyes in wastewater and subsequently in water resources, even at very low concentrations, is
52 easy to observe visually as the result of textile industry activities. They may increase effluent
53 toxicity and lead to environmental damage (Robinson et al., 2002). In addition, many
54 synthetic dyes are poorly biodegradable. In some dyehouse effluents, dye concentration can
55 reach up to 400 mg/l (O'Neill et al., 1999). A specific study has even demonstrated that these
56 concentrations can exceed 600 mg/L in Nigeria (Yusuff and Sonibare, 2004). Their presence
57 in water reduces light penetration and has a negative impact on photosynthesis. Moreover, the
58 dyeing process itself generally also contributes to the water body contamination by chromium,
59 zinc and copper which are all toxic to aquatic plants and fish below 1.0 mg/L (Eremektar et
60 al., 2007; Sharma et al., 2007; Verma, 2008). The research on textile wastewater toxicity,
61 which has been carried out so far, shows how the action of toxic dyes occurs at different
62 levels of the food chain, from food supply (i.e. algae and plants) to consumers (i.e.
63 crustaceans and fishes) (Manu et al., 2003; Robinson et al., 2002; Sharma et al., 2007; Soni et
64 al., 2006; Tigini et al., 2011). Most research effort has been devoted towards elucidating or

65 improving the degradation mechanisms of textile dyes with the hope to reduce their toxicity
66 levels (Phugare et al., 2011a,b). Environmental regulations in most countries (i.e. EU
67 directive 91/271) have prioritized in wastewater dye decontamination in order to minimize
68 environmental damage (Robinson et al., 2001). Although the Environmental Protection
69 Agency (EPA) has emitted new practice guidelines for environmental management, the
70 presence of pollution dyes remains a serious environmental issue specifically for small textile
71 industries in various countries (e.g. China, India, Taiwan) where working conditions and low
72 economic status does not allow an efficient wastewater treatment before disposal into water
73 sources (Mathur et al., 2005; Gregory et al., 2007; You et al., 2009). Since the mid nineties,
74 the links between dyes, environmental impact and cancer emergence have been the subject of
75 considerable interest both from researchers and from the general public. However, the causal
76 relationship between dyes and certain types of cancer is difficult to establish. Several studies
77 have been conducted on the toxicity, mutagenicity and genotoxicity of textile dyes (Bakshi et
78 al., 2003; Ben Mansour et al., 2007; Dogan et al., 2005; Durnev et al., 1995; Mathur et al.,
79 2007; Schneider et al., 2004). In the case of azo dyes, the increase in bladder cancer
80 incidence, observed among textile industry workers, has been linked to prolonged exposure to
81 these dyes. A report of experts from the “Cosmetic Ingredient Review Committee” confirmed
82 that some anthraquinone dyes, like the disperse Blue 7 dye, used in the cosmetic industry as
83 hair colorant and in textile, induce genotoxicity in bacteria (Cosmetic ingredient, 2007). Since
84 then, research on these dyes confirmed their carcinogenic effects for humans and animals
85 (Tsuda et al., 2001). This toxic effect has been linked to their presence in the environment
86 (Dogan et al., 2005; Chou et al., 2007; Tigini et al., 2011). From an environmental point of
87 view, toxicity, genotoxicity and mutagenicity of industrial effluents have been demonstrated
88 (Alves de Lima et al., 2007; Grinevicius et al., 2009; Tigini et al., 2011). However, these
89 effects have generally not been linked to the presence of dyes. Chou and collaborators
90 associated the dioxin-like activity of some dyeing wastewater with the presence of specific
91 anthraquinone dyes (Chou et al., 2006, 2007). In the same studies, these authors demonstrated
92 that disperse blue 56 can bind the aryl hydrocarbon receptor (AhR), which is involved in
93 many physiological functions such as cell regulation and reproduction. Two Brazilian studies
94 have identified that the mutagenic activity of the Cristais River, a drinking water source of
95 São Paulo, is caused by the presence of three blue dyes; C.I. Disperse Blue 37 contributing to
96 55% of this effect (De Aragão et al., 2005).

