

1 **Progestagens for human use, exposure and hazard assessment for**
2 **the aquatic environment**

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23 **Capsule:**

24 Gestagens exposure and hazard assessment for the aquatic environment.

25
26 **Keywords:**

27 gestagens; metabolites; surface water; hazard; exposure; assessment

32 **Abstract:**

33 Little information is available on the environmental occurrence and ecotoxicological effects
34 of pharmaceutical gestagens released in the aquatic environment. Since eighteen different
35 gestagens were found to be used in France, preliminary exposure and hazard assessment were
36 done. Predicted environmental concentrations (PECs) suggest that if parent gestagens are
37 expected to be found in the ng.l^{-1} range, some active metabolites could be present at higher
38 concentrations, although limited data on metabolism and environmental fate limit the
39 relevance of PECs. The biological effects are not expected to be restricted to progestagenic
40 activity. Both anti-androgenic activity (mainly for cyproterone acetate, chlormadinone acetate
41 and their metabolites) and estrogenic activity (mainly for reduced metabolites of
42 levonorgestrel and norethisterone) should also occur. All these molecules are likely to have a
43 cumulative effect among themselves or with other xenoestrogens. Studies on occurrence,
44 toxicity and degradation time are therefore needed for several of these compounds.

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56 1. Introduction:

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58 It is now recognized that pharmaceutical compounds reach the aquatic environment. A wide
59 range of drugs (antibiotics, antidepressants, nonsteroidal anti-inflammatories, blood lipid-
60 lowering agents, anti-hypertensors and so on) have been found in wastewater treatment plant
61 (WWTP) effluents and surface waters, at concentrations ranging from the ng.l^{-1} to the $\mu\text{g.l}^{-1}$
62 (Halling-Sorensen et al., 1998; Ternes, 1998 ; Kolpin et al., 2002 ; Kümmerer, 2004).

63 Therefore, more and more studies are directed toward assessing the risk of human
64 pharmaceuticals in the aquatic environment. In this context, the question of the potential risk
65 of endocrine disruption due to hormones used in contraception and hormone replacement
66 therapy (HRT) needs to be addressed. It is well known now that many chemicals capable of
67 endocrine disruption are found in the aquatic environment (Colborn et al., 1993; Sumpter,
68 2005), but the contribution of human pharmaceuticals to this contamination has not yet been
69 defined.

70 A large number of studies investigating the occurrence and effects of natural and synthetic
71 estrogen steroids (ethinylestradiol, estradiol, estrone and estriol) and estrogen-like molecules
72 have been conducted, and the risk is now well documented. A few studies have been
73 conducted on the risk related to anti-androgens (Sumpter, 2005), and surprisingly, virtually no
74 studies have been conducted on the occurrence of gestagens in wastewater and surface water
75 and their effect on non-target organisms.

76 Gestagens (also called progestogens, progestagens or progestins) are hormones that produce
77 effects similar to those of progesterone (P4). Progesterone is a C-21 steroid hormone
78 involved in the female menstrual cycle, pregnancy and the embryogenesis of humans and
79 other species (Rozenbaum, 2001; Hardman et al., 1996). Since natural progesterone is

80 inactivated very rapidly in the organism, several synthetic progestins have been developed.
81 Progesterone and synthetic progestins act through nuclear receptors, mainly the progesterone
82 receptor (PR) but also through other receptors such as the androgen receptor (AR), estrogen
83 receptor (ER), glucocorticoid receptor (GR) and mineralocorticoid receptor (MR). Synthetic
84 progestins can have various hormonal activities: estrogenic, anti-androgenic and androgenic
85 (Table 1).

86 Gestagens are compounds that can pose a risk for the aquatic environment, at least for fish, in
87 which gestagens play a role in the control of spawning behavior (Kobayashi et al., 2002). In a
88 recent study (Kolodziej et al., 2003), the synthetic progestin medroxyprogesterone acetate
89 (MPA) and other steroid hormones were found in WWTP effluent samples. The authors
90 highlighted that, considering the levels found, these compounds were able to elicit
91 pheromonal responses in fish that could alter their behavior and interfere with their
92 reproduction (Kolodziej et al., 2003).

93 Reviewing the use of steroid hormones in France, we found that there were 18 different
94 natural and synthetic gestagens used but only few occurrence studies and no environmental
95 risk assessments. In this paper we therefore propose to review the knowledge on progestins
96 and to conduct a preliminary assessment of the risk for wastewaters and surface waters.

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98 **2. Pharmacology of gestagens:**

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100 2.1 Classification of gestagens:

101

102 Progesterone is the only natural gestagen: all other molecules are synthetic and are often
103 referred to as progestins or synthetic progestins. In this paper, the terms “gestagen” and

104 “progestin” will be used throughout to describe gestagens in general and synthetic progestins,
105 respectively. Synthetic progestins differ from progesterone by several chemical modifications
106 (Appendix A) and are classified accordingly. Briefly, there are derivatives of 17- α -
107 hydroxyprogesterone (pregnanes), of 17- α -norhydroxyprogesterone and 19-norprogesterone
108 (norpreganes), and of 19-nortestosterone (estranses and gonanes). There are large differences
109 in the biological effects of progestins: some can present estrogenic activity (norethisterone,
110 tibolone) while others are well known to display anti-androgenic activities (cyproterone and
111 chlormadinone acetate); these different properties are summarized in Table 1.

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113 2.2 Mechanism of action and activity of gestagens in mammals:

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115 Natural progesterone (P4) has progestagenic, anti-estrogenic, anti-aldosterone and mild anti-
116 androgenic activities. P4 mainly acts through the progesterone nuclear receptors PRA and
117 PRB and modifies the transcription of target genes (Rozenbaum, 2001). The main genomic
118 activities are:

- 119 • preparation of the uterus for nidation, then maintaining gestation.
- 120 • inhibition of the secretion of pituitary gonadostimulins,
- 121 • blastocyte implantation,
- 122 • anti-estrogenic effect by induction of 17-hydroxysteroid dehydrogenase, which
123 accelerates the conversion of estradiol into estrone, and by induction of estrogen
124 sulfotransferase.
- 125 • anti-mineralocorticoid action by inhibition of aldosterone receptors, which induces a
126 decrease in plasma sodium by increasing its urinary elimination.

127 P4 can also produce non-genomic effects. Such effects are reported to be faster than genomic
128 effects (hours versus days) and can trigger different metabolic reactions and receptors
129 (GABA, NMDA and acetylcholine receptors). P4 plays a role in oocyte maturation and
130 modulation of reproductive signaling in the brain, but also has a sedating action by
131 potentiating the GABA effect on GABA(A) receptors or can impair the glucose metabolism
132 (Hardman et al., 1996; Rozenbaum, 2001; Pharmacorama, 2008).

