RESEARCH ARTICLE

A comparison of effects of bisphenol A and bisphenol S on rat gut contractility *in vitro* after acute exposure

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ABSTRACT

Background: Prevailing standard of living allows generous usage of plastic containers to store, transport, and serve edibles. A chemical named bisphenol A (BPA) leaches from plastic containers into the edibles. Ingestion of BPA contaminated food is known to alter intestinal motility in addition to detrimental effects on fetal development, fertility, behavior, cognitive functions, immunity, and metabolism. These BPA-induced ill effects on health led to marketing regulation on BPA and introduction of bisphenol S (BPS) as a safer substitute of BPA. However, BPS is yet to be evaluated for its effects on gut motility. **Aims and Objectives:** Therefore, this study was aimed to assess comparative effects of BPS and BPA on gut motility. **Materials and Methods:** In an organ bath preparation, isometric contractions were recorded from segments of dissected gastric and small intestinal muscle strips prepared from rat gut, and cumulative concentration response of BPS and BPA on *in vitro* gut contractility was evaluated. **Results:** Both BPS and BPA treatment significantly diminished basal tone, maximum contractile tension, and the contractile frequency of spontaneous contractions in gastric as well as small intestinal muscle strips. **Conclusion:** From the present observation, it was apparent that both BPA and BPS have similar toxicity on gastric as well as small intestinal motility. Thus, the use of BPS as a substitute of BPA needs to be more critically evaluated.

KEY WORDS: Bisphenol A; Bisphenol S; Gut Motility; Plastic Toxins; *In vitro* Gut Contractility; Gastric Contractility; Small Intestinal Contractility; Albino Rats

INTRODUCTION

The use of plastic for storage, transport, and serving food has become an essential component of modern life. A chemical named bisphenol A (BPA) is used by industry for manufacturing the plastic items including containers for food and beverages.[1] BPA is known to leach from the plastic containers into edibles, especially when the container is heated up, repeatedly washed, or is exposed to high pH.[2]

Ingestion of BPA-polluted food exposes initially the gut and later all other body systems to this chemical. BPA is a well-documented xenoestrogen[3] and has been reported to impair *in vitro* intestinal contractility in animal studies.[4-9] Furthermore, it has been reported to cause defects in fetal development, metabolic dysfunction, reproductive disorders, carcinomas of prostate and breast, and dysfunctions of immune and nervous system.[10-15] Repeated reporting of BPA-induced ill health effects has elevated alarm about its suitability in food-related products. Several governments have banned the use of BPA in baby feeding bottles.[16] Due to increasing awareness of health hazards of BPA and regulation on its marketing, a BPA analog, bisphenol S (BPS) has been introduced by manufacturers as a safer alternative to BPA. Thermal paper marketed as BPA free may contain BPS.[17] BPS is being explored for its toxicity and has been found to be deleterious to human health as well.[18] Contrary to BPA,

the effect of BPS on gut motility has not been explored so far. It is possible that BPS has depressive effect on gut motility similar to BPA, as both belong to family of endocrine disrupting chemicals (EDCs) and have estrogenic activity. Estrogen has been documented to depress gut motility.

Therefore, the present study was aimed to compare the effects of BPS and BPA on gut motility. Accordingly, the objective was to compare, *in vitro*, cumulative concentration response of BPA and BPS on gastric and small intestinal muscle strips of adult albino rats.

MATERIALS AND METHODS

Animals

The animal experiments were performed as per guidelines of the institutional ethical clearance committee (Ref. No. Dean/2017/CAEC/245). Adult male albino rats of Charles foster strain (weighing 175–225 g) were fed standard rat feed, and water supply was *ad libitum*. The animals were housed in an environment of controlled temperature (25 ± 1°C), light (12:12 h light dark) and humidity. A total of 18 rats were recruited for the present study.

Dissection and Recording

The method for the dissection and recording contractility of gut smooth muscle preparations has been described earlier.[19] In brief, the rats were sacrificed by cervical dislocation after overnight fasting. After opening the abdomen, the stomach and small intestine were dissected out and immediately placed in a petri dish containing 100% oxygenated freshly prepared Krebs–Ringer solution. The intestinal contents were cleaned by Krebs–Ringer solution. Similarly, the stomach was opened along the greater curvature and the contents were removed.

At the start and end of each recording, calibration for the tension (0–10 g) was performed.

