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1 Invertebrates facing environmental contamination by endocrine disruptors: novel evidences and 2 recent insights

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11 **Abstract**

12 The crisis of biodiversity we currently experience raises the question of the impact of anthropogenic chemicals
13 on wild life health. Endocrine disruptors are notably incriminated because of their possible effects on
14 development and reproduction, including at very low doses. As commonly recorded in the field, the burden they
15 impose on wild species also concerns invertebrates, with possible specificities linked with the specific physiology
16 of these animals. A better understanding of chemically-mediated endocrine disruption in these species has
17 clearly gained from knowledge accumulated on vertebrate models. But the molecular pathways specific to
18 invertebrates also need to be reckoned, which implies dedicated research efforts to decipher their basic
19 functioning in order to be able to assess its possible disruption. The recent rising of omics technologies opens
20 the way to an intensification of these efforts on both aspects, even in species almost uninvestigated so far.

21 **Keywords:** endocrine disruption; endocrine disrupting chemicals (EDCs); nuclear receptors; oestrogeno-
22 mimetics; ecdysteroids ; Juvenile hormone; omics; mollusks; annelids; arthropods

23

24 **1. Introduction**

25 Anthropogenic pollutants are seen as one of the main causes concurring to the ongoing collapse of global
26 biodiversity. Among them endocrine disrupting chemicals (EDCs) pervade virtually all ecosystems on Earth. They
27 are largely reported in marine as well as freshwater environments, notably since actual treatment systems fail to
28 completely remove them from wastewater (*e.g.* Aris et al., 2014; Huang et al., 2019; Salgueiro-González et al.,
29 2015). They also complex to sediments where they can accumulate and persist, sometimes leading to
30 permanent release in the milieu following soil erosion. At last, many EDCs (notably phthalates, polychlorinated
31 biphenyl (PCBs), polycyclic aromatic hydrocarbons (PAHs), brominated flame retardants, dioxins, alkylphenols,
32 perfluorinated chemicals (PFCs) and also some pesticides) have been shown to be present in the atmosphere at
33 worrying concentrations (Annamalai and Namasivayam, 2015), and maybe conveyed by the microplastic
34 particles recently detected in the atmospheric compartment (Dris et al., 2016). Although they belong to various
35 classes of synthetic and natural compounds, the common feature of EDCs lays in that they can interfere at
36 different levels of the endocrine system of animals, disrupting physiological, biochemical and /or molecular
37 processes that control development, growth or reproduction; recent works add neurological and immune
38 processes to the list (Jones et al., 2017). Toxicological studies have uncovered multiple effects of EDCs, many of
39 which are hard to predict due to non-monotonic dose-response, frequent cocktail effects and transgenerational
40 implications (Flint et al., 2012; Skinner, 2014; Vandenberg et al., 2012; Xin et al., 2015; Z. Xu et al., 2017). Beyond
41 actual concerns as regards human health, the large spread of these molecules, through all ecosystems, from

42 Arctic ices to primitive rainforests, also raises concern about the effects of such contamination on animal health
43 and, ultimately, on worldwide biodiversity (Lenoir et al., 2016; Lyons, 2006; Sonne, 2010). In this respect,
44 studying endocrine disruption in invertebrate species is meaningful. First, from an anthropocentric point of view,
45 invertebrates are tremendous tools to detect and quantify the presence of EDCs in natural substrates, and thus
46 may serve as useful bioindicators. They also allow us to gain knowledge about EDC toxicological properties at the
47 scale of the individual's lifetime, which is much more difficult with vertebrate models (Hutchinson, 2007).
48 Besides, due to their small size which make them easy to handle and stock in the laboratory, and to the large
49 quantity of material they provide when rearing is possible, they represent a convenient and quick option to set
50 up multigenerational assays to investigate potential transgenerational effects of EDCs. Second and most
51 importantly, from an ecological point of view, invertebrates represent a huge part of worldwide biodiversity and
52 fulfill many crucial ecological roles in every ecosystem (Wilson, 2006); this implies that their present decline is a
53 clear worrying issue that requires to be handled urgently.

54 Because of their specific physiology (that diverged from that of vertebrates more than 600 MA ago) it appears
55 necessary to develop and use invertebrate models allowing to detect and predict the whole set of effects of
56 environmental EDCs on invertebrates wild populations. Contamination routes are likely multiple, with
57 proportions depending on species' life history, and often different from that of vertebrates. In particular,
58 because of their small size and thus relatively high surface/volume ratio compared to vertebrates, invertebrates
59 suffer from higher relative exposure levels, which can imply a higher bioaccumulation, or the need to develop
60 greater excretion capacities. In the aquatic environment, absorption coefficients may be higher compared to
61 vertebrate species due to the properties of invertebrates body wall, which is often a functional surface of
62 exchange, for respiratory gases for instance. As regards terrestrial invertebrates, direct contact and oral route
63 are so far considered as the most probable ways of contamination. However, the possible role of arthropods
64 cuticle as a trap for atmospheric pollutants just begins to be studied. It could constitute an effective way of
65 contamination disregarded so far (Lenoir et al., 2014, 2012). Noteworthy, invertebrates generally lack the blood-
66 gonad barrier that provide some protection to reproductive organs in mammals. At last, as for fishes and
67 amphibians, mature gametes are in most cases directly released in the environment, which implies an exposure
68 of organisms all along the life cycle – from gametogenesis to adulthood – including the highly vulnerable first
69 stages of development. In short, apprehending the consequences of invertebrates exposure to EDC implies to
70 take into account many of their peculiarities, and specific researches are required in this field, which is reflected
71 by the growing body of literature dedicated to the subject.

72 In this review, we will first try to gauge the burden environmental EDCs place on invertebrate populations in
73 nature, focusing as much as possible on studies that evaluate disruption in wild caught animals, exposed to EDCs
74 in real situations. Then, we will produce an overview of the most recent molecular insights that may enlighten
75 our comprehension of endocrine disruption in invertebrates. Finally, we will discuss the contribution of the
76 recent approaches that rely on omics, to get a wider view of EDCs impact on invertebrate physiology. Note that
77 the vast majority of our current knowledge relates to protostome species (mainly arthropods, mollusks and
78 annelids), which explains that the present review will mainly focus on this taxonomic group.

79 80 **2. Delineating the burden of endocrine disruption on wild invertebrates**

81 Risk assessment procedures have been using invertebrate models (mainly aquatic species) for long, both to
82 detect potential EDC activities in mixtures, and to gain a basic understanding of the ecological effects of known
83 EDCs. Conversely, field studies demonstrating EDCs effects on invertebrate wild populations and communities
84 are scarcer, in aquatic as well as in terrestrial ecosystems (Amiard and Amiard-Triquet, 2015).

