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Effects of agricultural pesticides on the reproductive system of aquatic wildlife species, with crocodilians as sentinel species

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15 KEY WORDS: Environmental Contamination; Atrazine; Endosulfan; Crocodile, Endocrine
16 Disrupters; *Caiman latirostris*.

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18

19 ABBREVIATIONS

20 11β HSD2, 11-beta-hydroxysteroid dehydrogenase type 2; 17α HSD12, 17-alpha-
21 hydroxysteroid dehydrogenase 12; AD4BP/SF-1, Ad4 binding protein/steroidogenic factor-
22 1; amh: antimüllerian hormone; ANVISA, Agência Nacional de Vigilância Sanitária
23 (Brazil); APVMA, Australian Pesticides and Veterinary Medicines Authority; AR,
24 androgen receptor; ATZ, atrazine; BPA, bisphenol A; CAR, constitutive androstane
25 receptor; DACT, diaminochlorotriazine; dnmt-1: DNA (cytosine-5)-methyltransferase 1;
26 DDT, Dichlorodiphenyltrichloroethane; dmrt-1, doublesex and mab-3 related transcription
27 factor 1; DTI, The Department of Trade and Industry; E2, 17β -estradiol; EDCs, endocrine-
28 disrupting compounds; END, endosulfan; EPA: Environmental Protection Agency; ER,
29 estrogen receptor; $ER\alpha$, estrogen receptor alpha; fshr, follicle-stimulating hormone receptor
30 gene; GAM, gonadal adrenal mesonephros; IUCN, International Union for Conservation of
31 Nature; ND: not detected; NR2C1, Nuclear Receptor Subfamily 2 Group C Member 1;
32 OCP, organochlorine pesticide; OJEU, Oficial Journal of the European Union; P450C17,
33 cytochrome P450-17A1; PPQS, Directorate of Plant Protection, Quarantine & Storage
34 (India); PR, progesterone receptor; PXR, pregnane X receptor; SENASA, Servicio
35 Nacional de Sanidad y Calidad Agroalimentaria (Argentina); sox9a, SRY-box transcription
36 factor 9a; srd5b: 5-beta reductase; *star*, steroidogenic acute regulatory; T, testosterone;
37 THR, Thyroid Hormone Receptor; TSD, temperature-dependent sex determination; wt-1,
38 Wilms' tumor protein 1.

39 **ABSTRACT**

40 Agricultural pesticides represent a significant class of endocrine-disrupting chemicals
41 (EDCs) to which non-target organisms around the world are constantly exposed.
42 Laboratory studies have found strong evidence showing the endocrine-disruptive potential
43 of these pesticides at environmentally relevant exposure levels. Since the field of endocrine
44 disruption continues to grow in richness and complexity, this review aims to provide an
45 update on the effects of two agricultural pesticides that act as EDCs: atrazine and
46 endosulfan. We will focus mainly on the effects on crocodylians due to their worldwide
47 occurrence in tropical and sub-tropical wetland ecosystems and their ecological and
48 physiological features, which render them vulnerable to exposure to pesticides with
49 endocrine-disrupting action at all life stages. The results here reviewed provide important
50 insights into the effects of hormonally active agricultural pesticides at cellular, tissue, and
51 organ levels in the reproductive system of crocodiles. A better understanding of the effects
52 of exposure to environmentally relevant doses of EDCs on the reproductive system of
53 crocodylians will contribute to protect and improve the health of both wildlife species and
54 humans.

55

56 **Outline**

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78 **1. INTRODUCTION**

79 Agriculture is the main source of food for humans and domestic animals. Major
80 tasks to ensure crop production include increasing crop yield and reducing crop loss from
81 pest injury. To increase crop yield, herbicide-resistant crops, such as glyphosate-resistant
82 soybean, have gained widespread acceptance. Although the use of herbicide-resistant crops
83 initially met this purpose, it has led to pesticide (herbicide) overuse and increasing pesticide
84 environmental pollution. Nowadays, new approaches for crop disease management, such as
85 the development of genetically modified pest-resistant crop varieties (Li et al., 2016) and
86 the use of engineered nanomaterials as pesticides (Adisa et al., 2019), may reduce pesticide
87 use and pollution. However, the use of conventional pesticides (insecticides, fungicides and
88 bactericides) is currently the strategy of choice (Zhang, 2018).

89 Soil and water bodies adjacent to agricultural fields sustain complex living
90 communities interacting within large food webs. Although these communities are not
91 considered the target of weed control practices, they can be either directly or indirectly
92 exposed to pesticides. Pesticide application techniques and pesticide overuse constitute a
93 substantial source of contamination for non-target systems (Pereira et al., 2009). Therefore,
94 non-target organisms are of primary interest when addressing the potential adverse effects
95 of pesticides.

96 The habitat fragmentation due to increased conversion of natural habitats into
97 agricultural fields and the use of pesticides classified as endocrine-disrupting compounds
98 (EDCs) influence the distribution and abundance of wildlife species. The concept of EDCs
99 emerged from observing that few natural compounds and a large number of man-made
100 chemicals released into the environment impact on the wildlife population and have the

101 potential to disrupt the endocrine system of animals, including humans (Bern et al., 1992).
102 Exposure to EDCs can cause different effects according to the developmental stage at
103 which the exposure occurs. Since the endocrine system exhibits an organizing effect on the
104 developing embryo, a disruption of the normal hormonal signals can permanently modify
105 the organization and future function of the reproductive system (Guillette et al., 1995).

106 Amphibians and reptiles are experiencing a worldwide decline, and environmental
107 pollutants are one of the putative causes (Gibbons, 2000; Hopkins, 2000.; Whitfield et al.,
108 2007). The ubiquitous distribution of endocrine-disrupting contaminants and their
109 documented, nonlethal, multigenerational effects on the reproduction, development,
110 behavior and immune systems of several species have led to concern about the impact of
111 EDC exposure on wildlife worldwide (Bergman, 2012). The reproductive disorders in
112 wildlife reported to date include reduced fertility, reduced hatchability, reduced viability of
113 offspring, impaired hormone secretion and activity, and modified reproductive organ
114 histoarchitecture.

115 Certain wildlife taxa such as amphibians are especially vulnerable to chemical
116 perturbation and, as mentioned above, are suffering alarming population declines. One
117 possible reason for this is that the breeding season of many amphibian species coincides
118 with the seasonal application of pesticides in spring and early summer. A mechanism that
119 may account for these population declines is the disruption of the amphibian pheromonal
120 systems by agrochemical pesticides (Park et al., 2001). Since the pheromonal system plays
121 a critical role in the conspecific recognition, migration, social behavior, and reproduction of
122 amphibians, agrochemical disruption may compromise important aspects of the amphibian
123 behaviour regulated by pheromones, such as mate choice and mating success. In addition, it

124 has been demonstrated that the greater the agricultural intensity, the greater the number and
125 severity of defects in toad populations (Hamlin and Guillette, 2010).

126 Understanding the developmental consequences of endocrine disruption in wildlife
127 can lead to the discovery of new indicators of exposure to EDCs. Thus, wildlife serves as
128 an important sentinel of ecosystem health. Eco-epidemiological approaches examining
129 wildlife reproductive abnormalities combined with traditional toxicological studies
130 identifying causation can provide the best model to predict ecosystem concerns due to
131 environmental contamination (Tabor and Alonso Aguirre, 2004). Wildlife has proven
132 valuable to our understanding of the potential effects of EDCs on human health by
133 considerably contributing to our understanding of the mechanisms and consequences of
134 EDC exposure. However, the threats posed by EDCs to populations of wildlife species are
135 also significant, particularly to endangered species whose existence is vulnerable to any
136 reproductive perturbation. Recently, Tubbs and McDonough (2018) addressed this subject
137 and reviewed the literature concerning EDC effects on endangered species.

138 Since the richness and complexity of the field of endocrine disruption continues to
139 grow, the aim of this review is to provide an update on the effects of two agricultural
140 pesticides that act as EDCs: atrazine (ATZ) and endosulfan (END) (Luque et al., 2018). We
141 focus mainly on crocodylians due to their worldwide occurrence in tropical and sub-tropical
142 freshwater ecosystems (Martin, 2007) and their ecological and physiological features,
143 which render them vulnerable to exposure to pesticides with endocrine-disrupting action at
144 all life stages. Crocodylians are widely considered as critical components of freshwater
145 ecosystems, but nearly half of the 25 crocodylian extant species are listed as threatened
146 (IUCN, 2017). As an example, Somaweera et al. (2019) summarized the existing
147 knowledge on the various direct and indirect threats faced by *Crocodylus johnstoni*. In

148 addition, a growing body of literature has demonstrated that the growth, reproduction and
149 survival of crocodilians are influenced by the wide array of xenobiotic compounds
150 introduced into the environment by agricultural activities (Beldomenico et al., 2007;
151 Gonzalez-Jauregui et al., 2012; Milnes et al., 2008; Stoker et al., 2011). Crocodilians, due
152 to their long life-span and carnivorous food habits, bioaccumulate contaminants,
153 particularly lipophilic organochlorine pesticides (OCPs). Studies have shown the presence
154 of OCPs in eggs and tissues of several species of crocodilians (Bouwman et al., 2014;
155 Guillette et al., 2000; Guillette et al., 1999; Nilsen et al., 2017; Rainwater et al., 2002;
156 Stoker et al., 2011; Wu et al., 2006; Yoshikane et al., 2006), as well as the resulting
157 reproductive impairment and population declines (Crain and Guillette, 1998; Guillette and
158 Guillette, 1996; Stoker et al., 2011; Stoker et al., 2013).

159

160 **2. AGRICULTURAL PESTICIDES AS ENDOCRINE-DISRUPTING** 161 **COMPOUNDS**

162 **2.1. Environmental concentrations of ATZ and END**

163 Most pesticide application techniques drive agricultural pesticides directly or
164 indirectly into soils. Thus, agricultural soils are the primary recipients of these pesticides,
165 and water bodies adjacent to agricultural areas are usually the ultimate recipients of
166 pesticide residues. Table 1 summarizes the environmental concentrations of ATZ and END.
167 ATZ is a widespread used herbicide, whereas END is an OCP widely used in the past
168 decades, which have been classified as an EDC. Although many OCPs have been banned,
169 wildlife is still currently exposed (Jurgens et al., 2016). Due to their lipophilic properties,
170 OCPs accumulate in adipose tissues and reach the organisms not only by direct exposure
171 but also through the diet and from the mother to the eggs during vitellogenesis in oviparous

172 species (Muñoz-de-Toro et al., 2006; Stoker et al., 2011). Although not a lipophilic
173 compound itself, ATZ and its metabolite diaminochlorotriazine (DACT), have been found
174 in tissues of turtle at concentrations similar to those found in water (Douros et al., 2015).

175 2.2 Mechanisms involved in ATZ and END endocrine disruptive actions

176 END and ATZ are important EDCs, whose mechanisms of action are summarized in Table
177 2. END is an OCP with long persistence in the environment, which has been classified both
178 as a xenoestrogen and a xenoandrogen. *In vitro* and *in vivo* studies have demonstrated that
179 its estrogenic actions are mediated mainly via estrogen receptor (ER) signaling pathways
180 (Lemaire et al., 2006; Soto et al., 1994; Varayoud et al., 2008). On the other hand, Lemaire
181 et al. (2004) reported that END endocrine activities *in vivo* probably result from direct and
182 specific binding to the androgen receptor (AR) ligand-binding domain, while Viswanath et
183 al. (2010) described END as a potent anti-androgenic compound identified in an *in vitro*
184 assay. Recently, Sebastian and Raghavan (2015) using docking studies described the three-
185 dimensional structure of END binding pattern with AR. END has also been shown to
186 inhibit progesterone receptor (PR) binding in American alligators (Vonier et al., 1996).
187 END endocrine disruptive actions could also be the consequence of an alteration in
188 metabolism pathways (Coumoul et al., 2002).

189 ATZ is known to behave both as an antiandrogen (Hayes et al., 2011) and as a
190 xenoestrogen (Fan et al., 2007; Holloway et al., 2008; Luque et al., 2018). Although ATZ is
191 estrogenic, it seems unable to bind ER (Mizoguchi and Valenzuela, 2016) and is considered
192 an estrogen agonist due to its ability to induce aromatase activity, which in turn leads to
193 inappropriate and excessive estrogen production (Luque et al., 2018; Roberge et al., 2004).
194 ATZ induces aromatase in the gonads of fish (Suzawa and Ingraham, 2008) and amphibians
195 (Hayes et al., 2010; Keller and McClellan-Green, 2004) and in human cell lines (Fan et al.,

196 2007; Holloway et al., 2008; Sanderson et al., 2001). Moreover, ATZ interferes with
197 thyroid homeostasis, as evidenced by its effects on amphibian metamorphosis, a well-
198 known thyroid-regulated process. Although other factors, such as estrogenic or
199 glucocorticoid pathways, cannot be ruled out in the metamorphosis of anuran species
200 (Brodeur et al., 2013), the fact that ATZ binds to the thyroid hormone receptor (Xiang et
201 al., 2017) provides strong evidences supporting its thyroid-disrupting activity. Both ATZ
202 and END can also disrupt the endocrine pathways by epigenetic modulation of gene
203 expression, altering key molecules, such as receptors or steroidogenic enzymes (Clearly et
204 al., 2019; McBirney M et al., 2017; Milesi et al., 2017). When epigenetic changes occur
205 during certain stages of development, they become permanent and can be inherited by the
206 offspring, even leading to transgenerational effects (Clearly et al., 2019; Nilsson et al.,
207 2018)

208

209 **3. CAIMAN LATIROSTRIS: A SENTINEL OF WETLAND HEALTH**

210 *Caiman latirostris*, the broad-snouted caiman, is a crocodile species widely
211 distributed in South America. It resides in coastal freshwater and marsh habitats of
212 Uruguay, northeastern Argentina, Paraguay, Bolivia and the southern region of Brazil. The
213 ecological and physiological features of *C. latirostris*, such as its terrestrial and aquatic
214 habits, longevity, apex predator position in the food web, and sex determination plasticity
215 (Stoker et al., 2003), make it particularly vulnerable to the exposure to agrochemicals. At
216 embryonic stages, caimans may be exposed to contaminants by maternal transfer and
217 through the eggshell. After hatching, the main routes of contaminant exposure are the
218 remaining yolk sac, the diet, and the aqueous environment. The diet of caimans, which
219 consists mainly of fish, is an important source of exposure to persistent lipophilic

220 compounds such as OCPs (Muñoz de Toro et al., 2006), similarly to that reported for Nile
221 crocodiles eating tilapia (Buah-Kwofie et al., 2018). After ingestion, OCPs are absorbed
222 through the digestive tract, transported to other tissues, and deposited into lipid storage
223 (Bargar et al., 1999).

224 Effective monitoring of new and ever-changing environmental and human health
225 hazards requires diverse wildlife indicator species. In this context, since top predators as
226 caimans are essential to maintain the integrity of food webs, biomagnification of EDCs can
227 affect entire ecosystems by harming species at the highest trophic levels. Caiman
228 population stability is critical to keep the balance and health of wetland ecosystems.
229 Therefore, conducting research on the effects of pesticide exposure on *C. latirostris* is
230 especially important, not only to assess the impact on caiman populations but also to further
231 characterize this species as a sentinel of ecosystem health. The characterization of *C.*
232 *latirostris* as a sentinel species may allow the detection of regions with high contamination
233 burdens. To this aim, the high site fidelity of caimans is a useful characteristic (Stoker et
234 al., 2011). It has been proposed that emerging sentinel species should signal more than a
235 “cause and effect” relationship specific to the organism (Tabor and Alonso Aguirre, 2004).
236 Instead, the chosen species should highlight ecological relationships between the impacted
237 sentinel and its ecosystem relevant to the well-being of local human populations (Tabor and
238 Alonso Aguirre, 2004).