133 Synthetic progestins are mainly used in association with an estrogen in oral contraception.
134 Progestin activity may differ from natural P4 (Table 1): some can display estrogenic activities
135 (tibolone [TBL], levonorgestrel [LNG]), while others are stronger anti-androgens
136 (cyproterone acetate [CPA]). Synthetic progestins are generally more potent than P4; as an
137 example they are reported to be much more effective inhibitors of the secretion of
138 gonadostimulins (Pharmacorama, 2008). Progestins also have several other metabolic effects,
139 depending on their chemical structure. For example, ethinylated gestagens, particularly
140 gestodene (GSD), have been demonstrated to inhibit cytochrome P450 enzymes (Rozenbaum,
141 2001; Kuhl, 1996).

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143 2.3. Structure activity relationships:

144

145 As noted above, progestins can have subtle differences in their mode of action. This is related
146 to chemical modifications at key points of the general structure of gestagens (Rozenbaum,
147 2001; Stanczyk, 1996). By acting on these key points, it is possible not only to modulate the
148 progestagenic activity but also to switch the activity to other steroid pathways. The key points
149 are shown in Figure 1 and the structure–activity relationships are summarized in Table 2.

150

151 **2. Material and methods:**

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153 This paper has two main goals: i) to review the ecotoxicological and pharmacological
154 knowledge on progestogens used in human medicine to provide an overview of the biological
155 effects of these molecules and ii) to assess the exposure to the aquatic environment by
156 calculating predicted environmental concentrations (PECs) and to provide a preliminary
157 characterization of the risk for gestagens.

158 To do so, the scientific literature was reviewed, as well as the following databases and books:
159 the Banque Claude Bernard (BCB), a complete, free French databank on human
160 pharmaceuticals (<http://www.resip.fr>), the BIAM database (www.biam2.org), the drugs.com
161 drug database (www.drugs.com), the Micromedex Drugdex® databank (from Thomson
162 Micromedex, available at www.micromedex.com/products/drugdex), the Martindale
163 compendium's *Complete Drug Reference* (Martindale 2002), *Les progestatifs* (Rozenbaum,
164 2001) and the Goodman and Gilman's *The Pharmacological Basis of Therapeutics* (Hardman
165 et al. 1996). Special attention was paid to the metabolism and active metabolites of gestagens.
166 As highlighted in previous studies on pharmaceuticals, metabolism is one of the most
167 important processes that can reduce the quantities of pharmaceuticals reaching the aquatic
168 environment. Moreover, human metabolism can give rise to metabolites (Figure 2) that must
169 be considered when assessing the environmental risk (Besse et al., 2008; Huschek et al.,
170 2003). Finally, as highlighted above in the structure–activity relationships, modifications in
171 chemical structure can result in important modifications in activity, both quantitatively and
172 qualitatively.

173 Preliminary exposure assessment was implemented by calculating PECs, as described in
174 Besse et al. (2008), using the following equation:

175

$$176 \quad PEC_{surfacewater} = \frac{consumption \times F_{excreta} \times F_{stp}}{WW_{inhab} \times hab \times Dilution \times 365}$$

177

178 PEC is expressed in mg.l^{-1} using the following parameters: consumption is the quantity
 179 (mg.year^{-1}) of an active molecule consumed by the population over 1 year in a defined zone
 180 (generally a country); hab is the number of inhabitants and 100 the correction factor for the
 181 percentages; 365 is the number of days per year (day.year^{-1}); WW_{inhab} is the volume of
 182 wastewater per person per day (default value = $200 \text{ l. inhabitants}^{-1} \text{ day}^{-1}$); dilution is the
 183 dilution factor from WWTP effluent to surface waters (default value set at 10); $F_{excreta}$ is the
 184 excretion fraction of the active molecule; F_{stp} is the fraction of emission of the drug from
 185 wastewater treatment plants (WWTPs) directed to surface water, which can be defined as (1-
 186 WWTP removal fraction).

187 PEC gestagens were calculated using the actual amounts of progestogens provided by the
 188 French Medical Product Safety Agency (Agence Française de Sécurité Sanitaire des Produits
 189 de Santé, AFSSAPS, Paris).

190

191 **3. Results**

192

193 *3.1. Metabolism data*

194

195 Metabolism and pharmacokinetic data for progestins are limited (Stanczyk, 2003). No reliable
 196 excretion fraction value could be determined, except for cyproterone acetate (CPA). However,
 197 reviewing metabolism data highlighted very valuable information on the metabolites of
 198 gestagens, particularly progestins, that can help describe the hazard for the aquatic

199 environment. All gestagens are metabolized extensively following the same general pathway:
200 in the liver, they are mainly submitted to reduction and hydroxylation. Reduction generally
201 acts primarily on a double-bond of ring A then on the ketone function of carbon 3. These
202 structural modifications lead to metabolites that can be pharmacologically active but whose
203 activity differs from the parent compounds. The parent compound and metabolites can
204 subsequently be sulfo- and glucuroconjugated prior to excretion (Stanczyk, 2003;
205 Rozenbaum, 2001). Information on gestagen metabolites is summarized in Table 3 and
206 detailed in Appendix B. Although some metabolites with progestagenic properties are formed,
207 it should be noted that some metabolites have a significant *in vitro* estrogenic activity (Garcia-
208 Becerra et al., 2002; Larrea et al., 2001). This has been shown for metabolites of gestagens
209 structurally related to testosterone (Appendix B) and has a strong implication for the hazard
210 and risk assessment related to gestagens. For medrogestone, nomegestrol acetate,
211 promegestone, norgestrienone and norelgestromin, no data were found.

212

213 *3.2. PEC calculation and comparison with field measurements:*

214

215 Since no quantitative excretion data were available other than for CPA, the calculation of
216 PEC values for progestogens remains limited and only conservative PEC values, assuming no
217 metabolism, could be calculated. Moreover, since very few studies have been conducted on
218 progestagens in the environment, no data exist on the WWTP removal rates for these
219 molecules. Therefore, to limit the uncertainties, we calculated PEC values for the WWTP
220 influent, without considering the WWTP removal rate and dilution factor. The results are
221 displayed in Table 4. Since lynestrenol and norgestimate are prodrugs, their consumption
222 amounts were summed with the amounts of norethisterone (NET) and levonorgestrel (LNG),

223 their corresponding active metabolite, respectively, to calculate a more accurate PEC. PEC
224 values for progestins range from less than the ng.l^{-1} to the hundred ng.l^{-1} level.
225 Norelgestromin and norgestrienone show very low PEC values, even with conservative
226 assumptions; consequently, these two molecules are not expected to be present in the aquatic
227 environment. P4 showed a high PEC of more than $2 \mu\text{g/l}$; however, like all other gestagens,
228 P4 is submitted to extensive metabolism and only traces of the parent compound are excreted;
229 therefore, lower concentrations are expected in the aquatic environment. Moreover, it has
230 been shown that P4 was highly removed in WWTPs, with a high proportion sorbed on sludge.
231 (Esperanza et al., 2007).