The gut segments of 1–1.5 cm length were placed in an organ bath filled with Krebs–Ringer solution (37°C ± 0.5°C) and continuously supplied with 100% O_2. The tissue segment was fastened to a tissue holder on the one end and force transducer (MLT 0210, AD instruments, Australia) on the other end. The vertically mounted strips helped in recording of mainly the longitudinal muscle contractions. An optimum resting tension 0.5 g for intestinal segments and 1.0 g[20] for gastric corpus segments was applied. The intestinal and gastric segments were left to equilibrate for 30 and 45 min, respectively. Replacement of Krebs–Ringer solution was done every 15 min.

After recording of contractions, the segment of tissue was removed from the organ bath and placed on blotting paper for lightly soaking the extra water from the tissue. The two ends of the strips were cut to remove the injured parts. The wet tissue was then weighed to express the tension per unit weight of tissue (g/g wet tissue).

Isometric contractions were recorded and analyzed by polygraph PowerLab 4/ST system and suitable software (chart-5 for Windows, ADInstruments, Sydney, Australia).

Drugs and Solutions

The physiological solution (Krebs–Ringer solution) was prepared with following composition (in mmol): NaCl, 119; KCl, 4.7; $CaCl_2.2H_2O$, 2.5; KH_2PO_4, 1.2; $MgSO_4.7H_2O$, 1.2; $NaHCO_3$, 5; and glucose, 11, with pH adjusted to 7.4. BPA and BPS were obtained from Sigma Aldrich, US.

Bisphenols were dissolved in 100% dimethyl sulfoxide (DMSO) to have stock solution (100 mM), and the required concentrations were prepared from dilution of stock with double distilled water.

Grouping of Animals

A total of 18 rats were divided into 3 groups (*n* = 6). One gastric corpus and one small intestinal muscle strips were prepared from each rat. The gut muscle strips were exposed to BPA, BPS, and vehicle in Groups 1, 2, and 3, respectively.

Experimental Protocol

After stabilization period, the spontaneous contractile activity of stabilized tissue was recorded for 10 min followed by exposure of gut tissue to cumulative bath concentration of BPA (0.1–100) µM in Group 1 and BPS (0.1–100) µM in Group 2. For each concentration, the exposure time was 10 min, followed by exposure to the next higher concentration, without wash. In Group 3, the tissue was exposed to equivolume of DMSO present in respective BPA/BPS concentrations. This group served as control.

Parameters Studied and Statistical Analysis

From recording of 10 min, data of 1 min were analyzed (9th to 10th min). Parameters studied were basal tone, maximum contractile tension (MCT), and contractile frequency (CF). The maximum height of contractions was converted to tension (g) with the help of chart-5 software and was expressed as MCT per unit mass (g/g wet tissue) using the tissue weight. In similar manner, the basal level of tension (g) after each contraction was expressed as basal tone per g (BT). Contractions per min were calculated as CF. The initial values all the parameters before giving any treatment were considered 100%, and changes after application of BPA/BPS/vehicle were considered as % of initial. The values

were then pooled to calculate mean ± standard error of mean (SEM). The concentration dose–response relationship was compared using two-way and one-way analysis of variance (ANOVA) as required.

RESULTS

Gastric Contractile Activity

Characterization of stabilized spontaneous contractile activity

The spontaneous contractile activity of gastric corpus muscle strips started within 45 min during stabilization period. The contractions observed were slow and of tonic type. The contractions varied from strip to strip in their amplitude and frequency. The mean ± SEM values of initial (before any treatment) BT, MCT, and CF in different groups are presented in Table 1. Figure 1a shows the original sample recording of spontaneous contractile activity of gastric tissue without any treatment.

DMSO (vehicle) did not alter spontaneous contractions

There was no statistically significant ($P > 0.05$, one-way ANOVA) change in the BT, MCT, and CF after application of different (0.0001–0.1 v/v %) concentrations of DMSO used to prepare different bath concentrations (0.1–100 μM) of BPA/BPS [Figure 2a] as compared to pretreatment values. This group served as control.

BPA inhibited spontaneous contractile activity

There was statistically significant ($P < 0.05$, one-way ANOVA) decline in the MCT, BT, and CF, after application of 100 μM bath concentration of BPA, and the pattern of spontaneous contractions got abolished eventually and frequency became zero [Figures 2b and 3].

