TJPS 40 (2), April-June 2016: 61-69

Thai Journal of Pharmaceutical Sciences (TJPS)

Journal homepage: http://www.tjps.pharm.chula.ac.th

Effects of Continuous Exposure to Bisphenol A on Male and Female Mice from Prenatally to Adulthood

Kannikar Chatsantiprapa[1*] , Tip-arpa Sophon[1] , Jintana Sattayasai[2]

[1] *Kannikar Chatsantiprapa*
Faculty of Pharmaceutical Sciences, Khon Kaen University, M.S.Toxicology Program, Khon Kaen, 40002, Thailand
[2] *Jintana Sattayasai*
Faculty of Medicine, Khon Kaen University, Department of Pharmacology, Khon Kaen, 40002, Thailand

ARTICLE INFO
Article history:
Received: 21 January 2016
Revised: 3 March 2016
Accepted: 5 May 2016

Available online: 17 June 2016

Keywords:

Bisphenol A, continuous exposure, mice, reproductive system, TDI

ABSTRACT
BisphenolA (BPA), a monomer estrogenic chemical of many plastic products has controversial biological effects. This study assessed the effects at 0.05 mg/kg BW/day (Tolerable Daily Intake, TDI) and 0.5 mg/kg BW/day (10TDI), in male and female mice continuously exposed from embryo to adulthood. Results found BPA at the expected safe dose (TDI) decreased anogenital distance index (AGDI), motile sperm count, and ejaculation frequency, increased cauda epididymis weight and ejaculation latency in male mice, increased lordosis quotient in female mice. 10TDI decreased motile sperm counts, decreased intromission latency, unable to perform ejaculation in male mice, shortened AGDI in female mice. Other systems, at TDI, male mice had increased aggressive scores. At 10TDI, both male and female mice had increased body weights at 4 weeks and aggressive scores at 8-9 weeks. Mated female mice had increased internal organ weights at 13 weeks after pup delivery. The adverse effects on reproductive and related systems were confirmed.

Introduction

Bisphenol A (BPA) is a chemical used in the production of polymer science products such as polycarbonate (PC) plastic and epoxy resin. PC or BPA-based plastic is clear and tough and is made into a variety of household products, such as water bottles, sport equipment, CDs, DVDs, and baby feeding bottles. Epoxy resins containing BPA are used to line water pipes, beverage cans and in making thermal paper as that used in sales receipts. In 2003, the U.S. consumption [1] was 856,000 tons, 72% of which used to make PC plastic and 21% going into epoxy resin. It is considered to be an estrogenic substance. The effects of BPA on reproductive system were reported such as effects on anogenital distance (AGD) in Sprague-Dawley female rats by Kobayashi et al. [2] and effects in male children by Miao et al.[3] The National Toxicology Program1 concluded that there was some concern for effects on the brain, behavior, and prostate gland in fetuses, infants, and children and minimal concern or negligible concern on other effects at current human exposure. The safety of BPA has been controversial for decades. Despite all the disputes BPA-based baby products have been withdrawn from the markets in several countries.[4] The US FDA assessment released in 2014, however, said that BPA is safe at the current levels occurring in foods.[5] The

**Correspondence:*
Kannikar Chatsantiprapa, Faculty of Pharmaceutical Sciences,
Khon Kaen University, M.S.Toxicology Program, Khon Kaen, 40002,Thailand.
Email: kannikar@kku.ac.th

European Food Safety Authority (EFSA)'s experts also concluded in 2015 that BPA poses no health risk to consumers because current exposure to the chemical is too low to cause harm.[6] Despite the conclusion of its safety, the estimated safe level, known as tolerable daily intake (TDI) has been lowered in 2015 to 4 µg/kg BW/day or 0.004 mg/kg BW/day6 from that TDI of 0.05 mg/kg BW/day which had been used for many years.[7] This new TDI is stated as temporary pending the outcome of an on-going long-term study in rats involving prenatal as well as postnatal exposure to BPA.[6] Due to these controversies, this study was conducted to confirm the effects of BPA on reproductive and related systems from continuous exposure, from prenatal to adulthood, to the EFSA's recommended TDI dose at 0.05 mg/kg BW/day which was suggested as a safe dose as this study started before the new temporary TDI of EFSA4 was announced. The continuous long-term exposure came from the idea that mothers use many BPA containing products in their daily life. Therefore, babies' exposure to BPA starting from the prenatal period and then continuous exposure until adulthood comes from the use of many common BPA-based consumer products in their daily lives.

Materials and methods

The study was approved by Khon Kaen University's ethics committee on research using animals (AEKKU 08/2556).

Bisphenol A solution preparation

Bisphenol A (BPA) powder (99.9%, Sigma-Aldrich, U.S.) was dissolved in a few drops of ethanol then diluted with distilled water to make 100 ml BPA solution.

