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Effect of bisphenol S and bisphenol A on morphometric and hormonal changes of thyroid gland and iodine concentration in urine of Wistar rats

Efekt Bisfenolu S a Bisfenolu A na morfometrické parametry štítné žlázy, změny hormonů a koncentrace jodu v moči potkanů Wistar

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ABSTRACT

Bisphenol S (BPS) is the major substitute of the endocrine disruptor bisphenol A (BPA). Due to the presence of strong double bonds, BPS is more resistant to biodegradation and therefore more BPS remains in the environment. Numerous studies show that BPS disrupts the reproductive, nervous, and cardiovascular systems and could have an impact on thyroid hormones. The study aimed to analyze the effects of a 10-week exposition of BPS and BPA on lipid markers, morphometric parameters of the rat thyroid gland, the thyroid stimulating hormone (TSH) levels and the influence of BPS on urine iodine concentration. Male Wistar rats received BPS in sunflower oil daily by gavage. The control group (GI) received only the vehicle. The BPS experimental group two (GII) received 4 ug/kg/day, group three (GIII) received 50 ug/kg/day, and group four (GIV) received 100 mg BPS/kg/day. Group five (GV) received 100 mg BPA/kg/day. Groups four and five were made to compare the influence of high concentration between BPS and BPA. Results show the influence of BPS and BPA on body weight, triacylglycerols, cholesterol and total protein concentration. Morphometric changes in the size of thyroid gland follicles show a bigger influence of BPS than BPA. Results show also increasing in TSH concentrations in all groups with bisphenols up to physiology standards of Wistar rats (GI 3.14 ± 1.28 ng/ml, GII 5.12 ± 1.16 ng/ml, GIII 5.55 ± 2.39 ng/ml, GIV 5.56 ± 1.98 ng/ml, GV 4.47 ± 1.09 ng/ml) and influence of BPA and BPS on higher iodine concentrations in urine.

Keywords: thyroid, iodine, follicles, triacylglycerols, cholesterol, protein, TSH

ABSTRAKT

Bisfenol S (BPS) je hlavní náhradou endokrinního disruptoru bisfenolu A (BPA). Díky přítomnosti silných dvojných vazeb je BPS odolnější vůči biologickému rozkladu, a proto zůstává více v prostředí. Četné studie ukazují, že BPS narušuje reprodukční, nervový a kardiovaskulární systém a může mít vliv na hormony štítné žlázy. Cílem studie bylo analyzovat účinky 10 týdenní expozice BPS a BPA na lipidové markery, morfometrické parametry štítné žlázy potkana, hladiny tyreoidálního stimulačního hormonu (TSH) a vliv BPS na koncentraci jódu v moči. Samci potkanů Wistar dostávali BPS ve slunečnicovém oleji denně jícnovou sondou. Kontrolní skupina (GI) obdržela pouze nosič. Experimentální skupina dvě (GII) dostávala 4 ug BPS/kg/den, skupina tři (GIII) dostávala 50 ug BPS/kg/den, skupina čtyři (GIV) dostávala 100 mg BPS/kg/den. Skupina pět (GV) dostávala 100 mg BPA/kg/den. Čtvrtá a pátá skupina byly vytvořeny pro porovnání vlivu vysoké koncentrace BPS a BPA. Výsledky ukazují vliv BPS a BPA na tělesnou hmotnost, triacylglyceroly, cholesterol

a celkovou koncentraci bílkoviny. Morfometrické změny velikosti folikulů štítné žlázy vykazují větší vliv BPS než BPA. Výsledky také ukazují zvýšení koncentrací TSH ve všech skupinách s bisfenoly nad fyziologické standardy potkanů Wistar (GI 3,14 ± 1,28 ng/ml, GII 5,12 ± 1,16 ng/ml, GIII 5,55 ± 2 ,39 ng/ml, GIV 5,56 ± 1,98 ng/ml, GV 4,47 ± 1,09 ng/ml) a vliv BPA a BPS na zvýšené koncentrace jódu v moči.