BPS inhibited spontaneous contractile activity

There was a significant decrease in MCT, BT, and CF after application of 100 μM dose of BPS [Figures 2c and 3]. The dose responses of BPA and BPS were mutually not significantly different ($P > 0.05$, two-way ANOVA).

Small Intestinal Contractile Activity

Characterization of stabilized spontaneous contractile activity

The spontaneous contractile activity started within 30 min during stabilization period. The contractions observed were fast and of mostly phasic type. As observed with gastric muscle strips, the contractions varied from strip to strip in their amplitude and frequency. The mean ± SEM values of initial (before any treatment) BT, MCT, and CF in different groups are presented in Table 1. Figure 1b shows the original
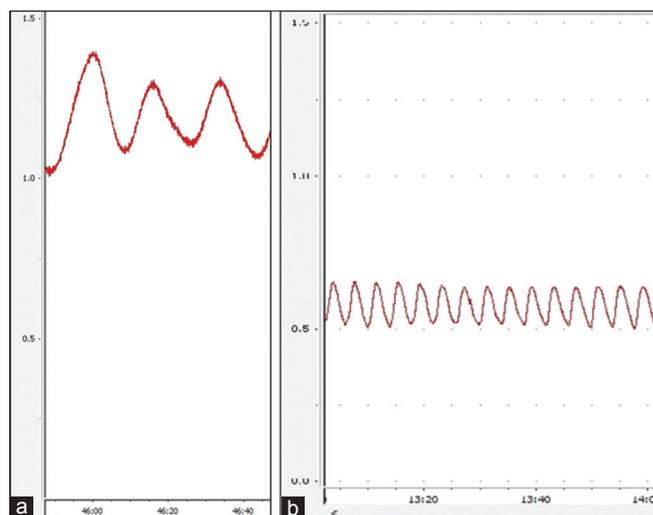

Figure 1: (a-b) Sample of original recordings showing spontaneous contractions of intestinal and gastric corpus longitudinal muscle strips, respectively. Vertical and horizontal calibrations represent the tension (g) and time (min), respectively
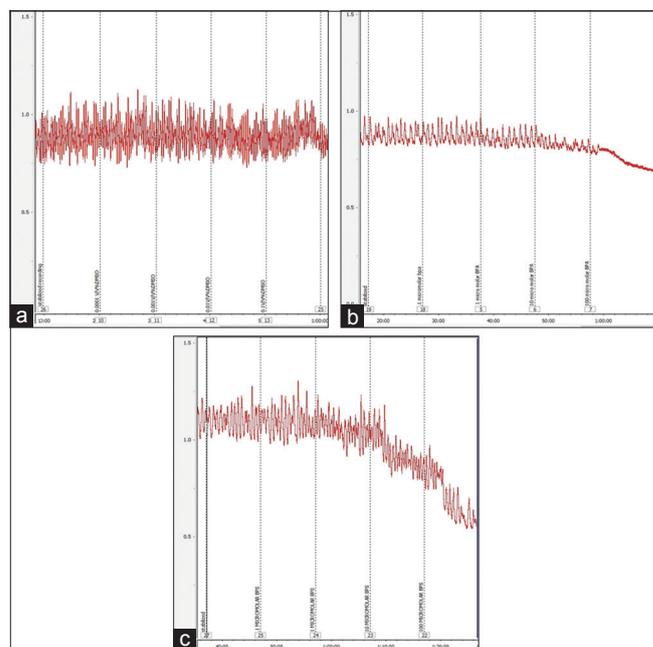

Figure 2: (a-c) Sample of original recordings from gastric corpus longitudinal muscle strips showing cumulative dose response (0.1–100 μM) to vehicle, bisphenol A, and bisphenol S, respectively. Dotted lines indicate the point of application of vehicle/drugs, and vertical and horizontal calibrations represent the tension (g) and time (min), respectively

sample recording of spontaneous contractile activity of intestinal tissue without any treatment.

DMSO (vehicle) did not alter spontaneous contractions

There was no statistically significant ($P > 0.05$, one-way ANOVA) change in the BT, MCT, and CF after application of different (0.0001–0.1 v/v %) concentrations of DMSO used to prepare different bath concentrations of BPA/BPS [Figures 4a and 5]. This group served as control.

