Potential effects for environmental xeno-oestrogens

Pollution and fertility

Xeno-oestrogens are man-made compounds that have oestrogenic-like activity, and exposure to xeno-oestrogens in critical periods of development and during adulthood may adversely affect both the male and female reproductive systems and fertility. For example, some studies suggest semen quality is declining globally due to xeno-oestrogen exposure, although this remains a controversial issue.

Endogenous oestrogens act by binding to and activating the oestrogen receptor (ER), a ligand-activated transcription factor that regulates transcription of oestrogen-responsive genes. Two forms of ER have been identified: ERα and ERβ. Both ERs are found throughout the male and female reproductive tracts and provide a transcriptional mechanism through which endocrine disruptors may alter male and female fertility. There are numerous examples of endocrine-disrupting chemicals affecting different endocrine organ systems. In this review, we focus on three xeno-oestrogens of current interest – bisphenol A (BPA), genistein and 2,3,7,8-tetrachloro-dibenzo-p-dioxin (TCDD) (Table 1) – and evidence for their effect on human fertility.

BPA

BPA is a high-production volume chemical used to manufacture polycarbonate plastics and epoxy resins. Polycarbonate plastic is widely found in infant feeding bottles, storage containers, and other food and drink containers, whereas epoxy resins are used in metal products such as food and beverage cans and water supply pipes. Humans are exposed to BPA primarily through food, but also through inhalation and skin exposure to air, dust and water. BPA leaches from polycarbonate bottles and epoxy resins and can be detected in over 90% of the US population. Many expert panels have evaluated the scientific evidence for reproductive and developmental effects of BPA at typical human exposure levels, but consensus is lacking on whether there is cause for concern. Many controversies surround BPA’s potential impact on human health, because studies have not examined the effects of BPA on humans, but have been conducted with immortalized cell lines, primary cells and rodents.

Although BPA stimulates several molecular pathways, its most characterized activity is as a non-steroidal oestrogen that binds both ERs and interferes with the activity of endogenous estrogens. BPA’s affinity for the ERs is 10000-fold weaker than oestradiol and it acts as both an ER agonist and antagonist. This section focuses on ‘low dose’ studies (less than 50 mg/kg per day), which in the US is considered the lowest dose at which adverse effects occur. Low-dose studies are generally considered to be environmentally relevant doses (doses resulting in serum levels similar to those found in human serum).

Prenatal and adult BPA exposure in males can decrease levels of circulating testosterone levels in mice and rats (" and references therein). Some studies report increased adult prostate size in offspring of pregnant mice fed with BPA, whereas decreased adult prostate, epididymal and seminal vesicle weight have also been reported. Poor sperm motility and an increased incidence of malformed sperm were observed in the same study. Adult mice and rats exposed to BPA produce less sperm than controls, and in mice this is associated with decreased fertility. A decrease in testis and seminal vesicle weight is observed in BPA-exposed adult mice and rats, whereas prostate weight in rats is reported to either increase and/or decrease. Spermatid abnormalities are seen in adult mice and rats exposed to BPA. Contradictory to these studies, men working in epoxy resin manufacturing with higher levels of BPA compared with non-workers exhibited no differences in serum testosterone or andro-stenedione hormone levels or any abnormal clinical signs, clinical chemistry or hepatic function.

In female mice, prenatal BPA exposure induces early puberty, as determined by oestrous cycle length, vaginal cytology, age of vaginal opening and age of first oestrus. Decreased adult vaginal wet weight and altered vaginal

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Sex hormones

Pollution and fertility

morphology is observed in mice and rats respectively. Prenatal exposure to BPA increased adult uterine expression of Hoxa10, a gene necessary for uterine development, suggesting that early BPA exposure can affect imprinting of developmentally critical genes. Leaching of BPA from polycarbonate drinking bottles resulted in meiotic abnormalities such as aneuploidy in developing mouse oocytes, and oral exposure of controlled doses had similar effects. In a follow-up study, pregnant mice treated with low, environmentally relevant, doses of BPA displayed fetal oocyte meiotic aberrations, which resulted in increased aneuploid eggs and embryos in the adult. In contrast, a similar study of adult mice exposed chronically to BPA did not observe a significant induction of hyperploidy or polyploidy in oocytes and zygotes.

In direct contrast with these studies demonstrating both organizational and activational effects of BPA on the rodent reproductive tract, in a large well-controlled two-generation mouse study, Tyl et al. found “no BPA-related effects on adult mating, fertility, or gestational indices, ovarian primordial follicle counts, oestrous cyclicity, precoital interval, offspring sex ratios or postnatal survival, sperm parameters or reproductive organ weights or histopathology.” Similarly, in a three-generation study in rats, the same authors observed no low-dose BPA-related effects on any of the above parameters listed across the generations for either sex. Finally, a rat two-generation study by Ema et al. did not find significant BPA-related changes in reproductive or developmental parameters; however, significant changes (within 5% of control) in anogenital distance in both sexes were reported. These results are consistent with studies of BPA in drinking water conducted in pregnant rats by Koon et al. and in mice by Cagen et al., in which no evidence was found for disruption in either female pubertal development or reproductive function.