Klíčová slova: štítná žláza, jod, folikuly, triacylglyceroly, cholesterol, bílkovina, TSH

INTRODUCTION

Bisphenols are chemicals used in large quantities for the production of polycarbonate plastics and epoxy resins. These products are often used as food and beverage packaging materials (European Commission, 2011). Bisphenol A is the main bisphenol, and a lot of research indicates the possibility of its toxicity. In case of long-term exposure to higher concentrations, bisphenol A works as a strong endocrine disruptor (Ghisari and Bonefeld-Jorgensen, 2005; Bonefeld-Jorgensen et al., 2007). Based on these findings developed countries have been creating conditions to reduce exposure to bisphenol A, in particular by limiting the use of bisphenol A in food and beverage storage containers, in particular those intended for infants (FDA, 2013; Chen et al., 2016).

Currently, Bisphenol S is used as the main alternative to BPA (Wu et al., 2018). BPS is a structural analogue of BPA, but with higher resistance to sunlight and high temperature (Naderi et al., 2014).

Nevertheless, its ever-increasing use leads to its higher emissions to the environments where it has already been detected, for example, in food and beverages, rivers, sewage sludge or human tissues (Wu et al., 2018; Yamazaki et al., 2015; Lee et al., 2015). According to Yanan et al. (2023), BPS was the most detected BPA analogue (91%) in the urine of the Chinese population. In Japan, BPS was detected in urine in 97% of monitored individuals compared to 81% in the U.S. (Liao et al., 2012; Lehmler et al. 2018)

According to Wei et al. (2018) BPS, just like BPA, is a very strong endocrine disruptor. Studies have indicated its significant influence in the area of reproduction, e.g. Grandin et al. (2019) recorded BPS transmission between placenta and fetus, Lu et al. (2023) refers to the impact of bisphenols especially on pregnant women and newborn babies, Lee et al. (2019) observed a significant impact of BPS on Danio Rerio hatching time. In several zebrafish and rat studies, BPS disrupted sex hormones – it increased estrogen concentration, decreased testosterone production, and suppressed GnRH transcript expression in the hypothalamus (Ji et al., 2013; Naderi et al., 2014; Ullah et al., 2016).

According to many *in vitro* and *in vivo* studies (Zhang et al., 2018; Guo et al., 2021) it has become obvious that bisphenol A analogues have a similar negative effect on the thyroid gland and its hormones. It can therefore be assumed that BPS, just like BPA, significantly affects the proper functioning of the thyroid gland, interferes with thyroid hormone synthesis and inhibits synthesis in several ways: it is an antagonist of thyroid hormone receptors (Freitas et al., 2011; Moriyama et al., 2002; Sun et al., 2009; Kudo and Yamauchi, 2005), alters the gene expression of proteins involved in the synthesis of thyroid hormones – thyroid peroxidase (TPO), thyroglobulin (TG) and SLC5a5 (sodium iodide symporter – NIS (Kim et al., 2019).

Cao et al. (2011) found that bisphenols can also bind to the transport protein TH transthyretin and compete with TH for binding sites. Da Silva et al. (2019) describes the reduction of iodothyronine deiodinase type I activity in the liver, which interferes with the activation of the prohormone T4 to the active hormone T3.

Our study aimed to compare the effects of BPS and BPA at different concentrations, including their effects on thyroid hormonal function, iodine metabolism and thyroid morphometric changes. There is a lack of sufficient studies dealing with the effect of BPS on changes in the structure of the thyroid gland.

MATERIALS AND METHODS

Animals

For our experiment were used 30 male Wistar rats (AnLab s.r.o. Prague, Czech Republic) of the same age and with no statistically significant difference in weight (Table 1). After one week of acclimatization, we started a ten-week experiment. Animals were maintained in a temperature-controlled room (23 ± 1 °C) in a 24h light/dark cycle (lights on: 07:00; lights off: 19:00), with free access to water and standard chow. All animals were housed in the same bisphenol-free polysulfone (PSU) cages. All materials and consumables used in their maintenance were also bisphenol-free to avoid contamination.

Experimental groups received BPS (O2S(C6H4OH)2) (Sigma Aldrich 80-09-1) in sunflower oil (vehicle) once daily (in the morning) by intragastric gavage. We have five experimental groups. Group one (GI) was the control group and received only the vehicle. Experimental group two (GII) received 4 ug BPS/kg/day, group three (GIII) received 50 ug BPS/kg/day, group four (GIV) received 100 mg BPS/kg/day and group five (GV) received 100 mg BPA/kg/day. The dose of bisphenols was controlled during the experiment according to the current weight of the rats.