Table 1: Mean±SEM of absolute values of contractile parameters (MCT expressed as g/g wet tissue, BT [g/g wet tissue], and CF) of gastric and small intestinal contractions in Group 1–3 before any treatment

Parameters	Gastric			Intestinal		
	Pre-BPA (Group 1)	Pre-BPS (Group 2)	Pre-DMSO (Group 3)	Pre-BPA (Group 1)	Pre-BPS (Group 2)	Pre-DMSO (Group 3)
MCT	7.70±1.60	6.69±1.57	8.85±1.88	8.56±1.37	7.85±1.04	8.12±1.20
BT	7.15±1.59	5.97±1.31	7.52±1.59	7.17±1.10	6.54±0.81	7.35±1.13
CF	2.12±0.51	1.93±0.29	2.21±0.37	13±0.89	12.58±0.88	14.3±1.81

MCT: Maximum contractile tension, BT: Basal tone, CF: Contractile frequency, BPA: Bisphenol A, BPS: Bisphenol S, DMSO: Dimethyl sulfoxide, SEM: Standard error of mean

BPA inhibited spontaneous contractile activity

There was statistically significant ($P < 0.05$, one-way ANOVA) decline in the MCT, BT, and CF, after application of 100 µM bath concentration of BPA [Figures 4b and 5].

BPS inhibited spontaneous contractile activity

There was a significant decrease in MCT, BT, and CF after application of 100 µM dose of BPS [Figures 4c and 5].

The dose response of BPA and BPS was mutually not significantly different ($P > 0.05$, two-way ANOVA).

DISCUSSION

This study examined the concentration (0.1–100 µM) response of BPS and BPA on gastric and small intestinal contractility *in vitro* to know differential impact of BPS and BPA on contractility of gastric and small intestinal smooth muscle. Our findings show that the *in vitro* exposure of BPA decreased the spontaneous contractile activity in both small intestine and stomach. It was apparent that the inhibition was by *per se* action of BPA and not the vehicle (DMSO) because our vehicle control experiments clearly showed that the amount of DMSO used for dissolving various concentration of BPA did not alter the smooth muscle contractile activity significantly. Furthermore, *in vitro* exposure of BPS inhibited the spontaneous contractile activity in both the gut tissues. Some studies report the impairment of *in vitro* intestinal contractility in rats after exposure to varying bath concentrations of BPA. This study for the 1st time revealed that *in vitro* exposure BPA may depress gastric contractility as well. Furthermore, we are first to report the depression of contractility of gut smooth muscle after acute exposure of BPS, an alternative to BPA. The comparison of effects of BPA and BPS indicated that both the agents have potential to impair spontaneous contractility in small intestine and stomach. The attenuation of spontaneous contractile activity by both the bisphenols was characterized by decrease in MCT, BT, and CF. The reduction of tension or tone suggested that the components of contractile machinery are likely to be affected, whereas the depression of frequency may be due to impairment of function of interstitial cells of Cajal which are the determinant of CF.[21]