232 Only a few studies on the occurrence of progestagens in the environment have been
233 conducted to date and LNG and NET are the main progestagens that have been investigated.
234 Kuch and Ballschmitter (2000) did not find NET acetate in WWTP effluents, whereas LNG
235 was found in only one sample, at the low concentration of 1 ng.l^{-1} . A study in French rivers
236 did not detect LNG or NET, (Labadie and Budzinski, 2005a). Another study (Solé et al.,
237 2000) did not reveal the presence of LNG, NET or progesterone in surface waters and WWTP
238 effluents. Other studies (Petrovic et al., 2002; Lopez de alda et al., 2002) report the presence
239 of NET, LNG and P4 in WWTP effluents or in sediment rivers (Table 5). Three very recent
240 studies also report the occurrence of gestagens in different environmental samples (Table 5):
241 P4 has been found in hospital and urban WWTP effluents in concentrations up to 33 ng.l^{-1}
242 (Pauwels et al., 2008); several gestagens have been measured (Chang et al., 2008) in the low
243 ng.l^{-1} range in effluents and surface waters; P4, LNG and NET have been found in surface
244 water and groundwater samples at the ng.l^{-1} range (Vulliet et al., 2008). Finally,
245 medroxyprogesterone acetate (MPA) was found in effluent samples (Kolodziej et al., 2003).
246 MPA was detected in four of eight effluents tested, at concentrations ranging from 1 ng.l^{-1} to

247 15 ng.l⁻¹. MPA was also detected at low concentrations (0.4–0.7 ng.l⁻¹) in wetland samples.
248 For MPA, the maximum effluent value of 15 ng.l⁻¹ found in one effluent sample (Kolodziej et
249 al., 2003) is equal to the conservative PECs determined for influents, suggesting that this
250 molecule could be excreted in significant amounts. Since PECs remain conservative because
251 of limited data, the calculated values are much higher than the range of the measured values
252 (i.e., ng.l⁻¹). This difference could be mainly related to extensive metabolism and low
253 excretion amounts of the unchanged parent molecule.

254

255 **4. Discussion:**

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257 *4.1. Concentrations entering the aquatic environment*

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259 Given the limited metabolism data, most particularly the lack of quantitative excretion data, it
260 was only possible to calculate conservative PECs, which limits their environmental relevance.
261 Although it was not possible to calculate the excretion fraction for gestagens, some evidence
262 (Verhoeven, 2001; Stanczyk, 1990; Vos, 2003) suggest that natural progesterone and some
263 progestins related to testosterone (i.e., LNG, NET, ETO and GTD) have excretion fractions of
264 a free and/or conjugate unchanged molecule ranging from about 5 to 10%. Applying this
265 excretion fraction range to calculated PECs gives refined values in the range of field
266 measurements. Field measurements from the study of Kolodziej et al. (2003) suggest that
267 MPA could be excreted in higher amounts as an unchanged molecule.
268 Some gestagen metabolites could be present at higher concentrations. Although no excretion
269 value could be calculated, there is some evidence in the literature for higher excretion rates
270 than parent compounds.

271 Concentrations of pregnanediol (main metabolite of P4) entering the aquatic environment
272 could be of environmental concern. Assuming a 15% excretion fraction value for this
273 compound (Grady et al., 1952; Sommerville and Marrian, 1950) gives a PEC for WWTP
274 influent of about 340 ng.l^{-1} . Pregnanediol is also physiologically excreted in urine, in daily
275 rates ranging from 1 mg for men to 70 mg for pregnant women (Hardman et al., 1996), giving
276 a cumulated excretion over a year of about 150 kg for France (data not shown). Added to the
277 assumed PEC levels described above, this gives concentrations entering WWTPs of about 370
278 ng.l^{-1} . This metabolite is reported to be inactive. Moreover, it has undoubtedly been present in
279 the environment for decades, and there is no evidence that any biological effect is related to it.
280 However, considering the rates of its release into the environment through WWTPs, which
281 should have considerably increased with the use of P4 in HRT, concerns are being raised and
282 studies should investigate the fate and toxicity of this molecule.

283 Other metabolites with estrogenic activity could be found in surface waters at higher levels
284 than parent compounds. Following an oral administration of NET (Stanczyk and Roy, 1990),
285 there was five times more $3\alpha\text{-}5\beta\text{-tetrahydro-NET}$ (free and conjugated) than NET (free and
286 conjugated) in urine. The same study showed that total urinary amounts of $3\alpha\text{-}5\beta\text{-tetrahydro-}$
287 LNG (free and conjugated) were 15 times higher than total amounts of LNG. Although there
288 is a need for more studies, especially to determine WWTP removal rates of these molecules,
289 these results indicate that wastewater effluent levels for $3\alpha\text{-}5\beta\text{-tetrahydro-NET}$ and $3\alpha\text{-}5\beta\text{-}$
290 tetrahydro-LNG could be up to 45 ng.l^{-1} and 240 ng.l^{-1} , respectively (by taking into account
291 maximum values for NET and LNG reported in Table 5 and assuming comparable removal
292 rates for parent compounds and metabolites). Therefore, there is evidence that most relevant
293 compounds according to the environmental exposure and biological activity have not been
294 targeted yet.

295

296 *4.2. Fate and behavior in the aquatic environment*

297

298 Except for P4, for which high removal rates in WWTPs and rapid degradation in surface
299 water are expected (Esperanza et al., 2007; Labadie and Budzinski, 2005b), no data are
300 available on the environmental half-lives and degradation pathways for gestagens. This is a
301 significant limitation for the environmental assessment of gestagens and limits the relevance
302 of the environmental concentrations of metabolites calculated above. It is not possible to draw
303 any conclusion on the degradability of these molecules, but the following assumptions can be
304 made. First, the degradability of synthetic progestins could be lower than for natural P4,
305 although this is an indirect assumption. Synthetic progestins are specifically designed to have
306 higher body half-lives than P4, which is rapidly inactivated. Moreover, a parallel can be
307 drawn between estrogens and gestagens. Synthetic ethinylestradiol (EE2) is reported to be
308 more resistant to bacterial biodegradation than natural estradiol (E2) (Ying et al., 2003;
309 Jürgens et al., 2002), whereas the photodegradation times are similar (Jürgens et al., 2002).
310 Resistance to bacterial degradation could be related to differences in stereochemistry and the
311 presence of a C17 ethinylated group in EE2. Similarly, synthetic progestins (and their
312 metabolites) could be more resistant to bacterial biodegradation, especially NET, LNG, TBL
313 and norgestimate, which also have an ethinylated function on C17 (Appendix A) but also
314 CPA, MPA and CMA, which display acetate groups on C17 (Appendix A). Second,
315 pharmaceuticals are often considered to be pseudo-persistent contaminants (Daughton, 2003),
316 because they are introduced in the aquatic environment on a continual basis through WWTPs.
317 Given that gestagens are used throughout the year for oral contraception and HRT, this
318 continuous release could balance a possible rapid degradation.