85 **2.1 In aquatic ecosystems**

86 In aquatic ecosystems, the most relevant case certainly remains the imposex phenomenon that affects
87 gastropod mollusks living in areas contaminated by organotin: tributyltin [TBT] and triphenyltin [TPT], at levels
88 as low as few ng per liter (DeFur, 1999). These organometallic compounds have been used as antifouling agents

89 for several decades until their progressive ban by most countries, in the 80s. However, incomplete observation
90 of worldwide legislation, as well as organotins desorption from contaminated sediments explain that organotins
91 contamination is still a major toxicological concern (Laranjeiro et al., 2018). At the individual level, endocrine
92 disruptive effects in contaminated areas result in the imposition of male sex characteristics on female snails,
93 with a clear relationship between the extent of the masculinization process and the dose of organotins detected
94 in the environment (Graceli et al., 2013). At some point, affected females suffer from impaired reproduction
95 ending up to sterility and even to death (DeFur, 1999). Individual defects due to environmental contamination
96 thus translate into population-level concerns, with a demographic fall and acute risk of local extinction in the
97 worst polluted areas (Bryan et al., 1986; Langston et al., 2015; Roach and Wilson, 2009). In the field, TBT was
98 also shown to affect other invertebrates, disturbing shell calcification and reproduction in bivalve mollusks, and
99 affecting crustacean growth, carbohydrate and lipid metabolism and sexual maturation (Graceli et al., 2013;
100 Vogt et al., 2018). More recently, concerns were raised over the toxicity of TBT in terrestrial ecosystems that can
101 be contaminated notably through soil enrichment with marine sediments or sewage sludge. Silva and colleagues
102 (2014) assessed TBT toxicity in terrestrial invertebrates and observed negative effects on the food consumption
103 and assimilation capabilities in isopods (*Porcellionides pruinosus*), and on juvenile production and/or mortality in
104 collembolans (*Folsomia candida*). But additional studies in field conditions are still required, notably in terms of
105 TBT doses, especially in soils continuously enriched with sewage sludge.

106 In line with the vertebrate (and especially fish) situation, field exposure cases to estrogenic compounds (e.g.
107 xeno-estrogens such as 17 α -ethinylestradiol (EE2), bisphenol A (BPA), nonylphenols and octylphenols) provide
108 another illustration of endocrine disruption in wild aquatic invertebrates (Amiard and Amiard-Triquet, 2015; Jin
109 et al., 2012), with effects including sex-ratio modification, delayed sexual maturity and intersexuality. Numerous
110 studies have reported disruptive effects of estrogens in invertebrate models, including at concentrations
111 compatible with doses measured in the field (ng/l) (Aris et al., 2014; Bovier et al., 2018; Flint et al., 2012; Herrero
112 et al., 2015; Leonard et al., 2017; Liu et al., 2012; Morales et al., 2018; Oehlmann et al., 2009, 2007; Oetken et
113 al., 2004; Wright-Walters et al., 2011). Experimental studies have also demonstrated that the reproduction of
114 the *Potamopyrgus antipodarum* snail (a freshwater mollusk quite abundant in Europe) is impaired by the
115 exposure to a mixture of environmental estrogens. This response was found to be comparable to that of four
116 species of freshwater fishes, with a similar sensitivity to these molecules (Jobling et al., 2004), suggesting that
117 EDC concerns extend to wild invertebrate populations exposed to estrogenic effluents, especially as regards
118 sessile species. In good agreement with this study, when caged downstream of an effluent discharge, the same
119 snail species was found to bioaccumulate more alkylphenols, BPA, Estradiol and Testosterone than when
120 animals were located upstream. These experiments also demonstrated a sharp decrease of reproductive
121 parameters in these animals (reduction in the number of embryos produced) after 6 weeks of exposure (Gust et
122 al., 2014). Concomitant measures of egg proteins and mRNA levels allowed authors to demonstrate that
123 reproductive disruption did not affect oocyte development, but rather targeted early embryonic development,
124 suggesting the disruption of estrogen signaling pathways (Gust et al., 2014). Recent *in situ* surveys on the marine
125 mussel *Mytilus trossulus* also evidenced some reproductive impairment in bivalve wild populations exposed to
126 presumed estrogenic water wastes. Individuals living in the vicinity of a sewage purification plant outlet showed
127 an increased frequency of gonadal regression and atresia and malformations, which were successfully mimicked
128 by an experimental exposure to 50 or 500 ng/dl of EE2 (Smolarz et al., 2017). Such experimental exposure in
129 natural conditions is a powerful way to evidence local contamination by EDCs, but requires a careful choice of
130 the biomarkers recorded. The level of yolk protein vitellogenin, most often estimated by the Alkali Labile
131 Phosphate method, has been commonly used as a biomarker of feminization in several aquatic species. Both the
132 method (Sánchez-Marín et al., 2017) and its results (Boulangé-Lecomte et al., 2017; Short et al., 2014) have
133 recently been questioned by proteomic and transcriptomic approaches, respectively.

134 In freshwater ecosystems, chironomids are increasingly imposing themselves as a convenient and suited model
135 of study. Studies carried out in *Chironomus riparius* recently contributed to reveal the endocrine disruptive
136 effects of complex mixtures of heavy metals in experimental settings in which larvae were reared in sediments
137 (more or less contaminated) originating from abandoned mines, polluted shores or reference sites. Among other
138 negative effects recorded as regards biomass and respiration rate, exposed individuals showed disrupted

139 expression of ecdysone-related genes, impaired reproduction and growth, both at the larval and adult stages
140 (Arambourou et al., 2020, 2019).