Table 1. Characteristics of experimental groups of Wistar rats

Group	Body weight (g)	BPS/BPA kg/day
GI (n = 6)	172.97 ± 12.36	Control – sunflower oil (vehicle)
GII (n = 6)	177.87 ± 10.27	Oil + 4 ug BPS/kg/day
GIII (n = 6)	174.45 ± 12.89	Oil + 50 µg BPS/kg/day
GIV (n = 6)	173.05 ± 6.60	Oil + 100 mg BPS/kg/day
GV (n = 6)	172.23 ± 7.39	Oil + 100 mg BPA/kg/day

All experimental procedures complied with the law of the Czech Republic (Act No 246/1992 Coll., on the protection of animals against cruelty). The study design was approved by ethical committees at the Biology Centre of CAS and the Central Commission for Animal Welfare under protocol no. 82/2021. Euthanasia was done by cardiac puncture.

Biochemical analysis

On the final day of the experiment, rats were weighed and were put under inhalation anaesthesia (by anaesthetic gas Isoflurane). Blood was collected by cardiac puncture and parameters were determined by an ELLIPSE analyzer (DIALAB spol. s.r.o.).

Determination of TSH in serum

Blood collected by cardiac puncture was used. After clot formation, the samples were centrifuged and the serum fraction was kept at – 20 °C.

TSH levels were determined by commercially available ELISA kits for rats (TSH MBS2508625 MyBioSource, San Diego, USA).

Histology and morphometry

Thyroid glands were fixed in 10% neutral buffered formalin (Diapath S.p.A., Marinengo, Italy). After fixation, the thyroid glands were trimmed, processed, embedded in paraffin, sectioned at a thickness of 4 µm, and stained with hematoxylin and eosin (HE) and Periodic acid–Schiff (PAS). The measurements were conducted using a Leica IM 500 Version 4.0 visual analysis program and Leica DC 320 camera in combination with a Leica DM 2500 microscope. Follicle area, follicle lumen area, length and width of follicles and Height of thyreocyte were measured (Figure 1). In each thyroid gland was measured 40 follicles.

Analysis of iodine content in urine

Iodine content in urine was determined by a spectrophotometric method according to Sandell-Kolthoff.

Statistical analysis

For statistical analysis, IBM SPSS Statistics 21 and R statistic software were used.

