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Review

Sources, concentrations, and exposure effects of environmental gestagens on fish and other aquatic wildlife, with an emphasis on reproduction

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ABSTRACT

Fish and other aquatic wildlife, including frogs, turtles, and alligators, have been used as vertebrate sentinels for the effects of endocrine disrupting and other emerging chemicals of concern found in aquatic ecosystems. Research has focused on the effects of estrogenic, androgenic, and thyroidogenic compounds, but there is a growing body of literature on the reproductive health exposure effects of environmental gestagens on aquatic wildlife. Gestagens include native progestogens, such as progesterone, and synthetic progestins, such as gestodene and levonorgestrel, which bind progesterone receptors and have critically important roles in vertebrate physiology, especially reproduction. Roles for progestogen include regulating gamete maturation and orchestrating reproductive behavior, both as circulating hormones and as secreted pheromones. Gestagens enter the aquatic environment through paper mill effluent, wastewater treatment plant effluent, and agricultural runoff. A number of gestagens have been shown to negatively affect reproduction, development, and behavior of exposed fish and other aquatic wildlife at ng/L concentrations, and these compounds have been measured in the environment at single to 375 ng/L. Given the importance of endogenous progestogens in the regulation of gametogenesis, secondary sex characteristics, and reproductive behavior in vertebrates and the documented exposure effects of pharmaceutical progestins and progesterone, environmental gestagens are an emerging class of contaminants that deserve increased attention from researchers and regulators alike. The potential for environmental gestagens to affect the reproductive health of aquatic vertebrates seems evident, but there are a number of important questions for researchers to address in this nascent field. These include identifying biomarkers of gestagen exposure; testing the effects of environmentally relevant mixtures; and determining what other physiological endpoints and taxa might be affected by exposure to environmental gestagens.

1. Introduction

Fish, amphibians, and aquatic reptiles have been used as indicator species for the effects of exposure to native and synthetic steroid hormones and other emerging chemicals of concern in the aquatic environment (Bergman et al., 2012; Damstra et al., 2002; Kloas et al., 2009; Richardson et al., 2005). Past research efforts have focused on the effects of estrogenic, androgenic, and thyroidogenic compounds and some antagonists of receptors for these compounds on fish and other wildlife. The research on exposure effects of gestagens is an emerging field with a relatively small but impactful set of studies. Gestagens include natural progestogens (e.g., progesterone) and synthetic progestins (e.g., gestodene and levonorgestrel), compounds whose physiological functions are mediated through nuclear and membrane progesterone receptors (nPR and mPR, respectively) and potentially through progesterone membrane receptor component (PGMRC) proteins. There are six known PRs in fishes, the two isoforms of nPR in some species (PR-A and PR-B) Conneely et al., 2002; Zapater et al., 2013 and four mPRs (mPRα, mPRβ, mPRγ-1, and mPRγ-2) (Thomas, 2012), as well as PGMRC-1 and PGMRC-2 (Cahill, 2007; Mourot et al., 2006; Thomas, 2008).

From studies extending back to the 1990s, we have learned that an estrogenic component of oral contraceptives and hormone replacement therapeutics, ethinylestradiol, negatively affects the reproductive health of fish and other aquatic wildlife

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(Larsson et al., 1999; Purdom et al., 1994). Progestins are also components of most oral contraceptives and hormone replacement therapy pharmaceuticals, as well. Similar to estrogens and androgens, gestagens enter the aquatic environment through paper mill plant effluent, wastewater treatment plant effluent, and animal agricultural runoff (Chang et al., 2009; Jenkins et al., 2003; Mansell et al., 2011). While the number of environmental measurements of gestagen concentration is limited, most of the laboratory exposure studies to date have used environmentally relevant concentrations.

Progestins function as oral contraceptives or in hormone replacement therapeutics (Africander et al., 2011; Sitruk-Ware and Nath, 2013). Additionally, melengestrol acetate is an anabolic gestagen that is administered to beef cattle in the US to suppress estrus on concentrated animal feeding operations (Schiffer et al., 2001; Upjohn, 1996). Researchers categorize human progestins in generations that are generally defined by the parent compound from which that generation of progestins was derived (Africander et al., 2011; Sitruk-Ware and Nath, 2013). First through third generations are older progestins mostly based on 19-nortestosterone, with the exception of medroxyprogesterone acetate based on 17-OH-progesterone. Examples include norethindrone, levonorgestrel, desogestrel, and gestodene and all are relatively nonspecific and show cross-reactivity with the human androgen receptors. Drospirenone is a fourth generation progestin that produces little androgenic side effects as it is derived from spironolactone, the mineralocorticoid receptor agonist. The most recent progestins, such as nestorone, nomogesterol acetate, and trimegestone are derived from 19-norpregnane and are designed to be the most specific for the human nPR and to produce negligible side effects (Africander et al., 2011; Sitruk-Ware and Nath, 2013).