239

240 **4. EFFECTS OF AGRICULTURAL PESTICIDES ON SEX DETERMINATION** 241 **AND GONADAL DIFFERENTIATION**

242 Sex determination is the process that guides bipotential gonads to develop into
243 testicles or ovaries. It can be initiated by the activation of a specific gene, by the influence

244 of the environment, or by both. The process of sex determination leads to gonadal
245 differentiation (i.e. the development of ovaries or testes) and ends when the gonad is
246 irreversibly compromised to be a testicle or an ovary (Warner, 2011). The sex of the
247 offspring of all crocodylians, some lizards, and many turtle species is determined by the
248 environment (Bull, 1980). The main factor that determines the sex of the progeny in the
249 absence of sex chromosomes is the incubation temperature of the eggs during a critical
250 period of development (thermosensitive period) (Valenzuela et al., 2014). This process is
251 known as temperature-dependent sex determination (TSD) (Gilbert, 2000; Lang and
252 Andrews, 1994). In species with TSD, temperature initiates a cascade of molecular events
253 that favors the development of male or female gonadal organs by altering gene expression
254 and cellular signaling of steroid hormones, hormone receptors, and steroidogenic enzymes
255 (Mizoguchi and Valenzuela, 2016). From the *eco-evo-devo* perspective, TSD is a prime
256 example of phenotypic plasticity (Martinez-Juarez and Moreno-Mendoza, 2019;
257 Matsumoto and Crews, 2012).

258 The sex determination of crocodylians and turtles involves the critical role of
259 estrogens. In this sense, several researchers have found that the administration of 17 β -
260 Estradiol (E2) during the thermosensitive period overrides the effects of the male
261 incubation temperature, producing phenotypic females in *Trachemys scripta*, *Alligator*
262 *mississippiensis*, and *C. latirostris*, among other species (Crain et al., 1997; Crews et al.,
263 1996; Milnes et al., 2002; Stoker et al., 2003). This effect has been defined as sex reversal
264 or estrogen-induced sex determination (Canesini et al., 2018; Crews et al., 1991; Crews et
265 al., 1991; Holleley et al., 2016; Tousignant and Crews, 1994; Wibbels et al., 1992). The
266 role of estrogens in sex determination is important to be evaluated because it is known that
267 most EDCs exhibit estrogenic activity and are classified as xenoestrogens (Sonnenschein

268 and Soto, 1998). The actions of estrogens are mediated by a plethora of molecular
269 mechanisms that constitute different pathways through which xenoestrogens trigger their
270 effects. In caimans, studies have shown that bisphenol A (BPA), a classical EDC with
271 estrogenic activity, induces sex reversal when caiman embryos from eggs incubated at the
272 male-producing temperature (33°C) are exposed to an environmentally relevant dose
273 (Stoker et al., 2003). In addition, Beldoménico et al. (2007) reported that, despite the
274 estrogenic activity demonstrated for ATZ, exposure of caiman embryos to an ecologically
275 relevant dose of ATZ topically administered to eggs prior to the thermosensitive period
276 failed to produce females at the male-producing temperature (33°C). These authors also
277 observed that, conversely, all eggs treated with E2 and incubated at 33°C were females.
278 Following the protocol of Beldoménico et al. (2007), Canesini, (2018) showed that this
279 ecologically relevant dose of ATZ increased the expression of genes intimately involved in
280 testicular development, such as *amh* and *sox-9*, as well as the expression of proteins
281 involved in the estrogenic pathway, such as estrogen receptor alpha (ER α), PR and
282 aromatase.

283 In *A. mississippiensis*, Crain et al. (1997) reported that the gonadal adrenal
284 mesonephros complex (GAM) from embryos exposed in ovo to ATZ showed a tendency to
285 produce higher than normal levels of aromatase in developing testis in vitro, suggesting that
286 ATZ could alter embryonic steroidogenesis. In male African clawed frogs, *Xenopus laevis*,
287 a species with genetic sex determination, (Hayes et al., 2010) found that exposure to ATZ
288 decreased testosterone levels, testicular size, number of Sertoli cells, sperm production,
289 fertility, and the number of germ cells and increased the levels of aromatase expression as
290 compared with control males. Since both aromatase expression and androgen levels are

291 critical for sex differentiation, the results of Hayes et al. allow concluding that this process
292 is clearly affected by exposure to ATZ.

293 In *Rana pipiens* and *Acris blanchardi*, studies have shown that ATZ can alter the
294 sex ratios and reduce metamorphic success, prolonging the time of this process (Hoskins
295 and Boone, 2018; Langlois et al., 2010). Furthermore, in the turtle *Chelydra serpentina*,
296 hatchlings born from eggs exposed to ATZ, either experimentally or environmentally, have
297 been shown to develop oocytes in the testis, demonstrating that ATZ could affect gonadal
298 development, even without causing complete sex reversal (de Solla et al., 2006).

299 ATZ exposure also affects reproduction in fishes. As an example, Cleary et al.
300 (2019) have recently reported that the F2 generation of *Medaka* (*Oryzias latipes*) exposed
301 to an environmentally relevant dose of ATZ showed a reduced fertilization rate. These
302 authors also found significant transgenerational differences in the expression patterns of
303 genes that play critical roles in gametogenesis, steroidogenesis and DNA methylation
304 (Cleary et al., 2019). These results suggest that, although early life exposure to ATZ did not
305 cause significant phenotype changes in the directly exposed *Medaka* F0 generation,
306 subsequent generations of fish were at greater risk of reproductive dysfunction (Cleary et
307 al., 2019).

308 Female and male caiman reproductive tissues are highly sensitive to the effects of
309 END (Durando et al., 2016; Durando et al., 2013; Rey et al., 2009; Stoker et al., 2008).
310 Several studies have shown that *in ovo* exposure to a single high dose of END (20 ppm) did
311 not override the temperature effect on *C. latirostris* males in sex determination, but
312 modified the testis histoarchitecture in hatchlings and increased the expression of sex-
313 determining genes (*amh*, *Sf1* and *Sox-9*) in neonates (Beldomenico et al., 2007; Durando et
314 al., 2013). Studies in marine crustacean species, such as *Palaemonetes pugio* and *Daphnia*

315 *magna*, have reported that the embryos treated with END showed a delay in developmental
316 processes: increased hatching time in *P. pugio* (Wirth et al., 2001) and delayed molting
317 process in *D. magna* (Palma et al., 2009). In the male Asian catfish (*Clarias batrachus*),
318 Rajakumar et al. (2012) found that exposure to a very low dose of END (2.5 µg/L) at 50
319 days post-hatch modulated testis growth by decreasing the progression of differentiation of
320 spermatogonia to spermatocytes. These authors also found that END decreased the
321 expression of several genes, including testis-related transcription factors (*dmrt1*, *sox9a* and
322 *wt1*), which play a vital role in catfish testicular differentiation/development. Similarly,
323 they detected a low expression of genes related to steroidogenesis (*11β-hsd2*, *17β-hsd12*,
324 *P450c17* and *star*) and orphan nuclear receptors (*nr2c1* and *Ad4BP/SF-1*) (Rajakumar et
325 al., 2012).

326 Although ATZ and END behave as estrogen agonists, their potencies are lower than those
327 of E2 or BPA since neither ATZ nor END affect reptile sex determination (de Solla et al.,
328 2006; Beldomenico et al., 2007). On the other hand, many reports (summarized in Tables 3
329 and 4) support the influence of both agrochemicals on gonadal development/differentiation
330 in a vast array of aquatic wildlife species.

331

332 5. EFFECTS OF AGRICULTURAL PESTICIDES ON GONAD 333 HISTOARCHITECTURE AND FUNCTIONS

334 Estrogen receptor alpha is expressed in the gonad of caiman embryos from stages 22
335 to 24, when the gonad is still bipotential and differentiation is at its onset (Canesini et al.,
336 2018). Thus, the caiman gonad could be disrupted by *in ovo* exposure to EDCs that act as
337 xenoestrogens through ERα, such as END, or by EDCs that increase the endogenous
338 estrogen synthesis, such as ATZ.

339 **5.1 Males**

340 Rey et al. (2009) showed that *in ovo* exposure to an environmentally relevant dose
341 of ATZ disrupted the testicular histoarchitecture in caiman hatchlings. These authors
342 observed disorganized and tortuous seminiferous tubules that exhibited a loss of
343 intratubular intercellular connections (which play a critical role in the control of cell
344 proliferation and differentiation during spermatogenesis) (de Montgolfier et al., 2007;
345 Sridharan et al., 2007) and either emptied tubular lumens or luminal cellular detritus. Rey et
346 al. (2009) also observed altered organization of the peritubular myoid cells, providing
347 evidence that ATZ endocrine-disrupting effects on the caiman testis may be mediated in
348 part through alterations in mesenchymal–epithelial cell interactions. Thus, collectively,
349 long-lasting effects on caiman reproductive performance could be expected.

350 Hayes et al. (2011) reviewed the effects of ATZ on male gonads across vertebrate
351 classes and concluded that ATZ demasculinizes male gonads, producing testicular lesions
352 associated with reduced germ cell numbers in fish, amphibians, reptiles, and mammals, and
353 induces partial and/or complete feminization in fish, amphibians, and reptiles. The authors
354 stated that plausible and coherent mechanisms that may explain these effects include
355 decreasing androgen levels and induction of estrogen synthesis (Hayes et al., 2011).

356 The histoarchitecture of the testes of *C. latirostris* is also disrupted by experimental
357 embryonic exposure to END during the critical period of sex determination. In this species,
358 (Rey et al., 2009) found that the balance between the proliferation and apoptosis of
359 testicular cells of hatchlings was altered. Moreover, Durando et al. (2013; 2016), following
360 the same exposure protocol as Rey et al., demonstrated that the histoarchitecture of the
361 testes was altered not only in 10-day-old hatchlings, but also in 90-day-old and juvenile
362 caimans. As described in ATZ-exposed caimans, these authors also found that testes from

363 caimans exposed to END showed disorganized seminiferous tubules, with loss of
364 intratubular intercellular connections and either emptied tubular lumens or luminal cellular
365 detritus. Besides, Durando et al. (2013) observed thickening of the peritubular connective
366 tissue, a fact that could interfere with the paracrine signaling of the testis, leading to an
367 impaired testicular function. The changes observed in caiman testes long after exposure
368 ended highlight the vulnerability of the embryo to a single dose of END. Stoker et al., 2011,
369 detected END residues in caiman eggs harvested in areas with high agricultural activity.
370 Thus, *C. latirostris* could be naturally exposed to END and thus suffer the consequences
371 described above. Durando et al. (2013) also found that, besides disrupting the
372 histoarchitecture of the testes, *in ovo* exposure to END increases mRNA expression of *amh*,
373 *sox9* and *sf-1* in GAM complexes of 10-day-old males. Similarly, Kohno et al. (2008)
374 described that juvenile American alligators living in a lake polluted with a complex mixture
375 of environmental contaminants exhibited an increased expression of *sf-1* mRNA compared
376 to alligators from a non-contaminated lake. Kohno et al. (2008) also suggested that, in
377 males, *sf-1* regulates the transport of cholesterol needed for steroid synthesis, whereas in
378 females *dax-1* regulates transcription of P450arom, which is essential for the conversion of
379 androgens to estrogens. Therefore, a sexually dimorphic pattern of alterations of
380 interactions between steroid hormones, their receptors and related genes in polluted
381 environments could be expected.

382 The fact that caimans exposed to END show decreased levels of testosterone (Rey et
383 al., 2009) and increased levels of *sf-1* expression, even if these seem opposed, suggests that
384 END can modify other steps in the steroidogenic pathway such as StAR or steroidogenic
385 enzymes, as reported for other xenoestrogens or in other species (Guyot et al., 2004;
386 Rajakumar et al., 2012).

387 5.2 Females

388 As known, ovarian follicle development and progression through successive stages
389 of folliculogenesis are complex processes finely regulated by cell–cell and cell-extra
390 cellular matrix interactions that require appropriately timed endocrine signals (Van
391 Voorhis, 1999); thus, exposure to EDCs could impair follicular dynamics. In caimans, the
392 ovaries of neonatal individuals are characterized by the presence of abundant germ cells
393 clusters and follicles in stages I–III, whereas those of pre-pubertal juvenile caimans present
394 fewer cell clusters and exhibit follicles not only at stages I–III, but also at advanced pre-
395 vitellogenic stages (Stoker et al., 2008; Stoker et al., 2003). Stoker et al. (2008) found that
396 the exposure of the embryonic ovary of *C. latirostris* to ATZ or END accelerated follicular
397 dynamics, showing a higher proportion of type III follicles, which is the most advanced
398 follicular stage seen in neonatal controls. On the other hand, E2- or BPA-exposed caimans
399 exhibited multioocyte follicles, whereas un-exposed caimans and ATZ- or END-exposed
400 ones occasionally present follicles with two oocytes (Stoker et al., 2008). Studies of wild
401 populations of alligators from a pesticide-contaminated area reported high incidence of
402 multioocyte follicles in females (Guillette et al., 1994). These results allow concluding that
403 follicular dynamics could be disrupted by low doses of EDCs and that it is a useful
404 biomarker for EDCs with estrogenic actions even of low potency (Luque et al., 2018). On
405 the other hand, the disruption of follicular assembly that leads to multioocyte follicles
406 require, at least in caimans, exposure to estrogenic agonists of higher potency such as E2 or
407 BPA or to a mixture of EDCs, as reported in alligators (Guillette et al., 1994).

408

409 6. EFFECTS OF AGRICULTURAL PESTICIDES ON THE REPRODUCTIVE 410 TRACT AND EXTERNAL GENITALIA

411 **6.1. The Reproductive Tract**

412 The female reproductive tract –called the oviduct in oviparous species- of caimans
413 shows important postnatal development and differentiation (Galoppo et al., 2016), as
414 described in other vertebrates. The ontogeny of changes in the caiman oviduct from the
415 neonatal to the pre-pubertal juvenile stage has been established using a score system
416 designed to quantify histological features and identify biomarkers of the histofunctional
417 differentiation (Galoppo et al., 2016). Desmin filaments are responsible for stabilizing
418 sarcomeres, a fact required to maintain the mechanical integrity of smooth muscle cells.
419 The expression of desmin has been proposed as a biomarker of the organization and
420 maturity of the oviductal muscular layer (Galoppo et al., 2016). On the other hand, the
421 expression of ER α , PR and AR shows that the caiman oviduct could be a target of the
422 action of EDCs early in life (Galoppo et al., 2017; Galoppo et al., 2016). Moreover, it has
423 been shown that early postnatal exposure to E2 decreases oviduct ER α expression and
424 induces AR translocation from the cytoplasm to the nucleus. In addition, early postnatal
425 exposure to BPA has been found to modify the temporal pattern of oviduct postnatal
426 development, advancing the adenogenesis process (Galoppo et al., 2017).

427 In oviparous species, such as *C. latirostris*, a prominent function of the oviducts is
428 to provide the ovulated eggs with the egg white proteins and eggshell. In this regard, Stoker
429 et al. (2013) assessed the relationship between the presence of OCPs that behave as EDCs
430 in caiman eggs and eggshell features. Their results suggest a direct effect of the exposure to
431 OCPs on the maternal oviductal functions, evidenced by decreased eggshell porosity
432 (Stoker et al., 2013). These authors also found that the decreased eggshell porosity led to
433 decreased hatchling survival at 10 days of age (Stoker et al., 2013).