In conclusion, the extent to which BPA may affect rodent or human fertility remains a controversial topic; even though numerous studies have been reported, no clear consensus has been developed, thereby requiring further investigations to definitively resolve these issues.

Genistein

The isoflavone genistein is a dietary phyto-oestrogen that is found in the environment as genistein (aglycone in unconjugated form) and also genistin (glucoside form). Soya beans and soya products, such as tofu, soya bean paste and soy sauce, are dietary sources of genistein. Genistein activates gene transcription by interacting with ERs; however, from binding assays, it has a preference for ERβ rather than ERα. At low doses (<1–10 μM), genistein acts as an ER agonist, whereas at high concentrations (>10 μM), it is shown to be an ER antagonist. Genistein can affect the reproductive system by altering growth of oestrogen-dependent cells, oestrogen synthesis and availability, and synthesis of other human reproductive hormones. Exposure can occur from gestational and lactational exposure and during adulthood through soya food consumption.

Postnatal exposure to genistein did not alter the fertility of male rats, as no alteration in sperm count, serum testosterone or gonadal histopathological was seen. Long-term exposure of male mice to genistein during gestation and lactation did not affect body weight, seminal vesicle and testicular weight, or sperm counts and motility. However, ductile branching during mammary gland development was enhanced. Low concentrations of genistein exposure throughout gestation and lactation and during puberty had no effect on testosterone levels, but high concentrations significantly decreased serum testosterone in male rodents without affecting aggressive behaviour. Follicle-stimulating hormone (FSH) levels remained unchanged, but luteinizing hormone (LH) and adrenocorticotropic hormone levels were augmented, indicating that exposure to high concentrations of genistein during puberty affects steroidogenesis in male mice. Interestingly, a recent study in men with subfertile partners demonstrated an association between high soya food intake and low sperm concentrations, suggesting a link between high genistein levels and adverse male reproductive effects.

Table 1. Relative binding affinity (RBA) of ERs for xeno oestrogens and 17β oestradiol

<table>
<thead>
<tr>
<th>Structure</th>
<th>Name</th>
<th>RBA (ERα)</th>
<th>RBA (ERβ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>17β-Oestradiol</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Bisphenol A</td>
<td>0.01</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Genistein</td>
<td>4</td>
<td>87</td>
<td></td>
</tr>
<tr>
<td>TCDD</td>
<td>N/A</td>
<td>N/A</td>
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Soy (Glycine max)
In females, prenatal exposure to genistein at low doses did not alter the time of vaginal opening, whereas high concentrations disrupted fertility by affecting ovarian and uterine histology in rats. In addition, the treatment with a low dose of genistein during postnatal days 1–5 causes prolonged oestrous cycles, small ovaries with no corpus lutea and a lower lordosis quotient than in control rats. A similar study in mice showed that genistein treatment caused abnormal oestrous cycles, early vaginal opening, subfertility and altered ovarian function. High concentrations of genistein during gestational and lactating periods decreased maternal weight gain in rats and caused behavioural changes both in male and female offspring. Moreover, genistein-treated mice failed to maintain pregnancy owing to a decrease in implantation site size coupled with increased re-absorptions. Pup survival decreased in a concentration-dependent manner. Oral treatment with genistein at low concentrations inhibited mammary gland regression in ovariectomized rats and showed uterotrophic activity in a dose-dependent manner. In humans, women who consume soya isoflavone have higher circulating levels of genistein than women without soya in their diets. They also show delayed menstruation and suppressed LH and FSH surges, as well as increased follicular phase length. Infants fed on soya-based formulas had higher circulating levels of isoflavone than those fed on cow’s milk or human breast-milk. Vegetarian mothers have greater exposure to phyto-oestrogens, and a vegetarian diet has been associated with increased risk of hypo-spadias in boys. Therefore genistein in soya-based diets and infant formulas may be associated with defects in reproductive development.

In conclusion, exposure to genistein from soya-based diets at normal consumption levels appears not to significantly affect male and/or female fertility. Only exposure to high doses of genistein in both animals and human diets may alter adult reproductive function and/or reproductive development of the fetus. The reproductive functions of females appear to be more sensitive than those of males to genistein exposure.

