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Oxidative and Hormonal Disruptions Underlie Bisphenol A - Induced Testicular Toxicity in Male Rabbits

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***Abstract*—**The presence of endocrine-disrupting compounds, such as bisphenol A (BPA), in the environment can cause serious health problems. However, there are controversial opinions. This study investigated the reproductive, metabolic, oxidative and immunologic-disrupting effects of bisphenol A in male rabbits. Rabbits were divided into five groups. The first four rabbit groups were administered oral BPA (1, 10, 50, or 100 mg/kg/day) for ten weeks. The fifth group was administered corn oil as the vehicle. BPA significantly decreased serum testosterone, estradiol and the free androgen index (FAI) and significantly increased sex hormone binding globulin (SHBG) compared with the placebo group. The higher doses of BPA showed a significant decrease in follicular stimulating hormone (FSH) and luteinizing hormone (LH). A significant increase in blood glucose levels was identified in the BPA groups. The non-significant difference in insulin levels is a novel finding. The cumulative testicular toxicity of BPA was clearly demonstrated by the dose-dependent decrease in absolute testes weight, primary measures of semen quality and a significant increase in testicular malonaldehyde (MDA). Moreover, BPA significantly decreased total antioxidant capacity (TAC) and significantly increased immunoglobulin G (IgG) at the highest concentration. Our results suggest that BPA, especially at higher doses, is associated with many adverse effects on metabolism, oxidative stress, immunity, sperm quality and markers of androgenic action.

***Keywords*—**Bisphenol A, oxidative stress, rabbits, semen quality, steroidogenesis.

I. Introduction

SEVERAL thousand anthropogenic chemicals currently released in the environment are endocrine-disrupting compounds (EDCs) [1]. EDCs are defined as exogenous chemicals or chemical mixtures that impact endocrine system structure or function and cause adverse effects [2]. Endocrine systems regulate a multitude of developmental, metabolic, and reproductive processes, including embryonic development, gonadal formation, sex differentiation, growth, and digestion. EDCs may affect these processes by binding to or blocking hormone receptors, thereby triggering or preventing a hormonal response [3]. Chemicals implicated in endocrine disruptions include biocides, industrial compounds, surfactants, and plasticizers, including bisphenol A (BPA) [4]. BPA (4,4′-isopropylidenediphenol) has become ubiquitous in the environment within the past 80 years because of its presence in a multitude of products, including polycarbonate plastics used in the manufacture of baby formula bottles, water bottles, and other clear plastic containers [5]. Vandenberg also reported that BPA is also used in the epoxy resin that lines the inside of metal food and soda cans, medical devices, and credit card receipts. BPA is consumed when it leaches from plastic containers into foods or drinks when they are heated or when they become broken or cracked. Other potential routes of exposure to BPA include air, dust, water, 5-gallon water coolers, printer inks, toners and thermal receipt paper (used by most gas stations and supermarkets), where BPA can rub off paper onto our hands and into our mouths [6].

Furthermore, these authors have shown that more than 93 percent of the general population has some BPA in their bodies, and infants and children have been estimated to have the highest daily intake of BPA because “they eat, drink, and breathe more than adults on a pound for pound basis”. Several metabolic pathways of BPA have been identified. Metabolism of BPA is predominately characterized by phase II conjugation reactions in the gastrointestinal tract and liver.

During the first-pass metabolism, the major metabolic pathway involves a chemical reaction, which includes the rapid conjugation by uridine diphosphate glucuronyl trasferases (UGT) to form BPA-glucuronide. The second minor metabolic pathway involves the chemical reaction sulfatation to form BPA-sulfate by sulfotransferase in rats and humans [7]. BPA functions similar to a synthetic estrogen (diphenyl compound that contains two hydroxyl groups in the ‘para’ position, which makes it remarkably similar to synthetic estrogen) [1] In animal studies, BPA has been associated with reproductive abnormalities, hormonal changes, enlarged prostate glands, abnormalities in the number of chromosomes in eggs, and pre-cancerous changes in the breast and prostate [8]; it also has been associated with obesity and insulin resistance, a condition that commonly precedes the development of diabetes. We aimed to address the gaps that exist in understanding how environmental exposures and multiple BPA-induced adverse effects are related; furthermore, because recent research has indicated a paradoxical phenomenon with BPA and other chemicals that affect the endocrine system and their impact on health is sometimes greater at low doses compared with high doses [9], we investigated the effects of low and high doses of BPA on

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metabolism, immunity, oxidative stress, and reproductive parameters, including semen quality and reproductive hormones, in male rabbits.

