A new chapter in the bisphenol A story: bisphenol S and bisphenol F are not safe alternatives to this compound

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Bisphenol A (BPA) is a widely studied typical endocrine-disrupting chemical, and one of the major new issues is the safe replacement of this commonly used compound. Bisphenol S (BPS) and bisphenol F (BPF) are already or are planned to be used as BPA alternatives. With the use of a culture system that we developed (fetal testis assay [FeTA]), we previously showed that 10 nmol/L BPA reduces basal testosterone secretion of human fetal testis explants and that the susceptibility to BPA is at least 100-fold lower in rat and mouse fetal testes. Here, we show that addition of LH in the FeTA system considerably enhances BPA minimum effective concentration in mouse and human but not in rat fetal testes. Then, using the FeTA system without LH (the experimental conditions in which mouse and human fetal testes are most sensitive to BPA), we found that, as for BPA, 10 nmol/L BPS or BPF is sufficient to decrease basal testosterone secretion by human fetal testes with often nonmonotonic dose-response curves. In fetal mouse testes, the dose-response curves were mostly monotonic and the minimum effective concentrations were 1,000 nmol/L for BPA and BPF and 100 nmol/L for BPS. Finally, 10,000 nmol/L BPA, BPS, or BPF reduced *Insl3* expression in cultured mouse fetal testes. This is the first report describing BPS and BPF adverse effects on a physiologic function in humans and rodents. (Fertil Steril® 2015;103:11–21. ©2015 by American Society for Reproductive Medicine.)

Key Words: Bisphenol A, bisphenol S, bisphenol F, BPA, BPS, BPF, Insl3, endocrine disruptors, environmental health, fetus, testis, Leydig cells, testosterone, human, organotypic culture, species differences

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BISPHENOL A IS A TYPICAL ENDOCRINE-DISRUPTING CHEMICAL

The field of environmental health emerged historically from the observation of the decreased reproductive success of wildlife populations in relation to industrial chemicals (1). The concept of endocrine-disrupting chemicals (EDCs) was then proposed after various reproductive function alterations in wildlife and humans were linked to the intensive use of pesticides in agriculture (2). Thus, reproduction has a central place in the EDC field. One of the most studied EDCs is bisphenol A (BPA; 2,2-bis

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(4-hydroxyphenol) propane). A survey of the Pubmed database with the use of "bisphenol A" or "BPA" as keywords provided more than 10,000 articles published up to August 2014, including clinical, epidemiologic, and experimental studies. Moreover, BPA played and still has a major role in the emergence of new concepts in the EDC field.

BPA was first synthesized by Dianin in 1891, and its estrogenic activity was discovered in 1936 (3). It is therefore one of the oldest synthetic compounds known for its endocrine activity, although diethylstilbestrol (DES) has stronger estrogenic activity. In the 1950s, it was observed that BPA could be polymerized to make polycarbonate plastic, a miraculous cheap product that is lightweight, transparent, colorable, resistant to impact, heat, and chemicals, inalterable with time, and easy to mold and thermoform. BPA rapidly became one of the most produced and used chemicals worldwide, even though it was a recognized synthetic estrogen. About 70% of BPA production (3.4 million tons per year) is used to produce polycarbonate plastics used in a variety of common products (optical, media, automotive, electrical and electronics, housewares and appliances, construction, medical, packaging, etc.). About 20% of BPA is used as an essential component of epoxy resins that are mainly used to coat the inner surface of food and beverage metallic cans. Finally, BPA is used as antioxidant or inhibitor of polymerization in some plasticizers, polyvinyl chloride, and thermal cash register paper.

