Chronic exposure to low doses of bisphenol A alters hydromineral responses in rats

Paula Nuñez [a,b,*], Juan Arguelles [a,b], Carmen Perillan [a,b]

[a] *Departamento de Biología Funcional, Facultad de Medicina y Ciencias de la Salud, Universidad de Oviedo, Spain*
[b] *Instituto de Neurociencias del Principado de Asturias (INEUROPA), Oviedo, Spain*

ARTICLE INFO

ABSTRACT

Bisphenol A (BPA) is a chemical commonly used in the industrial sectors, hence humans are exposed to the compound repetitively. BPA is an endocrine disruptor and has been anticipated to interfere on chemical estrogen receptor functions and other nuclear hormone receptors. Estrogens are steroid hormones that, in addition to their neuroendocrine roles, affect water and salt intakes in numerous species, including humans and rodents. Changes in the hydrosaline balance produce compensatory behavioral and physiological responses, which serve to preserve or restore osmolarity and blood volume to optimal levels, thus preventing cardiovascular disease. The aim of the present work was to determine for first time the effect of long-term and low-dose BPA treatment on thirst and sodium appetite. Wistar rats were exposed to BPA via drinking water to mimic the most likely route of human exposure, and different dipsogenic and natriorexigenic stimuli were assessed. The BPA-treated rats tend to drink less water that control rats following 24-h fluid restriction, but there was no statistically significant decrease. Perhaps the BPA dose does not have enough estrogenic potency to affect water intake. In the extracellular fluid depletion test, the control rats significantly increased 2.7% NaCl solution intake on repeated testing, showing sodium appetite sensitization, i.e. the capacity to enhance sodium intake produced by stimulus repetition; whereas BPA-treated rats did not. In this study, fluid and electrolyte balance in BPA-treated rats is generally adequate but impaired in osmotic challenges, for example by sodium depletion. Thus, neuroendocrine systems involved in maintaining body fluid and electrolyte homeostasis were altered in BPA-treated rats.

1. Introduction

Bisphenol A (BPA) is a chemical commonly used in the industrial sector, hence humans are exposed to the compound repetitively. Although there are BPA-free products available, the compound is still generally found in consumer products (Ma et al., 2019). These contaminants leach from those consumer products into the environment, including draining into water, food and air. BPA has been detected in human urine, blood, and milk (Azzouz et al., 2016). Furthermore, recent epidemiological studies have reported the association of BPA exposure with the development of different metabolic diseases, especially cardiovascular diseases. These studies demonstrated the association of high urinary BPA levels with an increased risk of coronary artery disease, hypertension, and myocardial infarction (Zhang et al., 2020).

Many studies have investigated the mechanism through which BPA produces its effects. BPA is an endocrine disruptor. The term Endocrine

Disrupting Chemicals defines a group of chemicals with very different origins, structures, and uses. These are natural or synthetic substances that are exogenous to the body, and interfere with the production, release, transport, metabolism, binding, biological action or elimination of hormones responsible for maintaining homeostasis and regulating development. Since BPA has a structure similar to the potent estrogen receptor (ER) agonist diethylstilbestrol, it has been anticipated to interfere with ER functions and other nuclear hormone receptors (MacKay & Abizaid, 2018). This feature has caused BPA to be thought of as a weak environmental estrogen (E2). However, recent studies on BPA action mechanisms at low concentrations have revealed that BPA can trigger an entire battery of cellular estrogenic responses of equal or greater efficiency and power than estradiol by itself. In this sense, BPA can directly affect neuroendocrine function and cause physiological effects in target organs (Ma et al., 2019).

Estrogens are steroid hormones that, in addition to their neuroendocrine roles, affect water and salt intakes in numerous species,

Abbreviations

angiotensin II (Ang-II)
Ang-II type 1 receptor (AT1)
bisphenol A (BPA)
estrogen (E2)
estrogen receptor (ER)
ovariectomised (OVX)
renin-angiotensin-aldosterone system (RAAS)

including humans and rodents (Barron et al., 1986; Stachenfeld, 2008). Changes in hydrosaline balance produce compensatory behavioral and physiological responses, which serve to preserve or restore osmolarity and blood volume to optimal levels, thus preventing cardiovascular disease (Santollo & Daniels, 2015). Thirst and sodium appetite are fundamental for the maintenance of body fluid homeostasis (Curtis, 2009). Studies in male and female rats, including ones conducted during the estrous cycle and in ovariectomised (OVX) rats with hormone replacement, suggest that estrogens inhibit water (anti-dipsogenic effect) and sodium (anti-natriorexigenic effect) consumption (Santollo et al., 2013). We found in a previous study that BPA acute exposure (administered subcutaneously) had similar inhibitory effects on saline intake in males and OVX females, regardless of whether it was spontaneous or stimulated intake. Males were more sensitive to the anti-dipsogenic effect of BPA than OVX females, and there was no inhibitory effect of BPA on deprivation-induced water intake in males or OVX females (Nuñez et al., 2018).

Therefore, the aim of the present work was to determine for first time the effect of long-term and low-dose BPA treatment on thirst and sodium appetite in adult male Wistar rats. These rats were exposed to BPA via drinking water to mimic the most likely route of human exposure. The capacity of BPA-treated rats to maintain body fluid homeostasis in response to different dipsogenic and natriorexigenic stimuli was assessed.

2. Materials and methods

2.1. Chemicals

Bisphenol A (BPA Sigma-Aldrich, purity ≥99%, CAS no. 80–05-7) was dissolved in ethanol and subsequently diluted with purified water to final concentration (ethanol comprising 1% of the final solutions).