141 **2.2 In terrestrial ecosystems**

142 In terrestrial ecosystems, the main cases for which endocrine disruption effects are well identified under field
143 conditions relate to insect growth disruptors (IGD, formerly named insect growth regulators or IGR). Such
144 substances have been designed by purpose to affect insect growth and development through a specific
145 disruption of their endocrine regulatory pathways, including juvenile hormone analogues (JHA), and steroidal
146 ecdysone agonists (Pener and Dhadialla, 2012). For these molecules, the drop of target insect populations is the
147 hallmark of their efficacy. Unfortunately, although the design of these molecules was intent to produce more
148 selective pesticides (they are often sold as “reduced-risk insecticides”), non-target species sharing similar
149 hormone systems can also be affected. Pener and Dhadialla (2012) reviewed several studies demonstrating
150 lethal or sublethal effects of IGD on non-target species, especially predators or parasitoids of the target species,
151 proving the diffusion of IGD in food webs. Lawler (2017) recently reviewed the environmental effects of
152 methoprene use for mosquito control and pinpointed some negative issues at realistic environmental levels,
153 mainly on aquatic invertebrate species: lobster larvae, mysid shrimp embryos, small diptera and zooplankton-
154 sized crustacean. Although often disregarded, these species, and especially planktonic species which occupy key
155 positions in aquatic food webs, for sure deserve further attention on the subject of endocrine disruption in field
156 context. Regarding IGD’s use for agronomic purposes, the potential threatening of pollinators and other
157 beneficial insects’ survival is a big subject of concern, due to the huge ecosystemic services they fulfill. This
158 concerns domestic honeybees but also Lepidoptera, parasitic wasps, Coleoptera and numerous wild bees that
159 play crucial roles in plant-insect mutualistic networks (Quesada and Sadof, 2019; Steffan-Dewenter et al., 2005).
160 Beyond direct mortality provoked by many pesticides, some IGD are responsible of sublethal effects that may
161 contribute to the actual decline of (wild and managed) pollinators and beneficial insects populations (Johnson et
162 al., 2010; Mommaerts and Smagghe, 2011). Bees illustrate how complex and multiple the routes of exposure can
163 be: in addition to direct contact associated with spraying as well as the interaction with treated plants, foragers
164 collect nectar and pollen susceptible to be contaminated. They also bring it back to the nest to feed all the
165 members of the colony (social bees) or their larvae (solitary bees). For brood as well as for hive bees,
166 contamination thus probably mainly arise through food intake, which may strongly lead to specific toxic effects
167 (Sanchez-Bayo and Goka, 2014; Stanley and Preetha, 2016). Recent studies evidenced a clear contamination of
168 the pollen stored into beehives, by many pesticides and herbicides at sublethal concentrations, among which
169 ecdysone analogues (tebufenozide, methoxyfenozide) and JHA (fenoxycarb, pyriproxyfen, methoprene) (Böhme
170 et al., 2018; Calatayud-Vernich et al., 2018; Hakme et al., 2017). Experimental work had previously
171 demonstrated that exposure of micro-colonies of *Bombus terrestris* to JHA (pyriproxifen or kinoprene), through
172 pollen contamination (e.g. not through sugar water contamination) at the maximum field recommended
173 concentration, has no acute toxicity on workers but enhances larval mortality. This effect was hypothesized to
174 be linked with a lethal blocking of development before metamorphosis (Mommaerts et al., 2006). In the same
175 study, authors evidenced a stimulatory effect of a very low dose of kinetoprene (0.0650 mg/l) on the
176 development of the female reproductive system, together with a higher reproductive output, strongly arguing in
177 favor of an endocrine nature of the disruption. Even though neurotoxic pesticides – and in the first place all
178 neonicotinoids – are the most incriminated molecules in the complex interactions that lead to bee CCD (colony
179 collapse disorder; Sanchez-Bayo and Goka, 2014), IGD chronic exposure should not be disregarded, as regards its
180 potential sublethal effects and possible involvement in synergistic effects. Furthermore, neonicotinoids
181 themselves were recently shown to exert EDC-like effects in bees, generating sublethal effects suspected to
182 largely contribute to population extinctions over a long time scale (Woodcock et al., 2016; Baines et al., 2017).
183 More field studies are now needed to assess and cross-reference several key parameters of pollinators’ potential
184 poisoning by pesticides. These include (1) the dynamics of pollen contamination in relation with its species of
185 origin and the nature of the contaminants; (2) the persistency of chemicals under specific conditions inside real
186 hives and along seasons; (3) the link between external exposure (direct contact and/or chronic ingestion) and
187 potential risk; (4) the possible interaction between contaminants, which needs to be addressed by testing

188 realistic pesticide mixture (see Böhme et al., 2017); (5) the direct effects on bee survival and activity but also
189 sublethal effects (fertility, abnormal larval development) that may affect colony productivity; (6) the side-effects
190 that may arise through the disturbance of other functions such as olfactory performance (see e.g. Chakrabarti et
191 al., 2015), orientation and learning capacities, which are essential to foraging process and feeding, flight activity
192 and foraging efficiency (see Prado et al., 2019), pheromonal communication (notably blurring of nestmate
193 identification) and social interactions (see e.g. Fourrier et al., 2015); and (7) the indirect adverse effects that may
194 ensue from microbiota modifications.

195 More generally, all herbivorous invertebrates may come across EDCs through the contamination of their food:
196 some well identified or emerging organic contaminants including BPA, nonylphenol or triclosan are largely
197 distributed in the water cycle and end up in reclaimed water eventually used for food crop irrigation. This can
198 lead to their accumulation in leafy vegetables (Dodgen et al., 2013) and to a direct exposure of herbivorous
199 species. In the insect crop pest *Spodoptera littoralis*, Maria and colleagues (2019) evidenced a higher mortality at
200 pupal stage and an increase in some larval instar duration associated with modifications of ecdysteroid titers
201 and nuclear receptor expression, after consumption of food contaminated with BPA concentrations similar to
202 that found in plants. At last, atmospheric contamination also potentially concerns all epigeic species. Although
203 this route of exposure has barely been considered so far, we recently evidenced a chronic contamination of
204 several species of aerial insects (mainly ants but also crickets and honeybees) by phthalates, which are potent
205 EDCs (Oehlmann et al., 2009; e.g. Mankidy et al., 2013). These phthalates impregnate the cuticle and mix with
206 the cuticular components (Lenoir et al., 2012). In these studies, all tested individuals were found to be
207 contaminated, with varying contamination levels depending on the species. Phthalates accounted for 0.11 to
208 2.66% of the cuticular compounds in the different ant species tested, 0.73% in honey bees and 2.76% in the
209 wood cricket *Nemobius sylvestris*. This contamination was clearly established to originate from the atmosphere.
210 It seems that, due to its biochemical properties, insects cuticle can trap the phthalates present in both the
211 vapor phase and the particulate phase (adsorbed to atmospheric particles; Lenoir et al., 2014; Teil et al., 2006).
212 The insect cuticle keeps trace of this contamination in the form of a basal level of phthalates mixed to cuticular
213 compounds, which can be measured by GC-MS. In addition, phthalates were also clearly demonstrated to be
214 able to cross the epidermal barrier in these insects, as they were detected inside the body of animals, mainly in
215 fat. Experimental contaminations by environmental doses spiked onto the cuticle induced a reduction of queens'
216 eggs output and disturbed immune gene expression in workers of the black garden ant (*Lasius niger*), suggesting
217 possible endocrine disruptive effects in natural populations (Cuvillier-Hot et al., 2014). Considering that these
218 observations may potentially apply to all invertebrate species possessing a cuticle or a lipophilic integument, it
219 appears necessary to pursue investigations on other species, in order to examine whether it is necessary to
220 include this mode of contamination in risk assessment protocols for terrestrial invertebrates.
221

222 3. New insights in invertebrate molecular disruption

223 As an emerging science, EDC ecotoxicology first based on a vertebrate-centered approach, taking advantage of
224 our better knowledge of vertebrates physiology, to detect and identify disturbances linked with environmental
225 exposure to contaminants. Then, knowledge acquired from vertebrate models was transposed to invertebrates
226 and researches have focused on susceptible endocrine pathways shared between invertebrates and vertebrates,
227 and in a first place on the possible disruption of the estrogen receptor (ER)-associated cascades. But, as more
228 than 600 Million years have elapsed since the split between protostomes and deuterostomes, the former
229 acquired endocrine systems of regulation unique to them (e.g. ecdysteroids, Juvenile hormones), which are
230 potential targets for environmental endocrine disruption and which call for dedicated researches. Even
231 nowadays, while substantive knowledge has been accumulated for some groups (e.g. insects), a main gap of our
232 current knowledge over endocrine disruption in invertebrates remains our lack of detailed comprehension of
233 endocrine signaling pathways in many disregarded groups of invertebrates (Castro and Santos, 2014;
234 Hutchinson, 2007). This concerns receptors and ligands, associated cascades and regulation, but also the
235 dynamics of bioaccumulation and elimination of potential disruptors. This basic knowledge is necessary to
236 ascertain that an adverse effect observed in the field is indeed the result of an endocrine disruption mechanism