Both BPA and BPS are known as EDC.[22] They have estrogenic actions.[3] Estrogen has been reported to depress gut contractility.[23] Estrogen mediates its actions through two types of estrogen receptors (ER), namely ERα and ERβ. The ERβ is known to be expressed in the intestine.[24] Hence, the diminished contractility of gut may be attributed to the estrogen-like actions of bisphenols. However, earlier reports showed that pretreatment with tamoxifen, an ER antagonist, failed to block the BPA-induced depression of gut contractility in rat ileum and colon.[7] Thus, it is not clear if the mechanism of depression of smooth muscle contractility involves ER receptor, at least in rat
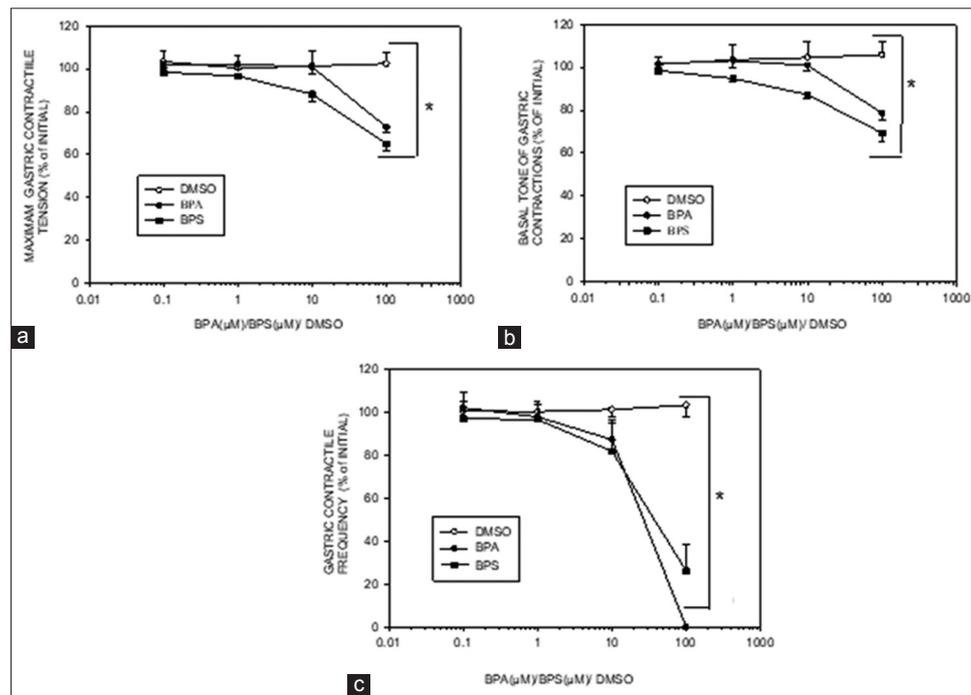
Figure 3: (a-c) The effect of bisphenol A, bisphenol S (0.1–100 μM) concentration, or vehicle on mean±standard error of mean values of percentage initial of maximum contractile tension, basal tone, and contractile frequency, respectively, in gastric tissue. (n = 6 in each group). The asterisk indicates statistically significant ($P < 0.05$, two-way analysis of variance) difference from (control) dimethyl sulfoxide fed group

gut. The cellular actions of estrogen are mediated by genomic (transcription dependent) and non-genomic pathways. The non-genomic pathway involves activation of membrane or cytosolic ER[25] which tamoxifen possibly failed to block.[7] The non-genomic action of estrogen might be affecting pathways involved in the gut contractility. Furthermore, estrogen has some ER independent which involves activation of potassium channels or inhibition of calcium channels[26] which could be the cause of depressive effect of bisphenols on gut contractility. Further, BPA has also been reported to inhibit duodenal movement by increasing AChE activity and decreasing the availability of free Ca^{2+} in smooth muscle cells.[4] Furthermore, the involvement of nitric oxide-mediated soluble guanylyl cyclase and α-adrenergic signaling pathways in visceral smooth muscle cells has been reported in decreased duodenal contractility caused by BPA.[9] However, in another study, NO inhibitor L-NAME synthase failed to block the inhibitory effect of BPA on gut contractility.[18] The precise mechanism through which BPA inhibits gut contractility is so far not established. This study revealed that BPS impaired the gut contractility in a way similar to that of BPA. Therefore, it may affect the same contractile machineries of gut smooth muscle by similar pathways.[4,9] BPS, unlike to BPA, increased 17a-OH progesterone levels[27] which is known to decrease gut motility.[28] Therefore, reduced gut contractility by BPS may be by some different or additional mechanism.

Strength and Limitation

This study for the 1st time revealed depression of *in vitro* gastric contractility after exposure of BPA, depression of
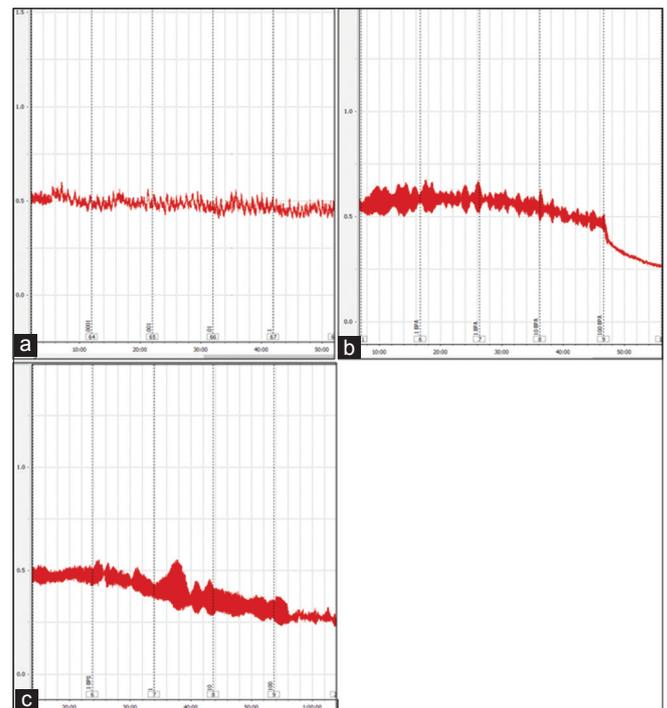