**TCDD**

TCDD or dioxin is found naturally from volcanic eruptions and forest fires, and anthropogenically from waste incineration, chlorination processes and plastic manufacturing (and references therein). Because TCDD is a by-product of polychlorinated phenol manufacturing, all chlorophenoxy herbicides have been banned in the US since 1983 (and references therein); however, TCDD is highly persistent and a widespread environmental contaminant. TCDD is listed as a human carcinogen, but is not believed to be mutagenic. TCDD is a ligand for the aryl hydrocarbon receptor (AhR) which forms a heterodimeric transcription factor complex with the aryl hydrocarbon receptor nuclear translocator (ARNT). Activated AhR–ARNT complexes bind ERα and ERβ, causing recruitment of these unliganded ERs to ER-responsive promoters, resulting in adverse oestrogen-related actions of dioxin. Conversely, ERα mediates transrepression of AhR-dependent gene regulation.

The effect TCDD may have on male rodent fertility is uncertain, as older reports suggest that TCDD causes a spectrum of effects in the male reproductive system, such as decreased sperm count and decreased weight of the seminal vesicles, prostate and epididymis (and references therein). However, studies designed with similar endpoints in rats yielded conflicting findings. Bell et al. (and see references therein) demonstrated that developmental exposure to TCDD, acutely or chronically, has no adverse effects on epididymal sperm levels or the weights of accessory sex organs, but TCDD given chronically at a low dose delayed puberty Testis development and male gonadotropin secretion were resistant to gestational and postnatal TCDD exposure. Prepubertal rats exposed to a single dose of TCDD demonstrated a disturbed testicular proteome profile along with histological changes in the testis, impaired spermatogenic parameters and increased oestriadiol levels, but decreased testosterone levels. Overall, rodent studies pertaining to TCDD’s effect on male fertility are inconclusive.

Few epidemiological studies exist that evaluate semen quality and reproductive outcome after TCDD exposure; however, US Vietnam veterans exposed to TCDD from aerial herbicide spraying were examined 20–30 years following exposure and, although they had detectable serum TCDD levels, reproductive parameters including serum testosterone, FSH, LH and testicular abnormalities were normal. Another study indicated that serum dioxin-like activity increases with age and was associated with decreased seminal fluid volume and lower total testosterone levels without changes in LH, inhibin B, FSH, total sperm number or sperm morphology. Reports have indicated both a positive (and references therein) or undetectable association between lowered male/female sex ratio in offspring and paternal serum TCDD levels.
More studies are required to examine the effects of dioxin on male fertility.

The female response to TCDD is markedly different than that in the male and is more complex owing to effects on both the ovaries and uterus. Female rat pups exposed to TCDD in utero and from lactation exhibited disrupted steroidogenesis. Faqi et al. report delayed vaginal opening and reduced uterine weight. Chronic TCDD exposure in rats leads to delayed puberty, loss of reproductive cyclicity with age and decreased serum oestradiol concentrations, leading to the conclusion that chronic TCDD exposure disrupts reproductive function with age. Immature female rats treated with dioxin shed fewer ova than controls and exhibit attenuated folliculogenesis, whereas direct application of TCDD to the ovary blocks ovulation and references therein). At environmentally relevant concentrations, TCDD decreases oestradiol secretion from granulosa cells, which express AhR, and this may predispose females to TCDD-related fertility defects. AhR-null mice are subfertile from impaired folliculogenesis and ovulation owing to reduced synthesis of oestradiol, which is consistent with the observation that Cyp19a1, a key enzyme in oestrogen synthesis, is a target gene of AhR in the ovary.

Endometriosis, a disease defined as the growth of endometrial glands and stroma outside of the uterus is estimated to affect 10–30% of reproductive-age women, and up to 50% of infertile women have endometriosis. TCDD exposure is considered a risk factor for endometriosis. Rier et al. observed spontaneous endometriosis in rhesus monkeys that had been chronically dosed with TCDD. TCDD impacts endometriosis lesion growth and formation in rodent models and references therein). An association between TCDD exposure and endometriosis has not firmly been established, but is suggested to play a role. Higher dioxin levels are found in the peritoneal fluid of women with endometriosis, where it may induce inflammation that promotes endometriosis. However, several studies find no association of TCDD with endometriosis and references therein). Additional well-controlled studies need to be performed to determine the role that TCDD plays in endometriosis and infertility.

At the current time, the effects of TCDD on male and female fertility appear inconclusive; therefore studies designed to examine male and female fertility after chronic low-dose exposure and bioaccumulation are warranted.

Conclusion

Overall, conclusive data is lacking to adequately determine how or whether these environmental xeno-oestrogens will affect reproduction, reproductive organ formation, response to endogenous hormones and potentially undiscovered effects. Large well-controlled experimental animal and epidemiological studies are needed to ascertain the more precise effects and mechanisms of action of these xeno-oestrogens.
References