434 Preliminary results obtained at our lab (Galoppo et al. 2017b) have demonstrated
435 that *in ovo* exposure of caimans to a single dose of ATZ or END increased the expression
436 of desmin, suggesting that these pesticides induce the organization and maturation of the
437 oviductal muscular layer (Figure 1). We have also found that embryonic exposure to ATZ
438 and END modified the oviductal expression of steroid hormone receptors at pre-pubertal
439 juvenile stage: exposure to END reduced ER α expression, whereas exposure to ATZ
440 reduced nuclear AR expression in the luminal epithelium of the *C. latirostris* oviduct
441 (Galoppo et al., 2017b). Additionally, prenatal exposure to END increased collagen
442 remodeling in prepubertal juvenile caimans (Tavalieri et al., 2019b,c), a feature related to
443 adenogenesis (Galoppo et al., 2016), and increased the mesenchymal area of the oviduct
444 occupied by glands and gland density (Figure 2) (Tavalieri et al., 2019b,c). These results
445 suggest that embryonic END exposure modifies the temporal pattern of oviductal
446 differentiation, namely adenogenesis.

447 Collectively, the results above-described demonstrate that the caiman oviduct is
448 highly sensitive to EDC exposure at prenatal or early postnatal developmental stages.
449 Moreover, changes in histomorphological features, in the temporal and spatial pattern of
450 steroid hormone receptors, in collagen remodeling and in muscle-associated protein
451 expression suggest a precocious oviduct histofunctional differentiation following ATZ and
452 END exposure. The modification of the temporal pattern of oviductal biomarkers suggests
453 that organizational changes could impair *C. latirostris* oviductal functions and/or
454 reproductive health later in life.

455

456 **6.2. External Genitalia**

457 In humans, the prevalence of genital malformations and pathologies is increasing in
458 many countries, more prominently in those of the western hemisphere (Main et al., 2010).
459 Several reports have linked the emergence of congenital anomalies such as hypospadias,
460 cryptorchidism and micropenis, with exposure to EDCs (Agopian et al., 2013; Botta et al.,
461 2014; Hsieh et al., 2008; Main et al., 2010; Wang and Baskin, 2008). Agrochemical
462 pesticides can be carried off the application site, affecting people, domestic animals and
463 wildlife. Regarding the effects of pesticides classified as EDCs on wildlife, a high
464 incidence of cryptorchidism has been reported in the Florida panther (*Puma concolor coryi*)
465 related to the presence of EDCs in the environment (Facemire et al., 1995) however a;
466 genetic component could be contributing to this phenomenon (Mansfield and Land, 2002).

467 Juvenile *A. mississippiensis* alligators living in Lake Apopka, a contaminated lake in
468 the state of Florida (USA), have been found to exhibit reduced phallus size and lower
469 plasma concentrations of testosterone than those of alligators of similar size living in lakes
470 with low anthropic activity (Guillette et al., 1999; Guillette and Guillette, 1996). The
471 alligators living in Lake Apopka showed increased concentrations of DDT stored in their
472 fat tissues (EPA, 1994). Similar results have been observed within the Kissimmee-
473 Everglades drainage of Florida, where the alligators living in the most contaminated areas
474 exhibit reduced phallus size as compared with that of alligators living in moderate or low
475 contaminated sites (Gunderson et al., 2003). A possible explanation for these results is that
476 both ATZ and dichlorodiphenyldichloroethylene, a metabolic product from DDT, were
477 detected among the sediment and water contaminants found in the drainage area
478 (Gunderson et al., 2003).

479 In crocodylians, the external genitalia (i.e. phallus or clitero-penis) are composed of
480 an unpaired organ that is considered a sexually dimorphic structure (Allsteadt and Lang,

481 1994; Nuñez-Otaño et al., 2010; Tavalieri et al., 2019a). In males, the phallus serves
482 functions of intromission and insemination into the female cloaca during copulation,
483 whereas, in females, profuse innervation suggests that the phallus could be associated with
484 sex arousal and/or involved in a neuroendocrine response needed for successful copulation
485 (Tavalieri et al., 2019a). The phallus size is the most reliable anatomic characteristic used
486 to differentiate adult and juvenile crocodylian males from females (Nuñez-Otaño et al.,
487 2010). In *C. latirostris*, males can be distinguished from females when caimans attain a
488 total length of about 50 cm (Tavalieri et al., 2019a). At our lab (Tavalieri et al. 2017), we
489 also found that, since the phallus is a hormone-dependent organ, its size may be altered by
490 exposure to EDCs. Our experiments showed that *in ovo* exposure to environmentally
491 relevant doses of ATZ did not affect the phallus length/caiman condition index ratio in
492 prepubertal juvenile male and female caimans (Figure 3). However, correlations between
493 male phallus length and caiman body mass showed that the slope of control caimans was
494 steeper than the slope of ATZ-exposed caimans (Figure 4), suggesting that *in ovo* exposure
495 to ATZ slows down phallus growth (Tavalieri et al., 2017). We also found that *in ovo*
496 exposure to ATZ alters the protein expression of sex hormone receptors in the phallus of
497 prepubertal juvenile male and female caimans. As shown in Table 5, male caimans showed
498 significant decreased expression of AR, evidencing a demasculinization effect. In contrast,
499 females showed increased AR expression. Our results also showed that the phallus of male
500 and female caimans exhibited increased ER α expression (Tavalieri et al., 2017). This
501 altered AR and ER α expression and the decreased testosterone levels in ATZ-exposed
502 caiman males reported by Rey et al. (2009) suggest that the phallus sensitivity to hormones
503 and its subsequent hormone-mediated growth may be compromised at adulthood.

504

505 Besides their effect as endocrine disruptors, ATZ and END have also been classified
506 as immune disruptors (Brodkin et al., 2007; Christin et al., 2004; Kumari et al., 2017;
507 Mizota and Ueda, 2006; Pushpanjali et al., 2005; Singh et al., 2016). At our lab, we found
508 that exposure to ATZ increases the phallic transversal area occupied by lymphocyte
509 aggregates (structures comprised of small, highly basophilic, mononuclear cells) in pre-
510 pubertal juvenile caimans (Figure 5 and Table 6) (Tavalieri et al., 2019a). The presence of
511 lymphocyte aggregates has been described in the phallus of *C. latirostris* (Tavalieri et al.,
512 2019a) and *A. mississippiensis* (Moore et al., 2012). Considered as common structures
513 needed to prevent infections under healthy conditions, lymphocyte aggregates can also be
514 associated with lesions or pathological conditions (Govett et al., 2005). Increased
515 lymphocyte aggregates in ATZ-exposed caimans could be the result of increased
516 lymphocyte proliferation activity, as reported in 21-day-old *A. mississippiensis* in ovo
517 exposed to the xenoestrogens tetrachlorodibenzo-p-dioxin or ethynyl estradiol (Keller et al.,
518 2005). On the other hand, since immune responses are known to be modulated by sex
519 hormones (Kovats et al., 2016; Lai et al., 2012), the ATZ immune-disrupting properties
520 observed in the crocodylian phallus may be a consequence of the alterations caused by ATZ
521 in hormone levels and/or in the expression of sex hormone receptors.

522 7. CONCLUSIONS

523 Endocrine disruption is a potential global problem observed not only in heavily polluted
524 areas but also in those considered “pristine”. There is particular concern about the
525 exposures of wildlife to mixtures of biologically active chemicals, which, combined with
526 other stressors, may play an even greater role in reproductive disorders. This global concern
527 has been increased by recent evidences supporting the classification of agricultural
528 pesticides such as ATZ and END as EDCs.

529 Published results highlight the usefulness of crocodiles, an order with TSD, not only to
530 assess estrogenic activity *in vivo* and as a sentinel to monitor EDCs in wetland
531 environments, but also as model species to study endocrine disruption and development at
532 the lab and the consequences of real-life scenarios of exposure to EDCs in the field. As
533 shown in Figure 6, pesticide application techniques and their massive use constitute a
534 substantial source of contamination for non-target organisms. This figure also illustrates the
535 role of the foodweb in this top predator vertebrate. Further studies are required to better
536 understand the pathways involved and the long-term consequences of the disruption in the
537 crocodile reproductive system here reviewed. If this is accomplished, we may begin to
538 mitigate the reproductive impacts of EDCs on wildlife through better-informed policy
539 decisions.

540 Mitigating the ecological impacts of chemical pollution from agricultural activities will
541 require the protection of wildlife from chemical contaminants that already exist in the
542 environment, removal of these contaminants where feasible, and increasing efforts to
543 prevent further additions to terrestrial and freshwater ecosystems.

544

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552 **9. REFERENCES**

- 553 Adisa, I.O., Pullagurala, V.L.R., Peralta-Videa, J.R., Dimkpa, C.O., Elmer, W.H., Gardea-
554 Torresdey, J.L., White, J.C., 2019. Recent advances in nano-enabled fertilizers and
555 pesticides: A critical review of mechanisms of action. *Environmental Science:*
556 *Nano*. 6, 2002-2030.
- 557 Agopian, A.J., Lupo, P.J., Canfield, M.A., Langlois, P.H., 2013. Case-control study of
558 maternal residential atrazine exposure and male genital malformations. *Am. J. Med.*
559 *Genet. A*. 161A, 977-982.
- 560 Allsteadt, J., Lang, J.W., 1994. Sexual dimorphism in the genital morphology of young
561 American Alligator, *Alligator mississippiensis*. *Herpetologica*. 51, 314-325.
- 562 ANVISA, 2020a. [http://portal.anvisa.gov.br/documents/111215/117782/a14.pdf/76773817-](http://portal.anvisa.gov.br/documents/111215/117782/a14.pdf/76773817-be41-4334-9846-77bd559f9e80)
563 [be41-4334-9846-77bd559f9e80}](http://portal.anvisa.gov.br/documents/111215/117782/a14.pdf/76773817-be41-4334-9846-77bd559f9e80)
- 564 ANVISA, 2020b. [http://portal.anvisa.gov.br/registros-e-](http://portal.anvisa.gov.br/registros-e-autorizacoes/agrotoxicos/produtos/monografia-de-agrotoxicos/excluidas)
565 [autorizacoes/agrotoxicos/produtos/monografia-de-agrotoxicos/excluidas](http://portal.anvisa.gov.br/registros-e-autorizacoes/agrotoxicos/produtos/monografia-de-agrotoxicos/excluidas)
- 566 APVMA, 2016.
567 [https://portal.apvma.gov.au/pubcris?p_auth=4UqIVydd&p_p_id=pubcrisportlet_W](https://portal.apvma.gov.au/pubcris?p_auth=4UqIVydd&p_p_id=pubcrisportlet_WAR_pubcrisportlet&p_p_lifecycle=1&p_p_state=normal&p_p_mode=view&p_p_col_id=column-1&p_p_col_pos=2&p_p_col_count=4&_pubcrisportlet_WAR_pubcrisportlet_id=81685&_pubcrisportlet_WAR_pubcrisportlet_javax.portlet.action=viewProduct)
568 [AR_pubcrisportlet&p_p_lifecycle=1&p_p_state=normal&p_p_mode=view&p_p_c](https://portal.apvma.gov.au/pubcris?p_auth=4UqIVydd&p_p_id=pubcrisportlet_WAR_pubcrisportlet&p_p_lifecycle=1&p_p_state=normal&p_p_mode=view&p_p_col_id=column-1&p_p_col_pos=2&p_p_col_count=4&_pubcrisportlet_WAR_pubcrisportlet_id=81685&_pubcrisportlet_WAR_pubcrisportlet_javax.portlet.action=viewProduct)
569 [ol_id=column-](https://portal.apvma.gov.au/pubcris?p_auth=4UqIVydd&p_p_id=pubcrisportlet_WAR_pubcrisportlet&p_p_lifecycle=1&p_p_state=normal&p_p_mode=view&p_p_col_id=column-1&p_p_col_pos=2&p_p_col_count=4&_pubcrisportlet_WAR_pubcrisportlet_id=81685&_pubcrisportlet_WAR_pubcrisportlet_javax.portlet.action=viewProduct)
570 [1&p_p_col_pos=2&p_p_col_count=4&_pubcrisportlet_WAR_pubcrisportlet_id=81](https://portal.apvma.gov.au/pubcris?p_auth=4UqIVydd&p_p_id=pubcrisportlet_WAR_pubcrisportlet&p_p_lifecycle=1&p_p_state=normal&p_p_mode=view&p_p_col_id=column-1&p_p_col_pos=2&p_p_col_count=4&_pubcrisportlet_WAR_pubcrisportlet_id=81685&_pubcrisportlet_WAR_pubcrisportlet_javax.portlet.action=viewProduct)
571 [685&_pubcrisportlet_WAR_pubcrisportlet_javax.portlet.action=viewProduct](https://portal.apvma.gov.au/pubcris?p_auth=4UqIVydd&p_p_id=pubcrisportlet_WAR_pubcrisportlet&p_p_lifecycle=1&p_p_state=normal&p_p_mode=view&p_p_col_id=column-1&p_p_col_pos=2&p_p_col_count=4&_pubcrisportlet_WAR_pubcrisportlet_id=81685&_pubcrisportlet_WAR_pubcrisportlet_javax.portlet.action=viewProduct)
- 572 APVMA, 2010.
573 [https://portal.apvma.gov.au/pubcris?p_auth=4UqIVydd&p_p_id=pubcrisportlet_W](https://portal.apvma.gov.au/pubcris?p_auth=4UqIVydd&p_p_id=pubcrisportlet_WAR_pubcrisportlet&p_p_lifecycle=1&p_p_state=normal&p_p_mode=view&p_p_col_id=column-1&p_p_col_pos=2&p_p_col_count=4&_pubcrisportlet_WAR_pubcrisportlet_id=44305&_pubcrisportlet_WAR_pubcrisportlet_javax.portlet.action=viewProduct)
574 [AR_pubcrisportlet&p_p_lifecycle=1&p_p_state=normal&p_p_mode=view&p_p_c](https://portal.apvma.gov.au/pubcris?p_auth=4UqIVydd&p_p_id=pubcrisportlet_WAR_pubcrisportlet&p_p_lifecycle=1&p_p_state=normal&p_p_mode=view&p_p_col_id=column-1&p_p_col_pos=2&p_p_col_count=4&_pubcrisportlet_WAR_pubcrisportlet_id=44305&_pubcrisportlet_WAR_pubcrisportlet_javax.portlet.action=viewProduct)
575 [ol_id=column-](https://portal.apvma.gov.au/pubcris?p_auth=4UqIVydd&p_p_id=pubcrisportlet_WAR_pubcrisportlet&p_p_lifecycle=1&p_p_state=normal&p_p_mode=view&p_p_col_id=column-1&p_p_col_pos=2&p_p_col_count=4&_pubcrisportlet_WAR_pubcrisportlet_id=44305&_pubcrisportlet_WAR_pubcrisportlet_javax.portlet.action=viewProduct)
576 [1&p_p_col_pos=2&p_p_col_count=4&_pubcrisportlet_WAR_pubcrisportlet_id=44](https://portal.apvma.gov.au/pubcris?p_auth=4UqIVydd&p_p_id=pubcrisportlet_WAR_pubcrisportlet&p_p_lifecycle=1&p_p_state=normal&p_p_mode=view&p_p_col_id=column-1&p_p_col_pos=2&p_p_col_count=4&_pubcrisportlet_WAR_pubcrisportlet_id=44305&_pubcrisportlet_WAR_pubcrisportlet_javax.portlet.action=viewProduct)
577 [305&_pubcrisportlet_WAR_pubcrisportlet_javax.portlet.action=viewProduct](https://portal.apvma.gov.au/pubcris?p_auth=4UqIVydd&p_p_id=pubcrisportlet_WAR_pubcrisportlet&p_p_lifecycle=1&p_p_state=normal&p_p_mode=view&p_p_col_id=column-1&p_p_col_pos=2&p_p_col_count=4&_pubcrisportlet_WAR_pubcrisportlet_id=44305&_pubcrisportlet_WAR_pubcrisportlet_javax.portlet.action=viewProduct)
- 578 Bargar, T.A., Sills-McMurry, C., Dickerson, R.L., Rhodes, W.E., Cobb, G.P., 1999.
579 Relative distribution of polychlorinated biphenyls among tissues of neonatal
580 American alligators (*Alligator mississippiensis*). *Arch. Environ. Contam. Toxicol.*
581 37, 364-368.
- 582 Beldomenico, P.M., Rey, F., Prado, W.S., Villarreal, J.C., Muñoz-de-Toro, M., Luque,
583 E.H., 2007. In ovum exposure to pesticides increases the egg weight loss and