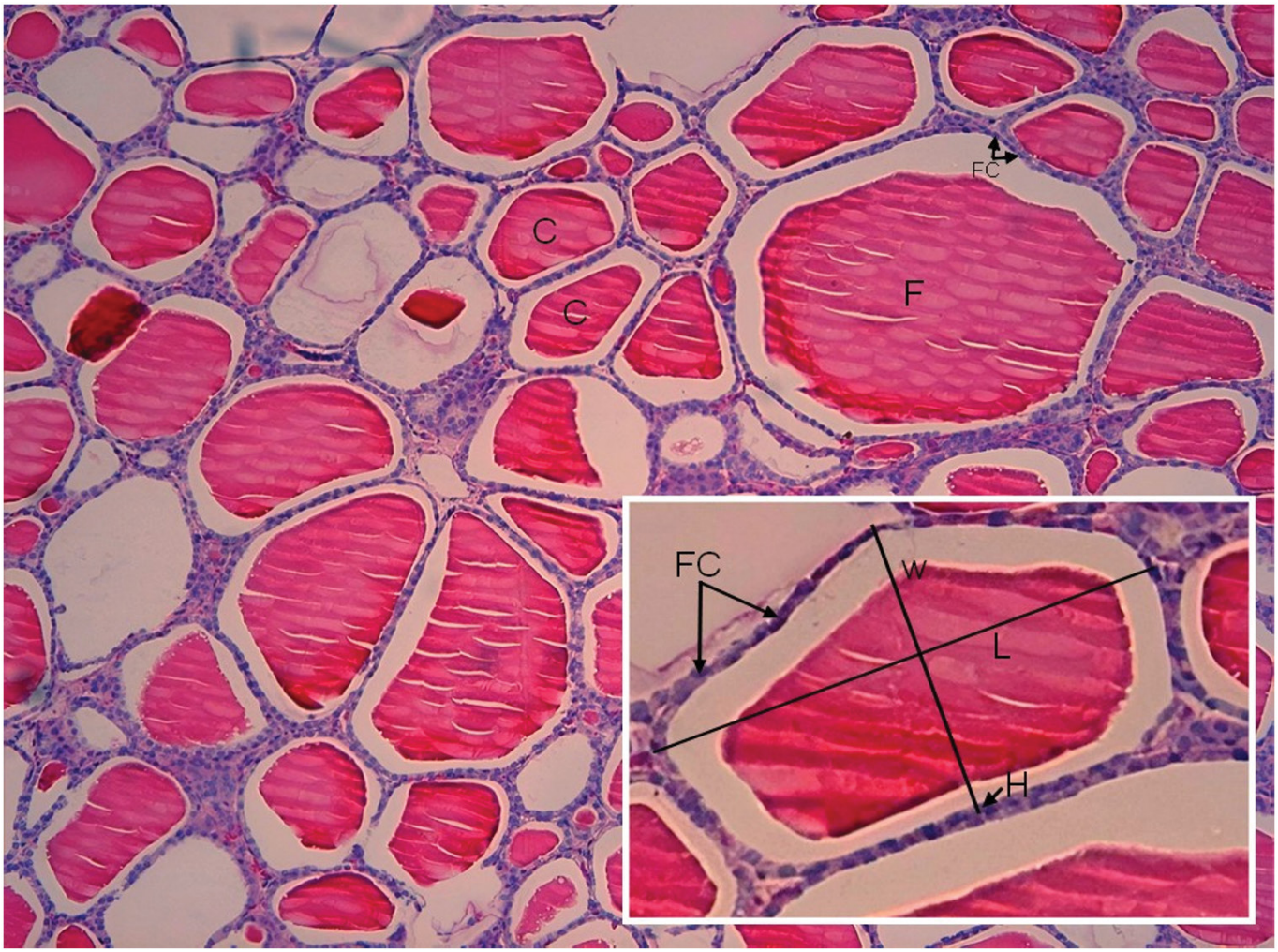

Figure 1. A photomicrograph of a section in the thyroid gland of a control group showing the follicle area with colloid and follicular cells (F), follicle lumen area (C), follicular cells area (FC), height of thyreocyte (H) and length (L) and width (W) of follicles

The Kruskal-Wallis test was used to determine whether at least one group was statistically significantly different. If so, it was used post hoc tests – the Bonferroni correction for multiple comparisons. Furthermore, Microsoft Excel 2016 was used.

RESULTS AND DISCUSSION

Effects of Bisphenol S and Bisphenol A on rat gains and lipid biomarkers

Given the claims of several authors (Qin et al.2020; Shi et al. 2017) on increasing weight of studied individuals after exposure to various concentrations of bisphenols, we expected this trend would manifest in our experiment.

However, we observed decreasing trends in weight gain with increasing BPS concentration (Figure 2). Triacylglycerol concentrations also decreased significantly with increasing BPS concentration and decreasing gain (Figure 3), in the 100 mg BPS and 100 mg BPA groups ($P < 0.017$ and $P < 0.022$). Values of all bisphenol groups were below the physiological standard for triacylglycerols (GII-0.58 mmol/l, GIII-0.63 mmol/l, GIV-0.36 mmol/l, GV-0.35 mmol/l). Our findings contradict those of Ejaz ul Haq et al. (2020), who measured statistically significantly elevated ($P < 0.0001$) triacylglycerol levels with high BPA concentrations. Elevations of triacylglycerols in both BPA and BPS were also reported by Héliès-Toussaint et al. (2014).