2.2. Animals

All experimental procedures followed the guidelines set forth in the Care and Use of Animals, and The University of Oviedo's Experimental Animal Use Committee approved all experimental procedures (PROE 15/2016). Adult male (300–400 g, aged 4 months) Wistar rats were housed individually under standard conditions at $22 \pm 3\,°C$ with light/dark periods of 12h and a minimum relative humidity of 40%. Rats were maintained on 2014 Teklad global 14% protein rodent maintenance diet (Envigo Laboratories, Barcelona, Spain), which does not contain alfalfa or soybean meal (chow diet). The composition of the diet was as follows: calories from protein, 18%; calories from fat, 11%; calories from carbohydrate, 71%; and an energy content of 2.9 kcal/g. Purified water was supplied ad libitum in glass bottles with rubber stoppers. Fluid intakes were measured by weighing the drinking bottles on an electric balance during the early light phase once a week. The spillage and evaporation is < 0.5 g/day (Nuñez Martinez et al., 2016). We did not attempt to correct this source of error. However, we kept records of visible spillage. Individual body weight and food intake was recorded weekly. Rats were habituated to housing conditions for at least 15 days.

2.3. Exposure

Rats were randomly assigned to the control (n = 6) or BPA (n = 6) group. Control rats had access to purified drinking water (containing 1% ethanol, vehicle), whereas the BPA group received purified drinking water containing BPA (5 mg/L; BPA Sigma-Aldrich, purity ≥99%, CAS no. 80–05-7, and ethanol comprising 1% of the final solution) to mimic the most likely route of human exposure (Desai et al., 2018). Water and BPA solutions were freshly made and changed once every week during the 3 months. Treated rats were always in BPA state, even during the experiments. At the end of the experiment, animals were euthanized under intraperitoneal ketamine (75 mg/kg b.w.)/xylazine (10 mg/kg b. w.) injection. Blood was collected by cardiac puncture, heparinized, centrifuged, and plasma stored at −20 °C. The analyses of plasma BPA levels were conducted at the Environmental Testing Unit of the University of Oviedo. A highly sensitive gas chromatography-mass spectrometry (GC-MS) method for the determination of BPA plasma samples was used (Azzouz et al., 2016). When the rats had already been exposed to BPA for 12 weeks, we used two ingestive-behaviour experiments: the 24-h fluid restriction test followed 8 day later by the extracellular fluid depletion test.

2.4. Experiment 1: 24-h fluid restriction

Water deprivation produces thirst by causing both intracellular and extracellular dehydration. Water and 2.7% NaCl solution was supplied simultaneously from two separate drinking bottles, reversing their location daily during six days. We then performed a water deprivation test on day seven to assess water and 2.7% NaCl solution induced intakes. The rats were maintained for 24h on the standard food diet with no drinking fluids available after which, 2.7% NaCl solution and water were made available. Fluid intakes were then measured. Intake of the two fluids was recorded after 5, 10, 15, 30, 60 and 120 min (Nuñez et al., 2018).

2.5. Experiment 2: extracellular fluid depletion

Furosemide treatment causes hypovolemic dehydration. Rats were weighed in the morning and placed in standard metabolism cages without drinking tubes. Furosemide (Seguril, Sanofi-Aventis Laboratories, Barcelona, Spain) was injected (10 ml/kg body wt sc) to induce natriuresis and diuresis. After the 1st hour after furosemide injection, urine was collected and access to water was provided. Food was not present. On the next morning, urine was collected for the remainder of 19h after furosemide, and urine volume was recorded simultaneously with water intake (Thunhorst & Johnson, 2003). This urine volume was calculated as 1 g = 1 mL. Samples were refrigerated for later analysis of sodium content. The rats were then returned to their home cages with drinking tubes. Water and 2.7% NaCl solution was recorded every 30 min for 4 h. Rats received two such tests separated by 8 days to study sodium appetite sensitization.

2.6. Urine analysis

Urine volume was measured (UV). Urinary concentration of sodium (Una) was determined by reflectance spectrophotometry and used for calculation of urinary excretion of sodium (UnaV). Relative water balance was calculated by subtracting total UV collected from total amount of fluid (water + 2.7% NaCl solution) ingested over the course of an experiment. Relative sodium balance was calculated by subtracting the total UnaV collected from the total amount of sodium ingested in the form of 2.7% NaCl solution over the course of an experiment. We use the term "relative" for the balance measures, inasmuch as respiratory and fecal losses of water and sodium were not considered.

2.7. Statistical analysis

The results are expressed as the mean ± the standard error of the mean (SEM). Statistical software (IBM SPSS v. 24, IBM Analytics) was used for statistical analyses. All intake data were normalized for body-weight. The 2.7% NaCl preference was calculated as the ratio of 2.7% NaCl solution intake to total fluid intake expressed as a percentage. The Shapiro–Wilk test showed that the variables follow a normal distribution of data for p>0.05. Data were analyzed by ANOVA with group (control, BPA) as the between-subjects factor and with test and time (i.e., hours, days) as within-subjects repeated measures. Whenever the analysis showed either main effects, or statistically significant interactions, post hoc comparisons were performed using Least Significant Difference (LSD) test, with significance levels set at p < 0.05. The unpaired Student's t-test was used to analyze BPA levels (control rats vs. BPA-treated rats), after controlling for homogeneity of variance via the Levene test. Values of p < 0.05 were deemed as statistically significant.

3. Results

3.1. Body weight and food intake

Mean body weight and body weight gain of BPA-treated rats did not differ significantly from controls (Table 1). We studied weekly between-group variance in intake of food (Fig. 1). There was no effect on food intake associated with BPA exposure (F (1,10) = 1.19, p>0.05). Food intake over the course of 12 weeks was similar in BPA-treated and

Table 1
Values are expressed as the mean ± SEM.