- 584 decreases hatchlings weight of *Caiman latirostris* (Crocodylia: Alligatoridae).
585 *Ecotoxicol. Environ. Saf.* 68, 246-251.
- 586 Benedusi, V., Martini, E., Kallikourdis, M., Villa, A., Meda, C., Maggi, A., 2015.
587 Ovariectomy shortens the life span of female mice, *Oncotarget* 6, 10801-10811
- 588 Bergman, Å.H., J. J.; Jobling, S.; Kidd, K. A.; Zoeller, R. T., State of the science of
589 endocrine disrupting chemicals, World Health Organization and United Nations
590 Environment Programme: World Health Organization Library Cataloguing-in-
591 Publication Data, 2012.
- 592 Bern, H., Blair, P., Brasseur, S., Colborn, T., Cunha, G., Davis, W., Dohler, K.D.,
593 McLachlan, J., Myers, J.P., Peterson, R.E., Reijnders, P.J.H., Fox, G., Fry, M.,
594 Gray, E., Green, R., Hines, M., Kubiak, T.J., Soto, A., Van Der Kraak, G., vom
595 Saal, F., Whitten, P., 1992. Statement from the work session on chemically-induced
596 alterations in sexual development: the wildlife/human connection. pp 1-8 in
597 *Chemically-Induced Alterations in Sexual and Functional Development: The*
598 *Wildlife/Human Connection.* eds T. Colborn and C. Clement, Princeton Scientific
599 Publishing Co., NJ, U.S.
- 600 Botta, S., Cunha, G.R., Baskin, L.S., 2014. Do endocrine disruptors cause hypospadias?
601 *Transl Androl Urol.* 3, 330-339.
- 602 Bouwman, H., Booyens, P., Govender, D., Pienaar, D., Polder, A., 2014. Chlorinated,
603 brominated, and fluorinated organic pollutants in Nile crocodile eggs from the
604 Kruger National Park, South Africa. *Ecotoxicol Environ Saf.* 104, 393-402.
- 605 Brodeur, J.C., Sassone, A., Hermida, G.N., Codugnello, N., 2013. Environmentally-
606 relevant concentrations of atrazine induce non-monotonic acceleration of
607 developmental rate and increased size at metamorphosis in *Rhinella arenarum*
608 tadpoles. *Ecotoxicol. Environ. Saf.* 92, 10-17.
- 609 Brodtkin, M.A., Madhoun, H., Rameswaran, M., Vatnick, I., 2007. Atrazine is an immune
610 disruptor in adult northern leopard frogs (*Rana pipiens*). *Environ. Toxicol. Chem.*
611 26, 80-84.
- 612 Buah-Kwofie, A., Humphries, M.S., Combrink, X., Myburgh, J.G., 2018. Accumulation of
613 organochlorine pesticides in fat tissue of wild Nile crocodiles (*Crocodylus niloticus*)
614 from iSimangaliso Wetland Park, South Africa. *Chemosphere* 195,463-471.

- 615 Bull, J.J., 1980. Sex determination in reptiles *The Quarterly Review of Biology*. 55, 3-21.
- 616 Canesini, G., 2018. Determinación sexual y diferenciación gonadal en yacaré overo. Genes
617 involucrados en su regulación y efecto de la exposición a perturbadores endocrinos.
618 PhD Thesis.
619 [https://ri.conicet.gov.ar/bitstream/handle/11336/87983/CONICET_Digital_Nro.0f2](https://ri.conicet.gov.ar/bitstream/handle/11336/87983/CONICET_Digital_Nro.0f238c1a-ef08-44f2-862e-f8577226bccc_A.pdf?sequence=2&isAllowed=y)
620 [38c1a-ef08-44f2-862e-f8577226bccc_A.pdf?sequence=2&isAllowed=y](https://ri.conicet.gov.ar/bitstream/handle/11336/87983/CONICET_Digital_Nro.0f238c1a-ef08-44f2-862e-f8577226bccc_A.pdf?sequence=2&isAllowed=y).
- 621 Canesini, G., Stoker, C., Galoppo, G.H., Durando, M.L., Tschopp, M.V., Luque, E.H.,
622 Muñoz-de-Toro, M.M., Ramos, J.G., 2018. Temperature- vs. estrogen-induced sex
623 determination in *Caiman latirostris* embryos: Both females, but with different
624 expression patterns of key molecules involved in ovarian development. *Gen. Comp.*
625 *Endocrinol.* 259, 176-188.
- 626 Christin, M.S., Menard, L., Gendron, A.D., Ruby, S., Cyr, D., Marcogliese, D.J., Rollins-
627 Smith, L., Fournier, M., 2004. Effects of agricultural pesticides on the immune
628 system of *Xenopus laevis* and *Rana pipiens*. *Aquat Toxicol.* 67, 33-43.
- 629 Cleary, J.A., Tillitt, D.E., Vom Saal, F.S., Nicks, D.K., Claunch, R.A., Bhandari, R.K.,
630 2019. Atrazine induced transgenerational reproductive effects in medaka (*Oryzias*
631 *latipes*). *Environ Pollut.* 251, 639-650.
- 632 Coumoul, X., Diry, M., Barouki, R., 2002. PXR-dependent induction of human CYP3A4
633 gene expression by organochlorine pesticides. *Biochem. Pharmacol.* 64,1513-
634 1519.Crain, D.A., Guillette, L.J., Jr., 1998. Reptiles as models of contaminant-
635 induced endocrine disruption. *Anim. Reprod. Sci.* 53, 77-86.
- 636 Crain, D.A., Guillette, L.J., Jr., Rooney, A.A., Pickford, D.B., 1997. Alterations in
637 steroidogenesis in alligators (*Alligator mississippiensis*) exposed naturally and
638 experimentally to environmental contaminants. *Environ Health Perspect.* 105, 528-
639 533.
- 640 Crews, D., Bull, J.J., Wibbels, T., 1991a. Estrogen and sex reversal in turtles: a dose-
641 dependent phenomenon. *Gen. Comp. Endocrinol.* 81, 357-364.
- 642 Crews, D., Bull, J.J., Wibbels, T., 1991b. Estrogen and sex reversal in turtles: a dose-
643 dependent phenomenon. *Gen Comp Endocrinol.* 81, 357-364.
- 644 Crews, D., Cantu, A.R., Rhen, T., Vohra, R., 1996. The relative effectiveness of estrone,
645 estradiol-17 beta, and estriol in sex reversal in the red-eared slider (*Trachemys*

- 646 scripta), a turtle with temperature-dependent sex determination. *Gen Comp*
647 *Endocrinol.* 102, 317-326.
- 648 Cruze, L., Roark, A.M., Rolland, G., Younas, M., Stacy, N., Guillette, L.J., Jr., 2015.
649 Endogenous and exogenous estrogens during embryonic development affect timing
650 of hatch and growth in the American alligator (*Alligator mississippiensis*). *Comp.*
651 *Biochem. Physiol. B Biochem. Mol. Biol.* 184, 10-18.
- 652 de Montgolfier, B., Dufresne, J., Letourneau, M., Nagler, J.J., Fournier, A., Audet, C., Cyr,
653 D.G., 2007. The expression of multiple connexins throughout spermatogenesis in
654 the rainbow trout testis suggests a role for complex intercellular communication.
655 *Biol Reprod.* 76, 2-8.
- 656 de Solla, S.R., Martin, P.A., Fernie, K.J., Park, B.J., Mayne, G., 2006. Effects of
657 environmentally relevant concentrations of atrazine on gonadal development of
658 snapping turtles (*Chelydra serpentina*). *Environ. Toxicol. Chem.* 25, 520-526.
- 659 Douros D.L., Gaines K.F., Novak J. M., 2015 Atrazine and glyphosate dynamics in a lotic
660 ecosystem: the common snapping turtle as a sentinel species. *Environ. Monit.*
661 *Assess.* 187,114
- 662 DTI,2017. http://www.dti.gov.za/industrial_development/docs/Agricultural_Remedies.pdf
- 663 Durando, M., Canesini, G., Cocito, L.L., Galoppo, G.H., Zayas, M.A., Luque, E.H.,
664 Muñoz-de-Toro, M., 2016. Histomorphological changes in testes of broad-snouted
665 caimans (*Caiman latirostris*) associated with in ovo exposure to endocrine-
666 disrupting chemicals. *J. Exp. Zool. A Ecol. Genet. Physiol.* 325, 84-96.
- 667 Durando, M., Cocito, L., Rodriguez, H.A., Varayoud, J., Ramos, J.G., Luque, E.H., Muñoz-
668 de-Toro, M., 2013. Neonatal expression of amh, sox9 and sf-1 mRNA in *Caiman*
669 *latirostris* and effects of in ovo exposure to endocrine disrupting chemicals. *Gen.*
670 *Comp. Endocrinol.* 191, 31-38.
- 671 EPA, U.S., Tower Chemical Company Superfund Site Biological Assessment March 1994.
672 U.S. Environmental Protection Agency, Biological Assessment, 1994.
- 673 EPA, 2019. [https://www.epa.gov/sites/production/files/2019-10/documents/rup-report-](https://www.epa.gov/sites/production/files/2019-10/documents/rup-report-oct2019.pdf)
674 [oct2019.pdf](https://www.epa.gov/sites/production/files/2019-10/documents/rup-report-oct2019.pdf)
- 675 EPA, 2010. [https://archive.epa.gov/pesticides/reregistration/web/html/endosulfan-](https://archive.epa.gov/pesticides/reregistration/web/html/endosulfan-agreement.html)
676 [agreement.html](https://archive.epa.gov/pesticides/reregistration/web/html/endosulfan-agreement.html)

- 677 Facemire, C.F., Gross, T.S., Guillette, L.J., Jr., 1995. Reproductive impairment in the
678 Florida panther: nature or nurture? *Environ Health Perspect.* 103 Suppl 4, 79-86.
- 679 Fan, W., Yanase, T., Morinaga, H., Gondo, S., Okabe, T., Nomura, M., Komatsu, T.,
680 Morohashi, K., Hayes, T.B., Takayanagi, R., Nawata, H., 2007. Atrazine-induced
681 aromatase expression is SF-1 dependent: implications for endocrine disruption in
682 wildlife and reproductive cancers in humans. *Environ. Health Perspect.* 115, 720-
683 727.
- 684 Galoppo, G.H., Canesini, G., Tavalieri, Y.E., Stoker, C., Kass, L., Luque, E.H., Muñoz-de-
685 Toro, M., 2017a. Bisphenol A disrupts the temporal pattern of histofunctional
686 changes in the female reproductive tract of *Caiman latirostris*. *Gen. Comp.*
687 *Endocrinol.* 254, 75-85.
- 688 Galoppo, G.H., Stoker, C., Canesini, G., Schierano-Marotti, G., Durando, M., Luque, E.H.,
689 Muñoz-de-Toro, M., 2016. Postnatal development and histofunctional
690 differentiation of the oviduct in the broad-snouted caiman (*Caiman latirostris*). *Gen.*
691 *Comp. Endocrinol.* 236, 42-53.
- 692 Galoppo, G.H., Tavalieri, Y.E., Canesini, G., Luque, E.H., Muñoz-de-Toro, M., 2017b.
693 Long lasting effects of prenatal agrochemical exposure on caiman latirostris oviduct
694 development and differentiation. *Medicina Buenos Aires* 77, 293-294.
- 695 Galoppo, G.H., Tavalieri, Y.E., Schierano-Marotti, G., Osti, M.R., Luque, E.H., Muñoz-de-
696 Toro, M.M., 2020. Long-term effects of in ovo exposure to an environmentally
697 relevant dose of Atrazine on the thyroid gland of *Caiman latirostris*. *Environ. Res.*
698 186. doi: 10.1016/j.envres.2020.109410.
- 699 Gibbons, J., 2000. The global decline of reptiles, de´ ja` vu amphibians. *Bioscience.* 50,
700 653–666.
- 701 Gilbert, S.F., *Environmental sex determination.* Dev. Biol., 6 ed, Sinauer Associates,
702 Sunderland (MA). 2000.
- 703 Gonzalez-Jauregui, M., Valdespino, C., Salame-Mendez, A., Aguirre-Leon, G., Rendon-
704 vonOsten, J., 2012. Persistent Organic Contaminants and Steroid Hormones Levels
705 in Morelet’s Crocodiles From the Southern Gulf of Mexico. *Arch. Environ.*
706 *Contam. Toxicol.* 62, 445-454.

- 707 Govett, P.D., Harms, C.A., Johnson, A.J., Latimer, K.S., Wellehan, J.F., Fatzinger, M.H.,
708 Christian, L.S., Kelly, T.R., Lewbart, G.A., 2005. Lymphoid follicular cloacal
709 inflammation associated with a novel herpesvirus in juvenile alligators (*Alligator*
710 *mississippiensis*). *J Vet Diagn Invest.* 17, 474-479.
- 711 Guillette, L.J., Crain, D.A., Gunderson, M.P., Kools, S.A.E., Milnes, M.R., Orlando, E.F.,
712 Rooney, A.A., Woodward, A.R., 2000. Alligators and endocrine disrupting
713 contaminants: a current perspective. *Am. Zool.* 40, 438-452.
- 714 Guillette, L.J., Jr., Brock, J.W., Rooney, A.A., Woodward, A.R., 1999. Serum
715 concentrations of various environmental contaminants and their relationship to sex
716 steroid concentrations and phallus size in juvenile American alligators. *Arch.*
717 *Environ. Contam. Toxicol.* 36, 447-455.
- 718 Guillette, L.J., Jr., Crain, D.A., Rooney, A.A., Pickford, D.B., 1995. Organization versus
719 activation: the role of endocrine-disrupting contaminants (EDCs) during embryonic
720 development in wildlife. *Environ. Health Perspect.* 103 Suppl 7, 157-164.
- 721 Guillette, L.J., Jr., Gross, T.S., Masson, G.R., Matter, J.M., Percival, H.F., Woodward,
722 A.R., 1994. Developmental abnormalities of the gonad and abnormal sex hormone
723 concentrations in juvenile alligators from contaminated and control lakes in Florida.
724 *Environ Health Perspect.* 102, 680-688.
- 725 Guillette, L.J., Jr., Guillette, E.A., 1996. Environmental contaminants and reproductive
726 abnormalities in wildlife: implications for public health? *Toxicol. Ind. Health.* 12,
727 537-550.
- 728 Gunderson, M.P., Kools, S.A., Milnes, M.R., Guillette, L.J., Jr., 2003. Effect of acute stress
729 on plasma beta-corticosterone, estradiol-17 beta and testosterone concentrations in
730 juvenile American alligators collected from three sites within the Kissimmee-
731 Everglades drainage basin in Florida (USA). *Comp Biochem Physiol C Toxicol*
732 *Pharmacol.* 135C, 365-374.
- 733 Guyot, R., Odet, F., Leduque, P., Forest, M.G., Le Magueresse-Battistoni, B., 2004.
734 Diethylstilbestrol inhibits the expression of the steroidogenic acute regulatory
735 protein in mouse fetal testis. *Mol. Cell. Endocrinol.* 220, 67-75.