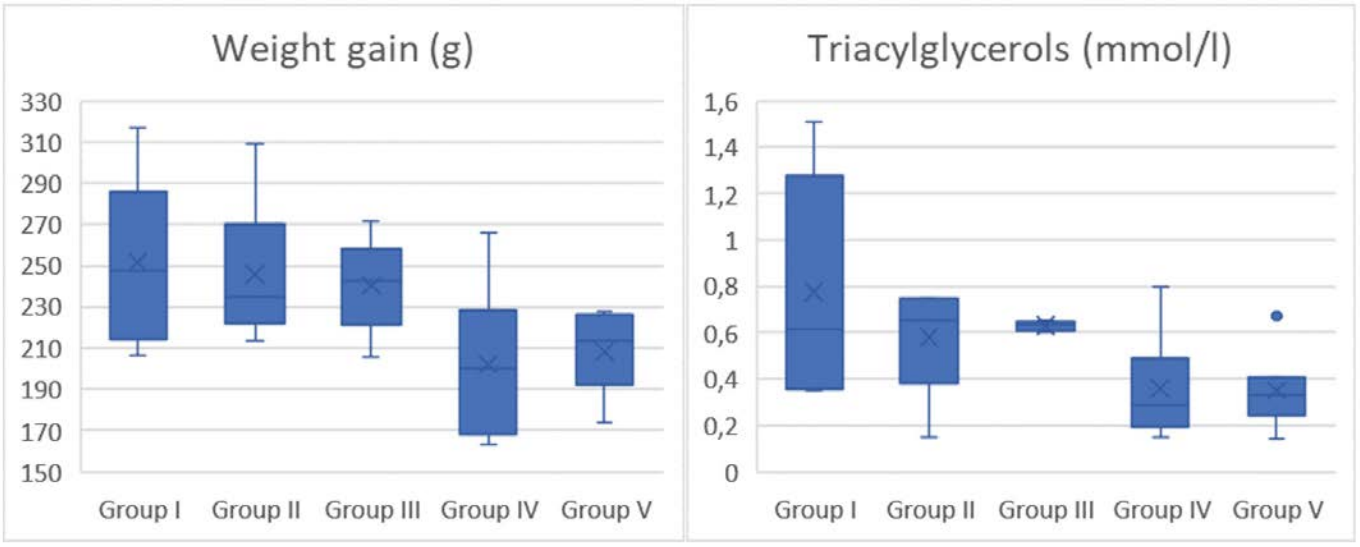

Figure 2 and Figure 3. Weight gain at the end of the experiment and levels of triacylglycerols (normal range 1.44 – 8.05 mmol/l)

We only observed a decrease in cholesterol level (Figure 4) in group V (100 mg BPA), where a statistically significant negative effect of BPA was observed compared to 50 ug/kg/day BPS and 100 mg/kg/day BPS ($P < 0.005$ and $P < 0.035$). Total protein levels also showed a negative effect of BPA compared to groups with various BPS concentrations (Figure 5). Group V (100 mg/kg/day BPA) and Group III (50 ug/kg/day BPS) differed statistically significantly ($P < 0.022$).

Influence of Bisphenol S and Bisphenol A on morphological changes of follicles

The results in Table 2 show a difference in the height of thyrocytes ($P < 0.0001$). All groups at this level of significance manifest a decrease in thyroid length compared to the control group. In the follicular cell area parameter, the follicular cell area was reduced compared to the control in all groups ($P < 0.0001$) except group GIII ($P < 0.01$).