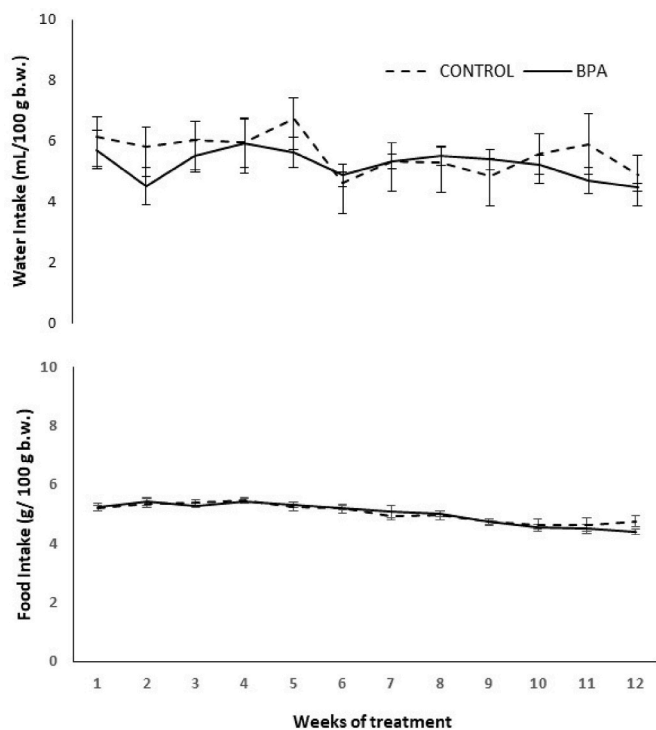|	Initial body weight (g)	Final body weight (g)	Body weight gain
Control	329.17 ± 22.38	432.83 ± 14.16	103.67 ± 14.20
BPA	345.67 ± 25.37	470.50 ± 27.43	124.83 ± 20.33

Fig. 1. Ad libitum amount of food eaten (g/100g of b.w./day) and water drunk (mL/100g of b.w./day) in control and BPA-treated groups was monitored for 12 weeks. N = 6 animals/group. Results are expressed as the mean ± SEM.

control rats (BPA = 60.4 ± 1.3 g/100 g bw/day; control = 60.5 ± 1.3 g/ 100g bw/day).

3.2. BPA effects on plasma BPA levels

The average water consumption over the course of 12 weeks was similar in BPA-treated and control rats (BPA = 5.22 ± 0.64 ml/100 g bw/day; control = 5.59 ± 0.43 ml/100 g bw/day). There was no effect on water intake associated with BPA exposure (F (1,10) = 6.19, p>0.05). Water intakes by week are presented in Fig. 1. The amount of BPA consumed by rats via drinking water was approximately 250 μg/kg/ day. The daily intake of BPA in adult humans was found to be approximately 500 μg/kg/day (Taylor et al., 2011). The European Food Safety Authority (2011) has set a regulatory specific migration limit for BPA at 600 μg/kg. Therefore, in the present situation, BPA migration from materials and articles shall not exceed a specific migration limit of 0.05 mg BPA/kg of food (Regulation (EU) 2018/213, 2018). BPA-treated rats had higher (t (5) = -1.09, p < 0.05) plasma BPA levels (34.34 ± 3.11 ng/mL) as compared to the detectable levels in control rats (15.71 ± 12.02 ng/mL). The limit of quantification for this method (GS-MS) was 1 ng/mL for BPA. Since 1999, more than a dozen studies using a variety of different analytical techniques have measured BPA concentrations in human plasma (Vanderberg at al., 2007). A recent study observed that plasma BPA level was 2.22 ± 9.91 ng/mL (Wiraagni et al., 2019).

3.3. Experiment 1: 24-h fluid restriction

The water intake, 2.7% NaCl solution intake and 2.7% NaCl preference at 120 min after a 24h period of water deprivation is shown in Fig. 2. A significant main effect of time (water, F (1,10) = 197.37, p < 0.001; 2.7% NaCl solution, F (1,10) = 102.91, p < 0.001) revealed that fluid intake for both groups increased during the test. As expected, 2.7% NaCl solution intake was significantly lower than water intake (F (3,20) = 193.77, p < 0.001). There was no significant interaction between groups and solutions (F (1,10) ≤ 4.61, p>0.05). Water and 2.7% NaCl solution intakes and 2.7% NaCl preference did not differ between control and BPA-treated rats.

3.4. Experiment 2: extracellular fluid depletion

In the 20h period after furosemide injection and before the drinking tests, BPA-treated rats tended to drink less water and excrete the same urine volume as control rats. UV was similar in BPA-treated rats and control rats for tests 1 and 2 (Table 2). The generally less UV values of rats were accompanied by smaller over-night water intakes so that water balance immediately before tests (1 and 2) was similar between the groups. Rats drank enough fluid during the 4-h test to replace more than the amount lost during the 20h depletion.

The total amount of fluid ingested during the 4-h salt appetite tests is presented in Fig. 3. A significant main effect of time (water, F (1,9) = 51.54, p < 0.001; 2.7% NaCl solution, F (1,9) = 209.58, p < 0.001) revealed that fluid intake for both groups increased during the tests. Control rats augmented intakes of water and 2.7% NaCl solution on repeated testing (test 2 vs. test 1), while BPA-treated rats did not. Pairwise comparisons revealed that water and 2.7% NaCl solution intake in control rats was significantly higher (p < 0.05) in test 2 than in test 1 at times 3, 4, 5, 6, 7 and 8. There was no effect of BPA treatment on 2.7% NaCl preference in any of the test times. In test 2, the average preference for 2.7% NaCl was significantly higher (p < 0.05) in the control and BPA-treated rats at time 1 when compared to the rest of the time points.