- 736 Hamlin, H.J., Guillette, L.J., Jr., 2010. Birth defects in wildlife: the role of environmental
737 contaminants as inducers of reproductive and developmental dysfunction. *Syst Biol*
738 *Reprod Med.* 56, 113-121.
- 739 Hayes, T.B., Anderson, L.L., Beasley, V.R., de Solla, S.R., Iguchi, T., Ingraham, H.,
740 Kestemont, P., Kniewald, J., Kniewald, Z., Langlois, V.S., Luque, E.H., McCoy,
741 K.A., Muñoz-de-Toro, M., Oka, T., Oliveira, C.A., Orton, F., Ruby, S., Suzawa, M.,
742 Tavera-Mendoza, L.E., Trudeau, V.L., Victor-Costa, A.B., Willingham, E., 2011.
743 Demasculinization and feminization of male gonads by atrazine: consistent effects
744 across vertebrate classes. *J. Steroid Biochem. Mol. Biol.* 127, 64-73.
- 745 Hayes, T.B., Khoury, V., Narayan, A., Nazir, M., Park, A., Brown, T., Adame, L., Chan,
746 E., Buchholz, D., Stueve, T., Gallipeau, S., 2010. Atrazine induces complete
747 feminization and chemical castration in male African clawed frogs (*Xenopus*
748 *laevis*). *Proc Natl Acad Sci U S A.* 107, 4612-4617.
- 749 Holleley, C.E., Sarre, S.D., O'Meally, D., Georges, A., 2016. Sex Reversal in Reptiles:
750 Reproductive Oddity or Powerful Driver of Evolutionary Change? *Sex Dev.* 10,
751 279-287.
- 752 Holloway, A.C., Anger, D.A., Crankshaw, D.J., Wu, M., Foster, W.G., 2008. Atrazine-
753 induced changes in aromatase activity in estrogen sensitive target tissues. *J. Appl.*
754 *Toxicol.* 28, 260-270.
- 755 Hopkins, W., 2000. Reptile toxicology: challenges and opportunities on the last frontier in
756 vertebrate ecology. *Environ. Toxicol. Chem.* 19, 2391-2393.
- 757 Hoskins, T.D., Boone, M.D., 2018. Atrazine feminizes sex ratio in Blanchard's cricket frogs
758 (*Acris blanchardi*) at concentrations as low as 0.1 mug/L. *Environ. Toxicol. Chem.*
759 37, 427-435.
- 760 Hsieh, M.H., Breyer, B.N., Eisenberg, M.L., Baskin, L.S., 2008. Associations among
761 hypospadias, cryptorchidism, anogenital distance, and endocrine disruption. *Curr*
762 *Urol Rep.* 9, 137-142.
- 763 IUCN, 2017. International Union for Conservation of Nature.
764 <http://www.iucn.org/pages/Conservation-Status.html>.

- 765 Jurgens, M.D., Crosse, J., Hamilton, P.B., Johnson, A.C., Jones, K.C., 2016. The long
766 shadow of our chemical past - High DDT concentrations in fish near a former
767 agrochemicals factory in England. *Chemosphere*. 162, 333-344.
- 768 Keller, J.M., McClellan-Green, P., 2004. Effects of organochlorine compounds on
769 cytochrome P450 aromatase activity in an immortal sea turtle cell line. *Mar.*
770 *Environ. Res.* 58, 347-351.
- 771 Keller, J.M., Peden-Adams, M.M., Aguirre, A.A., Immunotoxicology and implications for
772 reptilian health. 2005 In: *Toxicology of Reptiles* ed by Susan C. M. Gardner, Eva
773 Oberdorster, CRC press
- 774 Kohno, S., Bermudez, D.S., Katsu, Y., Iguchi, T., Guillette, L.J., Jr., 2008. Gene expression
775 patterns in juvenile American alligators (*Alligator mississippiensis*) exposed to
776 environmental contaminants. *Aquat Toxicol.* 88, 95-101.
- 777 Kovats, S., Turner, S., Simmons, A., Powe, T., Chakravarty, E., Alberola-Ila, J., 2016.
778 West Nile virus-infected human dendritic cells fail to fully activate invariant natural
779 killer T cells. *Clin. Exp. Immunol.* 186, 214-226.
- 780 Kumari, U., Singh, R., Mazumder, S., 2017. Chronic endosulfan exposure impairs immune
781 response rendering *Clarias gariepinus* susceptible to microbial infection. *Aquat*
782 *Toxicol.* 191, 42-49.
- 783 Lai, J.J., Lai, K.P., Zeng, W., Chuang, K.H., Altuwaijri, S., Chang, C., 2012. Androgen
784 receptor influences on body defense system via modulation of innate and adaptive
785 immune systems: lessons from conditional AR knockout mice. *Am J Pathol.* 181,
786 1504-1512.
- 787 Lang, J.W., Andrews, H.V., 1994. Temperature dependent sex determination in
788 crocodylians. *J. Exp. Zool.* . 270, 28-44.
- 789 Langlois, V.S., Carew, A.C., Pauli, B.D., Wade, M.G., Cooke, G.M., Trudeau, V.L., 2010.
790 Low levels of the herbicide atrazine alter sex ratios and reduce metamorphic success
791 in *Rana pipiens* tadpoles raised in outdoor mesocosms. *Environ Health Perspect.*
792 118, 552-557.
- 793 Lemaire, G., Mnif, W., Mauvais, P., Balaguer, P., Rahmani, R., 2006. Activation of alpha-
794 and beta-estrogen receptors by persistent pesticides in reporter cell lines. *Life Sci.*
795 79, 1160-1169.

- 796 Lemaire, G., Terouanne, B., Mauvais, P., Michel, S., Rahmani, R., 2004. Effect of
797 organochlorine pesticides on human androgen receptor activation in vitro. *Toxicol.*
798 *Appl. Pharmacol.* 196, 235-246.
- 799 Li, Y., Hallerman, E.M., Liu, Q., Wu, K., Peng, Y., 2016. The development and status of Bt
800 rice in China. *Plant Biotechnol. J.* 14, 839-848.
- 801 Li, Y., Luh, C.J., Burns, K.A., Arao, Y., Jiang, Z., Teng, C.T., Tice, R.R., Korach, K.S.,
802 2013. Endocrine-Disrupting Chemicals (EDCs): In vitro mechanism of estrogenic
803 activation and differential effects on ER target genes. *Environ Health Perspect*, 121,
804 459-466
- 805 Luek, J.L., Dickhut, R.M., Cochran, M.A., Falconer, R.L., Kylin, H., 2017. Persistent
806 organic pollutants in the Atlantic and southern oceans and oceanic atmosphere. *Sci.*
807 *Total Environ.* 583,64-71.
- 808 Luque, E.H., Muñoz-de-Toro, M., Ramos, J.G., Estrogenic Agonist. In: M.K. Skinner,
809 (Ed.), *Encyclopedia of Reproduction* 2nd ed, Academic Press: Elsevier2018, pp.
810 753–759.
- 811 Main, K.M., Skakkebaek, N.E., Virtanen, H.E., Toppari, J., 2010. Genital anomalies in
812 boys and the environment. *Best Pract Res Clin Endocrinol Metab.* 24, 279-289.
- 813 Mansfield, K.G., Land, E.D., 2002. Cryptorchidism in Florida panthers: prevalence,
814 features, and influence of genetic restoration. *J. Wildl. Dis.* 38, 693-698.
- 815 Martin, S., Global diversity of crocodiles (Crocodylia, Reptilia) in freshwater. In: Lévêque
816 C. Balian E.V., Segers H., Martens K. , (Ed.), *Freshwater Animal Diversity*
817 *Assessment. Developments in Hydrobiology*, Springer, Dordrecht2007.
- 818 Martinez-Juarez, A., Moreno-Mendoza, N., 2019. Mechanisms related to sexual
819 determination by temperature in reptiles. *J. Therm. Biol.* 85, 102400.
- 820 Matsumoto, Y., Crews, D., 2012. Molecular mechanisms of temperature-dependent sex
821 determination in the context of ecological developmental biology. *Mol. Cell.*
822 *Endocrinol.* 354, 103-110.
- 823 McBirney M, King SE, Pappalardo M, Houser E, Unkefer M, Nilsson E, Sadler-Riggleman
824 I, Beck D, Winchester P, Skinner MK., 2017. Atrazine induced epigenetic
825 transgenerational inheritance of disease, lean phenotype and sperm epimutation
826 pathology biomarkers. *PLoS One*;12(9):e0184306.

- 827 Milesi, M.M., Durando, M., Lorenz, V., Gastiazoro, M.P., Varayoud, J., 2020. Postnatal
828 exposure to endosulfan affects uterine development and fertility. *Mol Cell*
829 *Endocrinol*, 511, e110855.
- 830 Milesi M.M., Varayoud J., Ramos J.G., Luque E.H. 2017. Uterine ER α epigenetic
831 modifications are induced by the endocrine disruptor endosulfan in female rats with
832 impaired fertility. *Mol Cell Endocrinol*.454, 1-11
- 833 Milnes, M.R., Bryan, T.A., Katsu, Y., Kohno, S., Moore, B.C., Iguchi, T., Guillette, L.J.,
834 Jr., 2008. Increased posthatching mortality and loss of sexually dimorphic gene
835 expression in alligators (*Alligator mississippiensis*) from a contaminated
836 environment. *Biol Reprod*. 78, 932-938.
- 837 Milnes, M.R., Jr., Roberts, R.N., Guillette, L.J., Jr., 2002. Effects of incubation temperature
838 and estrogen exposure on aromatase activity in the brain and gonads of embryonic
839 alligators. *Environ Health Perspect*. 110 Suppl 3, 393-396.
- 840 Mizoguchi, B.A., Valenzuela, N., 2016. Ecotoxicological Perspectives of Sex
841 Determination. *Sex Dev*. 10, 45-57.
- 842 Mizota, K., Ueda, H., 2006. Endocrine disrupting chemical atrazine causes degranulation
843 through Gq/11 protein-coupled neurosteroid receptor in mast cells. *Toxicol Sci*. 90,
844 362-368.
- 845 Montiel-León, J.M., Muñoz, G., Vo Duy, S., Do, D.T., Vaudreuil, M.A., Goeury, K.,
846 Guillemette, F., Amyot, M., Sauvé S., 2019. Widespread occurrence and spatial
847 distribution of glyphosate, atrazine, and neonicotinoids pesticides in the St.
848 Lawrence and tributary rivers. *Environ. Pollut*. 250, 29-39.
- 849 Moore, B.C., Roark, A.M., Kohno, S., Hamlin, H.J., Guillette, L.J., Jr., 2012. Gene-
850 environment interactions: the potential role of contaminants in somatic growth and
851 the development of the reproductive system of the American alligator. *Mol Cell*
852 *Endocrinol*. 354, 111-120.
- 853 Muñoz-de-Toro, M., Beldomenico, H.R., Garcia, S.R., Stoker, C., De Jesus, J.J.,
854 Beldomenico, P.M., Ramos, J.G., Luque, E.H., 2006. Organochlorine levels in
855 adipose tissue of women from a littoral region of Argentina. *Environ Res*. 102, 107-
856 112.

- 857 Nilsen, F.M., Kassim, B.L., Delaney, J.P., Lange, T.R., Brunell, A.M., Guillette, L.J., Jr.,
858 Long, S.E., Schock, T.B., 2017. Trace element biodistribution in the American
859 alligator (*Alligator mississippiensis*). *Chemosphere*. 181, 343-351.
- 860 Nilsson E.E, Sadler-Riggelman I, Skinner M.K., 2018 Environmentally induced epigenetic
861 transgenerational inheritance of disease. *Environ Epigenetics*, 4:1–13
- 862 Nuñez-Otaño, N., Imhof, A., Bolcatto, P.G., Larriera, A., 2010. Sex differences in the
863 genitalia of hatching *Caiman latirostris* *Herpetological Review*. 41, 32-35.
- 864 OJEU, 2004. Official Journal of the European Union.. [https://eur-lex.europa.eu/legal-](https://eur-lex.europa.eu/legal-content/ES/TXT/PDF/?uri=CELEX:32004D0248&from=EN)
865 [content/ES/TXT/PDF/?uri=CELEX:32004D0248&from=EN](https://eur-lex.europa.eu/legal-content/ES/TXT/PDF/?uri=CELEX:32004D0248&from=EN)
- 866 OJEU, 2005. Official Journal of the European Union.. [https://eur-lex.europa.eu/legal-](https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32005D0864&from=EN)
867 [content/EN/TXT/PDF/?uri=CELEX:32005D0864&from=EN](https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32005D0864&from=EN)
- 868 Palma, P., Palma, V.L., Matos, C., Fernandes, R.M., Bohn, A., Soares, A.M., Barbosa, I.R.,
869 2009. Effects of atrazine and endosulfan sulphate on the ecdysteroid system of
870 *Daphnia magna*. *Chemosphere*. 74, 676-681.
- 871 Park, D., Hempleman, S.C., Propper, C.R., 2001. Endosulfan exposure disrupts pheromonal
872 systems in the red-spotted newt: a mechanism for subtle effects of environmental
873 chemicals. *Environ Health Perspect*. 109, 669-673.
- 874 Pereira, J.L., Antunes, S.C., Castro, B.B., Marques, C.R., Goncalves, A.M., Goncalves, F.,
875 Pereira, R., 2009. Toxicity evaluation of three pesticides on non-target aquatic and
876 soil organisms: commercial formulation versus active ingredient. *Ecotoxicology*.
877 18, 455-463.
- 878 PPQS,
879 2017: [http://ppqs.gov.in/sites/default/files/banned_restricted_phased_out_31.10.20](http://ppqs.gov.in/sites/default/files/banned_restricted_phased_out_31.10.2019.pdf)
880 [19.pdf](http://ppqs.gov.in/sites/default/files/banned_restricted_phased_out_31.10.2019.pdf)
- 881 PPQS, 2019: <http://ppqs.gov.in/statistical-database>
- 882 Pushpanjali, P.A.K., Prasad, R.L., Prasad, A., Singh, S.K., Kumar, A., Jadhao, S.B., 2005.
883 In ovo embryotoxicity of a-endosulfan adversely influences liver and brain
884 metabolism and the immune system in chickens. *Pestic. Biochem. Physiol.*, 103-
885 114.