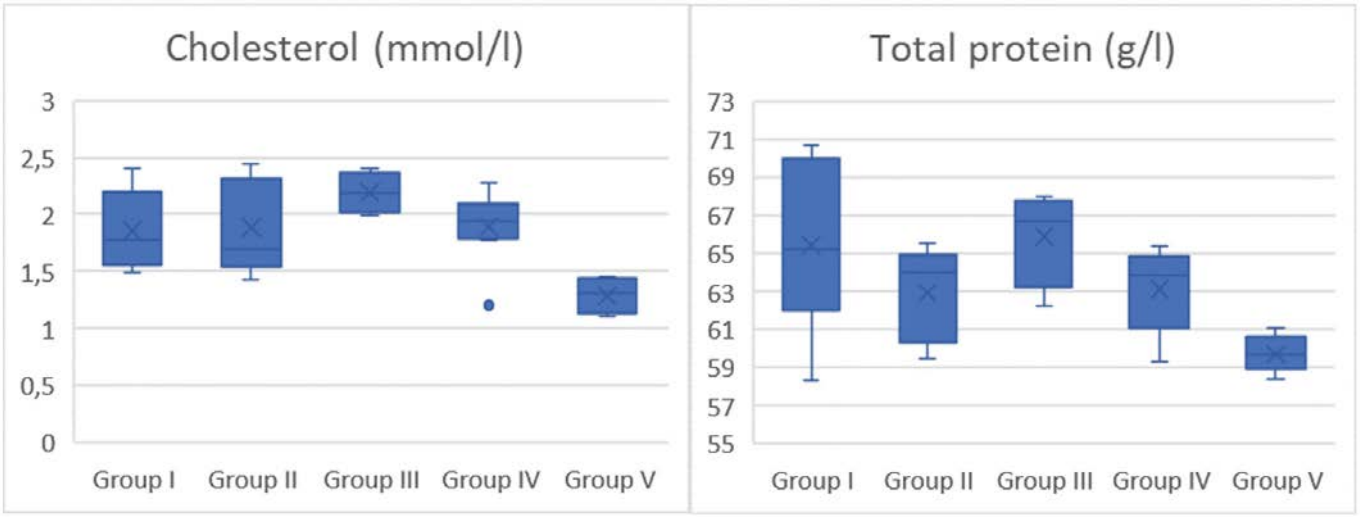

Figure 4 and Figure 5. Levels of cholesterol (normal range 0.55 - 3 mmol/l) and total protein (normal range 51 - 76 g/l)

Table 2. Morphometric parametrs of periphery thyroid gland follicles

Group	Length of follicle (µm)	Width of follicle (µm)	Follicle area (µm2)	Follicular cells area (µm2)	Height of thyreocyte (µg/l)	Follicle lumen area (µm2)
GI (n = 6)	121.74 ±11.70	89.34 ±7.08	9320.09 ±1640.18	32.42 ± 2.72	10.39 ± 1.24	7258.64 ± 1264.73
GII (n = 6)	138.20 ±4.90**	98.87 ±3.33	11292.34 ±552.14	27.63 ± 0.71****	8.51 ± 0.59****	8377.65 ±262.51
GIII (n = 6)	138.25 ±4.90 *	98.87 ±3.33**	11388.73 ±2455.38	30.03 ± 3.16**	8.79 ±1.28****	8295.61±2414.77
GIV (n = 6)	139.11 ± 9.23***	102.12 ±4.76***	11444.41 ±1286.7 *	30.43 ± 2.39****	8.82 ±1.15****	8550.21 ± 1306.69
GV (n = 6)	129.62 ± 5.63	100.78 ±4.57	10656,56 ±922.44	28.98 ±1.41****	8.85 ±0.30****	7870.10 ± 862.01

Statistically significant differences between the control group and groups with bisphenols *$P < 0.05$ ** $P < 0.01$ *** $P < 0.001$ **** $P < 0.0001$

As for the results of the width of the follicle and length of the follicle parameter, the greatest magnification occurred in group IV ($P < 0.001$). With group GII, there is a statistically significant difference in the length of the follicle ($P < 0.01$). The GIII group follicles also expanded at this level of significance and their length also increased statistically significantly ($P < 0.05$). The follicle area only increased significantly in the group IV ($P < 0.05$).

These results lead to the conclusion that even small concentrations of BPS have a significant effect on follicular morphology (Table 2), especially on the reduction of follicular cell area and the reduction of the height of thyreocyte. On the other hand, not even a high concentration of BPA affects the length, width and overall size of follicles significantly. According to our investigation, the effect of BPA only manifests in the reduction of the follicular cell area and the height of thyreocyte.

Both BPS and BPA influenced central follicle morphology less than marginal follicles (Table 3). Only GIII ($P < 0.01$) differed statistically significantly out of the BPS groups, namely in the greater length of the follicle and width of the follicle. The group, to which BPA was administered, differed ($P < 0.05$) in greater width of the follicle, and also the follicle lumen area increased here.

Our results are consistent with those of Lee et al. (2017), which also reports a smaller effect of BPA on thyroid morphology compared to its BPS analogue. As well Chen et al. (2016) observed increased thyroid follicle activity when exposed to BPS. Hypertrophy and hyperplasia of the follicular epithelium have been observed. Nevertheless, unlike our experiment, he observed colloidal depletion.