II. Materials and Methods

A. Chemicals and Reagents

The bisphenol A was obtained from Aldrich Chemical Company, Inc., Wisconsin, USA. The 100% corn oil was purchased from a local market. All other reagents were of analytical, high-performance liquid chromatography (HPLC) or the best available pharmaceutical grade.

B. Animals

The present study was conducted on 30 male rabbits (4-5 months old, body weights from 1600-2000 g). Handling of the animals occurred in compliance with the Guidelines for the Care and Use of Animals for Scientific Purposes. The animals were caged in a well-ventilated animal room with a 12 h dark/light cycle and a controlled temperature; all animals had free access to a standard diet and drinking water *ad libitum*.

C. Experimental Design

To investigate the toxicity of BPA, 30 rabbits were randomly divided into five groups, which consisted of six rabbits per group. Different groups of rabbits were administered freshly prepared BPA orally via gavage at specific concentrations between 1 and 100 mg/kg with corn oil as the vehicle [10]. The animals were treated via oral gavage once daily for ten weeks as follows. For group one, the control group, all rabbits were administered corn oil (vehicle). Group two was orally administered 1 mg/Kg/day of BPA. Group three was administered 10 mg/Kg/day of BPA. Group four was administered 50 mg/Kg/day of BPA. Group five was administered 100 mg/Kg/day of BPA. Body weights were recorded, and clinical observations were made daily. Twelve hours after the last dose was received, the animals were fasted and blood samples (2.5 ml) were obtained from the ear vein. The sera were separated for measurement of testosterone, estradiol, FSH, LH, SHBG, insulin, glucose, TAC, IgM and IgG. Immediately following the blood sample collection, the animals were then sacrificed and their testes were rapidly excised and weighed with an electronic analytical balance. The testes were used to determine the malonaldehyde (MDA) content.

D. Hormonal Measurement

The measurement of testosterone, estradiol, FSH, LH and SHBG was conducted using enzyme immunoassay (EIA) kits. The serum collected from the animals was allowed to thaw prior to the hormone assay using an EIA reader. The free androgen index (FAI) was calculated as total T × 100/SHBG.

E. Sperm Characteristics

As described by Saalu et al. [11], sperm motility, concentration and progressive motility were determined by removing the caudal region of the epididymis and placing it in a beaker that contained 1 ml of physiological saline solution; each section was then quickly incised with a pair of sharp scissors and maintained for several minutes to release its spermatozoa into the saline solution. Semen drops were placed on a slide, and two drops of warm 2.9% sodium citrate were added. The slide was covered with a cover slip and examined with a light microscope using a 40× objective to determine sperm motility. The sperm concentration was identified using a hemocytometer (Improved Neubauer; Hauser Scientific Inc., Horsham, PA, USA). In this analysis, the percent motile sperm refers to the percentage of sperm with any flagellar movement, whether twitching or progressive. The motility was also analyzed using the World Health Organization (WHO) 1999 a, b, c, and d method, with forward motile sperm classified as a + b [12]. The sperm morphology was performed using a staining technique.

F. Statistical Analysis

All statistical analyses used ANOVAs and were conducted with the SPSS 10.0 (SPSS Inc., Illinois, USA) computer program. Values of $P < 0.05$ were considered significant.

III. Results

In the analyses adjusted for age, sex and body weight, the absolute testes weights, relative testes weights and final body weights of the male rabbits in the control and treatment groups are presented in Table I. There was a significant decrease in the absolute testes weights of the BPA groups in a concentration dependent manner compared with the control group. There was a significant decrease in the body weight differences in the BPA groups at low and high doses compared with the control group. During treatment, a reddish secretion was observed around the nose of the BPA (50 mg mg/kg) group, but not in the other groups.

Serum testosterone, estradiol and FAI were significantly decreased, while SHBG was significantly increased in the BPA groups compared with the control group (P <0.05; Tables II and III). Table IV shows the effects of BPA on serum FSH and LH levels. The highest concentrations of BPA (50 and 100 mg/kg) showed a significant decrease in FSH and LH. On the other hand, all doses ≤10 mg/kg showed nearly the same hormonal level compared with the control group.