97 Up to now, the estrogenic activity of dyes has seldom been studied. Rare studies
98 mainly report the effects of food colors, such as tartazine (E102) and erythrosin B (E127).
99 Both compounds affect chromosome structure and increase Estrogen Receptor (ER) site-
100 specific DNA binding to Estrogen Response Element (ERE) in HTB 133 cells (Roychoudhury
101 et al., 1989) and in the E-screen test (Datta et al., 2008). The present manuscript investigates
102 both the estrogenic and anti-estrogen activities of twenty three pure commercial textile dyes
103 using the Yeast Estrogen Screen (YES). This *in vitro* assay has been developed for the
104 detection of endocrine disrupting compounds (EDCs). In order to indentify estrogen and anti-
105 estrogen activity of textile dyes, the YES assay has been used to probe two different modes of
106 action. First, a reporter gene assay measures the impact of dye binding on ER on its ability to
107 promote binding-dependent transcriptional and translational activity. Second, an ER
108 competitive binding assay measures how a dye competes with 17 β -estradiol (E2)-dependent
109 ER activation. In addition, the endocrine activity (estrogenic and anti-estrogenic activity) has
110 been assessed on a textile effluent coming from a blue jean manufacturing Tunisian industry
111 in an attempt to correlate the endocrine activity observed for commercial compounds and that
112 obtained for dyeing wastewater.

113

114 2. Materials and methods

115

116 2.1. Selected dyes

117

118 All the dyes selected (23) in this study are used in the textile industry (Table 1 and 2).
119 Only, 17 on 23 dyes have a known or communicated structure. The structure of the 6 other
120 dyes were confidential and not available in the chemical abstracts service. However, the
121 safety and chemical information certificate of analysis usually indicates the nature of the dye
122 (azoic or anthraquinone-type chemical structure). For each dye, a stock solution (10 g/L) was
123 prepared by dissolving in distilled water, followed by filtration through Whatmann No. 5 filter
124 paper. All these dyes appeared perfectly soluble in water at this concentration as assessed by
125 the absence of precipitate. For each dye, the endocrine activity was assessed at four
126 concentrations in the range of 1×10^{-5} g/L to 1 g/L. The natural fluorescence of the dyes and
127 their interference on the fluorescence emitted by yeast was determined beforehand in order to
128 avoid spurious signals generated by the YES test in our experimental conditions. The
129 fluorescence of each dye has been measured in the absence of yeast cells and subtracted from

130 the data obtained for estrogenic tests. The quenching potential of the dyes was also assessed
131 on the yeast fluorescence emission during the antiestrogenic test as follows. The fluorescence
132 of yeast cell has been measured after 6 h of incubation of the reference compound E2 (from
133 Sigma-Aldrich, St Quentin-Fallavier, France). Then, each dye was added to the medium at
134 various concentrations and the fluorescence quenching was measured immediately.
135 Compounds inducing more than 10% of fluorescence quenching were not investigated further
136 for anti-estrogenic activity (noted by an asterisk in Table 2). The cytotoxicity of each dye was
137 evaluated by measuring yeast growth at an O.D. of 600 nm.

138

139 2.2. Wastewater sampling and extraction

140

141 Wastewater samples (1 L) were collected from the textile effluent of an industrial blue
142 jeans factory in Tunisia. Raw water samples were centrifuged (2000 g, 15 min) to eliminate
143 solids in suspension. Supernatants were then extracted by solid-phase extraction as described
144 by Pillon et al. (2005). Briefly, aqueous samples were concentrated on reverse-phase C18 (5
145 g, 20 mL) cartridges (Sigma-Aldrich, St Quentin-Fallavier, France) preconditioned with
146 methanol. Compounds elution from the column was triggered using methanol followed by
147 hexane. Eluates were dried at 37°C in a rotary evaporator and residues were taken up in 2 mL
148 methanol (concentration factor: 500).