319 Concentrations of gestagens in sediment could be higher than those found in the water
320 column. This is suggested by the results of a study (Jenkins et al., 2003) in which P4 levels
321 found in sediment were 20 times higher than those found in the water column. Moreover, Kd
322 values for NET and P4 have been calculated (Lopez de alda et al., 2002) and the results
323 indicate that gestagens may have a general tendency to accumulate in sediments, but this
324 remains to be confirmed.

325

326 *4.3. Biological effects on aquatic species*

327

328 Several progesterone receptors have been found in fish (Pinter and Thomas, 1995; Todo et al.,
329 2000; Zhu et al., 2003) and gestagens play major roles in fish reproduction. They trigger
330 oocyte maturation (Lutes, 1985; Truscott et al., 1992) and ovulation in female fish (Scott et
331 al., 1983; Pinter and Thomas, 1999; Venkatesh et al., 1991). They also play a role in the
332 spermiation in males (Barry et al., 1990). Therefore, there is evidence that synthetic
333 progestins could interfere with endogen gestagens in fish and adversely affect their
334 reproduction. Moreover gestagens also act as pheromones in fish and are involved in the
335 control of spawning behavior (Kobayashi et al., 2002). At environmental concentrations,
336 gestagens could interfere with natural pheromones and therefore impair the physiological
337 responses and spawning behavior in fish (Kolodziej et al., 2003; Sorensen et al., 1990).
338 However, except for CPA, which was assessed for its anti-androgenic properties, no
339 ecotoxicological data in fish were found.

340 Very few data are available on the ecotoxicity of gestagens on aquatic invertebrates. A very
341 recent study, assessing the effects of estradiol, EE2 and MPA on the cladoceran *Ceriodaphnia*
342 *dubia*, showed no effect on reproduction at concentrations ranging from 5 µg.l⁻¹ to 5 mg.l⁻¹

343 (Jukowski et al., 2008). On the contrary, P4 was shown to induce the production of more
344 male-dominated broods in a 25-day assay at the concentration of $100 \mu\text{g.l}^{-1}$ (Kashian and
345 Dodson, 2004). Another study (Goto and Hiromi, 2003) determined a 48-h EC_{50} for NET on
346 immobilization on *Daphnia magna* of 6.41 mg.l^{-1} , which is very higher than environmental
347 concentrations. No chronic toxicity was observed for NET on the total number of offspring,
348 reproduction frequency, total number of male offspring, and total number of molts at tested
349 concentrations ($< 500 \mu\text{g.l}^{-1}$), but the results suggested that there was a synergistic effect
350 between EE2 and NET and the tested parameters.

351 No ecotoxicological data are available for metabolites of progestins; however,
352 pharmacological data i) show that some of these metabolites have a pharmacological activity
353 and ii) suggest that they could be found at higher concentrations than parent compounds in the
354 environment. Therefore, there is a potential for biological effects on non-target organisms.

355 The risk could be linked to mixture of gestagens. Gestagens used by humans are only found at
356 very low concentrations in aquatic samples; however, a number of different molecules (18 in
357 France) are used. Moreover, since parent molecules show a main similar mode of action, there
358 is a real risk of additive or synergistic effects in the environment. Finally, there is a risk of
359 synergistic effects between gestagens and estrogens, as shown for P4 and EE2 (Goto and
360 Hiromi, 2003) and as such effects have been shown in humans (Rozenbaum, 2001).

361

362 4.4. Hazard characterization:

363

364 Although ecotoxicological data are still too limited to provide information for the hazard
365 assessment of gestagens, the review of pharmacological data can give valuable hints on the
366 biological effects of gestagens and their metabolites. Surprisingly, the potential hazard posed

367 by these molecules may not be limited only to the progestagenic activity, but also to
368 estrogenic and to anti-androgenic activity. The different types of biological activities to the
369 different molecules are discussed here and are summarized in Figure 3. The hazard
370 characterization provided here is limited to aquatic species that display similar receptors to
371 those found in mammals; for other species, other effects (if any) should occur.

372

373 *Progestagenic activity.* The risk of progestagenic activity is obviously the first to consider.
374 Natural progesterone and parent progestins have a significant binding affinity and
375 transcriptional activity on the PR. Synthetic progestins have a higher activity than natural
376 progesterone. Dihydro metabolites of progestins have substantially less binding affinity to PR
377 than the parent compound but within the same range as progesterone. This has been shown *in*
378 *vitro* for GES, NES and LNG (Larrea et al., 2001; Lemus et al., 2000; García-Becerra et al.,
379 2002). Some metabolites that can be excreted in higher amounts than parent compounds also
380 have a significant activity: DHD, 17-hydroxyprogesterone and 20 α -dihydroprogesterone, Δ 4-
381 tibolone, and to a lesser extent, reduced metabolites. Therefore, there is a potential hazard of
382 progestagenic activity in the environment. Considering the low measured concentrations in
383 the environment, the risk posed by progestagenic activity could be mainly related to the
384 mixture of gestagens and their metabolites.

385 A very recent study (Van der Linden et al., 2008) used several modified CALUX assays to
386 detect hormonal activities in water samples. These authors showed that there were multiple
387 hormonal activities in effluent samples. Progestagenic activity was found in only half of the
388 samples, whereas estrogenic activity and glucocorticoid activity was found in all the samples.
389 Higher progestagenic activity was found in an industrial effluent sample than in municipal
390 effluents, suggesting that the contribution of gestagens used by humans to the aquatic

391 contamination is not the major one. No activity was found in paper mill effluent, whereas a
392 previous study (Jenkins et al., 2003) detected natural progesterone in sediment and surface
393 waters exposed to paper mill effluent in concentrations 100 times higher than at a reference
394 site. No progestagenic activity was found in hospital effluents, perhaps because most of the
395 progestagen consumption is used for hormone replacement therapy or contraceptives and is
396 therefore used outside hospitals. Nevertheless, P4 has been found in hospital WWTPs
397 (Pauwels et al., 2008).