237 rather than a more general direct or indirect toxic effect (Barata et al., 2004; Hutchinson, 2007; Lagadic et al.,
238 2007; Weltje and Schulte-Oehlmann, 2007). In the past years, some attempts have been made to develop
239 arthropods-specific *in vitro* assays dedicated to screen environmental contaminants for ecdysteroid
240 agonist/antagonist activities (Dinan et al., 2001; Kontogiannatos et al., 2015; Soin et al., 2009; Swevers et al.,
241 2004; Yokota et al., 2011; Zotti et al., 2013). However, it appears that ecdysteroid receptors have ligand-binding
242 properties that can substantially vary between invertebrate species, even among members of a same order
243 (Graham et al., 2007). This suggests that the results of such molecular assays can hardly be generalized among
244 species on a large scale and involve the need to develop taxa-specific tests (Santos et al., 2018; Yokota et al.,
245 2011).

246 EDCs can interfere with the endocrine pathways of animals in many ways. They can affect endogenous hormonal
247 levels, or those of hormone receptors by disturbing their specific synthesis and/or catabolism; they can also
248 disturb hormonal receptor functions by spatially mimicking endogenous hormones, thus affecting receptors
249 availability or leading to unexpected signalization (agonist or antagonist). For decades, physiological and
250 developmental endpoints have been used to detect and characterize EDCs in invertebrates, all the more that
251 several model species, easy to breed and possessing a short generation time are available in several taxonomic
252 groups. Many reviews have reported about lists of suspected EDCs and proposed modes of action based on the
253 examined studies (e.g. Hutchinson, 2002; Lagadic et al., 2007; LeBlanc, 2007; Oehlmann et al., 2007; Oetken et
254 al., 2004; Rodríguez et al., 2007; Soin and Smaghe, 2007; Zou, 2005). More recently, molecular approaches
255 allowed a deeper investigation of the mode of action of these EDCs in invertebrates, which we will focus on in
256 the next chapter. Figure 1 sums-up the main molecular regulatory pathways hypothesized or demonstrated to
257 be affected by chemicals in protostomes.

258 **3.1 Molecular pathways susceptible to endocrine disruption and shared with vertebrates**

259 3.1.1. Estrogen receptors and relatives

260 In vertebrates, many EDCs exert their action as agonists of estrogen receptors (ER) or/and antagonists of
261 androgen receptors (AR). The invertebrate orthologs of these receptors are thus potential targets for
262 environmental endocrine disruption. To date, the only steroid receptor evidenced in protostomes belongs to the
263 ER family and shares a common ancestor with vertebrate steroid receptors (SR) named AncSR, which would
264 have arose before protostome and deuterostome cleavage (Castro and Santos, 2014; Jones et al., 2017; Markov
265 et al., 2009). Absent from the Ecdysozoa, ERs orthologs have been characterized in mollusks (Hultin et al., 2014;
266 e.g. Keay et al., 2006; Matsumoto et al., 2007; Raingeard et al., 2013; Thornton, 2003; Zhang et al., 2012),
267 annelids (Keay and Thornton, 2009; Lv et al., 2017) and recently in rotifers (Jones et al., 2017). As in vertebrates,
268 these receptors have genomic signaling pathways that activate gene transcription through the direct binding of
269 the nuclear ER complex to ERE (estrogen-response elements) sites (or indirectly through AP-1 or Sp-1 binding
270 sites) upstream of target genes (Hamilton et al., 2017). However, the modalities of ER commitment clearly differ
271 among invertebrate groups. Indeed, the Mollusca receptor was shown to have been vestigialized through some
272 decisive substitutions that froze the ER in an active conformation, resulting in its constitutive activity.
273 Subsequent mutations then filled the ligand pocket with bulky residues precluding any ligand binding and
274 rendering unlikely any reversal to the ancestral function (Bridgham et al., 2014). ERs in mollusks are thus ligand-
275 independent transcriptional activators, which implies that mechanisms of endocrine disruption by estrogenic
276 environmental contaminants in these species are necessarily independent of any nuclear ER activation (Canesi
277 and Fabbri, 2015). On the contrary, annelids and rotifers ERs display the classic properties of vertebrates ER,
278 including apparent sensitivity to estrogens (Castro and Santos, 2014; Jones et al., 2017; Keay and Thornton,
279 2009). However, the exact nature of their endogenous ligand is still unknown. Steroidogenesis exists in
280 protostomes but has evolved independently from vertebrate cascades, partly through the recruitment of
281 cytochrome P450 enzymes formerly involved in xenobiotic detoxification (Markov et al., 2009). Recent work by
282 Blalock and collaborators (2018) evidenced, for the first time in a protostome (the mussel *Mytilus edulis*), a
283 partial protein equivalent to vertebrate CYP11A enzyme, responsible for the side-chain cleavage of cholesterol
284 to pregnenolone. Hence, within the whole steroid biosynthesis pathway, only the last step of aromatization of
285 testosterone into estradiol would still be missing in lophotrochozoa, the aromatase CYP19A appearing restricted

286 to chordates (Goldstone et al., 2016; Markov et al., 2009). For this reason and also because unbiased methods to
287 detect steroids in invertebrate tissues so far failed to find estrogens, some authors refute the idea of an
288 estrogen-like endogenous ligand for protostome ERs (Holzer et al., 2017a; Scott, 2013). But whatever their origin
289 – endogenous or exogenous – estrogens clearly affect the physiology of many invertebrates, including mollusks,
290 paving the way to possible endocrine disruption by oestrogeno-mimetics. In vertebrates, ERs can be activated by
291 several estrogenic endogenous ligands (estradiol, Δ^5 -androstenediol, 5 α -androstenediol, and 27-
292 hydroxycholesterol). They also are responsive to several plant-derived compounds with estrogenic activity such
293 as genistein, coumestrol, and resveratrol (Baker and Lathe, 2018). This promiscuous responsiveness is proposed
294 to explain their response to synthetic chemicals such as BPA and phthalates (Engel et al., 2017; Flint et al., 2012).
295 Similar cross-binding may be considered for ligand-sensitive ER described in protostomes. Keay and Thornton
296 (2009) for example report an agonist effect of genistein and an antagonist effect of BPA on *Platynereis dumerilii*
297 ER.