Gestagens have critical roles in vertebrate reproduction, including regulation of gamete maturation and behavior (Norris and Carr, 2013). Results of gestagen exposure studies in fish and other aquatic wildlife demonstrate alteration in reproduction, development, and behavior (Kloas et al., 2009; Ogawa et al., 2011; Orlando, 2008; Runnalls et al., 2013; Wibbels and Crews, 1995; Zeilinger et al., 2009) (Table 1). In teleost fishes, the final stages of egg and sperm maturation as well as the early events in spermatogenesis are controlled by the progestogens 17α,20β-dihydroxy-4-pregnenone (DHP) Miura et al., 2006; Senthilkumaran et al., 2004; Zhu, 2003 or 17α,20β,21-trihydroxy-4-pregnen-3-one (20β-S) Thomas and Doughty, 2004; Thomas et al., 2002. Some progestogens, when excreted in their free or glucuronidated and sulfated conjugate forms, together with other hormones, function as pheromones that help coordinate reproductive behavior in many fishes (Hamdani and Døving, 2007; Kermen et al., 2013; Stacey et al., 2003). Clearly, disruption of PR signaling could have important effects on individual reproductive fitness and robustness of fish and other aquatic wildlife populations.

2. Sources and concentration of environmental gestagens

Gestagens enter the environment from at least three sources: paper mill plant effluent, wastewater treatment plant effluent, and runoff from animal agriculture (Fig. 1). Steroids and reproductive hormones, including gestagens such as progesterone and norethindrone, were measured in low ng/L concentrations at approximately half of the 139 sites tested in a national reconnaissance sampling study of US streams (Kolpin et al., 2002).

2.1. paper mill effluent

Plants are known to produce a great number of flavonoid and phytosterol metabolites in response to daily changes in sunlight intensity and day length, temperature, atmospheric carbon dioxide and ozone, and grazing (Weston and Mathesius, 2013). Some of these phytosterol metabolites either directly, or following microbial degradation, bind and activate animal steroid hormone receptors, including estrogen, androgen, and progesterone (Adlercreutz et al., 1993; Durhan et al., 2002; Jenkins et al., 2003; MacLatchy and Van Der Kraak, 1995; Patisaul and Jefferson, 2010). Stigmastanol, a plant steroid that can be microbially degraded to androstenedione (Jenkins et al., 2003) has been measured in approximately 50 of 139 freshwater stream samples collected across the US (Kolpin et al., 2002). Progesterone and androstenedione have been measured in nanomolar concentrations in the sediment downstream of a paper mill effluent pipe in the Fenholloway River, Florida (Jenkins et al., 2003). Progesterone has also been measured at ng per g dry wood of the loblolly pine (*Pinus taeda*), a tree that is commonly by the Southeast US paper mill industry (Carson et al., 2008). There are over 20 years of publications from Canada, Europe, and the US linking paper mill effluent and reproductive interference in exposed fishes ranging from neuroendocrine disruption (see review this issue by León-Olea et al. (2014)) to intersex fish and altered reproductive physiology (Basu et al., 2009; Munkittrick et al., 2013; Orlando et al., 2007). While there are only a few studies documenting environmental gestagen output by paper mill plants, these studies do suggest that more research should be done to investigate the extent to which phytogestagens contribute to these observed effects on fish and extend these studies to other aquatic wildlife.

2.2. Wastewater treatment plant effluent

Wastewater treatment plant effluent continually adds low concentrations of hormones, including estrogens, androgens, and gestagens, as well as other emerging contaminants such as neuroendocrine disrupting chemicals (see review this issue by León-Olea et al. (2014)) directly into lakes, rivers, streams, and, in some locations, pressure-injected underground. There are more than 21,000 publically owned wastewater treatment plant facilities in the US (USEPA, 2008). While most of lipophilic hormones partition to the biosludge, what leaves the plants is in the low to hundreds of ng/L concentration range in the effluent. This concentration is biologically active and negatively affects fish and other aquatic wildlife reproduction and development. Given the common use of oral contraceptives and hormone replacement therapy, wastewater treatment plants are a continual source of excreted pharmaceutical progestins and progesterone. In contrast, discharges from animal agriculture and leaking residential septic systems (Swartz et al., 2006) are more difficult to quantify, and runoff from these sources is either accidental or the result of poor management.

Gestagens have been measured in wastewater treatment plant effluent and in receiving waters at low to hundreds of ng/L (Chang et al., 2009; DeQuattro et al., 2012; Runnalls et al., 2013; Vulliet et al., 2007). Sixty-two water samples were collected on the River Wenyu and its tributaries in Beijing, China (Chang et al., 2009), a mostly urban region that covers the most densely populated area of Beijing and contains four municipal wastewater treatment plants. The authors developed a new method for measuring the concentration of multiple steroid hormones, including gestagens, and detected megestrol acetate, norethindrone, medroxyprogesterone, progesterone, 17α,20β-dihydroxy-4-pregnen-3-one, 21α-hydroxyprogesterone, and 17α-hydroxyprogesterone in the samples. As this area contains no livestock farms, it was concluded that the major source of these contaminants was the wastewater treatment plants. In Chengdu, China and Bangi and Kajang Towns, Malaysia, researchers measured the progestins norethindrone and levonorgestrel in the low hundreds of ng/L in the