In tests, urine was collected for the 1st hour after furosemide injection and again in the morning 19 hours later. In the 1st hour, UV, Una, and UnaV were similar between control and BPA-treated rats (all F (1,8) ≥ 4.89, not significant, Table 3A). Una and UnaV were significantly greater in test 2 than in test 1 (both F (1,8) ≥ 0.211, p < 0.05) in both groups. UV and UnaV were similar (F (1,8) ≥ 0.755, not significant) in
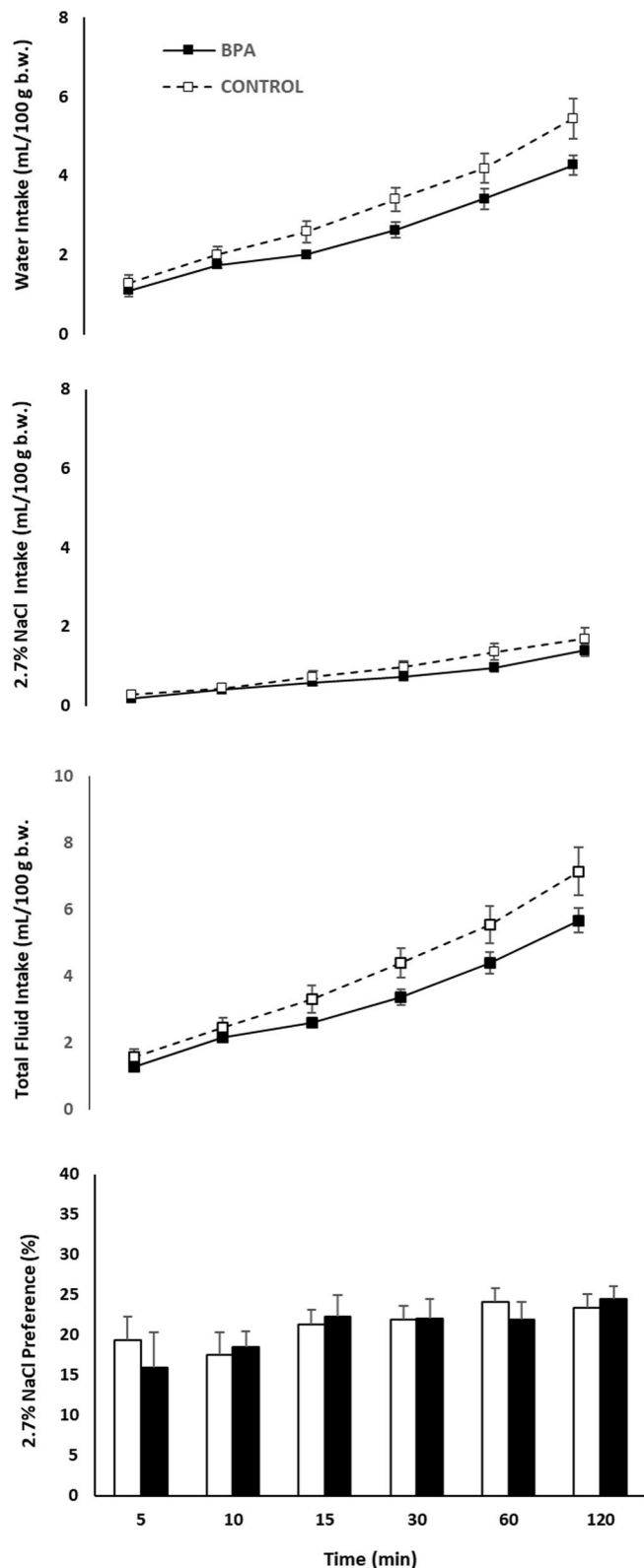
Fig. 2. Cumulative intake of water, 2.7% NaCl solution (mL/100g b.w.) and total fluid (water + 2.7% NaCl solution; mL/100g b. w.) and 2.7% NaCl preference (ratio of 2.7% NaCl solution intake to total fluid intake) at 120 min after a 24-h period of water deprivation in control and BPA-treated rats. N = 6 animals/group. Results are expressed as the mean ± SEM.

Table 2

Values are expressed as the mean ± SEM. Urine volume (UV), water intake and relative water balance were measured in the 20-h preceding the salt appetite test, total fluid intake during the 4-h salt appetite test, and relative water balance at the end of testing.

	20-h UV, mL/100 g body wt	20-h water intake mL/100 g body wt	Pretest water balance, mL/100 g body wt	4-h fluid intake, mL/100 g body wt	Relative water balance, mL/100 g body wt
Control					
Test I	4.78 ± 1.04	4.86 ± 1.18	−0.07 ± 0.35	8.43 ± 1.00	8.36 ± 0.70
Test_II	5.80 ± 2.2	6.02 ± 1.91	−0.22 ± 0.36	11.29 ± 1.56*	10.49 ± 1.97
BPA					
Test I	4.97 ± 0.36	4.42 ± 0.41	0.55 ± 0.23	7.79 ± 1.19	8.34 ± 0.71
Test_II	5.46 ± 1.09	5.00 ± 0.76	0.45 ± 0.35	8.67 ± 1.09	9.12 ± 1.76

*p < 0.05 test 2 vs. test 1.

control and BPA-treated rats in the morning (19 hours later). Una over the entire 19 hours depletion period was significantly lower (F (1,8) ≥ 0.559, p < 0.05) in rats in test 2 vs. test 1 in both groups (Table 3B). It appears that control rats reduced Una at some point after the 1st hour of depletion, presumably when the effects of furosemide began to decline, whereas BPA-treated rats did not.

Control rats ingested more sodium in test 2 than in test 1 (F (1,8) = 0.431, p < 0.05), whereas BPA-treated rats did not (Table 3C). In control rats, 4h sodium balance significantly decreased (F (1,8) = 0.337, p < 0.05) in test 2 and did not decrease for tests in BPA-treated rats as it did in control rats. Thus, BPA-treated rats drank similar amounts of sodium during the 4h drinking tests when compared to control rats. On repeated testing, control rats increased ingestion of sodium in amounts that far exceeded additional urinary losses of sodium on repeated testing. BPA-treated rats did not. We did not collect urine during the 4h drinking tests, so we cannot address the cumulative water and sodium balances at the end of testing, but it is evident that BPA-treated rats were refractory in their capacity to ingest sufficient water and 2.7% NaCl solution to restore deficits accrued during the 20 h preceding the drinking tests.

4. Discussion

Neuroendocrine and behavioural responses are critical for defending the body from challenges to water-salt balance that would lead to altering the cardiovascular system in a long-term manner, even in response to BPA doses below those regulatory agencies regard as safe for humans (Gore et al., 2019; Ma et al., 2019). In this study, fluid and electrolyte balance in BPA-treated rats is generally adequate but impaired against osmotic challenges, for example by furosemide deprivation. So, neuroendocrine systems involved in maintaining body fluid and electrolyte homeostasis could be altered in BPA-treated rats.