Table 3. Morphometric parameters of central thyroid gland follicles

Group	Length of follicle (µm)	Width of follicle (µm)	Follicle area (µm2)	Follicular cells area (µm2)	Height of thyreocyte (µg/l)	Follicle lumen area (µm2)
GI (n = 6)	60.46 ± 8.32	47.70 ± 6.68	2379.96 ± 680.14	32.14 ± 2	9.52 ± 0.85	1014.49 ± 397.58
GII (n = 6)	62.81 ± 2.36	51.80 ± 1.27	2612.49 ± 174.8	33.01 ± 0.69	9.42 ± 0.63	1208.18 ±21.51
GIII (n = 6)	70.56 ± 4.5**	54.49 ± 1.28**	3099.35 ± 251.39	34.67 ± 0.29	10.21 ± 0.87	1338.83 ± 49
GIV (n = 6)	62.82 ± 4.2	50.53 ± 4.59	2514.00 ± 338.21	34.42 ± 1.75	9.86 ± 0.84	1061.27 ± 199
GV (n = 6)	68.12 ± 4.37	53.80 ± 3.07*	2926.27 ± 349.59	31.49 ± 0.56	9.51 ± 0.71	1398.95 ± 259.19*

Statistically significant differences between the control group and groups with bisphenols (*$P < 0.05$; ** $P < 0.01$).

According to these authors, BPS acts on RH expression in the hypothalamus. This in turn stimulates TSH in the pituitary gland, which in turn acts on thyroid follicles (De Groef et al., 2006).

Bisphenol S and Bisphenol A affect TSH levels and thyroid function

The thyroid stimulating hormone (TSH) controls the production of T4 and T3 and therefore also affects the function of the thyroid gland. If T4 and T3 are low, the principle of negative feedback increases its production in the adenopituitary gland (Eghtedari and Correa, 2022). We observed an increasing trend in TSH with increasing BPS dose ($P < 0.05$). TSH values were elevated above the physiological norm for Wistar rats (0.85 - 3.23 ng/ml) in all bisphenol groups compared to the control group (Table 3). Elevated TSH was also observed by Hu et al. (2023) who observed increased concentrations of BPA (2 mg/kg/day) and BPS (20 mg/kg/day) up to 1.21 times compared to the control group. At the same time, Hu et al. (2023) and Holloway et al. (2021) draw attention to impaired thyroid hormone synthesis and thyroid function, which are associated with the regulation of TSHR, thyroid peroxidase, thyroglobulin and NIS expression. Our results indicate the risk of thyroid dysfunction from the concentration of 4 ug/kg/day. According to Peeters (2017), elevated TSH is a typical symptom of hypothyroidism.

Table 4. TSH concentration and iodine content in urine of Wistar rats

Group	TSH (ng/ml) *	Iodine in urine (µg/L) *
GI (n = 6)	3.14 ± 1.28	89.44 ± 86.02
GII (n = 6)	5.12** ± 1.16	197.04 ± 64.93
GIII (n = 6)	5.55** ± 2.39	171.2 ± 59.43
GIV (n = 6)	5.56** ± 1.98	336.76 ± 103.07
GV (n = 6)	4.48** ± 1.09	225.94 ± 134.7

* $P < 0.05$; **Higher than normal range of TSH for Wistar rats (0.85 – 3.23 ng/ml).

Bisphenol S and Bisphenol A affect urine iodine concentration

With increasing BPS concentrations (and high BPA concentrations), the iodine content of all groups increased, indicating decreasing iodine utilization by the thyroid (Table 4). A statistically significant difference ($P < 0.014$) was found between the control group and group IV (100 mg BPS/kg/day). In our experiment, BPS increased urine iodine at the lowest concentration (4 ug BPS/kg/day) by 220.3% compared to the control group. Our finding is consistent with Zhang et al. (2018) and Deal and Volkoff (2021) who report the ability of BPA analogues to act on thyroid peroxidase and sodium iodine symporter.

CONCLUSION

This study demonstrated the effect of BPS and BPA on the thyroid gland at several levels. Increased urinary iodine content indicated a significant effect of BPS and BPA on iodine metabolism and its supply to the thyroid gland. Even low concentrations of BPS (4 ug/kg/day) have a more significant effect on TSH concentrations than high concentrations of BPA (100 mg/kg/day). This shows that BPS is not a suitable substitute for BPA for use in food contact plastics. The study also showed a significant effect of both BPS and BPA on changes in thyroid morphometric parameters. The effect of BPS on some morphometric parameters is more significant than that of BPA. Our results show the necessity to establish a tolerable daily intake (TDI) also for BPS.

Because of the low number of studies directly studying the effect of BPA analogues on thyroid morphology, more studies looking at this issue would be needed.

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