298 In vertebrate models, it is well established that ER signaling can be modulated by estrogen-related receptors
299 (ERRs). These are also nuclear receptors sharing sequence similarity with ERs, but that respond very weakly to
300 endogenous estrogens while being stimulated by some xenoestrogens. They may thus interfere with ER signaling
301 upon environmental EDC exposure, along several modalities: (1) by directly interacting with ERs, (2) by
302 competing with ERs for ERE site binding or (3) by targeting similar genes as ERs via the presence of ERR-response
303 elements in their promoter region, alongside with ERE (Xu et al., 2017). As a consequence, influence of ERR
304 activation on ER signaling will clearly depend on the level of expression and tissue distribution of both receptors.
305 Sequences homologous to ERRs have been described in annelids (the marine worm *Capitella* and the leech
306 *Helobdella*, Baker, 2008) and in mollusks (the snail *Lottia*, Baker, 2008; the snail *Physa*, Morales et al., 2018; two
307 *Mytilus* species, Nagasawa et al., 2015). Quite expectedly, mRNA of ER homologues were abundantly detected in
308 reproductive tissues (ovary but also pedal ganglion) in the mussels *Mytilus edulis* and *M. galloprovincialis*, while
309 mRNA of ERR homologues, otherwise involved in metabolism regulation and mitochondrial function (Hubbard et
310 al., 2015), had high levels of expression in the gill and digestive gland ; however, both receptors had basal levels
311 of expression in all tissues examined and may thus interfere with each other (Nagasawa et al., 2015). In *M.*
312 *edulis*, ER expression in ovary appeared regulated by *in vitro* exposure to 17 β -estradiol, while that of ERR did not
313 (Nagasawa et al., 2015). In the snail *Marisa cornuarietis*, various vertebrate-like estrogens were tested for their
314 possible ER and ERR gene transcription modulation, but gave negative results for both genes. Only genistein and
315 BPA up-regulated gene expression of ER and ERR orthologs respectively (Bannister et al., 2013). In this species,
316 BPA exposure at environmentally relevant concentrations had clear disruptive effects, leading to a
317 superfeminization phenomenon characterized by enlargement and malformations of female organs, and higher
318 egg mass production (Oehlmann et al., 2006). Also in the snail *Physa acuta*, BPA exposure led to a significant
319 increase in the mRNA levels of both ER and ERR, suggesting that these receptors could be involved in molecular
320 events that regulate the endocrine disruptor activity of this chemical in Gastropods (Morales et al., 2018).
321 Interestingly, ERR gene homologues have also been characterized in insects and their expression appeared
322 modulated by EE2 (Bovier et al., 2018) and ethylparaben (Liu et al., 2014) in *Drosophila melanogaster*, and by
323 Bisphenol S (BPS), Triclosan, BPA, nonylphenol or diethylhexyl phthalate (DEHP) in *Chironomus riparius* larvae
324 (Herrero et al., 2018, 2015; Martínez-Paz et al., 2017; Park and Kwak, 2010). In these cases, disruption is
325 proposed regarding the 20-Hydroxyecdysone pathway (see § 3.2), highlighting the multi-target character of
326 many EDCs.

327 Alongside and apart from direct interactions of estrogeno-mimetics with nuclear estrogen receptors, many
328 studies in vertebrates pointed out more rapid effects of these molecules (within seconds to minutes) through
329 non-genomic signaling pathways (Xu et al., 2017). They may be initiated by estrogen-responsive non-nuclear
330 receptors and may lead to local effects such as modification of ion fluxes or to activation of cytosolic kinase
331 cascades that ultimately affect gene transcription. Several studies reviewed by Janer & Porte (2007) suggested
332 the involvement of such non-genomic processes in endocrine regulation also in invertebrates. Recent
333 contributions from Omics studies brought further support: individuals of the mudsnail *Potamopyrgus*
334 *antipodarum* exposed to an effluent discharge, while accumulating alkylphenols, BPA and vertebrate-like sex-
335 steroid hormones, showed an inhibition of the expression of genes involved in non-genomic signaling pathways,

336 together with an induction of the repressors of the genomic pathway; this led to a drastic decrease in embryo
337 production (Gust et al., 2014). Similarly, a transcriptomic approach of mussels exposed to EE2 or 4-nonylphenol
338 (leading notably to female-skewed sex-ratios) revealed similar patterns of gene dysregulation with main targets
339 belonging to the non-genomic estrogen signaling pathway (Blalock et al., 2018).

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3.1.2. Other nuclear receptors

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At the molecular level as at the individual and population scales, the case of endocrine disruption by organotins was much investigated and led to many relevant trails. In the imposex phenomenon, endocrine disruption is highly suspected at different levels. High levels of testosterone observed in TBT treated prosobranch females could ensue from an inhibition of enzymes involved in steroid metabolism (reviewed in Graceli et al., 2013, Lafont and Mathieu, 2007). TBT may also induce adverse neuromodulation through the production of APGWamide which was shown to induce imposex in mud snails (*Ilyanassa obsoleta*, Oberdörster and McClellan-Green, 2002). Finally Pascoal and colleagues (2013) strengthened the hypothesis of a disruption of the retinoid X receptor (RXR) – peroxisome proliferator-activated receptor- γ (PPAR γ) pathway by evidencing a strong transcriptional response of the retinoid receptors and of putative members of this signaling pathway after TBT treatment in *Nucella lapillus*. They also showed that activating this pathway with a vertebrate ligand of PPAR γ led to imposex in this species. As additional support to this third presumed mechanism, it had been previously established that TBT was able to bind and activate RXR–PPAR vertebrate heterodimers, by mimicking 9cis-retinoic acid interaction with RXR (le Maire et al., 2009). All these clues suggest a strong involvement of this signaling pathway, quite shared between vertebrates and invertebrates, in imposex physiopathology (André et al., 2014; Iguchi and Katsu, 2008).

RXRs are nuclear receptors involved in numerous signaling pathways and biological functions since they can signal as homodimers, but also form heterodimers with many partners, such as PPAR (as exemplified above), retinoic acid receptor (RAR) or thyroid hormone receptor (TR). In Ecdysozoans, RXRs homologues (known as ultraspiracle (USP) in insects) are the obligate heterodimeric partners of ecdysteroid receptors (EcR, see §3.2). RXRs are widely distributed among metazoans and show a great degree of homology between taxa. Furthermore, their sensitivity to 9-cis-retinoic acid (RA) appears as an ancestral character already present in the common ancestor of Cnidaria and Bilateria, widely shared among metazoans and secondarily lost in ecdysozoans (André et al., 2017). Their functional partner in RA sensing, RARs, have also been detected in some annelids and mollusks. In both cases, the essential of the signaling machinery is present so that functional RA signaling pathways is expected, but according to specific processes, given that the molluscan RAR were proved insensitive to retinoids (Gutierrez-Mazariegos et al., 2014; Handberg-Thorsager et al., 2018). Finally, genome data mining recently identified key players possibly involved in retinoid metabolism and storage process in some protostomes, providing new potential targets for disruption. All this lead André et al. (2017, 2014) to suggest that RXR-dependent modulation by organotins might be evolutionary conserved among lophotrochozoans and vertebrates, and foresee that many more metazoan species might be potential targets for EDCs through disruption of the retinoid system. The identification of the physiologic effects of such disruptions clearly deserves future research in all invertebrate groups.