These rats were exposed to BPA via drinking water to mimic the most likely route of human exposure. Other studies show that BPA can enter the body via gastrointestinal, respiratory and dermal routes and lead to multiple organ toxicity for humans. The Environmental Protection Agency (EPA) of the USA defined the lowest observed adverse effect level (LOAEL) for BPA as 50 mg/kg/day, and the 'safe dose' as 50 µg/kg/day. The European Food and Safety Authority recommends a temporary tolerable daily intake of 4 µg/kg/day (Ma et al., 2019). Pharmacokinetic studies indicate that BPA exposure of approximately 400–500 µg/kg/day yield blood concentrations of the unconjugated, bioactive form of BPA which is similar to that reported in humans (Vandenberg
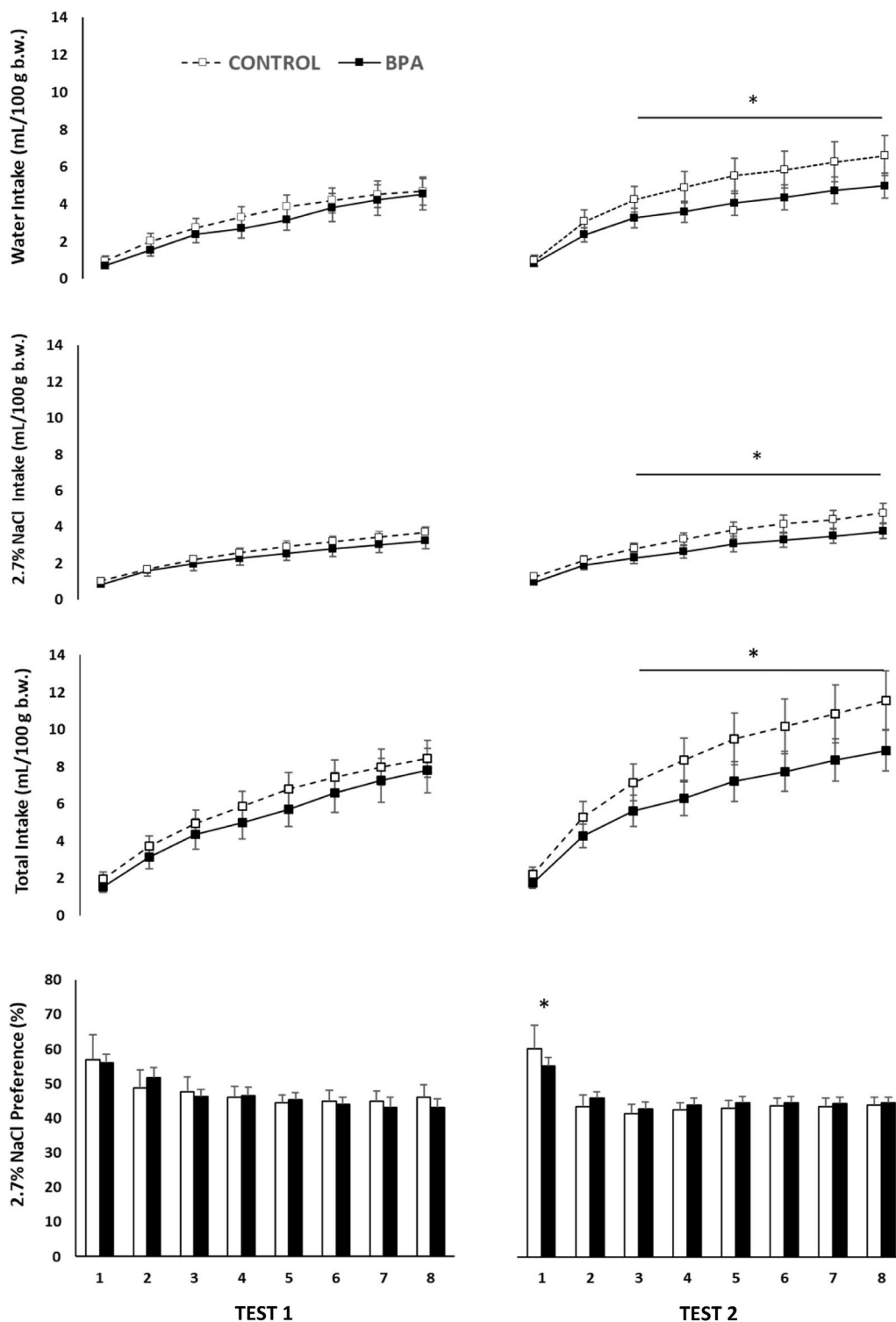
Fig. 3. Cumulative intake of water, 2.7% NaCl solution (mL/100g b.w.) and total fluid (water + 2.7% NaCl solution; mL/100g b. w.) and 2.7% NaCl preference (ratio of 2.7% NaCl solution intake to total fluid intake) in response to sodium depletion during the 4-h salt appetite test in control and BPA-treated rats in test 1 and test 2. In control rat intake: *p < 0.05 test 2 vs. test 1. In 2.7% NaCl preference: *p < 0.05 vs. the rest of the time points. N = 6 animals/ group. Results are expressed as the mean ± SEM.

et al., 2012). BPA-treated rats of the present study were exposed to a BPA dose of 250 µg/kg/day for 12 weeks. There are numerous outcomes across different organ systems that were disrupted in animals exposed to 250 µg BPA/kg/day. When results from several studies published thus far are combined, 51% appear only in low-dose groups (at or below 250 µg/kg bw/day), 21% in high-dose groups, and 27% across both low and high-dose exposure groups (Prins et al., 2019).

4.1. Body weight and food and water intake

In our study, body weight and food intakes were assessed for twelve weeks of BPA exposure. No significant differences in weight or food intakes were observed in BPA-treated rats compared to control rats. Previous reports indicate that BPA affects male rats' body weight under several conditions, but definitive conclusions about the influence of BPA and other EDCs on body weight are difficult because of the variety of exposure methods and exposure windows seen in the literature (Nuñez

Table 3
Values are expressed as the mean ± SEM. Urine volume (UV), urinary Na concentration (Una) and urinary Na excretion (UnaV) were measured in 1h (A) and 19h (B) after furosemide injection. Na intake during the 4-h salt appetite test, and relative Na balance at the end of testing are showed (C).