398 PR activity was only found in a small stream but not in river waters (Van der Linden et al.,
399 2008). This result suggests the absence or at least very low concentrations of gestagens in
400 surface waters. There is a need for more studies since this result may stem from several
401 factors: i) the low half-life for gestagens, as has been shown for P4 (Labadie and Budzinski,
402 2005b); ii) the sorption of gestagens in sediment or suspended matter (Lopez de alda et al.,
403 2002; Esperanza et al., 2007); and iii), a lack of sensitivity of the ER CALUX assay. The
404 reference compound used was org2058, reported to be 13 times more potent than
405 progesterone in this assay. Given that most gestagen metabolites have a progestagenic activity
406 similar to or lower than progesterone (Appendix B), it is possible that the use of org2058 as
407 the reference compound is not sensitive enough to detect an environmental progestagenic
408 activity.

409

410 *Estrogenic activity.* Progestins derived from testosterone appear to transform into
411 tetrahydroderivatives, which present an estrogenic activity. This had been shown *in vitro* for
412 GSD (Larrea et al., 2001; Lemus et al., 2000), NET (Larrea et al., 2001) and LNG, although
413 there are some contradictory results on this last molecule (Garcia-Becerra et al., 2002; Lemus
414 et al., 1992). The 3 β -5 α -tetrahydroderivatives from NET, GES and LNG have been shown to

415 bind with the α subunit of the ER and displayed *in vitro* transcriptional activities through the
416 $ER\alpha$ equivalent to about 90% of that of estradiol for tetrahydro-NET and tetrahydro-GES
417 (Larrea et al., 2001) and equivalent to about 80% for tetrahydro-LNG. Such derivatives are
418 structurally close to ethinylestradiol (Appendix A). Dihydroderivatives also display, but to a
419 lesser extent, an estrogenic activity through $ER\alpha$ (Larrea et al., 2001; Garcia-Becerra et al.,
420 2002). Moreover, TBL is metabolized into two main metabolites that are known to exhibit
421 estrogenic activity: 3α and 3β -hydroxy-TBL (Schindler et al., 2003, Rozenbaum, 2001).

422 Therefore, there is evidence that some gestagen metabolites could act as estrogenic
423 compounds in the aquatic environment. The environmental risk of estrogenic disruption
424 remains putative as *in vivo* human tetrahydro-isomers (3α - 5β , major isomer) are different
425 from those that have been tested *in vitro* (3β - 5α form). Since there can be differences in the
426 estrogenic activity of isomers (Larrea et al., 2001; Lemus et al., 2000), there is a need to
427 clearly assess the estrogenic activity of 3α - 5β -tetrahydroderivatives. Nevertheless, since NES
428 and GES are reported to have estrogenic activity (Table 1), there is strong evidence that their
429 metabolites display this activity.

430 As discussed above, concentrations of these metabolites entering the aquatic environment
431 could be higher than those of parent compounds; therefore, there is a potential risk of
432 estrogenic activity. They have a similar mode of action to estrogen: consequently,
433 tetrahydroderivatives and to a lesser extent, dihydroderivative metabolites from progestins
434 derived from testosterone are likely to contribute to an estrogenic risk for the aquatic
435 environment, by cumulative effect with each other and/or with other natural or xenoestrogens
436 such as ethinylestradiol.

437

438 *Anti-androgenic activity.* A risk of anti-androgenic activity cannot be excluded. CPA, CMA
439 and DSP are progestins that have an anti-androgenic activity. Hydroxylated metabolites of
440 CPA and CMA are reported to be anti-androgenic as well, so there is a possibility that the
441 mixture of these molecules is present in the aquatic environment in sufficiently high
442 concentrations to elicit anti-androgenic responses in non-target organisms. Exposure of snails
443 to nominal concentrations of 1.25 mg/l of CPA resulted in reduced length of male sex organs
444 (Tillmann et al., 2001); however, the authors concluded that anti-androgens could be of lesser
445 concern for snails compared with estrogens and androgens. The results of a study on exposure
446 of Japanese medaka to anti-androgens (Kiparissis et al., 2001) concluded that CPA had the
447 potential to alter testicular development and gametogenesis in fish. However, tested
448 concentrations were in the $\mu\text{g.l}^{-1}$ range, higher than what is expected in the aquatic
449 environment for CPA. In a 7-day experiment, exposure of fish to 250 ng.l^{-1} CPA resulted in
450 decreasing circulating levels of estradiol and testosterone (Sharpe et al., 2003). In the same
451 study, a significant decrease in plasma testosterone levels in males exposed to 100 ng.l^{-1} and
452 10 ng.l^{-1} in females was observed after 14 days. Vitellogenin plasma levels were not affected
453 by CPA exposure at the tested concentrations. Since the excretion fraction of unchanged CPA
454 are reported to range from 5 to 20% (Appendix B), the influent PEC ranges from 10 to 40
455 ng.l^{-1} , a comparable concentration that elicited effects in fish. Concentrations of CPA in
456 surface waters could be lower, depending on WWTP removal rates and degradation time. On
457 the other hand, other anti-androgenic compounds such as CMA and hydroxylated metabolites
458 of CMA and CPA could act additively. There is a potential risk of anti-androgenic activity
459 related to gestagens, although there is a need for more studies.

460

461 *4.5. Selection of relevant gestagens according to the environmental concern*

462

463 In previous work on pharmaceuticals (Besse and Garric 2008), a prioritization was conducted
464 on in order to identify the compounds of high concern for the environment. The prioritization
465 strategy previously implemented was not applicable to gestagens mainly because of the lack
466 of a reliable excretion fraction. Theoretically, it could be possible to rank gestagens and their
467 metabolites by comparing their consumption amounts and their relative binding affinities to
468 PR and ER, but even in this case, the available data are too limited and heterogeneous to
469 classify them accurately.

470 Nevertheless, it is possible to target a few compounds for which occurrence studies and
471 ecotoxicological assays should be implemented firstly, to assess the risk of endocrine
472 disruption, in accordance with each respective type of risk (i.e., progestagenic, estrogenic and
473 anti-androgenic). This risk of endocrine disruption for aquatic species is based on the
474 biological activity observed in mammals, therefore, it is limited to fishes and possibly to
475 invertebrates that display similar steroid receptors than in mammals.

476 MPA is the parent progestin that has been found in the highest amounts (Kolodziej et al.,
477 2003); therefore, other occurrence and toxicity studies should be directed toward MPA.

478 Occurrence studies should also be conducted on DHG, which is the third-ranked molecule in
479 terms of consumption amounts (Table 5). As DHG is mainly metabolized in the active DHD,
480 occurrence studies should also investigate this last molecule.

481 Tetrahydro-metabolites of LNG, GSD and NET, and the metabolites of TBL, are likely to
482 exert an estrogenic activity. Since GSD is used in low amounts (Table 5), concentrations of
483 this compound and its metabolites entering the environment are expected to be negligible. On
484 the contrary, occurrence and ecotoxicological studies should focus on $3\alpha,5\beta$ tetrahydro-
485 metabolites of LNG and NET and on 3α hydroxylated metabolite of TBL.