149

150 2.3. Yeast estrogen screen assay (YES)

151

152 2.3.1. Assay of estrogenic and anti-estrogenic activity

153

154 Both agonist and antagonist activities of chemical dyes were examined using the Yeast
155 strain BY4741 (Euroscarf, Frankfurt, Germany). This recombinant yeast strain carries the β -
156 galactosidase reporter gene under the control of the ERE and contains the human ER cloned
157 into the constitutive yeast expression vector pAAH5 (García-Reyero et al., 2001). The test
158 measures β -galactosidase activity (fluorescence at 460 nm and excitation at 355 nm) with a
159 fluorimeter (Fluoroskan Twinkle LB 970, BERTHOLD Technologies) after 6 h of exposure to
160 the compounds to evaluate. Tests were performed in 96-well plates. To determine the estrogen
161 agonist activity of dyes and effluent samples, E₂ was used as a positive control and distilled
162 water was used as a negative control. Four dye concentrations were tested in the range of

163 1×10^{-5} g/L to 1 g/L. For the effluent samples, a series of dilutions (1 to 1000 of concentrated
164 extract) were tested for their estrogenic activity. To determine the estrogen antagonist activity
165 of the dyes, 4-OHT was used as a positive control and E₂ (1 nM) was used as a negative
166 control. In the antagonist test, the ability of the dye to compete with E₂ for binding to ER
167 and/or inhibit the receptor functions was evaluated. The tested dyes or 4-OHT, the positive
168 control, were combined with 1 nM E₂ before the start of the assay. All experiments were
169 performed in triplicate. For estrogen agonist activity, the half maximal effective concentration
170 (EC₅₀) was calculated based on the sigmoidal dose–effect curve of E₂. For estrogen antagonist
171 activity, the half-maximal effective concentration (AC₅₀) was calculated based on the
172 sigmoidal dose–effect curve of 4-OHT.

173

174 2.3.2. Competitive binding assay

175

176 Competition between dyes and/or sample effluent and E₂ was measured at various
177 concentrations of E₂. Textile dye concentration used is 0.01 g/L and the textile dyeing effluent
178 sample at a dilution factor 5 of the concentrated extract. Thus any decrease of β-galactosidase
179 activity after 6 h of exposure indicates that the dye induces a decrease in E₂ binding.

180

181 2.4. UV/Visible spectrum deconvolution

182

183 The absorbency spectrum of a water sample can be decomposed into a few numbers of
184 spectra (reference spectra). The shape of the UV spectrum can be considered as a linear
185 combination of defined spectra (REF₁,..., REF_p) related to potential compounds present in
186 studied water sample (Thomas et al., 1996). $S_w = \sum a_i \times REF_i \pm r$, where S_w is the final
187 spectra, a_i and r are the coefficient of the i^{th} reference spectra and the admitted error,
188 respectively. The Secomam company (Alès, France) has developed the UVPro software based
189 on advanced UV spectral deconvolution (UV PRO, 2000) which allows creating dedicated
190 models and determination of reference spectra from a set of studied wastewater UV spectra.
191 The UVPro software has been applied to the textile effluent of an industrial blue jeans factory
192 of Tunisia. The reference spectra used for the deconvolution are obtained from the 23 selected
193 textiles dyes (Table 1). Using the deconvolution model, it is possible to assess the
194 contribution of studied dyes in the wastewater spectrum.

195

196 3. Results / Discussion

197

198 3.1. Estrogenicity / anti-estrogenicity studies of individual dyes

199

200 YES allows a fast determination of both endocrine agonist and antagonist actions. The
201 relative estrogenicity of each dye has been estimated using YES by comparison to the
202 estrogenicity of E2 as reference compound. Typical dose-response curves for E2 have been
203 established (data not shown). In the present study, we have essentially analyzed azo dyes.
204 These dyes are extensively used for dyeing cotton in textile industries. By using YES assay in
205 the present work, we have analyzed the degree of interference of textile dyes with the
206 endocrine system considering both the potential agonist and antagonist actions. A great
207 variability between dyes is observed. The YES assay data illustrate a dose-dependent estrogen
208 agonist activity from 1×10^{-5} g/L to 1 g/L for three dyes (Yellow Flavina CXL, Reactive dye
209 red 3BS and Solvent yellow 56) (Figure 1). All these dyes have weak estrogenic effects since
210 the maximum activity is obtained at 1 g/L. In addition, the estrogenic effect of the dyes did
211 not saturate precluding the determination of the EC₅₀ value, contrary to the positive control E2
212 (EC₅₀ value at 1 ng/L) (Figure 1). Higher dye concentrations were not investigated, since
213 water body dye concentration in the environment never exceeds 1 g/l. Table 2 also
214 summarizes the fact that the twenty other dyes had no estrogenic effect by themselves. These
215 results indicate that these dyes are not xenoestrogens that should highly impact the
216 environment, as compared to E2 or other known xenoestrogens (e.g. bisphenol A, paraben...)
217 (Routledge et al., 1998).