- 886 Rainwater, T.R., Adair, B.M., Platt, S.G., Anderson, T.A., Cobb, G.P., McMurry, S.T.,
887 2002. Mercury in Morelet's crocodile eggs from northern Belize. *Arch. Environ.*
888 *Contam. Toxicol.* 42, 319-324.
- 889 Rajakumar, A., Singh, R., Chakrabarty, S., Muruganankumar, R., Laldinsangi, C.,
890 Prathibha, Y., Sudhakumari, C.C., Dutta-Gupta, A., Senthilkumaran, B., 2012.
891 Endosulfan and flutamide impair testicular development in the juvenile Asian
892 catfish, *Clarias batrachus*. *Aquat Toxicol.* 110-111, 123-132.
- 893 Regaldo, L., Gutierrez, M.F., Reno, U., Fernández, V., Gervasio, S., Repetti, M.R.,
894 Gagneten, A.M., 2018. Water and sediment quality assessment in the Colastiné
895 Corralito stream system (Santa Fe, Argentina): impact of industry and agriculture on
896 aquatic ecosystems. *Environ. Sci. Pollut. Res. Int.* 25, 6951-6968
- 897 Rey, F., Gonzalez, M., Zayas, M.A., Stoker, C., Durando, M., Luque, E.H., Muñoz-de-
898 Toro, M., 2009. Prenatal exposure to pesticides disrupts testicular histoarchitecture
899 and alters testosterone levels in male *Caiman latirostris*. *Gen. Comp. Endocrinol.*
900 162, 286-292.
- 901 Roberge, M., Hakk, H., Larsen, G., 2004. Atrazine is a competitive inhibitor of
902 phosphodiesterase but does not affect the estrogen receptor. *Toxicol Lett.* 154, 61-
903 68.
- 904 Rotterdam Convention.
905 <http://www.pic.int/Procedures/NotificationsofFinalRegulatoryActions/Database/tabid/1368/language/en-US/Default.aspx>
906
- 907 Russart, K.L.G.; Rhen, T. 2016. Atrazine Alters Expression of Reproductive and Stress
908 Genes in the Developing Hypothalamus of the Snapping Turtle, *Chelydra*
909 *Serpentina*. *Toxicology* 366, 1-9.
- 910 Salvarani, P.I., Morgado, F., Vieira, L.R., Rendón-von Osten, J., 2019. Organochlorines
911 contaminants in eggs of Hawksbill (*Eretmochelys Imbricata*) and Green Sea Turtles
912 (*Chelonia Mydas*) from Mexico coast. *Arch. Environ. Contam. Toxicol.* 73, 425-
913 434.
- 914 Sanderson, J.T., Letcher, R.J., Heneweer, M., Giesy, J.P., van den Berg, M., 2001. Effects
915 of chloro-s-triazine herbicides and metabolites on aromatase activity in various

- 916 human cell lines and on vitellogenin production in male carp hepatocytes. *Environ.*
917 *Health Perspect.* 109, 1027-1031.
- 918 Sebastian, R., Raghavan, S.C., 2015. Endosulfan induces male infertility. *Cell Death Dis.* 6,
919 e2022.
- 920 SENASA, 2011. [http://www.senasa.gob.ar/normativas/resolucion-511-2011-senasa-](http://www.senasa.gob.ar/normativas/resolucion-511-2011-senasa-servicio-nacional-de-sanidad-y-calidad-agroalimentaria)
921 [servicio-nacional-de-sanidad-y-calidad-agroalimentaria](http://www.senasa.gob.ar/normativas/resolucion-511-2011-senasa-servicio-nacional-de-sanidad-y-calidad-agroalimentaria)
- 922 SENASA, 2020. [https://www.argentina.gob.ar/senasa/programas-](https://www.argentina.gob.ar/senasa/programas-sanitarios/productosveterinarios-fitosanitarios-y-fertilizantes/registro-nacional-de-terapeutica-vegetal)
923 [sanitarios/productosveterinarios-fitosanitarios-y-fertilizantes/registro-nacional-de-](https://www.argentina.gob.ar/senasa/programas-sanitarios/productosveterinarios-fitosanitarios-y-fertilizantes/registro-nacional-de-terapeutica-vegetal)
924 [terapeutica-vegetal](https://www.argentina.gob.ar/senasa/programas-sanitarios/productosveterinarios-fitosanitarios-y-fertilizantes/registro-nacional-de-terapeutica-vegetal)
- 925 Singh, P.P., Kumar, A., Chauhan, R.S., Pankaj, P.K., 2016. Effect of endosulfan on
926 immunological competence of layer birds. *Vet World.* 9, 777-782.
- 927 Somaweera, R., Brien, M.L., Sonneman, T., Didham, R.K., Webber, B.L., 2019. Absence
928 of evidence is not evidence of absence: Knowledge shortfalls threaten the effective
929 conservation of freshwater crocodiles *Global Ecology and Conservation.* 20,
930 e00773.
- 931 Sonnenschein, C., Soto, A.M., 1998. An updated review of environmental estrogen and
932 androgen mimics and antagonists. *J Steroid Biochem Mol Biol.* 65, 143-150.
- 933 Soto, A.M., Chung, K.L., Sonnenschein, C., 1994. The pesticides endosulfan, toxaphene,
934 and dieldrin have estrogenic effects on human estrogen-sensitive cells. *Environ*
935 *Health Perspect.* 102, 380-383.
- 936 Sridharan, S., Brehm, R., Bergmann, M., Cooke, P.S., 2007. Role of connexin 43 in Sertoli
937 cells of testis. *Ann. N.Y. Acad. Sci.* 1120, 131-143.
- 938 Stoker, C., Beldomenico, P.M., Bosquiazzo, V.L., Zayas, M.A., Rey, F., Rodriguez, H.,
939 Muñoz-de-Toro, M., Luque, E.H., 2008. Developmental exposure to endocrine
940 disruptor chemicals alters follicular dynamics and steroid levels in Caiman
941 latirostris. *Gen. Comp. Endocrinol.* 156, 603-612.
- 942 Stoker, C., Repetti, M.R., Garcia, S.R., Zayas, M.A., Galoppo, G.H., Beldomenico, H.R.,
943 Luque, E.H., Muñoz-de-Toro, M., 2011. Organochlorine compound residues in the
944 eggs of broad-snouted caimans (*Caiman latirostris*) and correlation with measures of
945 reproductive performance. *Chemosphere.* 84, 311-317.

- 946 Stoker, C., Rey, F., Rodriguez, H., Ramos, J.G., Sirosky, P., Larriera, A., Luque, E.H.,
947 Muñoz-de-Toro, M., 2003. Sex reversal effects on *Caiman latirostris* exposed to
948 environmentally relevant doses of the xenoestrogen bisphenol A. *Gen. Comp.*
949 *Endocrinol.* 133, 287-296.
- 950 Stoker, C., Zayas, M.A., Ferreira, M.A., Durando, M., Galoppo, G.H., Rodriguez, H.A.,
951 Repetti, M.R., Beldomenico, H.R., Caldini, E.G., Luque, E.H., Muñoz-de-Toro, M.,
952 2013. The eggshell features and clutch viability of the broad-snouted caiman
953 (*Caiman latirostris*) are associated with the egg burden of organochlorine
954 compounds. *Ecotoxicol. Environ. Saf.* 98, 191-195.
- 955 Sun, J.T., Pan L.L., Zhan, Y., Tsang, D.C., Zhu, L.Z., Li, X.D., 2017. Atrazine
956 contamination in agricultural soils from the Yangtze river delta of China and
957 associated health risks. *Environ. Geochem. Health* 39, 369-378.
- 958 Suzawa, M., Ingraham, H.A., 2008. The herbicide atrazine activates endocrine gene
959 networks via non-steroidal NR5A nuclear receptors in fish and mammalian cells.
960 *PLoS One.* 3, e2117.
- 961 Tabor, G.M., Alonso Aguirre, A., 2004. Ecosystem Health and Sentinel Species: Adding an
962 Ecological Element to the Proverbial “Canary in the Mineshaft”. *EcoHealth.* 1,
963 226-228.
- 964 Tavalieri, Y.E., Galoppo, G.H., Canesini, G., Truter, J.C., Ramos, J.G., Luque, E.H.,
965 Muñoz-de-Toro, M., 2019a. The external genitalia in juvenile *Caiman latirostris*
966 differ in hormone sex determinate-female from temperature sex determinate-female.
967 *Gen. Comp. Endocrinol.* 273, 236-248.
- 968 Tavalieri Y.E., Galoppo G.H., Canesini, G., Kass, L., Luque, E.H., Muñoz-de-Toro, M.,
969 2017. Effects of prenatal exposure to atrazine on the external genitalia of *Caiman*
970 *latirostris*. *Acta Toxicol. Argent.* 25 (Supl), 29
- 971 Tavalieri, Y.E., Galoppo, G.H., Mora, S.C., Luque, E.H., Muñoz-de-Toro, M., 2019b. Early
972 postnatal exposure to xenoestrogens alters the expression of molecules involved in
973 the postnatal differentiation of the oviduct of the broad-snouted caiman (*Caiman*
974 *latirostris*). *Medicina* 79, (Supl. IV), 288.
- 975 Tavalieri, Y.E, Galoppo, G.H., Alarcón, R.A., Luque, E.H., Muñoz-de-Toro, M., 2019c.
976 Effect of xenoestrogen exposure on the expression of FoxA2 in the oviduct of the

- 977 broad-snouted caiman (*Caiman latirostris*). P087. Proceeding of the Latin American
978 Society for Developmental Biology, LASDB Meeting 2019.
- 979 Tousignant, A., Crews, D., 1994. Effect of exogenous estradiol applied at different
980 embryonic stages on sex determination, growth, and mortality in the leopard gecko
981 (*Eublepharis macularius*). *J Exp Zool.* 268, 17-21.
- 982 Tubbs, C.W., McDonough, C.E., 2018. Reproductive Impacts of Endocrine-Disrupting
983 Chemicals on Wildlife Species: Implications for Conservation of Endangered
984 Species. *Annu Rev Anim Biosci.* 6, 287-304.
- 985 Ullah, R., Asghar, R., Baqar, M., Mahmood, A., Ali, S.N., Sohail, M., Schäfer, R.B., Eqani,
986 S.A.M.A.S., 2019. Assessment of organochlorine pesticides in the Himalayan
987 riverine ecosystems from Pakistan using passive sampling techniques. *Environ. Sci.*
988 *Pollut. Res.* 26, 6023-6037.
- 989 Valenzuela, N., Badenhorst, D., Montiel, E.E., Literman, R., 2014. Molecular cytogenetic
990 search for cryptic sex chromosomes in painted turtles *Chrysemys picta*. *Cytogenet.*
991 *Genome Res.* 144, 39-46.
- 992 Van Voorhis, B.J., Follicular development. In: E. Knobil, J.D. Neill, (Eds.), *Encyclopedia*
993 *of Reproduction*, Academic Press, New York, 1999, pp. 376–389.
- 994 Varayoud, J., Ramos, J.G., Bosquiazzo, V.L., Muñoz-de-Toro, M., Luque, E.H., 2008.
995 Developmental exposure to Bisphenol a impairs the uterine response to ovarian
996 steroids in the adult. *Endocrinology.* 149, 5848-5860.
- 997 Viswanath, G., Chatterjee, S., Dabral, S., Nanguneri, S.R., Divya, G., Roy, P., 2010. Anti-
998 androgenic endocrine disrupting activities of chlorpyrifos and piperophos. *J. Steroid*
999 *Biochem. Mol. Biol.* 120, 22-29.
- 1000 Vonier, P.M., Crain, D.A., McLachlan, J.A., Guillette, L.J., Jr., Arnold, S.F., 1996.
1001 Interaction of environmental chemicals with the estrogen and progesterone
1002 receptors from the oviduct of the American alligator. *Environ Health Perspect.* 104,
1003 1318-1322.
- 1004 Wang, M.H., Baskin, L.S., 2008. Endocrine disruptors, genital development, and
1005 hypospadias. *J Androl.* 29, 499-505.
- 1006 Warner, D., Sex determination in reptiles. . In: DO. Norris, KH. Lopez, (Eds.), *Hormones*
1007 *and Reproduction of Vertebrates.*, Elsevier Inc, San Diego, CA, 2011, pp. 1-38.

- 1008 Whitfield, S.M., Bell, K.E., Philippi, T., Sasa, M., Bolanos, F., Chaves, G., Savage, J.M.,
1009 Donnelly, M.A., 2007. Amphibian and reptile declines over 35 years at La Selva,
1010 Costa Rica. *Proc Natl Acad Sci U S A.* 104, 8352-8356.
- 1011 WHO, 2011. https://www.who.int/water_sanitation_health/dwq/chemicals/antrazine.pdf
1012 WHO, 2004. https://www.who.int/water_sanitation_health/dwq/chemicals/endosulfan.pdf
- 1013 Wibbels, T., Bull, J.J., Crews, D., 1992. Steroid hormone-induced male sex determination
1014 in an amniotic vertebrate. *J Exp Zool.* 262, 454-457.
- 1015 Wirth, E.F., Lund, S.A., Fulton, M.H., Scott, G.I., 2001. Determination of acute mortality
1016 in adults and sublethal embryo responses of *Palaemonetes pugio* to endosulfan and
1017 methoprene exposure. *Aquatic Toxicology* 53, 9-18.
- 1018 Wirth, E.F., Lund, S.A., Fulton, M.H., Scott, G.I., 2002. Reproductive alterations in adult
1019 grass shrimp, *Palaemonetes pugio*, following sublethal, chronic endosulfan
1020 exposure. *Aquatic Toxicology* 59, 93-99.
- 1021 Wu, T.H., Canas, J.E., Rainwater, T.R., Platt, S.G., McMurry, S.T., Anderson, T.A., 2006.
1022 Organochlorine contaminants in complete clutches of Morelet's crocodile
1023 (*Crocodylus moreletii*) eggs from Belize. *Environ Pollut.* 144, 151-157.
- 1024 Xiang, D., Han, J., Yao, T., Wang, Q., Zhou, B., Mohamed, A.D., Zhu, G., 2017. Editor's
1025 Highlight: Structure-Based Investigation on the Binding and Activation of Typical
1026 Pesticides With Thyroid Receptor. *Toxicol. Sci.* 160, 205-216.
- 1027 Xie, H., Wang, X., Chen, J., Li, X., Jia, G., Zou, Y., Zhang, Y., Cui Y., 2019. Occurrence,
1028 distribution and ecological risks of antibiotics and pesticides in coastal waters
1029 around Liaodong Peninsula, China. *Sci. Total Environ.* 656, 946-951.
- 1030 Yoshikane, M., Kay, W.R., Shibata, Y., Inoue, M., Yanai, T., Kamata, R., Edmonds, J.S.,
1031 Morita, M., 2006. Very high concentrations of DDE and toxaphene residues in
1032 crocodiles from the Ord River, Western Australia: an investigation into possible
1033 endocrine disruption. *J. Environ. Monit.* 8, 649-661.
- 1034 Zayas, M.A., Rodriguez, H., Galoppo, G.H., Stoker, C., Durando, M., Luque, E.H., Muñoz-
1035 de-Toro, M., 2011. Hematology and blood biochemistry of young healthy Broad-
1036 Snouted Caimans (*Caiman latirostris*). *J. Herpetol.* 45, 516-524.
- 1037 Zhang, W., 2018 Global pesticide use: Profile, trend, cost / benefit and more Proceedings of
1038 the International Academy of Ecology and Environmental Sciences. 8, 1-27.