486 CPA, CMA and their hydroxy metabolites should be tested for occurrence and toxicity
487 because they display a significant anti-androgenic activity.
488 Given that pregnanediol could be excreted in significant amounts, it should be tested for
489 ecotoxicity and occurrence.
490 Finally, for all the molecules cited above, degradation studies are needed to determine their
491 removal rates in WWTPs and their fate in the aquatic environment (sorption to sediment and
492 degradation time).

493

494 **Conclusion**

495

496 This study was the first one conducted on the environmental risk of gestagens used by
497 humans. Although it was not possible to conclude on the environmental risk due to limited
498 data on the fate of these molecules, it was possible to assess the hazard and the biological
499 effects of gestagens and their metabolites and also to target relevant metabolites for further
500 studies. The following conclusions, which remain to be confirmed, can be drawn.

501

- 502 • Synthetic progestins are expected to be found in effluent samples and possibly in
503 surface waters, mainly as metabolites, in concentrations possibly in the ng.l^{-1} and even
504 the 100 ng.l^{-1} range.
- 505 • Residual parent compounds and some active metabolites with progestagenic activity
506 could interfere with spawning behavior in fish.
- 507 • Metabolites of progestins derived from nortestosterone can act as estrogenic
508 compounds and therefore may act additively with other xenoestrogens such as
509 ethinylestradiol.

- 510 • Some progestins such as chlormadinone acetate and cyproterone acetate and their
511 metabolites have anti-androgenic properties and may pose a risk for aquatic species.
- 512 • Taken separately, progestins might not present a risk for the aquatic environment;
513 however, since they are expected be found in the environment as mixtures, there is a
514 risk of additive or even synergistic effects.

515

516 Therefore, as several other authors (Sumpter 2005; Johnson et al., 2008), we consider that
517 synthetic gestagens merit more attention than they have received to date. There is a need to
518 conduct other studies to clearly assess the risk for the aquatic environment, notably
519 occurrence studies and ecotoxicological assays for metabolites and degradation tests for some
520 parent gestagens and metabolites.

521

522

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524

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527 **References**

528

529 AFSSAPS 2006. Agence Française de Sécurité Sanitaire des Produits de Santé (French
530 Medical Product Safety Agency). Personal communication.

531

532 Barry, T.P., Santos, A.J.G., Furukawa, K., Aida, K., Hanyu, I. 1990. Steroid profiles
533 during spawning in male common carp. *Gen. Comp. Endocrinol.* 80, 223-231.

534

535 BCB 2008. Banque Claude Bernard. Available at <http://www.resip.fr>

536

537 Besse, J.-P., Kausch-Barreto, C., Garric, J., 2008. Exposure assessment of
538 pharmaceuticals and their metabolites in the aquatic environment: Application to the
539 French situation and preliminary prioritization. *J. Human Ecol. Risk Assess.* 14, 665-
540 695.

541

542 Besse, J.-P., Garric, J., 2008. Human pharmaceuticals in surface waters. Implementation
543 of a prioritization methodology and application to the French situation. *Toxicol. Lett.*
544 176, 104-123.

545

546 BIAM, 2006. Banque de données automatisée sur les médicaments.

547 <http://www.biam2.org>

548

549 Chang, H., Wu, S., Hu, J., Asami, M., Kunikane, S., 2008. Trace analysis of androgens
550 and progestogens in environmental waters by ultra-performance liquid chromatography-
551 electrospray tandem mass spectrometry. *J. Chromato A* 1195, 44-51.

552

- 553 Colborn, T., Vom Saal, F.S., Soto, A.M., 1993. Developmental effects of endocrine-
554 disrupting chemicals in wildlife and humans. *Environ. Health Perspec.* 101, 378-384.
555
- 556 D'Ascenzo, G., Di Corcia, A., Gentili, A., Mancini, R., Mastropasqua, R., Nazzari, M.,
557 Samperi, R. 2003. Fate of natural estrogen conjugates in municipal sewage transport
558 and treatment facilities. *Sci. Total Environ.* 302,199–209
559
- 560 Daughton C.G., 2003. Cradle-to-cradle stewardship of drugs for minimizing their
561 environmental disposition while promoting human health. I. Rational for and avenues
562 toward a green pharmacy. *Environ. Health Perspec.* 111, 757-774.
563
- 564 Drugdex©, 2008. Thomson Micromedex©. Healthcare series. <http://www.micromedex.com/products/drugdex/> Last accessed November 2008.
565
566
- 567 Drugs.com, 2006. Prescription Drug Information, Side Effects, Interactions. Available
568 at <http://www.Drugs.com>
569
- 570 Esperanza, M., Suidan, M.T., Marfil-Vega, R., Gonzalez, C., Sorial, G.A., McCauley,
571 P., Brenner, R., 2007. Fate of sex hormones in two pilot-scale municipal wastewater
572 treatment plants: Conventional treatment. *Chemosphere* 66, 1535-1544.
573
- 574 García-Becerra, R., Borja-Cacho, E., Cooney, A.J., Jackson, K.J., Lemus, A.E., Pérez-
575 Palacios, G., Larrea, F., 2002. The intrinsic transcriptional estrogenic activity of a non-
576 phenolic derivative of levonorgestrel is mediated via the estrogen receptor- α . *J Steroid*
577 *Biochem. Mol. Biol.* 82, 333-341.

578

579 Goto, T., Hiromi, J. 2003. Toxicity of 17 α -ethynylestradiol and norethindrone,
580 constituents of an oral contraceptive pill to the swimming and reproduction of
581 cladoceran *Daphnia magna*, with special reference to their synergetic effect . Marine
582 Poll. Bull. 47, 139-142.

583

584 Grady, H.J., Elliott, W.H., Doisy, E.A. Jr., Bocklage B.C., Doisy E.A., 1952. Synthesis
585 and metabolic studies of Progesterone-21-C14*. J Biol. Chem. 195, 755-762.

586

587 Halling-Sørensen, B., Nors Nielsen, S., Lanzky, P.F., Ingerslev, F., Holten-Lützhøft,
588 H.C., Jørgensen, S.E., 1998. Occurrence, fate and effects of pharmaceutical substances
589 in the environment- A review. Chemosphere 36, 357-393.

590

591 Hardman JG, Limbird LE, Molinoff PS, Ruddon RW, Goodman AG. (eds), 1996.
592 Goodman and Gilman's The Pharmacological Basis of Therapeutics. 9th edition New
593 York, NY: McGraw-Hill Professional.

594

595 Huschek G., Hansen P.D., Maurer H.H., Kregel D., Kayser A., 2004. Environmental
596 risk assessment of medicinal products for human use according to European
597 Commission recommendations. Environ Toxicol 19, 226-240.