218 In addition, some textile dyes have an anti-estrogenic activity. The anti-estrogenic
219 reference compound 4-OHT was used to demonstrate efficient anti-estrogenic activity when
220 incubated in the presence of 1 nM E2. The AC₅₀ value of 4-OHT was 0.5 μ M or 0.02 mg/L
221 (Table 2). Similar experiments were conducted with the dyes. Only some dyes have been
222 tested (Figure 2), the other ones, marked by an asterisks, could not be tested because they
223 quenched the β -galactosidase fluorescence. At a concentration of 1 g/L, antagonistic activities
224 of these dyes vary from 100% to 10% inhibition according to their colour (Table 2 and Figure
225 2A). Blue and red dyes show the highest inhibition potential. Everzol navy blue FBN, blue
226 HFRL, Direct Red 89 BNL 200% and Benzopurpurine 4B (Red 4B) are the most potent
227 inhibitors tested. Their inhibitory effects remain however inferior to that of 4-OHT. At 1 g/L,
228 these dyes are the only ones that induce a complete inhibition of the reporter gene in yeast

229 cells. Among these four dyes, Blue HFRL is the strongest antagonist; its anti-estrogenic
230 potency being approximately 750-fold less than that of 4-OHT. A reduction of 50% of β -
231 galactosidase activity is observed for concentrations as low as 15 mg/L. These concentration
232 values are thus considerably lower than the total color level found in the environment (400
233 mg/L). The antagonist effect of three other dyes (Benzopurpurine 4B (Red 4B), Everzol Navy
234 Blue FBN, and Direct Red 89 BNL) were, respectively, 3500-, 2500-, and 1250-fold less than
235 that of 4-OHT. In contrast, all other dyes were either weak ER inhibitors or non-ER
236 inhibitors. For instance, Everzol Blue ED, Red alpacide 3BL, Direct Blue 71, Blue ED 250,
237 Direct Black VSF and Blue DERF were weak ER inhibitors. The anti-estrogenic activity for
238 Blue DERF and Direct Blue 71 at 1 g/l, are 10% and 22%, respectively. Among these dyes,
239 most are of the azo class with sulfonated aniline (Benzopurpurine 4B, Direct Red 89 BNL
240 200%) and one is an anthraquinone dye, Everzol Blue ED. The Direct Black VSF AZO-
241 FREE, Blue ED 250, both being polyazo dyes, Everzo Yellow ED and Red ED (for which no
242 structures can be disclosed) induce inhibition of β -galactosidase expression by E2 with AC_{50}
243 > 1 g/L.

244 Competition between the textile dyes and E2 was also measured by varying the
245 concentration of E2 and maintaining a constant dye concentration (0.01 g/L, a concentration
246 that does not fully inhibit E2 effect, Figure 2A). Benzopurpurine 4B, Blue HFRL and Everzol
247 Navy Blue FBN can compete with E2 for binding to ER (Figure 2B). These dyes induces a
248 slight inhibition of E2 estrogenic activity when E2 is used at concentrations equal or lower
249 than 1.10^{-8} M. No effect is observed for higher concentrations of E2. For example, this
250 activity decreased from 200000 RLU without dye to 100000 RLU in presence of the Blue
251 HFRL at 10^{-8} M E2. This study also shows that the Red Alpacide 3BL induces a 44%
252 inhibition of estrogenic activity and the metal-complexed azo dye yellow 4G induces a 13%
253 inhibition (Table 2). The competitive binding assay in the YES assay showed that these dyes
254 can bind specifically to ER in order to induce these antagonist effects.

255

256 3.2. Estrogenicity / anti-estrogenicity studies of textile effluent

257

258 The textile effluent sample analyzed presents a weak estrogenic effect at the maximum
259 concentration tested (5-fold dilution). At higher concentrations of the sample, the sample
260 compounds reduce cell viability (data not show) precluding the use of the sample for the
261 estrogenic assay. For the dilution range (5- to 10000-fold), results from the YES bioassay

262 indicate that the industrial textile effluent presents a low (below 15%) but significant
263 estrogenic activity (Figure 3A). Also, the effluent sample inhibits 60% of estrogenic activity
264 (Figure 3B). In competition experiments, in which E2 concentration is varied and a single
265 dilution dose of the effluent sample is used (5-fold dilution), the anti-estrogenic effect is
266 observable until 10^{-5} M of E2 (Figure 3C). The competition effect of the sample is most
267 significant at 10^{-8} M.