1039

1040 **FIGURE LEGENDS**

1041 **Figure 1:** Effect of prenatal exposure to Atrazine (ATZ) and Endosulfan (END) on the
1042 oviductal desmin/ α -SMA ratio. Caiman eggs were treated with vehicle (ethanol), ATZ-0.2
1043 ppm or END-20 ppm topically applied to the eggshell at stage 20 of embryonic
1044 development (sex determination window) and incubated at the female-producing
1045 temperature (30°C) until hatching. Animals were raised under controlled conditions, as
1046 described in detail by Zayas et al. (2011). Females were euthanized at the pre-pubertal
1047 juvenile stage, a stage of development previously defined based on biometric parameters
1048 (Galoppo et al., 2016). The oviducts were dissected and processed until paraffin embedded.
1049 Desmin and alpha smooth muscle actin (α -SMA) expressions were assessed to evaluate the
1050 effects of exposures to ATZ and END on the thickness and organization of the oviductal
1051 muscle layers (Galoppo et al., 2017). Graph: END exposure significantly increases the
1052 desmin/ α -SMA ratio, whereas ATZ exposure shows a trend. Kruskal-Wallis followed by
1053 Mann Whitney post-test. Representative photomicrographs of oviducts showing the effects
1054 of END exposure on the oviduct muscular layer (desmin and α -SMA expression).
1055 Immunohistochemistry developed with DAB and counterstained with Mayer's
1056 hematoxylin.

1057

1058 **Figure 2:** Effect of prenatal exposure to ATZ and END on oviduct adenogenesis. Caiman
1059 eggs were topically treated at the sex determination window with vehicle, ATZ-0.2 ppm or
1060 END-20 ppm and incubated at the female-producing temperature until hatching. Animals
1061 were raised under controlled conditions (Zayas et al., 2011). Based on biometric
1062 parameters, pre-pubertal juvenile females were euthanized (Galoppo et al., 2016). The

1063 oviducts were dissected and processed until paraffin embedded. Morphometric analysis was
1064 performed following the same procedure used by Galoppo et al., 2020. Graphs: END
1065 significantly increases the mesenchymal area of the oviduct occupied by glands in pre-
1066 pubertal juvenile caimans. This is due, at least in part, to an increased number of glands.
1067 Kruskal-Wallis followed by Mann Whitney post-test. On the right, representative images of
1068 oviducts showing differences in gland number and size. PAS stain.

1069

1070 **Figure 3:** Effect of prenatal exposure to ATZ on the caiman phallus. In ovo exposure to
1071 ATZ did not affect sexual dimorphism of caiman phallus biometrics. Left panel:
1072 Representative digitalized photomicrograph used to measure phallus length, as indicated by
1073 the double-headed arrow (procedure details in Tavalieri et al., 2019a). Condition index, CI=
1074 $(BM/SVL^3) \times 100$ (Cruze et al., 2015). Phallus from control (VEH-exposed) male (n:17)
1075 and female (13) pre-pubertal juvenile caimans (samples from caimans of similar body
1076 condition). Right panel: The phallus length/condition index ratio is sexually dimorphic in
1077 control pre-pubertal juvenile caimans. In ovo exposure to ATZ did not affect the
1078 dimorphism, samples from 14 and 13 males and females, respectively. Different letters
1079 denote significant differences between groups. Kruskal-Wallis test was used to evaluate
1080 differences. Results were considered significant at $P < 0.05$.

1081

1082 **Figure 4:** Effect of prenatal exposure to ATZ on pre-pubertal juvenile male phallus length.
1083 The length of the phallus measured in control males (■—■) and ATZ-exposed males (▲ - -
1084 ▲) was positively correlated with caiman body mass. Spearman $r_S = 0.91$ for control
1085 (VEH-exposed) and $r_S = 0.84$ for ATZ. The slope of the curve in control males was steeper
1086 than the slope of ATZ-exposed caimans.

1087

1088 **Figure 5:** Effect of prenatal exposure to ATZ on lymphocyte distribution. In ovo exposure
1089 to ATZ increased the area occupied by lymphocyte aggregates (Table 6). Representative
1090 photomicrographs of the phallus shaft exhibiting lymphoid aggregates (Arrow). Phallus
1091 from pre-pubertal juvenile male caimans of similar body condition; (A) controls (VEH-
1092 exposed) and (B) ATZ-exposed. Hematoxylin-eosin staining.

1093

1094 **Figure 6:** Effects of agricultural pesticides on non-target wildlife species. The massive use
1095 of agricultural pesticides constitutes a substantial source of environmental contamination.
1096 Contaminants reach non-target organisms by the air, water, soil and food, affecting the
1097 health of the wetland ecosystems. As an example, ATZ and END are pesticides classified
1098 as EDCs, and crocodilians are apex semi-aquatic predators. The figure summarizes the role
1099 of the food web, the transfer of pesticides from the mother to the eggs, and the key role of
1100 crocodiles as sentinel of wetland health far from the primary sources of contaminants.

1101

Table 1 . Concentration of Atrazine and Endosulfan in the environment.

		Atrazine	References	Endosulfan*	References
Concentrations found in the environment	Soil	<1.0 – 113.0 ng/g	Sun et al., 2017	ND–120.7 pg/g	Ullah et al., 2019
	Fresh Water	<4.0 – 666.0 ng/L	Montiel-León et al., 2019	0.15-1.09 pg/L	Ullah et al., 2019
		0.13-1.73 µg/L	Regaldo et al., 2018	0.015-0.129 µg/L	Regaldo et al., 2018
		ATZ, 0.4-0.5 ppb DACT, 0.2-0.32 ppb	Douros et al., 2015		
	Sea Water	8.7 – 64.8 ng/L	Xie et al., 2019	<0.13-1.3 pg/L	Luek et al., 2017
	Eggs			<i>Caiman latirostris</i> , <5.0-46.0 ng/g lp	Stoker et al, 2011
			<i>Chelydra mydas</i> , 0.33-2.16 ng/g dry wt	Salvarani et al., 2019	
Fat Tissue			<i>Crocodilus niloticus</i> , 800-250 ng/g wet wt	Buah-Kwofie et al., 2018	
Mixed tissues (tail)	<i>Chelydra serpentina</i> ATZ: 0.03-0.07 ppb DACT: 0.2-0.32 ppb	Douros et al., 2015			
NOAEL		1.8 mg/Kg/day	WHO, 2011	0.6 mg/Kg/day	WHO, 2004
Restriction policies	USA	Restricted	EPA, 2019	Banned	EPA, 2010
	EU	Banned	OJEU, 2004	Banned	OJEU, 2005
	Argentina	Authorized	SENASA, 2020	Banned, July 2013	SENASA, 2011
	Australia	Authorized	APVMA, 2016	Banned	APVMA, 2010
	Brazil	Authorized	ANVISA, 2020a	Banned	ANVISA, 2020b
	China	Authorized	Rotterdam Convention	Severely Restricted	Rotterdam Convention
	India	Authorized	PPQS, 2019	Banned	PPQS, 2017
	South Africa	Restricted	DTI, 2017	Banned	DTI, 2017

Persistent or not		Persistent, relatively water soluble, relatively long half-life in soils	Bergman et al., 2012	Persistent lipophilic compound	Bargar et al., 1999
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*($\alpha+\beta$). **ANVISA**: Agência Nacional de Vigilância Sanitária, Brazil; **APVMA**: Australian Pesticides and Veterinary Medicines Authority; **DTI**: Department of Trade and Industry, South Africa; **EPA**: Environmental Protection Agency, **OJEU**: Official Journal of the European Union; **ppb**: parts per billion; **PPQS**: Directorate of Plant Protection, Quarantine & Storage, India; **SENASA**: Servicio Nacional de Sanidad y Calidad Agroalimentaria, Argentina; **DACT**: diaminochlorotriazine (ATZ metabolite); **ND**: Not detected.

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Table 2: Mechanisms involved in Atrazine and Endosulfan endocrine disruptive actions

	ATRAZINE	References	ENDOSULFAN	References
Mechanisms of action	Estrogen agonist (induces aromatase activity)	Fan et al., 2007; Holloway et al., 2008; Luque et al., 2018; Roberge et al., 2004	Xenoestrogen	Lemaire et al., 2006; Li et al., 2013 Milesi et al., 2020 Soto et al., 1994; Varayoud et al., 2008
	ER-independent induction of aromatase expression	Mizoguchi and Valenzuela, 2016	Antiandrogen	Lemaire et al., 2004; Viswanath et al., 2010 Sebastian & Raghavan, 2015.
	Antiandrogen	Hayes et al., 2011	Activate PXR and CAR	Coumoul et al., 2002;
	Binds to the THR	Xiang et al., 2017	PR antagonist	Vonier et al., 1996.
	Epigenetic disruption of steroid genic enzymes	Cleary et al., 2019	Epigenetic modulation of ER expression	Milesi et al., 2017

CAR: constitutive androstane receptor, ER: Estrogen Receptor, PR: Progesterone Receptor, PXR: pregnane X receptor, THR: Thyroid Hormone Receptor.

Table 3: Effects of natural or experimental exposure to atrazine (ATZ) on the reproductive system of aquatic/semi-aquatic wildlife

<i>Classes, Order or Species</i>	<i>ATZ Dose</i>	<i>Route of Exposure</i>	<i>Time of Exposure</i>	<i>Time of endpoint evaluation</i>	<i>Reported Effects</i>	<i>References</i>
<i>Oryzias latipes</i>	5 µg/L	Rearing water.	Embryo, for 12 days (from 8 hours to 12 days post fertilization; F0 generation).	Sexual maturity (90-120 days post hatching; F2 generation).	Effects on reproduction, fertility and expression of genes related to steroidogenesis and DNA methylation in gonads: -Males: Reduced fertilization rate, sperm count and total motile sperm. Increased expression of <i>star</i> and <i>fshr</i> genes in testis. -Females: Decreased expression of the methyltransferase <i>dnmt1</i> gene in ovaries.	Cleary et al., 2019
<i>Xenopus laevis</i>	2.5 ppb (2.5 µg/L)	Rearing water.	Lifelong, for 2 years (from hatching to sexually maturity).	Sexual maturity (2 years old).	Feminization of male-related external morphology. Decreased testosterone-dependent morphologies (demasculinized/feminized laryngeal morphology and decreased breeding gland size). Reduced plasma T levels. Increased expression of aromatase. Decreased frequency of tubules containing mature sperm. Reduced fertility rates. Altered behavior in mating choice (inability of atrazine-exposed males to compete with unexposed males for access to females).	Hayes et al., 2010

<i>Acris blanchardi</i>	0.1 µg/L	Rearing water.	Larval, for approximately 62 days (from free swimming stage to forelimb emergence).	Complete tail reabsorption (Gosner stage 46).	Feminized sex ratio.	Hoskins and Boone, 2018
<i>Rana pipiens</i>	1.8 µg/L	Rearing water.	Larval, for approximately 82 days (From Gosner stage 27 to metamorphosis climax).	Entering metamorphosis (Gosner stage 42).	Decreased survival rate. Induced female-biased sex ratio. Increased expression of <i>era</i> in brain. Abolished sexual dimorphism in liver expression of <i>srd5b</i> .	Langlois et al., 2010
<i>Chelydra serpentina</i>	2 ppb (2 µg/L)	<i>In ovo</i> (solution applied to the eggshell).	Embryo, exposure with a single dose applied at stage 20 of embryo development.	24 hours post treatment and 6 months post hatching.	Changes in the expression of genes related to steroidogenesis and hypothalamus-pituitary-gonad axis.	Russart and Rhen, 2016
<i>Caiman latirostris</i>	0.2 ppm (200 µg/L)	<i>In ovo</i> (solution applied to the eggshell).	Embryo, exposure with a single dose applied at stage 20 of embryo development.	At hatching (40-49 days post treatment).	Increased egg weight loss Decreased hatchling fractional weight	Beldoménico et al., 2007
	0.2 ppm (200 µg/L)	<i>In ovo</i> (solution applied to the eggshell).	Embryo, exposure with a single dose applied at stage 20 of embryo development.	Embryo stage 22 (5 days post treatment). Embryo stage 24 (14 days post treatment). Embryo stage 27 (29 days post treatment).	Male gonad, Increased amh and sox-9 gene expression. Increased ERα, PR and aromatase protein expression Increased cell proliferation. DNA damage in embryo male germ cells.	Canesini, 2018

	0.2 ppm (200 µg/L)	<i>In ovo</i> (solution applied to the eggshell).	Embryo, exposure with a single dose applied at stage 20 of embryo development.	Neonatal (10 days post hatching).	Females, Ovaries of exposed caimans showed an increase in type III follicles. E ₂ circulating levels were not altered T circulating levels significantly decreased	Stoker et al., 2008
	0.2 ppm (200 µg/L)	<i>In ovo</i> (solution applied to the eggshell).	Embryo, exposure with a single dose applied at stage 20 of embryo development.	Neonatal (10 days post hatching).	Males, Disrupted the testicular histoarchitecture, resulting in disorganized and tortuous seminiferous tubules. Increased seminiferous tubular perimeter, loss of intratubular intercellular connections and either emptied tubular lumens or luminal cellular detritus were observed. T circulating levels were not affected.	Rey et al., 2009
A. mississippiensis	0.14, 1.4 and 14 ppm (140, 1400 and 14000 µg/L)	<i>In ovo</i> (solution applied to the eggshell).	Embryo, exposure with a single dose applied at stage 21 of embryo development.	Neonatal (10 days post hatching).	GAM showed a tendency to produce <i>in vitro</i> higher than normal levels of aromatase in developing testis	Crain et al., 1997
Fish, amphibians, reptiles	Environmentally relevant doses	Different administration routes, times of exposure and times of endpoints evaluation.			Atrazine induces testicular lesions in all vertebrate classes examined	Revised by Hayes et al., 2011

Doses between parentheses, original ATZ doses were transformed to µg/L to allow proper comparisons. *fshr*: Follicular stimulating hormone receptor gene. *star*: Steroidogenic acute regulatory protein gene. *dnmt1*: DNA (cytosine-5)-methyltransferase 1 gene. ppb: parts per billion. T: testosterone. *era*: Estrogen receptor alpha gene. *srd5b*: 5-beta reductase gene. ppm: parts per million. *amh*: antimüllerian hormone gene. sox-9: SRY-box transcription factor 9. ER α : Estrogen receptor alpha. PR: Progesterone receptor. E₂: Estradiol.