Over the last years, the endocrine disruption of vertebrate biological processes controlled by thyroid hormone receptors (TRs) has been of growing concern, mainly because we progressively uncover the importance of this endocrine system on embryogenesis and early neurogenesis. Several EDCs including PCBs, the pesticide chlorpyrifos, BPA, poly-fluorinated surfactants and polybrominated diethyl ethers (PBDEs), are suspected to disrupt TH regulated pathways (Préau et al., 2015). TRs are also nuclear receptors functioning as ligand-dependent transcription factors; they mediate thyroid hormone (TH) effects by genomic and non-genomic mechanisms. If the genome of *Drosophila melanogaster* and *Caenorhabditis elegans* appeared deprived of TR-like sequences, lophotrochozoans on the contrary clearly possess TR orthologs (Orozco et al., 2017; Sainath et al., 2019). Note that putative TR orthologs have been proposed from *Daphnia pulex* and *D. magna* genomes, but their high sequence divergence poses question about their real origin (Sainath et al., 2019). Activating ligands are clearly different for protostome and deuterostome TRs, in agreement with the poor degree of conservation of their respective ligand-binding domains; and so far, endogenous ligands of protostome TRs remain unknown.

386 Still, endogenous synthesis of TH by the sea hare *Aplysia californica* is supported by its enzymatic equipment
387 (Heyland et al., 2006), T4 and T3 have been extracted from *Crassostrea gigas* hemolymph (Huang et al., 2015),
388 and TH were shown to induce settlement and metamorphosis in several mollusk species suggesting that TH
389 signaling is possible in Lophotrochozoa (Holzer et al., 2017b; Taylor and Heyland, 2017). Although up to now no
390 clear link has been established between TH and activation of TR in this group, the question remains open about
391 the possible effect of environmental thyroid disruptors on invertebrates development and physiology, and
392 urgently requires fundamental scientific work to understand the role of the thyroid endocrine system in
393 protostomes.

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3.2 Molecular pathways susceptible to endocrine disruption and specific to Protostomes

396 Protostomes have developed specific endocrine regulatory pathways that can also be affected by environmental
397 EDCs. In arthropods, there are three main classes of hormones: peptide hormones, ecdysteroids (mainly 20-
398 hydroxyecdysone (20E) in insects and crustaceans, together with ponasterone A in chelicerate species), and
399 terpenoids (mainly juvenile hormones (JH) in insects, methyl farnesoate (MF) in other arthropods). All three
400 systems interact to regulate notably growth, development and reproduction. The hormonal action of 20E exerts
401 via the activation of the ecdysone receptor (EcR), again a member of the superfamily of nuclear hormone
402 receptors, that regulates gene transcription through heterodimerization with RXR (called USP in insects) and
403 genomic binding to EcRE (ecdysone responsive element). It is now clear that the developmental events that lead
404 to prepupal and pupal molt depend on a tightly timed dynamic of hemolymphatic 20E titer. A sharp rise
405 followed by a clear decrease in the circulating levels of 20E are both important to regulate the expression of the
406 different waves of transcription factors that coordinate the different molting processes, trigger apolysis, timely
407 induce the expression of chitin synthase genes, regulate the production of the molting fluid needed to degrade
408 the old cuticle and provoke its shedding (Guittard et al., 2011; Song et al., 2017). Numerous EDCs, beginning
409 with IGDs that have been purposely developed to disrupt molting in insect pests (see §2.2), are able to
410 negatively affect synthesis or secretion of 20E, or to interfere with EcR (Soin and Smagghe, 2007). As a
411 consequence, it deregulates the fine-tuned signalization by 20E, causing notably molting failure and death, or
412 impairing gametogenesis or embryogenesis. All the adverse consequences of chemically mediated disruption of
413 molting in arthropods have recently been reviewed and illustrated by Song and colleagues (2017), highlighting
414 the multiplicity of potential EDCs targets in the process that can lead to mortality by ecdysis failure. In many of
415 the investigated cases, EDCs exert 20E agonistic effects, evidenced notably by an up-regulation of EcR or other
416 genes involved in the ecdysone cascade (e.g. four oestrogeno-mimetics in an amphipod, Gismondi, 2018; BPA in
417 the Lepidoptera *Sesamia nonagrioides*, Kontogiannatos et al., 2015; the insecticide fipronil in a copepod,
418 Gaertner et al., 2012; Triclosan, Martínez-Paz et al., 2017; Nonylphenol, Nair and Choi, 2012; several commonly
419 used UV-filters, Ozáez et al., 2014, 2013; the phthalate BBP, Planelló et al., 2011; BPA, Planelló et al., 2008; and
420 Bisphenol S, a substitute of BPA, in the Chironome aquatic larvae, Herrero et al., 2018). For most of these
421 chemicals, gene expression changes came along with affected developmental endpoints such as molting
422 malformations, increased pupation time and reduced emergence success in *S. nonagrioides* (Kontogiannatos et
423 al., 2015), emergence failures (Nonylphenol, Lee and Choi, 2006), delay in embryo hatching (Ozáez et al., 2014)
424 and reduced survival (Planelló et al., 2008) in *Chironomus riparius*. Noteworthy, a cell-based reporter assay in
425 *Bombyx mori* Bm5 cell line confirmed the agonistic effect of BPA in Lepidoptera (Kontogiannatos et al., 2015).
426 Less frequently, EDCs act as anti-ecdysteroids (e.g. 5-chloro-1H-benzotriazole, tamoxifen or testosterone in
427 *Daphnia magna*; resp. Giraud et al., 2017a; Jo et al., 2018; Mu and LeBlanc, 2002). It is clearly the case for the
428 phthalate DEHP, which was shown to induce a significant drop in EcR transcription both at short exposures to
429 high doses or at long exposures to low doses (i.e. environmental concentrations and below) in the Chironome
430 larvae (Herrero et al., 2017; Planelló et al., 2011). Similar antagonist effects have been observed in the
431 lepidopteran *Spodoptera littoralis*, also manifest through longer larval / pupal stages and global delay in adult
432 emergence (Aviles et al., 2019). On the other hand, very few is known over ecdysteroid function and possible
433 disruption outside of the arthropod group. EcR orthologs have been detected in the genome of mollusks,
434 annelids and nematods and *in silico* analyses predict possible binding to an ecdysone-related steroid (Laguerre

435 and Veenstra, 2010). Interestingly, while the exact role of such molecules in lophotrochozoa physiology is so far
436 unknown, the expression of EcR in the earthworm *Eisenia fetida* have been shown to be down-regulated in male
437 reproductive tissues after exposure to low doses of BPA (Novo et al., 2018), and up-regulated in full body
438 extracts after exposure to sublethal doses of 4-hydroxybenzophenone, a main product of degradation of
439 benzophenone-3 that is currently used as UV-filter in sunscreens (Novo et al., 2019). The endogenous roles of
440 such receptors in mollusks and annelids need clarification.