A)

	1h UV (mL)	1h Una (mmol/L)	1h UnaV (mmol)
Control			
Test I	7.66 ± 0.33	57.50 ± 5.5	0.43 ± 0.07
Test_II	8.83 ± 0.72	72.00 ± 11.7*	0.61 ± 0.04*
BPA			
Test I	8.83 ± 2.66	45.67 ± 8.17	0.35 ± 0.07
Test_II	9.00 ± 0.57	75.00 ± 18.68*	0.65 ± 0.12*

B)

	19h UV (mL)	19h Una (mmol/L)	19h UnaV (mmol)
Control			
Test I	19.83 ± 3.60	17.33 ± 9.40	0.28 ± 0.10
Test_II	24.33 ± 3.71	8.50 ± 0.5*	0.26 ± 0.08
BPA			
Test I	24.33 ± 3.71	16.00 ± 9.00	0.32 ± 0.12
Test_II	26.33 ± 6.61	13.33 ± 3.75[a]	0.31 ± 0.07

C)

	4h, 2.7% NaCl Intake (mL)	4h, Na Intake (mmol)	4h, Test Na balance (mmol)
Control			
Test I	15.17 ± 0.70	4.55 ± 0.21	4.01 ± 0.46
Test_II	19.16 ± 1.81*	5.75 ± 0.54*	5.03 ± 0.80*
BPA			
Test I	16.00 ± 2.25	4.80 ± 0.67	4.00 ± 0.01
Test_II	16.83 ± 2.15	5.05 ± 0.64	4.08 ± 0.97

*$p < 0.05$ test 2 vs. test 1.
[a]$p < 0.05$ control vs. BPA-treated rats.

at al., 2018). These studies indicate that BPA's effects on metabolism and body weight are dose and time sensitive, and that prenatal exposure seems to have a greater impact on body weight than postnatal exposure. The variability in results is likely related to the non-monotonic dose response curve that has been reported for many actions of BPA. However, the exact mechanisms through which BPA affects body weight are not fully understood and warrant further exploration (Adelawe at al., 2011; Vandenberg et al., 2009).

Daily water intake remains unchanged with BPA treatment. BPA-treated rats did not show an estrogenic effect of BPA on fluid homeostasis. Sex differences in fluid intake are known (Richter & Brailey, 1929). Studies in rats confirm that water intake fluctuates across the oestrous cycle. An 8–10% water intake reduction during oestrus demonstrates that estrogens have a clear anti-dipsogenic effect (Curtis, 2009; Xue et al., 2013). Several studies show decreased intake after E2 providing evidence for an activational effect of estrogens in female rats, but the lack of an effect of E2 in adult male rats (Jonklaas and Buggy, 1984, 1985) suggests that organizational effects of gonadal hormones are also necessary.

4.2. Experiment 1: 24-h fluid restriction

Dehydration is perhaps a more realistic method of experimental induction of thirst than hypertonicity or hypovolemia alone. 24-hour dehydration is associated with both systemic hypertonicity and hypovolemia, and therefore, dehydration of both intracellular and extracellular compartments (Santollo, 2017). In previous studies (Núñez, 2018) using BPA higher doses we demonstrated an anti-natriorexigenic and anti-dipsogenic effect in BPA-treated rats. Estrogen treatment was reported to decrease saline intake in both sexes (Stricker et al., 1991) by activation receptor beta (ERβ) in areas of the brain that control fluid

intake and decrease saline intake (Santollo et al., 2017), so sodium appetite-inhibitory response that we found may reflect BPA acting via ERβ. It is known that BPA has a stronger affinity for ERβ (Kuiper et al., 1998; Matthews et al., 2001, Routledge et al., 2000). We can also assume that all rats must return to the sodium balance through the sodium ingested in chow, because they do not appear to do so by ingesting the available 2.7% NaCl solution. Tests of water-deprived rats, however, indicate that estrogens also independently reduce water intake (Curtis, 2009). The BPA-treated rats tend to drink less water than control rats following dehydration, but we did not find statistically significant decreases. Perhaps the BPA dose did not have enough estrogenic potency to affect the control mechanisms of water intake.

4.3. Experiment 2: extracellular fluid depletion

Furosemide acts through the blockade of the $Na^+/K^+/2Cl^-$ co-transporter (NKCC2) in the thick ascending limb of the loop of Henle resulting in impaired reabsorption of Na^+, K^+ and Cl^-. As a consequence of body sodium and extracellular volume loss, the renin-angiotensin-aldosterone system (RAAS) and sodium appetite are activated (Fitzsimons, 1998). We use a single episode of furosemide-induced sodium depletion to evaluate the possibility of BPA effects on osmotic regulation prior to the establishment of behavioral fluid intake changes. A frequently used model to stimulate water and sodium intakes involves the administration of furosemide combined with sodium restriction (Thunhorst & Johnson, 2003). Furosemide causes diuresis and natriuresis. On the next morning, access to 2.7% NaCl solution is provided and the sodium-depleted animals consume substantial amounts of 2.7% NaCl solution. Several studies found that, with repeated testing rats drink significantly more sodium solution (Thunhorst et al., 1994). In the present experiment, the control rats significantly increased 2.7% NaCl solution intake on repeated testing, while BPA-treated rats did not.

Sodium depletion releases natriorexigenic hormones (angiotensin II, aldosterone), which not only induces sodium appetite, but also reorganizes the brain and future ingestive behaviour (Hurley and Johnson, 2013). The selective reorganization and enhancement of the ingestion of saline solutions may be in anticipation of future sodium depletions (Pereira et al., 2010; Sakai et al., 1987). It corresponds to sensitization, a kind of non-associative learning which is produced by stimulus repetition (Olsen, 2011). The capacity to enhance sodium intake presumably reflects an evolutionary adaptation to environmental sodium lack, thus reducing the risks associated with extracellular dehydration (Epstein, 1991). Previous studies have demonstrated that the development of sodium appetite sensitization is dependent on angiotensin II (Ang-II), aldosterone, and *N*-methyl-d-aspartate receptor signalling in the central nervous system. Administration of central Ang-II along with systemic aldosterone induces sensitization (Sakai et al., 1987); while antagonism of Ang-II type 1 (AT1) receptors or blockade of Ang-II synthesis concurrent with central mineralocorticoid receptor antagonism prevents the sensitization of sodium appetite (Pereira, 2010). In addition, repeated sodium depletions induced increased expression of molecular markers related to both the brain RAAS and neural plasticity that correlated with sodium intake (Hurley et al., 2014). Therefore, BPA-treated rats may have deficient water and salt appetite responses after sodium depletion on test 2 because they do not produce as much Ang-II as control rats.