Table 4. Effects of natural or experimental exposure to endosulfan (END) on the reproductive system of aquatic/semi-aquatic wildlife

Species	END Dose	Route of Exposure	Time of Exposure	Time of endpoint evaluation	Reported Effects	References
<i>Palaemonetes pugio</i>	0.2 µg/L	Maternal transfer (transfer from reproductive females previously exposed for 35 days through rearing water).	Embryo, for 24 hours.	Hatching.	Delayed hatching time.	Wirth et al., 2001
	0.2 µg /L	Rearing water.	Adult, for 27 days.	Adult.	Reduced number of gravid shrimps.	Wirth et al., 2002
<i>Daphnia magna</i>	45.8, 91.7, 229.3 and 458.7 µg/L	Rearing water.	Lifelong (from less than 24-hour-old specimens to sexual maturity).	Adult.	Decreased offspring number. Decreased female total length. Decreased brood size and delayed brood release. Decreased molt frequency.	Palma et al., 2009
	45.8, 91.7, 229.3 and 458.7 µg/L	Maternal transfer (transfer from reproductive females exposed from neonate to sexual maturity through rearing water).	Embryo, for 96 hours.	Neonatal.	Development of morphological abnormalities.	
	45.8, 91.7, 229.3 and 458.7 µg/L	<i>In vitro</i> (through embryo culture media).	Embryo, for 96 hours.	96 hour old embryos. Neonatal.	Decreased embryo survival rate. Development of morphological abnormalities.	
<i>Clarias batrachus</i>	2.5 µg/L	Rearing water.	Juvenile, for 50 days.	Juvenile (100 days post hatching).	Decreased RNA expression of testis-related transcription factors (<i>dmrt1</i> , <i>sox9a</i> and <i>wt1</i>). Decreased RNA expression of	Rajakumar et al., 2012

					steroidogenic enzymes (<i>11β-hsd2</i> , <i>17β-hsd12</i> and <i>P450c17</i>). Decreased testis somatic index. Decreased number of spermatocytes.	
<i>Caiman latirostris</i>	2 and 20 ppm (2000 and 20000 $\mu\text{g/L}$)	<i>In ovo</i> (solution applied to the eggshell).	Embryo, single dose applied at stage 20 of embryo development.	At hatching (40-49 days post treatment).	Increased egg weight loss. Decreased hatchling fractional weight.	Beldoménico et al., 2007
	0.02 ppm (END0.02), 2 ppm (END2) 20 ppm (END20) 50 μl ethanol (VEH) (20, 200 and 2000 $\mu\text{g/L}$)	<i>In ovo</i> (solution applied to the eggshell).	Embryo, single dose applied at stage 20 of embryo development.	Neonatal (10 days post hatching).	Females, With END2 and END20, a tendency to a higher proportion of type III follicles was observed. With END0.02, END2 and END20, E2 circulating levels were not altered. With END2 and END20, T circulating levels significantly decrease.	Stoker et al., 2008
	0.02 ppm (END0.02), 2 ppm (END2) 20 ppm (END20) 50 μl ethanol (VEH) (20, 200 and 2000 $\mu\text{g/L}$)	<i>In ovo</i> (solution applied to the eggshell).	Embryo, exposure with a single dose applied at stage 20 of embryo development.	Neonatal (10 days post hatching).	Males, With END0.02, END2 and END20, disrupted the testicular histoarchitecture, resulting in disorganized and tortuous ST, loss of intratubular intercellular connections and either emptied tubular lumens or luminal cellular detritus were observed. With END0.02 and END20, an increase in the ST perimeter was demonstrated. With END2 and END20, increased apoptosis in ST and decreased T circulating levels were observed. With END20, decreased cellular turnover in neonatal testis was observed.	Rey et al., 2009

	20 ppm (20000 µg/L)	<i>In ovo</i> (solution applied to the eggshell).	Embryo, exposure with a single dose applied at stage 20 of embryo development.	Neonatal (10 days post hatching). Late postnatal (90 days post hatching). Juvenile (older than 12 months post hatching).	Males, 90-day-old and juvenile, Altered histoarchitecture characterized by disrupted seminiferous tubules with empty lumens. Higher proportion of interstitial tissue, rich in extracellular matrix. 10-day-old, Increased expression of <i>amh</i> , <i>Sf1</i> and <i>Sox-9</i> genes.	Durando et al., 2013
	20 ppm (20000 µg/L)	<i>In ovo</i> (solution applied to the eggshell).	Embryo, exposure with a single dose applied at stage 20 of embryo development.	Neonatal (10 days post hatching). Late postnatal (90 days post hatching). Pre-pubertal juvenile (12 months post hatching).	Males, At 10-day-old, 90-day-old and pre-pubertal juveniles, disrupted testicular histoarchitecture, disorganized and tortuous ST. 90-day-old, lower proliferation index in the ST. Pre-pubertal juveniles, tendency to decreased ERα expression and a different distribution pattern of ERα in the ST.	Durando et al., 2016

Doses between parentheses, original END doses were transformed to µg/L to allow proper comparisons. *dmrt1*: Doublesex and mab-3 related transcription factor 1 gene; *sox9a*: SRY-box transcription factor 9a gene; *wt1*: Wilm's tumor 1 protein gene; *11β-hsd2*: 11β-hydroxysteroid dehydrogenase 2 gene; *17β-hsd12*: 17β-hydroxysteroid dehydrogenase 12 gene; *P450c17*: Cytochrome P450c17 gene; ppm: parts per million; *amh*: antimüllerian hormone gene; *Sf1*: steroidogenic factor-1 gene; ST, seminiferous tubules; ERα: Estrogen receptor alpha; T: testosterone; E2: estradiol.

Table 5: Effects of in ovo ATZ exposure on AR and ER protein expressions in two phallus regions

Phallus region ^a	Male VEH (n=9)	Male ATZ (n=6)	Female VEH (n=8)	Female ATZ (n=6)
Androgen receptor (% of AR+ epithelial nuclei)				
Glans sulcus epithelium	46.3 (35.0 - 73.3)	43.3 (15.1 - 82.8)	8.1 (1.4 - 30.7)	18.1 (9.8 - 33.8)
Glans cavity epithelium	71.7 (57.5 - 84.7)	37.9 (20.1 - 59.4) *	6.4 (0.7 - 48.8)	18.3 (9.8 - 33.8) *
Estrogen receptor alpha (% of ER α + epithelial nuclei)				
Glans sulcus epithelium	47.9 (43.4 - 70.6)	67.8 (35.7 - 71.9) *	7.9 (1.4 - 24.1)	40.6 (7.5 - 59.9) *
Glans cavity epithelium	54.7 (28.4 - 80.3)	82.1 (61.6 - 89.7) *	8.9 (0.5 - 52.9)	43.4 (2.6 - 46.9) *

^aPhallus regions as described in Tavalieri et al. (2019a). AR and ER α expressions were evaluated by immunohistochemistry. Results are expressed as percentage of positive stained nuclei, median and (range). Asterisks denote significant differences with the control. Mann-Whitney test was used to evaluate differences, results were considered significant at $P < 0.05$.

Table 6: *In ovo* exposure to ATZ increased the area occupied by lymphocyte aggregates in the phallus of both male and female juvenile caimans

Phallus region	Male VEH (n=9)	Male ATZ (n=6)	Female VEH (n=8)	Female ATZ (n=6)
Shaft	0.74 ± 0.30	6.87 ± 1.77 *	0.50 ± 0.32	2.7 ± 1.39
Glans	0.17 ± 0.06	0.73 ± 0.25	0.53 ± 0.24	2.82 ± 0.79 *

Values represent area of transverse section of the phallus (at least 3 sections/sample) occupied by lymphocyte aggregates. Number of samples, (n). Morphometric analysis was done following procedures previously described (Tavaliere et al., 2019a). Results are expressed as mean ± SEM. Asterisks denote significant differences with the control. Student's *t* test was used to evaluate differences, results were considered significant at $P < 0.05$.

FIGURE 2

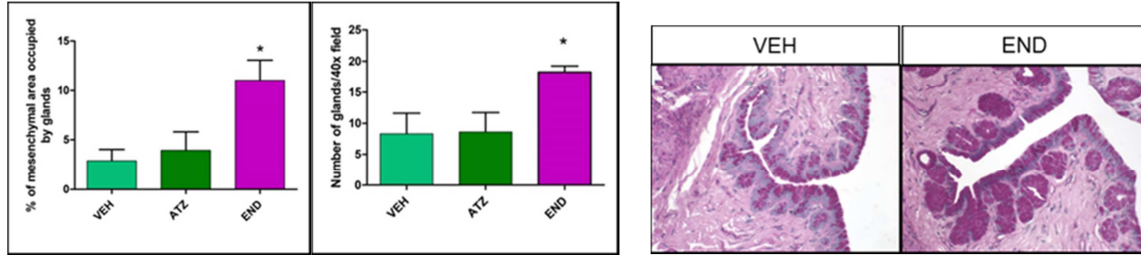


FIGURE 3

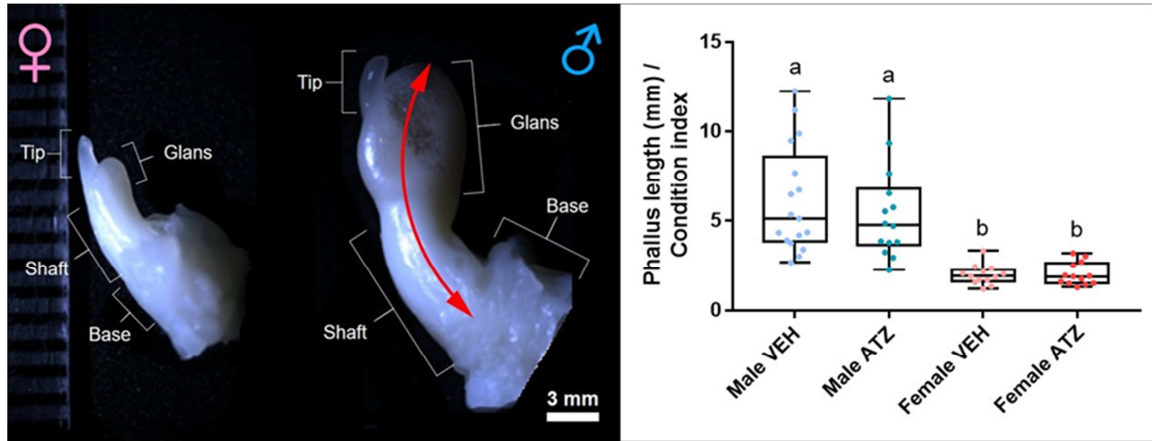


FIGURE 4

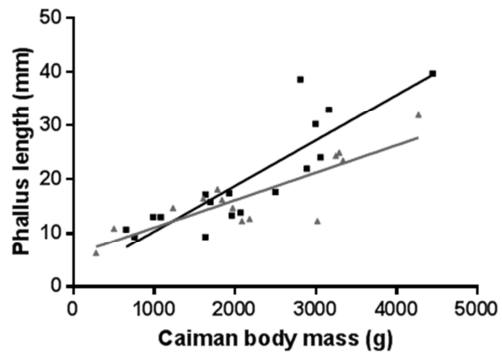
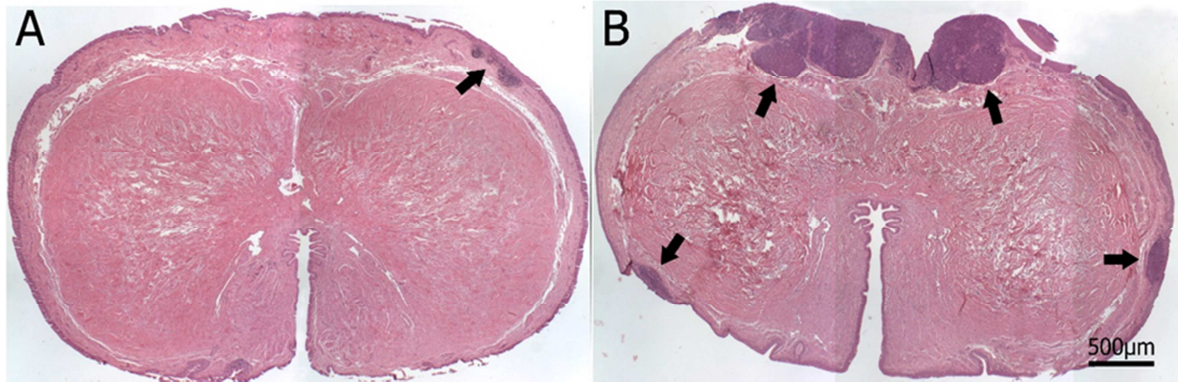
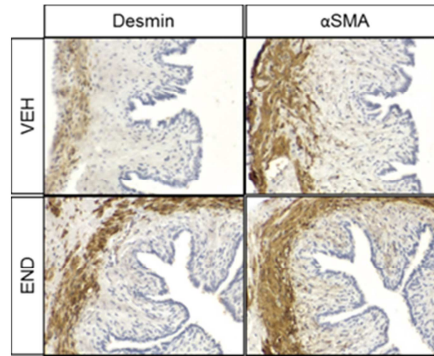
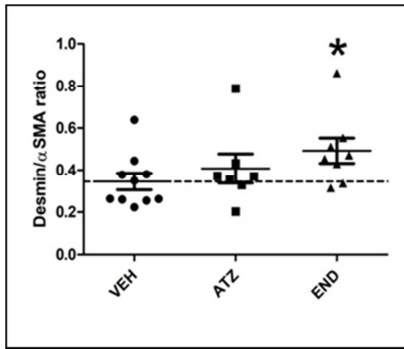
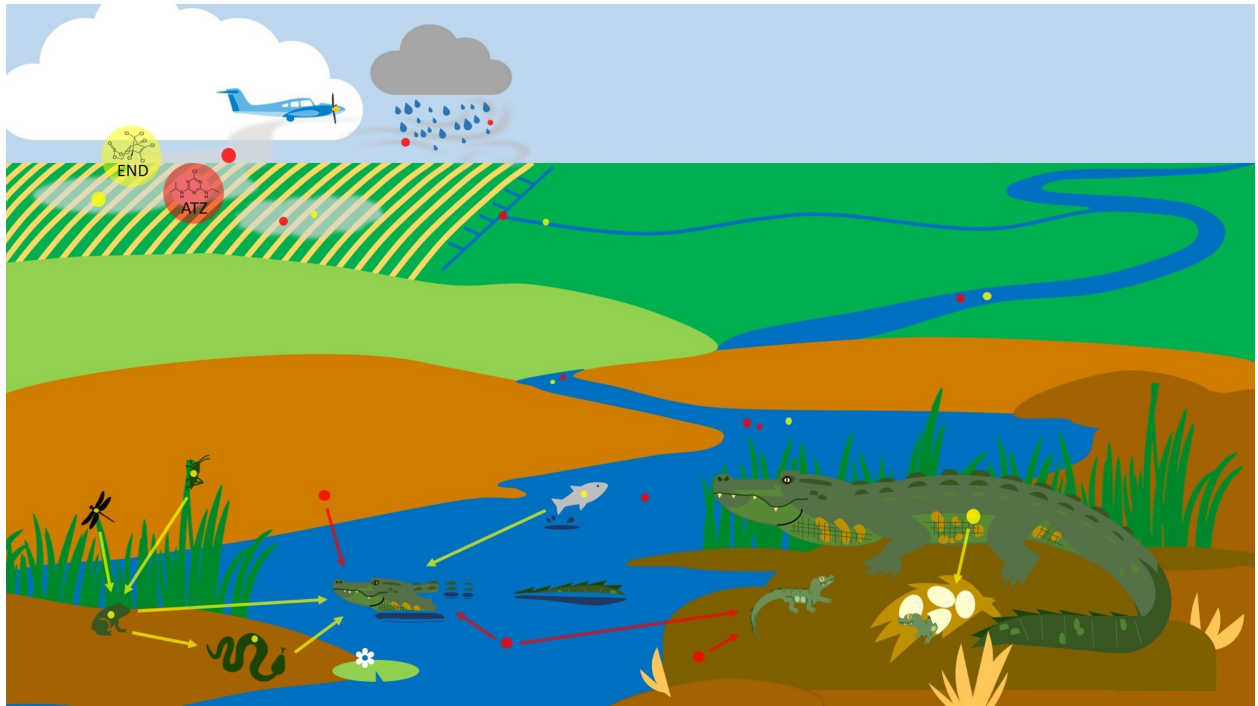


FIGURE 5







Journal Pre

Highlights

Evidence showing the endocrine-disruptive potential of ATZ and END is reviewed

Crocodylian ecological and physiological features make them a sentinel of wetland health

Apex predators are currently exposed to banned pesticides through the food web

Crocodylian reproductive tissues are highly sensitive to the effects of END and ATZ

Transgenerational consequences of ATZ and END exposure could be expected

Journal Pre-proof