441 The sesquiterpenoid hormones, JH and MF, are also key regulators in arthropods, regulating various
442 developmental and reproductive processes such as molting, growth, metamorphosis, and gonad maturation.
443 The elucidation of the signaling pathway of sesquiterpenoid hormones in the last decade evidenced
444 Methoprene-tolerant (Met; germ-cell expressed, Gce, in *Drosophila*) as their main receptor. The Met/Gce-
445 hormone complexes are translocated to the nucleus, where they bind, together with the co-activator SRC (Tai in
446 *Drosophila*), the specific JH response elements (JHRE) to activate transcription of the downstream target genes
447 (Qu et al., 2018). Easy screening of putative EDCs with JH-like activity is now possible with the OECD TG211
448 ANNEX 7 assay that use daphnids. Daphnid species are parthenogenetic species that produce only females in
449 controlled conditions, but may generate male offspring in response to JHs. The validated assay takes advantage
450 of this specificity to specifically detect JH analogs. Recently, Abe and colleagues (2015b) developed a derivative
451 short-term screening assay using adult *Daphnia magna* and with a chemical exposure of only 7 days. This test
452 notably ascertained the JH agonist effect of the IGD Diofenolan. In parallel, a two-hybrid assay evidenced that
453 diofenolan induced heterodimerization of Met and SRC, suggesting its direct binding to the JH receptor;
454 microarray analyses confirmed the dysregulation of many gene markers of JH action, evidencing this chemical as
455 a strong JH agonist (Abe et al., 2015a). The same study evidenced a concomitant disruption of the ecdysteroid
456 signaling pathway, reinforcing the hypothesis of a cross-talk between JH and ecdysteroid signaling pathways in
457 arthropod developmental processes, as previously suggested with other JH agonists such as fenoxycarb,
458 pyriproxyfen or Tris(2-butoxyethyl) phosphate, a flame retardant (Giraud et al., 2017b; Tuberty, 2005).
459 Sesquiterpenoid hormones had long been thought restricted to arthropods. However, Schenk and colleagues
460 (2016) recently established in *Platynereis dumerilii* that the hormone called nereidin, produced by head ganglia
461 and known to regulate growth and sexual maturation in annelids by inhibiting the switch to reproductive state,
462 corresponds to MF. They also evidenced that eleocytes, the cells that produce vitellogenin then captured by
463 maturing oocytes, express a Met ortholog, whose transcription is up-regulated both by nereidin and by
464 exogenous MF. Interestingly, the JHA methoprene and pyriproxyfen mimicked the effect of nereidin at similar
465 concentration (10nM), reducing vitellogenin expression in cultured eleocytes. Recent insights over EcR and JH
466 presence in annelids raise clear concerns about this group, notably as crucial members of the soil infauna. Their
467 endogenous way of life makes them largely exposed to IGD sprayed on fields and cultures. We need to acquire
468 background knowledge over the role of such putative regulatory pathways in this group so as to assess their
469 possible disruption in natural conditions.

470 **4. The promises of the high-throughput omics era**

471 Over the last decades, omics techniques became within reach of researchers to decipher how expressed genes
472 (transcriptomics), proteins (proteomics) and metabolites (metabolomics) are altered by exposure to
473 environmental pollutants (Colin et al., 2016). This gave rise to a new research field called ecotoxicogenomics
474 that contributes to an ongoing mutation of our comprehension of the effect of endocrine disruption. Indeed,
475 these recent techniques now make possible to compare different taxa on a large scale, to consider the
476 multiplicity of the endogenous targets of individual toxics, and to monitor endocrine disruption in natural
477 conditions much more precisely (Oliveira et al., 2016). Proteomics and metabolomics for instance enable to
478 investigate how entire pathways react to toxic exposure, giving more weight to subtle changes that may have
479 gone unnoticed if only few markers had been considered. Thereby, metabolomics profiling suggested that DEHP
480 has negative effects on energy production and more particularly on TCA cycle, as in vertebrates, in the crop pest
481 *Spodoptera littoralis* (Aviles et al., 2019). Tests on *Daphnia magna* metabolome confirmed that dissolved organic
482 matter specifically affects the bioavailability of some drugs present in waste water (Kovacevic et al., 2019), and
483 revealed convergent metabolic effects of some psychiatric drugs, despite different modes of action (Garreta-

484 Lara et al., 2018). Lastly, metabolomics approaches highlighted alterations in energy metabolism, amino acids
485 metabolism and glycerophospholipid metabolism in mussels exposed to a synthetic progestin while no changes
486 in gonad maturation and in steroids level were recorded (Cappello et al., 2017). For their part, coupled to NGS
487 technologies, genomics and transcriptomics offer via *de novo* approaches an unprecedented power of
488 description and analyses of gene sequences whose expression is altered after exposure, even in
489 environmentally-relevant species for which physiological and genomic knowledge had been historically poor. It
490 can reveal so far undetected disruption, and identify new biomarkers for the monitoring of given species in
491 specific environmental conditions. For example, the transcriptomic analysis of the response to mercury exposure
492 of two copepod species recently revealed an endocrine-disrupting potential for this metal, evidencing a
493 dysregulation of the estrogen signaling pathway in both species (Wang et al., 2017). Similarly, microarray
494 analyses of oysters exposed to microplastic particles revealed a molecular signature of endocrine disruption, in
495 the form of differential expression of hormone receptors or transcripts involved in hormonal pathways
496 (Sussarellu et al., 2016). Interestingly no EDC could have been detected in the biological samples of this study,
497 illustrating the pre-eminence of bio-assays over chemical quantification when incriminated substances are active
498 at very low doses and possibly below detection limits. Even more remarkable, omics approaches can unveil
499 endocrine disruption even in cases where compensatory mechanisms suppress any noticeable physiological
500 effects. For instance, in *Daphnia magna*, several studies screened the gene regulation changes after exposition
501 to different potent EDC (Giraud et al., 2015; Houde et al., 2016). Contrary to other benzotriazoles, 1H-
502 benzotriazole (BTR) has no effect on molting frequency but RNAseq analyses revealed the up-regulation of
503 cuticular proteins after BTR exposure, suggesting the latter could have compensated a possible molting
504 disruption (Giraud et al., 2017a).