The decreased behavioral responses of the BPA-treated rats are not likely to be due to significantly reduced water and sodium need when compared with control rats after depletion. Water balance immediately before salt appetite tests was similar between control and BPA-treated rats. Furthermore, the amounts of sodium lost by BPA-treated rats during the depletion periods were significantly greater than those of control rats in test 2, and were similar in test 1. However, the BPA-treated and control rats appeared to have an equivalent need for water and sodium at the start of the second salt appetite test and drank similar amounts of both fluids.

Numerous studies have also demonstrated that estrogen is involved

in regulation of sensitization in response to drugs like neurotransmitters or hormones. Xue et al. (2014) found a sex difference in the suppressor Ang II sensitizing effect on Ang II-induced hypertension showing that central infusion of E2 abolished Ang II-induced sensitization of hypertension in males and in OVX females. On the other hand, estrogen replacement in ovariectomised rats decreased AT1 receptor number in the anterior pituitary (Seltzer et al., 1992). Our results suggest that BPA-treated rats are protected from Ang II-induced sensitization through central like-estrogen BPA effect and its possible regulation of brain RAAS, perhaps due to a down regulation of Ang receptors. Estrogen inhibits Ang II-stimulated fluid intake via several estrogen receptor subtypes (Santollo et al., 2017) that could be modulated by BPA (McLachlan, 2016). Recent studies report that following BPA treatment, Ang II expression is upregulated and Ang II receptor antagonist attenuates the BPA-induced cell proliferation (Gao et al., 2019; Saura et al., 2014). Further studies are needed to clarify the potential role of BPA exposure on RAAS activity.

5. Conclusions

Overall, the data suggest that chronic BPA exposure at low doses may have small effects on daily fluid intake and the selection of saline solutions. Nevertheless, such small effects may cumulatively produce physiologically relevant long-term effects, especially against osmotic challenges, e.g. in cardiovascular health. Our results support the theory that environmental exposure (i.e. endocrine disruptor exposure) can modify activity in the neural network that controls fluid intake and hydrosaline homeostasis. Future studies need to be conducted to identify additional brain and kidney sites where estrogen interacts with RAAS components and to determine intracellular signalling pathways and neuroepigenetic mechanisms.

Ethical approval on animal research

All experimental procedures followed the guidelines set forth in the Care and Use of Animals, and The University of Oviedo's Experimental Animal Use Committee approved all experimental procedures (PROE 15/2016).

Funding sources

This work was supported by grants from the University of Oviedo (PAPI-20-PUENTE-12; PAPI-19-PUENTE-15).

Declaration of interests

None.

Authors' contributions

Paula Nuñez: investigation, formal analysis, writing–review & editing, funding acquisition. Juan Arguelles: formal analysis, writing -review & editing. Carmen Perillan: investigation, formal analysis, writing -review & editing.

References

Adewale, H. B., Todd, K. L., Mickens, J. A., & Patisaul, H. B. (2011). The impact of neonatal bisphenol-A exposure on sexually dimorphic hypothalamic nuclei in the female rat. *Neurotoxicology, 32*(1), 38–49.

Azzouz, A., Rascón, A. J., & Ballesteros, E. (2016). Determination of free and conjugated forms of endocrine-disrupting chemicals in human biological fluids by GC-MS. *Bioanalysis. 2016, 8*(11), 1145–1158.

Barron, W. M., Schreiber, J., & Lindheimer, M. D. (1986). Effect of ovarian sex steroids on osmoregulation and vasopressin secretion in the rat. *American Journal of Physiology, 250*, 352–361.

Curtis, K. S. (2009). Estrogen and the central control of body fluid balance. *Physiology & Behavior, 97*(2), 180–192.

Desai, M., Ferrini, M. G., Han, G., Jellyman, J. K., & Ross, M. G. (2018). In vivo maternal and in vitro BPA exposure effects on hypothalamic neurogenesis and appetite regulators. *Environmental Research, 164*, 45–52.

Fitzsimons, J. T. (1998). Angiotensin, thirst, and sodium appetite. *Physiological Reviews, 78*, 583–686.

Gao, F., Huang, Y., Zhang, L., & Liu, W. (2019). Involvement of estrogen receptor and GPER in bisphenol A induced proliferation of vascular smooth muscle cells. *Toxicology in Vitro, 56*, 156–162.

Gore, A. C., Krishnan, K., & Reilly, M. P. (2019). Endocrine-disrupting chemicals: Effects on neuroendocrine systems and the neurobiology of social behavior. *Hormones and Behavior, 111*, 7–22.

Hurley, S. W., Zhang, Z., Beltz, T. G., Xue, B., & Johnson, A. K. (2014). Sensitization of sodium appetite: Evidence for sustained molecular changes in the lamina terminalis. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology, 307*, 1405–1412.

Jonklaas, J., & Buggy, J. (1984). Angiotensin-estrogen interaction in female brain reduces drinking and pressor responses. *American Journal of Physiology, 247*, 167–172.

Jonklaas, J., & Buggy, J. (1985). Angiotensin-estrogen central interaction: Localization and mechanism. *Brain Research, 326*, 239–249.

MacKay, H., & Abizaid, A. (2018). A plurality of molecular targets: The receptor ecosystem for bisphenol-A (BPA). *Hormones and Behavior, 101*, 59–67.

Ma, Y., Liu, H., Wu, J., Yuan, L., Wang, Y., Du, X., Wang, R., Marwa, P. W., Petlulu, P., Chen, X., & Zhang, H. (2019). The adverse health effects of bisphenol A and related toxicity mechanisms. *Environmental Research, 176*, 108575.

Matthews, J. B., Twomey, K., & Zacharewski, T. R. (2001). In vitro and in vivo interactions of bisphenol A and its metabolite, bisphenol A glucuronide, with estrogen receptors alpha and beta. *Chemical Research in Toxicology, 14*, 149–157.

McLachlan, J. A. (2016). Environmental signaling: From environmental estrogens to endocrine-disrupting chemicals and beyond. *Andrology, 4*, 684–694.