Table V shows the cumulative testicular toxicity of BPA was clearly demonstrated by a dose-dependent decrease in sperm concentration, sperm motility, sperm progressivity and sperm morphology at different concentrations of BPA (10, 50 and 100 mg/kg). In contrast, 1 mg/kg showed nearly the same values compared with the control group. A highly significant difference in blood glucose levels was observed in all BPA groups compared with the control group. The non-significant difference (P >0.05) in insulin levels is a novel finding because previous studies have reported a significant decrease in insulin levels in laboratory animals treated with BPA, as shown in Table VI. The effects of BPA on the oxidative stress biomarkers are presented. In the BPA (10, 50 and 100 mg/kg) groups, there were significant decreases in TAC and significant increases in testicular MDA. In contrast, the 1 mg/kg group exhibited nearly the same values compared with

the control group, as shown in Table VII. In the BPA groups, there was a significant increase in IgG in a concentration dependent manner at 10, 50 and 100 mg/kg compared with the control group (P <0.05), with no significant difference in IgM at all doses (P > 0.05, Table VIII).

TABLE I
THE BODY WEIGHT (GM), SELECTED ABSOLUTE (GM), AND RELATIVE ORGAN WEIGHTS OF MALE RABBITS IN CONTROL AND BPA GROUPS

Parameters	Control	BPA (1 mg/kg)	BPA (10 mg/kg)	BPA (50 mg/kg)	BPA (100 mg/kg)
Initial body weight(g)	1600±15.67	1700±20.56	1750±10.45	1900±15.56	2000±20.40
Final body weight(g)	1850±10.20	1900±15.10	1900±10.30	2000±15.40	2050±10.20
Body weight difference(g)	250±8.60a	200±5.00b	150±6.22c	100±5.15d	50±7.13e
Absolute testes weight (g)	4.7±0.3a	4.6±0.2a	4.0±0.3b	3.8±0.2b	3.1± 0.2c
Relative testes weight (g)	0.0025a	0.0024a	0.0021ab	0.0019ab	0.0015bc

Means within the same column carrying different letters are significantly different (P<0.05). BPA: Bisphenol A.

TABLE II
THE EFFECT OF BPA ON SERUM TESTOSTERONE AND SERUM ESTRADIOL IN MALE RABBITS

Treatment group	Serum testosterone (ng/ml)	Serum estradiol (pg/ml)
Control	1.91±0.03a	119.34±3.67a
BPA 1 mg/kg	0.77±0.01b	117.34±4.65a
BPA 10 mg/kg	0.72±0.03b	92.45±4.76b
BPA 50 mg/kg	0.50±0.01c	75.67±3.67c
BPA 100 mg/kg	0.33±0.02d	51.78±3.26d

Means within the same column carrying different letters are significantly different (P<0.05). BPA: Bisphenol A.

TABLE III
THE EFFECT OF BPA ON SERUM SHBG AND FAI IN MALE RABBITS

Treatment group	SHBG (nmol/l)	FAI
Control	27.3±2.19c	6.99±1.50a
BPA 1 mg/kg	28.4±2.23c	2.71±0.97b
BPA 10 mg/kg	36.5±3.29b	1.97±0.39bc
BPA 50 mg/kg	48.7±4.38a	1.02±0.28c
BPA 100 mg/kg	49.2±3.62a	0.67±0.06cd

Means within the same column carrying different letters are significantly different (P<0.05). BPA: Bisphenol A, FAI: free androgen index, SHBG: sex hormone binding globulin.

TABLE IV
THE EFFECT OF BPA ON SERUM FSH AND SERUM LH IN MALE RABBITS

Treatment group	Serum FSH (mIU/ml)	Serum LH (mIU/ml)
Control	11.7±0.75α	22.1±3.47α
BPA 1 mg/kg	12.0±0.82α	21.7±3.11α
BPA 10 mg/kg	11.4±1.14α	17.2±1.87β
BPA 50 mg/kg	8.2±0.91β	16.8±1.39β
BPA 100 mg/kg	5.4±0.66χ	11.5±2.31χ

Means within the same column carrying different letters are significantly different (P<0.05). BPA: Bisphenol A, FSH: follicular stimulating hormone, LH: luteinizing hormone.