598

599 Jamin, C., 2003. Comment classer les progestatifs en 2003? XXIV^{èmes} Journées AFEM.
600 Available at <http://www.menopauseafem.com/doc/ppt/528.ppt>.

601

- 602 Jenkins, R.L., Wilson, E.M., Angus, R.A., Howell, W.M., Kirk, M., 2003.
603 Androstenedione and progesterone in the sediment of a river receiving paper mill
604 effluent. *Toxicol. Sci.* 73, 53-59.
605
- 606 Johnson, A.C., Ternes, T., Williams, R.J., Sumpter, J.P. 2008. Assessing the
607 concentrations of polar organic microcontaminants from point sources in the aquatic
608 environment: Measure or model? *Environ. Sci. Technol.* 42, 5390-5399
609
- 610 Johnson, A.C., Williams, R.J., 2004. A model to estimate influent and effluent
611 concentrations of estradiol, estrone, and ethinylestradiol at sewage treatment works.
612 *Environ. Sci. Technol.* 38, 3649-3658.
613
- 614 Jukosky, J.A., Watzin, M.C., Leiter, J.C., 2008. Elevated concentrations of
615 ethinylestradiol, 17 β -estradiol, and medroxyprogesterone have little effect on
616 reproduction and survival of *Ceriodaphnia dubia*. *Bull. Environ. Contam. Toxicol.* 81,
617 230-235.
618
- 619 Jürgens, M.D., Holthaus, K.I.E., Johnson, A.C., Smith, J.J.L., Hetheridge, M., Williams,
620 R.J., 2002. The potential for estradiol and ethinylestradiol degradation in English rivers.
621 *Environ. Toxicol. Chem.* 21, 480-488.
622
- 623 Kashian, D.R., Dodson, S.I. 2004. Effects of vertebrate hormones on development and
624 sex determination in *Daphnia magna*. *Environ. Toxicol. Chem.* 23, 1282-1288.
625

- 626 Kiparissis, Y., Metcalfe, T.L., Balch, G.C., Metcalfe, C.D., 2003. Effects of the
627 antiandrogens, vinclozolin and cyproterone acetate on gonadal development in the
628 Japanese medaka (*Oryzias latipes*). *Aquatic Tox.* 63, 391-403.
629
- 630 Kobayashi, M., Sorensen, P.W., Stacey, N.E. 2002. Hormonal and pheromonal control
631 of spawning behavior in the goldfish. *Fish Physiol Biochem* 26, 71-84.
632
- 633 Kolodziej, E.P., Gray, J.L., Sedlak, D.L., 2003. Quantification of steroid hormones with
634 pheromonal properties in municipal wastewater effluent. *Environ. Toxicol. Chem.* 22,
635 2622-2629
636
- 637 Kolpin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D., Barber, L.B.,
638 Buxton, H.T., 2002. Pharmaceuticals, hormones, and other organic wastewater
639 contaminants in U.S. streams, 1999-2000: A national reconnaissance. *Environ. Sci.*
640 *Technol.* 36, 1202-1211.
641
- 642 Kuch, H.M., Ballschmiter, K., 2000. Determination of endogenous and exogenous
643 estrogens in effluents from sewage treatment plants at the ng/L-level. *Fresenius J. Anal.*
644 *Chem.* 366, 392-395.
645
- 646 Kuhl, H., 1996. Comparative pharmacology of newer progestogens. *Drugs* 51, 188-215.
647
- 648 Kümmerer, K. 2004. *Kümmerer, Pharmaceuticals in the Environment* (2nd ed.),
649 Springer-Verlag.
650

651 Larrea, F., García-Becerra, R., Lemus, A.E., García, G.A., Pérez-Palacios, G., Jackson,
652 K.J., Coleman, K.M., Dace, R., Smith C.L., Cooney, A.J., 2001. A-ring reduced
653 metabolites of 19-nor synthetic progestins as subtype selective agonists for $E\alpha$.
654 *Endocrinology* 142, 3791-3799.

655

656 Labadie, P., Budzinski, H., 2005a. Determination of steroidal hormone profiles along
657 the Jalle d'Eysines River (near Bordeaux, France). *Environ. Sci. Technol.* 39, 5113-
658 5120.

659

660 Labadie, P., Budzinski, H., 2005b. Development of an analytical procedure for
661 determination of selected estrogens and progestagens in water samples. *Anal. Bioanal.*
662 *Chem.* 381, 1199-1205.

663

664 Lemus, A.E., Zaga, V., Santillán, R., García, G.A., Grillasca, I., Damián-Matsumura, P.,
665 Jackson, K.J., Cooney, A.J., Larrea, F., Pérez-Palacios, G., 2000. The oestrogenic
666 effects of gestodene, a potent contraceptive progestin, are mediated by its A-ring
667 reduced metabolites. *J Endocrinol.* 165, 693-702.

668

669 Lemus, A.E., Vilchis, F., Damsky, R., Chavez, B.A., Garcia, G.A., Grillasca, I., Perez-
670 Palacios, G., 1992. Mechanism of action of levonorgestrel: In vitro metabolism and
671 specific interactions with steroid receptors in target organs. *J Steroid Biochem. Mol.*
672 *Biol.* 41, 881-890.

673

- 674 López de Alda, M.J., Gil, A., Paz, E., Barceló, D., 2002. Occurrence and analysis of
675 estrogens and progestogens in river sediments by liquid chromatography-electrospray-
676 mass spectrometry. *Analyst* 127, 1299-1304.
677
- 678 Lutes, P.B. 1983. Oocyte maturation in white sturgeon, *Acipenser transmontanus*: some
679 mechanisms and applications. *Environ. Biol. Fish.* 14, 87-92.
680
- 681 Martindale. The complete drug reference. 2002. 33 Ed. Sean C Sweetman Ed.
682 Pharmaceutical Press. Great Britain.
683
- 684 Panter, G.H., Thompson, R.S., Beresford, N., Sumpter, J.P. 1999. Transformation of a
685 non-oestrogenic steroid metabolite to an oestrogenically active substance by minimal
686 bacterial activity. *Chemosphere* 38, 3579-3596.
687
- 688 Pauwels, B., Noppe, H., De Brabander, H., Verstraete, W., 2008. Comparison of steroid
689 hormone concentrations in domestic and hospital wastewater treatment plants. *J*
690 *Environ. Eng.* 134, 933-936.
691
- 692 Petrovic, M., Solé, M., López de Alda, M.J., Barceló, D., 2002. Endocrine disruptors in
693 sewage treatment plants, receiving river waters, and sediments: Integration of chemical
694 analysis and biological effects on feral carp. *Environ. Toxicol. Chem.* 21, 2146-2156.
695
- 696 Pharmacorama 2008. Pharmacorama, connaissance des médicaments. Available at
697 www.pharmacorama.com
698