BPA can leach from food or beverage containers and is then ingested. This is the main source of contamination, although its ubiquitous distribution leads also to contamination through the skin, especially in the case of thermal paper (4), or via inhalation of household dusts. Its concentration in human serum is a matter of debate, because biomonitoring and kinetic studies have reached seemingly conflicting conclusions (5). Most studies based on the analysis of blood samples from adults found that the concentration of unconjugated BPA, which is the biologically active form, ranges from 0.2 to 10 ng/mL (0.9–44 nmol/L), with an average concentration of ~1–3 ng/mL (4–13 nmol/L) (6–8). However, one study reported that unconjugated BPA represents no more than 2% of the total BPA in blood, leading to a plasma concentration of unconjugated BPA <0.02 ng/mL (<0.1 nmol/L) (9). No data are presently available on the BPA concentration in the plasma of human fetuses during the first trimester of pregnancy. BPA quantification in amniotic fluid and in umbilical cord blood samples during the second and the third trimesters of pregnancy reported a mean level of 1 ng/mL (4.3 nmol/L) with large interindividual variations (10). Furthermore, BPA might particularly accumulate in early fetuses because of lower metabolic clearance or conjugation at that development stage (11).

BPA has been associated with many human diseases, such as diabetes, obesity, cardiovascular, chronic respiratory and kidney diseases, breast cancer, behavioral troubles, tooth developmental defects, and reproductive disorders in both sexes (12–14). Considerable research effort on BPA toxicity during the last decades played a major role in raising two major concepts in the EDC field: low-dose effects (i.e., effects observed for concentrations in the range of human exposure) and nonmonotonicity (i.e., nonlinear relationship between dose and effect, where the slope of the curve changes sign somewhere within the range of the examined doses) (reviewed in 12, 15). Importantly, BPA appears to be one of the EDCs that most frequently shows a nonmonotonic dose-response curve (16).

FETAL TESTIS IS A MAJOR TARGET OF ENDOCRINE DISRUPTORS

One of the most studied functions in the EDC field is male reproduction. Many studies indicate that the incidence of male reproductive function abnormalities in humans has been increasing over the years (17–20). A decline in sperm quality was the first reported alteration (21) and was largely debated. Among the different papers dealing on this issue, published from 1995 up to now and including more than 1,000 participants, 16 found an unambiguous decline in sperm count, whereas ten reported no change and three an increase (22). A recent work showed that sperm concentration in France has been declining by 1.9% per year from 1996 to 2005 (23). It is now generally admitted that, despite geographic variations in semen quality, a global decrease in sperm count has occurred over the past five decades (24). Moreover, the incidence of testicular cancer, which is the most prevalent cancer in young men, has been steadily increasing in all studied countries. For example, it rose from 4 per 100,000 in 1960 to 10 per 100,000 in 2000 in Denmark (25). Finally, although the incidence of cryptorchidism and hypospadias shows large geographic variations (2%–9% and 0.2%–1% of male newborns, respectively), increasing trends have been reported in several countries (26).

In 1993, Sharpe and Skakkebaek hypothesized that EDCs, particularly EDCs with estrogenic effects, could be one of the causes of these disorders (27). This hypothesis has been strengthened by much epidemiologic, clinical, and experimental data over the years (18, 20, 28–31).

According to the "testis dysgenesis syndrome" (TDS) hypothesis, reduced sperm count, testicular cancer, hypospadias, and cryptorchidism result from defaults in testis development during fetal life (32, 33). Specifically, the higher occurrence of hypospadias and cryptorchidism may result from alterations of the function of fetal Leydig cells. Indeed, Leydig cells produce testosterone that is responsible for the masculinization of the male urogenital system and external genitalia (26, 34–37). An important finding in relation to the TDS hypothesis is that androgens must act before the phenotypic masculinization (38, 39). A reduction of testosterone synthesis or action between 15.5 and 18.5 days after conception (dpc) causes masculinization defects, such as hypospadias, cryptorchidism, incomplete development or agenesis of prostate and seminal vesicles, and reduction of the anogenital distance (AGD) and penis length in male rat fetuses, in which morphologic masculinization begins only at 18.5 dpc. It was therefore concluded that, in the rat, the development of genitalia is programmed between 15.5 and 18.5 dpc, a period that was named the "masculinization programming window". If such a window exists in other species, it would be between 13 and 17.5 dpc in the mouse and between the 6.5th and 14th gestational weeks (GW) in