TABLE V
SPERM CONCENTRATION, SPERM MOTILITY, SPERM PROGRESSIVITY AND SPERM MORPHOLOGY OF MALE RABBITS IN CONTROL AND BPA GROUPS

Parameters	Control	BPA (1 mg/kg)	BPA (10 mg/kg)	BPA (50 mg/kg)	BPA (100 mg/kg)
Sperm concentration ($x10^6$/ml)	76.29±3.67a	75.73±4.12a	61.41±3.76b	60.12±3.13b	48.71±2.67c
Sperm motility%	91.31±4.67a	92.38±5.29a	90.27±4.18a	72.56±3.71b	55.73±4.56c
Sperm progressivity	a	a	a	b	c
Sperm morphology(%Normal)	80.45±3.45a	81.34±5.67a	73.57±3.93b	61.29±3.19c	50.78±4.10d

Means within the same column carrying different letters are significantly different (P<0.05). BPA: Bisphenol A.

TABLE VI
THE EFFECT OF BPA ON SERUM INSULIN AND SERUM GLUCOSE LEVELS IN MALE RABBITS

Treatment group	Insulin	Glucose
Control	13.15±0.83	77.76±5.56d
BPA 1 mg/kg	12.76±0.91	79.39±4.76d
BPA 10 mg/kg	12.32±1.00	98.57±6.28c
BPA 50 mg/kg	12.18±0.87	158.59±6.29b
BPA 100 mg/kg	11.74±1.13	277.23±8.16a

Means within the same column carrying different letters are significantly different (P<0.05). BPA: Bisphenol A.

TABLE VII
THE EFFECT OF BPA ON SERUM TAC AND TESTICULAR MDA IN MALE RABBITS

Treatment group	TAC(mM/L)	MDA(nmol /g wet tissue)
Control	3.17±0.31a	72.4 ± 3.19d
BPA 1 mg/kg	3.03±0.43a	74.1 ± 2.67d
BPA 10 mg/kg	1.41±0.18b	93.6 ± 3.59c
BPA 50 mg/kg	1.38±0.21b	128.7 ± 6.91b
BPA 100 mg/kg	0.67±0.07c	169.2 ± 7.18a

Means within the same column carrying different letters are significantly different (P<0.05). BPA: Bisphenol A, MDA: Malonaldehyde, TAC: total antioxidant capacity.

TABLE VIII
THE EFFECT OF BPA ON SERUM IGM AND SERUM IGG IN MALE RABBITS

Treatment group	IgM(mg/dl)	IgG(mg/dl)
Control	70.29±4.19	306.32±8.13de
BPA 1 mg/kg	72.10±5.23	314.47±6.29d
BPA 10 mg/kg	68.42±3.58	338.15±8.49c
BPA 50 mg/kg	71.39±4.71	379.78±10.30b
BPA 100 mg/kg	69.78±5.18	411.61±9.87a

Means within the same column carrying different letters are significantly different (P<0.05). BPA: Bisphenol A, IgM: immunoglobulin M, IgG: immunoglobulin G.