268 The composition of the effluent sample in dyes has not been fully determined. Spectral
269 analyses indicate that most of its components are blue dyes. Indeed, UV/Visible
270 deconvolution of the spectrum of a blue jeans manufacturer's effluent of Tunisia indicates that
271 the sample contains mainly Blue DERF, Direct Blue 71, Everzol navy Blue FBN, and Grey
272 GGL dye according to the spectrum of each individual dye (Figure 4). The deconvolution of
273 the sample spectrum did not predict the presence of other dyes. Interestingly, our studies have
274 demonstrated that at least three of these dyes presented anti-estrogenic activity with the YES
275 assay. Most blue and navy blue dyes are derived from the Reactive Black 5 azo dye, which is
276 classified Xn (harmful). It is also suspected to be mutagenic and to be associated to bladder
277 cancer development (You et al., 2009). Nevertheless, without a complete characterization of
278 the nature of the dyes being released in the local environment of this factory, the anti-
279 estrogenic effect of the effluent sample can't be unequivocally be attributed to these blue
280 dyes. Since this effluent is essentially blue, and that some blue dyes are among those
281 presenting the highest anti-estrogenic effect, suspicion remains however high that this
282 Tunisian factory releases harmful components in the environment. In addition, it is worth
283 noting that the most active dyes have anti-estrogenic AC_{50} values (between 15 mg/L and 70
284 mg/L) that are well below the dye concentration that can be detected in some water bodies
285 (400 mg/L). If these same dyes are indeed present in the environment, they would present a
286 real anti-estrogenic activity to local water consumers.

287

288 **4. Conclusions**

289

290 Our results on the endocrine effects of textile dyes come in complement of those
291 already published on the carcinogenic and mutagenic effects of dyes. They point to the fact
292 that some dyes, mainly blue and red dyes, may be endocrine disruptor compounds. These
293 observations raise two issues. Concerning environmental pollution, some of these dyes may
294 be present at concentrations high enough that they may indeed affect life quality by promoting

295 tumor generation. Textile sewage is therefore a potential health hazard that should require a
296 better communication on the dyes used by the industry and its method of disposal. In that
297 respect, it appears as particularly important to efficiently treat industrial effluent containing
298 azo dyes before they get discharged into the environment. The second issue concerns the
299 pharmacological effect of these textile dyes. Besides 4-OHT, few compounds have anti-
300 estrogenic activity. It is therefore of interest to note that some of the dyes that we
301 characterized have similar anti-estrogenic activity, suggesting that they may be lead
302 compounds for the development of new 4-OHT like anti-estrogenic compounds.

303

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305

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407

408 **Figure legends**

409

410 **Figure 1:** Induction of estrogenic activities of some textile dyes. Average \pm standard
411 deviation (n=3).

412

413 **Figure 2:** Anti-estrogenic activity of textile (A) dyes as measured by the yeast estrogen screen
414 (B). Competitive binding essay of 3 textile dyes (0.01 g/l) against various concentrations of
415 E2. Average \pm standard deviation (n=3).

416

417 **Figure 3:** Estrogenic (A) and anti-estrogenic (B) activity of textile effluent, (C) competitive
418 binding assay of textile effluent (dilution factor 5-fold) against various concentrations of E2.
419 Average \pm standard deviation (n=3).

420

421 **Figure 4:** UV/Visible deconvolution of textile effluent spectrum.

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425

426 **Table 1:** Textile dyes analysed in this study. ^aDyes and informations obtained by Everlight
 427 Chemical SA.