- 699 Pinter, J., Thomas, P. 1999. Induction of ovulation of mature oocytes by the maturation-
700 inducing steroid 17,20 β ,21-trihydroxy-4-pregnen-3-one in the spotted seatrout. Gen.
701 Comp. Endocrinol. 115, 200-209.
702
- 703 Pinter, J., Thomas, P. 1995. Characterization of a progestogen receptor in the ovary of
704 the spotted seatrout, *Cynoscion nebulosus*. Biol. Reprod. 52, 667-675.
705
- 706 Raudrant, D., Rabe, T. 2003. Progestogens with antiandrogenic properties. Drugs 63,
707 463-492.
708
- 709 Rozenbaum, H., 2001. Les progestatifs. 2nd edition. ESKA, Paris.
710
- 711 Schindler, A.E., Campagnoli, C., Druckmann, R., Huber, J., Pasqualini, J.R., Schweppe,
712 K.W., Thijssen, J.H.H., 2003. Classification and pharmacology of progestins. Maturitas
713 46 (SUPPL. 1), S7-S16.
714
- 715 Sharpe, R.L., MacLatchy, D.L., Courtenay, S.C., Van Der Kraak, G.J., 2004. Effects of
716 a model androgen (methyl testosterone) and a model anti-androgen (cyproterone
717 acetate) on reproductive endocrine endpoints in a short-term adult mummichog
718 (*Fundulus heteroclitus*) bioassay. Aquatic Tox. 67, 203-215.
719
- 720 Sitruk-Ware, R., 2006. New progestagens for contraceptive use. Human Reproduction
721 Update 12, 169-178.
722

- 723 Schorderet M., 1998. Pharmacologie : Des concepts fondamentaux aux applications
724 therapeutiques" 3rd Ed. Frison roche. France.
725
- 726 Scott, A.P., Sumpter, J.P., Hardiman, P.A. 1983. Hormone changes during ovulation in
727 the rainbow trout (*Salmo gairdneri* Richardson). Gen. Comp. Endocrinol. 49, 128-134
728
- 729 Sommerville I.F., Marrian G.F., 1950. Urinary excretion of Pregnanediol* in human
730 subjects following the administration of Progesterone and of Pregnane-3a:20a-diol.
731 Biochem. J. 46. 255-289. Available at www.biochemj.org/bj/046/0285/0460285.pdf.
732
- 733 Sorensen, P.W., Hara, T.J., Stacey, N.E., Dulka, J.G., 1990. Extreme olfactory
734 specificity of male goldfish to the preovulatory steroidal pheromone 17a,20b-
735 dihydroxy-4-pregnen-3-one. J Comp. Physiol. Sens. Neural. Behav. Physiol. A 166,
736 373–383.
737
- 738 Stanczyk, F.Z., 2003. All progestins are not created equal. Steroids 68, 879-890.
739
- 740 Stanczyk, F.Z., 1996. Introduction: Structure-function relationships, metabolism,
741 pharmacokinetics and potency of progestins. Drugs of Today 32 (SUPPL. H), 1-14.
742
- 743 Stanczyk, F.Z., Roy, S., 1990. Metabolism of levonorgestrel, norethindrone, and
744 structurally related contraceptive steroids. Contraception 42, 67-96.
745
- 746 Sumpter, J.P., 2005. Endocrine disrupters in the aquatic environment: An overview.
747 Acta Hydrochem. Hydrobiol. 33, 9-16.

748

749 Ternes, T.A. 1998. Occurrence of drugs in German sewage treatment plants and rivers.
750 Water Res. 32, 3245–60.

751

752 Ternes, T.A., Kreckel, P., Mueller, J. 1999. Behaviour and occurrence of estrogens in
753 municipal sewage treatment plants—II. Aerobic batch experiments with activated
754 sludge. Sci. Total Environ. 225, 91–9.

755

756 Tillmann, M., Schulte-Oehlmann, U., Duft, M., Markert, B., Oehlmann, J., 2001.
757 Effects of endocrine disruptors on prosobranch snails (Mollusca: Gastropoda) in the
758 laboratory. Part III: Cyproterone acetate and vinclozolin as antiandrogens.
759 Ecotoxicology 10, 373-388

760

761 Todo, T., Ikeuchi, T., Kobayashi, T., Kajiura-Kobayashi, H., Suzuki, K., Yoshikuni, M.,
762 Yamauchi, K., Nagahama, Y., 2000. Characterization of a testicular $17\alpha,20\beta$ -
763 dihydroxy-4-pregnen-3-one (a spermiation-inducing steroid in fish) receptor from a
764 teleost, Japanese eel (*Anguilla japonica*). FEBS Letters 465, 12-17.

765

766 Truscott, B., So, Y.P., Nagler, J.J., Idler, D.R. 1992. Steroids involved with final oocyte
767 maturation in the winter flounder. J. Steroid Biochem. Mol. Biol. 42, 351-356.

768

769 Van Der Linden, S.C., Heringa, M.B., Man, H.-Y., Sonneveld, E., Puijker, L.M.,
770 Brouwer, A., Van Der Burg, B., 2008. Detection of multiple hormonal activities in
771 wastewater effluents and surface water, using a panel of steroid receptor CALUX
772 bioassays. Environ. Sci. Technol. 42, 5814-5820.

773

774 Venkatesh, B., Tan, C.H., Lam, T.J. 1991. Progestins and cortisol delay while estradiol-
775 17β induces early parturition in the guppy, *Poecilia reticulata*. *Gen. Comp. Endocrinol.*
776 83, 297-305.

777

778 Verhoeven, C.H.J, Gloudemans, R.H.M., Peeters, P.A.M., Van, J.J., Verheggen,
779 F.T.M., Groothuis, G.M.M., Rietjens, I.M.C.M., Vos, R.M.E., 2001. Excretion and
780 metabolism of desogestrel in healthy postmenopausal women. *J Steroid Biochem. Mol.*
781 *Biol.* 78, 471-480.

782

783 Vos, R.M.E., Krebbers, S.F.M., Verhoeven, C.H.J., Delbressine, L.P.C., 2002. The in
784 vivo human metabolism of tibolone. *Drug Metab. Dispos.* 30, 106-112.

785

786 Ying, G.-G., Kookana, R.S., Dillon, P., 2003. Sorption and degradation of selected five
787 endocrine disrupting chemicals in aquifer material. *Water Res.* 37, 3785-3791.

788

789 Zhu, Y., Bond, J., Thomas, P., 2003. Identification, classification, and partial
790 characterization of genes in humans and other vertebrates homologous to a fish
791 membrane progestin receptor. *Proc. Natl. Acad. Sci.* 100, 2237-2242.