IV. DISCUSSION

BPA is an endocrine disorder chemical released in the environment. Because of the limited information regarding the toxic effects of different concentrations on reproductive parameters, including semen quality and male reproductive hormones, metabolism, oxidative stress and immunity, our study used four different concentrations to evaluate the toxic effects of BPA on male rabbits. The Center for the Evaluation of Risks to Reproduction on BPA stated the lowest oral LD50 for rats is 3.25 g/kg bw for acute toxicity [13]. Because of the debates regarding the low-dose and high dose effects of BPA, this study included the low and high doses based on several reports and there was no incidence of animal death. In the current study, a significant decrease in the body weight difference and absolute organ weights were observed in a dose dependent manner with a remarkable bloody nasal secretion compared with the control group. A similar reddish secretion around the nose was observed in a study of chronic exposure to low doses of bisphenol A [14]. Another study showed a significant decrease in body weight in the group treated with ≥466 mg/kg/day BPA. Significant dose-related decreases in absolute (>22%) and relative liver weights (>10%) were observed following ≥466 mg/kg/day compared with controls [15]. The reproductive parameters, including semen quality and male reproductive hormones, evaluated the presence of testicular damage or disease. The data presented in our study demonstrate that BPA significantly decreased serum testosterone, estradiol, FAI, sperm concentration, sperm motility, sperm progressivity and sperm morphology. The serum FSH and LH levels were approximately normal in the lower concentration groups. Lahnsteiner et al. [16] observed a reduced sperm quality and delayed ovulation in brown trout following BPA exposure (1.75 mg/L). Altered sex steroid levels were also identified in the marine turbot following the exposure to BPA concentrations of 59 mg/L [17]. At BPA concentrations above environmentally relevant levels (274 mg/L), male guppies had reduced sperm counts [18]. Richter et al. [19] have noted that rodents exposed to BPA during the prenatal or perinatal periods exhibit a large variety of adverse reproductive outcomes, including decreased epididymal weight and daily sperm production. Regarding prepubertal or pubertal exposures, rodent studies have described a dramatic decrease in testosterone (T) levels [20]. Tohei et al. [21] reported that plasma concentrations of T were decreased and plasma concentrations of luteinizing hormone (LH) were increased in BPA-treated male adult rats compared with control rats. Juvenile male Sprague-Dawley rats treated with bisphenol A showed a decrease in circulating estradiol and testosterone levels [22]. Takahashi and Oishi [23] showed that BPA can cause a weight decrease in the testis, epididymis, prostate, and seminal vesicle and a decrease in sperm production. In pubertal mice and rats, BPA has been implicated in the abnormalities identified in the spermatid, spermatozoa, and the ectoplasmic specialization between the sertoli cells and the spermatid [24]. A study conducted that used two different strains of adult rats showed that estradiol benzoate three times per week for one month caused a decrease in testis weight, sperm production and degeneration of the seminiferous tubules in both strains [25]. Chickens that received oral doses of BPA as low as 2 mg/1000 g body weight every two days for up to 23 weeks exhibited reduced weight of the testes, with smaller seminiferous tubules and limited spermatogenesis. The authors suggest that an endocrine-disrupting mechanism may trigger these effects and that reproduction was likely to be impaired [26]. Hanaoka et al. [27] studied men occupationally exposed to BPA during the application of epoxy resins compared with matched control workers without exposure; the authors determined that BPA significantly decreased FSH levels after controlling for age and alcohol use. Meeker et al. [28] examined a male population that attended a fertility clinic and reported that BPA decreased the estradiol (E2)/T ratio and increased the FSH levels and the FSH/inhibin B ratio. It has been hypothesized that these changes may be the result, at least in