<i>Compound</i>	<i>CAS #</i>	<i>Family</i>	<i>Formula</i>
Benzopurpurine 4B (Red 4 B)	992-59-6	azo	C ₃₄ H ₂₆ N ₆ Na ₂ O ₆ S ₂
Direct Black VSF AZO-FREE (Direct Black 22)	6473-13-8	azo	C ₄₄ H ₃₂ N ₁₃ Na ₃ O ₁₁ S ₃
Yellow 4 G (Solvent yellow 19)	10343-55-2	azo	C ₁₆ H ₁₁ CrN ₄ O ₈ S
Direct Blue 71 (Blue BRR)	4399-55-7	azo	C ₄₀ H ₂₃ N ₇ Na ₄ O ₁₃ S ₄
Brown RL	12238-94-7	azo	C ₁₅ H ₁₄ O ₅
red Alpacide 3BL	12238-49-2	azo	C ₁₈ H ₁₀ N ₂ O ₂ Cl ₂
brown GV			C ₃₁ H ₂₁ N ₇ O ₆ Na ₂ S
Orange 7GL	12222-37-6	azo	C ₄₂ H ₂₈ N ₇ Na ₄ O ₁₅ S ₄
Direct Black PMSF		azo	C ₃₇ H ₂₅ N ₅ Na ₂ O ₆ S ₂
Everzol Navy Blue FBN ^a	93912-64-2	azo	C ₃₇ H ₂₉ ClN ₁₀ O ₂₂ S ₇ Na ₆
Blue HFRL ^b			
Direct Red 89 BNL 200% Blue DERF ^b		azo	
Everzol Yellow ED ^a	Confidential data		
Reactive dyes red 3BS (Red 195)	93050-79-4	azo	C ₃₁ H ₁₉ ClN ₇ O ₁₉ S ₆
Everzol Blue ED ^a	2580-78-1	antraquinone	C ₂₂ H ₁₈ N ₂ O ₁₁ S ₃ Na ₂
Yellow Flavina CXL ^b			
Everzol Navy ED	17095-24-8	azo	C ₂₆ H ₂₅ N ₅ O ₁₉ S ₆ Na ₄
Solvent yellow 56	2481-94-9	azo	C ₁₆ H ₁₉ N ₃
Yellow 3GF ^b			C ₃₀ H ₂₆ N ₄ Na ₂ O ₈ S ₂
Everzol Red ED ^a	Confidential data		
Grey GGL ^b			
Blue ED 250	89157-03-9	azo	C ₃₁ H ₂₄ ClN ₇ O ₁₉ S ₆ Na ₅

428 ^aDyes and informations obtained by Everlight Chemical SA. ^bReactive azo dye (copper
 429 complex, 70% pure for Blue HFRL) kindly provided by a textile industry unfortunately there
 430 is no available information on its structure (Baëta et al. 2011)

431

432 **Table 2:** Relative estrogen agonistic and antagonistic activities of industrial dyes. Estrogenic
 433 activity of textile dyes (1g/l). The anti-estrogenic activity of textile dyes has been determinate
 434 at 1g/l and expressed in 4-OHT%. The half maximal effective concentration (AC50) was
 435 calculated based on the sigmoidal dose–effect curve of 4-OHT.

<i>Compounds</i>	<i>Test Estrogen Screen agonist activity (E2%)</i>	<i>Test Estrogen Screen antagonist activity (4-OHT %)</i>	<i>Test Estrogen Screen antagonist AC 50 (g/l)</i>
E ₂ (10 ^{E-9} M)	100	-	-
OTH	-	100	2*10-5

Benzopurpurine 4B (Red 4 B)	0	100	0.07
Direct Black VSF AZO-FREE (Direct Black 22)	0	20	>1
Yellow 4 G (Solvent yellow 19)	0	13	>1
Direct Blue 71 (Blue BRR)	0	22	>1
*Brown RL	0	-	-
Red Alpacide 3BL	0	44	>1
*Brown GV	0	-	-
*Orange 7GL	0	-	-
*Direct Black PMSF	0	-	-
Everzol Navy Blue FBN	0	94	0.05
Blue HFRL	0	100	0.015
Direct Red 89 BNL 200%	0	100	0.025
Blue DERF	0	10	>1
*Everzol Yellow ED	0	-	-
Reactive dyes red 3BS (Red 195)	22	0	0
Everzol Blue ED	0	35	>1
Yellow Flavina CXL)	36	0	0
Everzol Navy ED	0	32	>1
Solvent yellow 56	11	0	0
Yellow 3GF	0	0	0
Everzol Red ED	0	30	>1
*Grey GGL	0	-	-
Blue ED 250	0	0	0

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