Núñez Martínez, P., Argüelles Luis, J., & Perillán Méndez, C. (2016). Stevia preferences in Wistar rats. *Psicothema, 28*(4), 442–447.

Nuñez, P., Arguelles, J., & Perillan, C. (2018). Short-term exposure to bisphenol A affects water and salt intakes differently in male and ovariectomised female rats. *Appetite, 120*, 709–715.

Olsen, C. M. (2011). Natural rewards, neuroplasticity, and non-drug addictions. *Neuropharmacology, 61*, 1109–1122.

Pereira, M. G., Becari, C., Oliveira, J. A., Salgado, M. C., Garcia-Cairasco, N., & Costa-Neto, C. M. (2010). Inhibition of the renin-angiotensin system prevents seizures in a rat model of epilepsy. *Clinical Science, 119*, 477–482.

Prins, G. S., Patisaul, H. B., Belcher, S. M., & Vandenberg, L. N. (2019). CLARITY-BPA academic laboratory studies identify consistent low-dose Bisphenol A effects on multiple organ systems. *Basic and Clinical Pharmacology and Toxicology, 3*(3), 14–31.

Regulation (EU) 2018/213. (2018). *Regulation (EU) 2018/213* (pp. L41/6–L4112). Official Journal of the European Union, 2018.

Richter, C. P., & Brailey, M. E. (1929). Water-intake and its relation to the surface area of the body. *Proceedings of the National Academy of Sciences, 15*(7), 570–578.

Sakai, R. R., Fine, W. B., Epstein, A. N., & Frankmann, S. P. (1987). Salt appetite is enhanced by one prior episode of sodium depletion in the rat. *Behav. Neurosci. 1987, 101*, 724–731.

Santollo, J., & Daniels, D. (2015). Control of fluid intake by estrogens in the female rat: Role of the hypothalamus. *Frontiers in Systems Neuroscience, 4*, 9–25.

Santollo, J., Torregrossa, A. M., & Daniels, D. (2017). Sex differences in the drinking response to angiotensin II (AngII): Effect of body weight. *Hormones and Behavior, 93*, 128–136.

Saura, M., Marquez, S., Reventun, P., Olea-Herrero, N., Arenas, M. I., Moreno-Gómez-Toledano, R., Gómez-Parrizas, M., Muñóz-Moreno, C., González-Santander, M., Zaragoza, C., & Bosch, R. J. (2014). Oral administration of bisphenol A induces high blood pressure through angiotensin II/CaMKII-dependent uncoupling of eNOS. *The FASEB Journal, 28*, 4719–4728.

Seltzer, A., Pinto, J. E., Viglione, P. N., Correa, F. M., Libertun, C., Tsutsumi, K., Steele, M. K., & Saavedra, J. M. (1992). Estrogens regulate angiotensin-converting enzyme and angiotensin receptors in female rat anterior pituitary. *Neuroendocrinology, 55*, 460–467.

Stachenfeld, N. S. (2008). Sex hormone effects on body fluid regulation. *Exercise and Sport Sciences Reviews, 36*(3), 152–159.

Stricker, E. M., Thiels, E., & Verbalis, J. G. (1991). Sodium appetite in rats after prolonged dietary sodium deprivation: A sexually dimorphic phenomenon. *American Journal of Physiology, 260*, 1082–1088.

Taylor, J. A., Vom Saal, F. S., Welshons, W. V., Drury, B., Rottinghaus, G., Hunt, P. A., Toutain, P. L., Laffont, C. M., & Van de Voort, C. A. (2011). Similarity of bisphenol A pharmacokinetics in rhesus monkeys and mice: Relevance for human exposure. *Environmental Health Perspectives, 19*, 422–430.

Thunhorst, R. L., & Johnson, A. K. (2003). Thirst and salt appetite responses in young and old Brown Norway rats. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology, 284*(2), 317–327.

Thunhorst, R. L., Lewis, S. J., & Johnson, A. K. (1994). Effects of sinoaortic baroreceptor denervation on depletion-induced salt appetite. *American Journal of Physiology, 267*, 1043–1049.

Vandenberg, L. N., Colborn, T., Hayes, T. B., Heindel, J. J., Jacobs, D. R., Jr., Lee, D. H., Shioda, T., Soto, A. M., vom Saal, F. S., Welshons, W. V., Zoeller, R. T., & Myers, J. P. (2012). Hormones and endocrine-disrupting chemicals: Low-dose effects and nonmonotonic dose responses. *Endocrine Reviews, 33*(3), 378–455.

Vandenberg, L. N., Hauser, R., Marcus, M., Olea, N., & Welshons, W. V. (2007). Human exposure to bisphenol A (BPA). *Reproductive Toxicology, 24*(2), 139–177.

Vandenberg, L. N., Maffini, M. V., Sonnenschein, C., Rubin, B. S., & Soto, A. M. (2009). Bisphenol-A and the great divide: A review of controversies in the field of endocrine disruption. *Endocrine Reviews, 30*, 75–95.

Wiraagni, I. A., Mohd, M. A., Bin Abd Rashid, R., & Haron, D. E. B. M. (2019). Validation of a simple extraction procedure for bisphenol A identification from human plasma. *PLoS One. 2019, 14*(10), Article e0221774.

Xue, B., Johnson, A. K., & Hay, M. (2013). Sex differences in angiotensin II- and aldosterone-induced hypertension: The central protective effects of estrogen.

American Journal of Physiology - Regulatory, Integrative and Comparative Physiology, 305(5), 459–463.

Xue, B., Zhang, Z., Beltz, T. G., Guo, F., Hay, M., & Johnson, A. K. (2014). Estrogen regulation of the brain renin-angiotensin system in protection against angiotensin II-induced sensitization of hypertension. *Am. J. Physiol. Heart Circ. Physiol. 2014, 307*, 191–198.

Zhang, Y. F., Shan, C., Wang, Y., Qian, L. L., Jia, D. D., Zhang, Y. F., Hao, X. D., & Xu, H. M. (2020). Cardiovascular toxicity and mechanism of bisphenol A and emerging risk of bisphenol S. *The Science of the Total Environment, 723*, 137952.