505 We seemingly are at the beginning of a new era of research possibilities, with much more tools to apprehend
506 complex toxic effects and intricate organism responses. This is especially true for invertebrates, so far neglected
507 with respect to the huge biodiversity they represent. Furthermore, constant progresses in high-throughput
508 sequencing techniques, both in terms of rapidity and prices, enhance the possibilities to compare multiple
509 species, populations, life-stages or tissues, expanding the scope of experimental possibilities (e.g. An et al., 2014
510 for a comparative study of testis and digestive gland response to estrogen exposure in *Chlamys farreri*). Finally,
511 combining genetic, proteic or metabolic changes detected through omics approaches with the description of
512 altered apical endpoints (e.g. body condition indexes, lipid content, fertility measures...) will undoubtedly
513 provide useful insights into the genes, proteins and metabolites possibly implicated in physiological disorders,
514 unveiling unknown molecular mechanisms of the physiological pathways affected (see Ciocan et al., 2012, and
515 Grilo and Rosa, 2017 for examples about intersex in invertebrates). A good example is probably that of the dog
516 whelk (*Nucella lapillus*), for which transcriptomics coupled to *in vitro* testing confirmed the alteration of some
517 processes already evoked (steroid metabolism, neuroendocrine regulation and retinoid mechanisms) following
518 TBT exposure, but also revealed the involvement in imposex of a regulatory pathway (peroxisome proliferator-
519 activated receptor or PPAR), not reported until then in invertebrates (Pascoal et al., 2013). The next challenge is
520 now to integrate omics in routine environmental monitoring studies notably to help regulatory agencies in their
521 management of environmental risks (Gouveia et al., 2019; Oliveira et al., 2016; Piña et al., 2018).

522

523 5. Conclusion

524 A growing research effort coupled with the use of ever more accurate and efficient methods and approaches
525 now allow us to better identify EDCs and refine our knowledge of their mode of action, even in non-model
526 animal species for which basic knowledge is limited. The disruption of hormonal regulatory pathways is of
527 course the hallmark of these harmful chemicals, but additional toxicological modes of action may also be
528 involved in sub-lethal effects (Flint et al., 2012). In addition to classical endpoints related to reproduction and
529 development, more and more studies pinpoint additional disturbed processes and pathways in invertebrates
530 exposed to EDCs, including immune deficiencies that may hamper the ability of individuals to fight their natural
531 pathogens and parasites (Canesi et al., 2007; Gagné et al., 2008; Lu et al., 2013) and behavioral changes that

532 may negatively affect food finding, habitat choice or sexual partner selection (Baglan et al., 2018; Clotfelter et
533 al., 2004; Lam et al., 2010; McCallum et al., 2013), with possible transgenerational effects (Giraud et al., 2017b;
534 Li et al., 2018; Oliveira-Filho et al., 2009). Epigenetic modifications are notably evoked as possible mechanism
535 entailed (Novo et al., 2018; Schwindt, 2015) but, as in vertebrates, the identification of the precise mechanisms
536 involved, as well as their implication in invertebrates require deeper investigations. Among future challenges, it
537 is necessary to accurately identify all involved mechanisms, in order to better characterize organisms as well as
538 populations response to multiple environmental stressors.
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540

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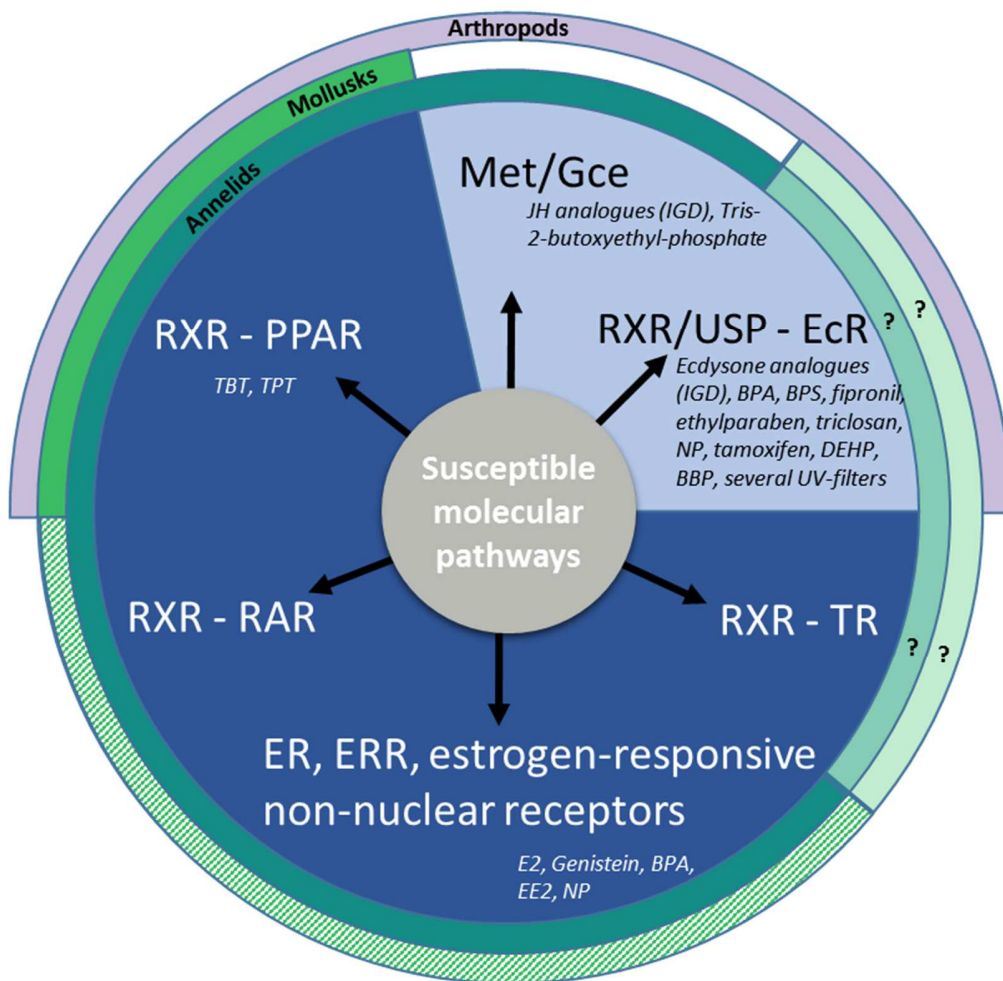
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1073 **Figure 1.** Main molecular regulatory pathways susceptible to endocrine disruption in protosomes;
1074 some are shared with deuterostomes (dark blue background), while others are specific to invertebrates
1075 (light blue background). Outer colored rings indicate the groups in which corresponding orthologs have
1076 been evidenced. Hatched display points out non-functional receptors (insensitivity to ligand), and a
1077 question mark signifies that an ortholog has been described but its functional activity needs further
1078 confirmation. In italic font below each molecular pathway, are listed examples of EDCs strongly
1079 suspected to interfere with the corresponding molecular pathway in protosomes (IGD, insect growth
1080 disruptors; BPA, bisphenol A; BPS, bisphenol S; NP, nonylphenol; DEHP, di-ethylhexyl phthalate; BBP,
1081 benzyl butyl phthalate; E2, 17 β -estradiol; EE2, 17 α -ethinylestradiol; TBT, tributyltin; TPT, triphenyltin).

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