Figure 4: (a-c) Sample of original recordings from intestinal longitudinal muscle strips showing cumulative dose response (0.1–100 μM) to vehicle, bisphenol A, and bisphenol S, respectively. Dotted lines indicate the point of application of vehicle/drugs, and vertical and horizontal calibrations represent the tension (g) and time (min), respectively

gastric and small intestinal contractility after acute exposure of BPS, and the comparison of effects of BPA and BPS
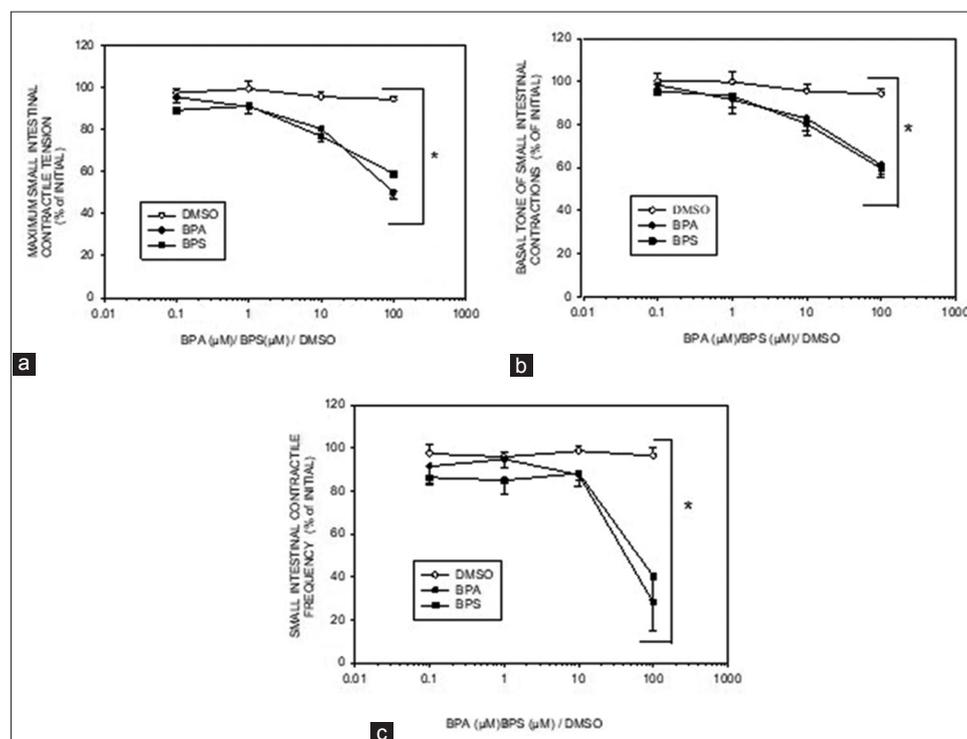
Figure 5: (a-c) The effect of bisphenol A, bisphenol S (0.1–100 µM) concentration, or vehicle on mean±standard error of mean values of percentage initial of maximum contractile tension, basal tone, and contractile frequency, respectively, in small intestinal tissue. ($n = 6$ in each group). The asterisk indicates statistically significant ($P < 0.05$, two-way analysis of variance) difference from (control) dimethyl sulfoxide fed group

on spontaneous *in vitro* gut contractility. Further, there is scope to determine if the effects of both the bisphenols in neonatal gut are similar to that of adult gut. Furthermore, the mechanism of action of BPA and BPS could be evaluated by pretreatment with antagonists.

CONCLUSION

The study suggests that both BPS and BPA have potential to impair the gut contractility which may cause gut dysmotility. Therefore, consideration of BPS as a safe alternative to BPA should be carefully evaluated.

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