part, of increased human exposure to EDCs [29]. BPA is a non-steroidal xenoestrogen that exhibits approximately 10^{-4} the activity of estradiol [30]. Previous work has indicated that BPA may be as effective as estradiol in triggering some receptor responses [31], and it may act as an androgen receptor antagonist [32]. Hanaoka et al. [28] examined men occupationally exposed to BPA and reported that BPA decreased serum FSH levels. Nevertheless, free testosterone levels did not differ between the two groups. Hanaoka et al. [28] speculated that BPA binds to the estrogen receptor (ER) in the pituitary gland, which results in the direct suppression of FSH secretion; this hypothesis is based on studies that have identified ERs in the pituitary gland [33] and that E2 directly inhibits gonadotropin secretion at the pituitary level [34]. Akingbemi et al. [35] described an inhibitory effect of BPA on testicular steroidogenesis at low exposure levels in pubertal rats, which they ascribed to an ER-mediated effect. In addition to its anti-androgenic effects through ER-mediated down-regulation of steroidogenesis (and thereby T production), BPA may also act as an androgen receptor antagonist, which prevents endogenous androgens from regulating androgen-dependent transcription [36]. The disruption of the androgen receptor–androgen interaction has been speculated to be significant in eliciting adverse effects on the male reproductive system, including sexual dysfunctions [37]. Our results suggest that FAI levels, which are a marker of biologically active T, may be somewhat decreased by BPA exposure. In the present study, we observed an inverse association between BPA exposure and FAI levels *in vivo* with no apparent compensatory increase in serum gonadotropins, as indicated by the lack of significant associations with LH and FSH. However, we cannot rule out a potential compensatory increase in serum gonadotropins in a larger study or a different study population. The regulation of SHBG is not completely understood, but androgen action lowers serum SHBG, whereas estrogen action increases it. The increase in SHBG levels that we reported here could be a direct result of the estrogenic action of BPA. Alternatively, it is possible that BPA acts by decreasing androgen action through an ER-mediated decrease in steroid production. We speculate that BPA *in vivo* may act at several levels. In addition to the alteration of markers of androgenic action, the endocrine feedback loop (mainly the hypothalamic–pituitary–target organ axis) could also be affected by BPA, which shows no compensatory mechanisms of increased LH or FSH. BPA has also been shown to decrease sperm motility and sperm count in animals by inducing oxidative stress and decreasing the cells' antioxidant defense system [38]. BPA generates significantly high concentrations of MDA in the brains and sperm of male rats treated orally and causes oxidative damage in the brains and testes of rats [39]. The main metabolic pathway of BPA is detoxification via glucuronidation. A minor route is oxidation via hydroxylation to a catechol followed by further transformation to an o-quinone. The catechol-o-quinone couple is capable of redox cycling with the generation of reactive oxygen species (ROS) and oxidative stress [40]. The current results revealed that BPA significantly increased testicular MDA, and significantly decreased TAC support these previous data. Our data showed that a high dose of BPA not only increases the free radical formation but also decreases its ability to detoxify reactive oxygen species. The present study is divergent from the studies previously reported by Takeuchi and Tsutsumi [41], who reported that BPA increased total T and FT levels in all subjects. Halldin et al. [42] found that quail eggs with BPA exposure at 67 and 200 mg/g per egg did not produce individuals with altered testicular weight symmetry, testosterone concentrations, male sexual behavior, or female fecundity. Nieminen et al. [43] stated that BPA exposure in wild mammals (field voles) at 250 mg/kg/day resulted in increased testosterone levels. The study conducted by Kato et al. [44] showed that neonatal BPA administration did not affect the normal reproductive parameters in rats. Murono et al. [45] used cultured Leydig cells and demonstrated that BPA did not decrease ambient testosterone levels. These controversial data may be the result of using different strains, exposure times and routes of administration. The current study showed that BPA caused a significant increase in the levels of blood glucose in a dose dependent manner with the absence of changes in insulin levels compared with the control group. A more recent study demonstrated that adult mice administered low doses of BPA twice per day for eight days did develop problems with their metabolism that would lead to type 2 diabetes [46]. At least two other articles published in 2012 concluded that BPA exposure places humans at risk for metabolic disorders and obesity [47], [48]. These findings were similar to a study published in the *Journal of the American Medical Association* in 2008, which identified a link between BPA levels and diabetes and heart disease, even when the data were statistically controlled for obesity [49]. A study published in *Circulation* in 2012, which was based on research in the UK, supported these findings [50]. Hugo et al. [51] determined that low-level exposure to BPA inhibited the release of adiponectin from human adipose (fat) tissue. Adiponectin increases insulin sensitivity and helps regulate glucose metabolism. The researchers hypothesized that environmental BPA exposure may increase the susceptibility to obesity and diabetes. Interaction with glucose transporter-2 and glucose transporter-8, oxidative stress and estrogenicity of BPA contributed to the observed effects [52]. In the current study, there was a significant increase in IgG, with no observed changes in IgM. Natural estrogen (17β-estradiol) has been shown to protect the main cells of the immune system from apoptosis and to regulate the synthesis of antibodies in the serum and female genital tract [53]. It is therefore plausible that EDCs, such as BPA, which possess estrogenic activity [54], may exert similar effects within the body. One potential mechanistic explanation for this association is that BPA may inhibit macrophage function [55]. If BPA disrupts the function of macrophages, it may increase the need for the host to increase antibody production to counteract this disruption. Further studies are needed to address the potential immune-boosting effects of BPA on immune systems. Nevertheless, the exposure to environmental toxicants, including EDCs, has been associated

with numerous disease outcomes, many of which involve underlying immune and inflammatory dysfunctions [56].

V. Conclusion

This novel study addressed the need to improve our understanding of how EDCs such as BPA affect health**.** Our results suggest that BPA, especially at higher doses, is associated with many adverse effects on metabolism, oxidative stress, immunity, sperm quality and markers of androgenic action. These results may reflect the estrogenic effects of BPA, which we hypothesize, could be related, in part, to an inhibitory effect on testicular steroidogenesis. The induction of oxidative stress by BPA may play an additional role in testicular toxicity. These results suggest that BPA poses a threat to endocrine and reproductive functions. BPA is found in polycarbonate (PC) plastics, which are typically clear and hard, marked with the recycle symbol "7" or may contain the letters "PC" near the recycle symbol. To avoid the risks of baby bottles with BPA or other questionable chemicals, packages that say “BPA-free” should be utilized, as well as the use of alternatives, such as glass bottles. To avoid warming up food in plastic containers with these chemicals, the use of stoneware, china, or glass dishes and containers in a microwave is recommended.

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