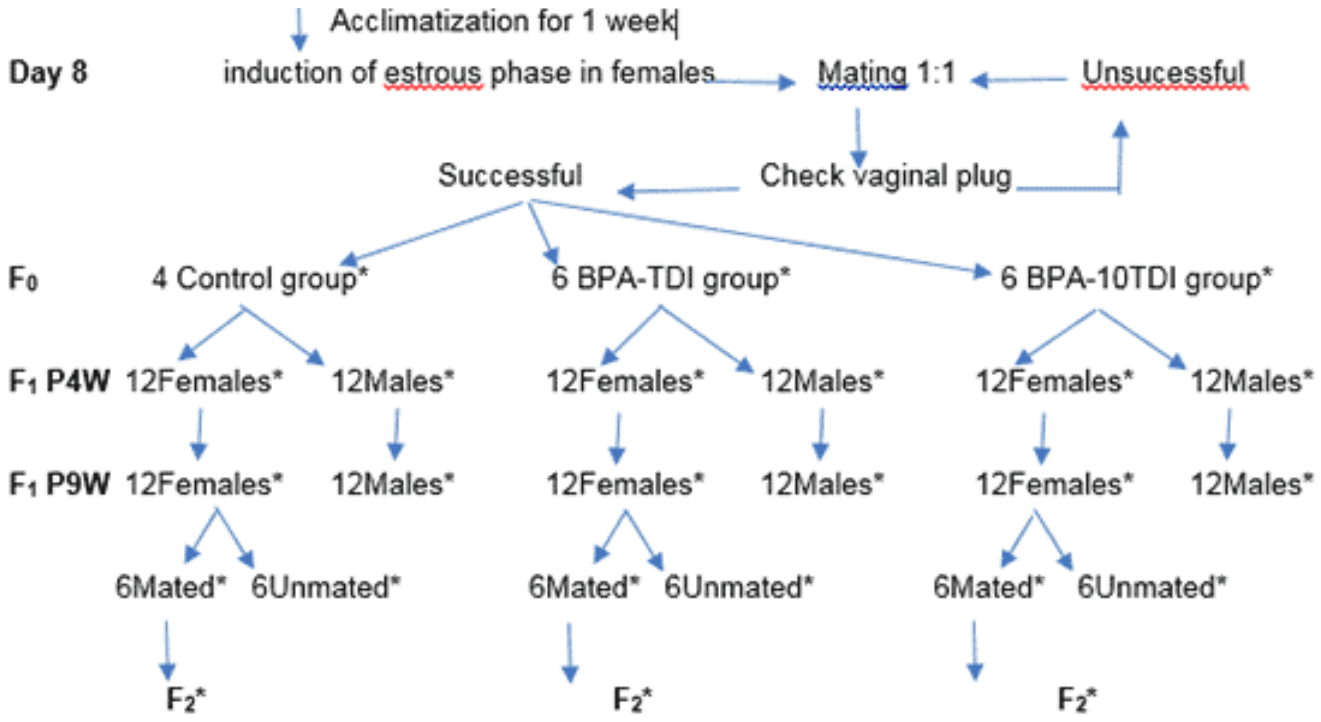

Figure 1 Flow chart of methodology and experimental procedures

Experimental procedures (see Figure 1)

Twenty-five ICR mice (purchased from National Laboratory Animal Center, Mahidol Univercity) at 5 weeks old (5 males and 20 females) acclimatized to the Northeast Animal Laboratory Centre, Khon Kaen University, for a week before starting the experiment under controlled conditions (temperature 23±2 ºC, relative humidity 30-60%, light intensity 350-400 Lux, ventilation 10-15 rounds per hour, light 12 h -06.00-18.00 h.:dark 12 h -18.00-06.00 h., noise under 85 db, free access to food and water ad libitum). The estrous phase of female mice was induced[8] by injecting 10 µg estradiol (0.15 48 h and 500 µg progesterone (0.15 ml) subcutaneously 4 h before placing with a male mouse 1:1 overnight for mating. On the next morning, the vaginal plugs of the female mice were examined. If the vaginal plug was not observed, the mouse was re-mated. If vaginal plug was observed the pregnancy was assumed to be started and it was counted the first gestational day. All the mated mice with vaginal plugs observed (F0) were then randomly assigned into 3 experimental groups: control, BPA-TDI, and BPA-10TDI (6-7 mice per group, however, only 4 mice in the control groups gave births to the pups). All pregnant mice were caged individually. Group 1 (control)

the pregnant mice (F0) were fed daily with vehicle, group 2 (BPA-TDI) fed with BPA solution at 0.05 mg/kg BW/day, group 3 (BPA-10TDI) fed with BPA solution at 0.50 mg/kg BW/day. All were fed as a single dose, approximately 0.1 ml in volume, via a feeding tube until the birth of the pups and until the pups were weaned (postnatal 4 weeks, P4W=pups receiving via mother's milk until 4 weeks old). After weanling, only 24 pups (F1, 12 males and 12 females) from each group were randomly selected from all pups born and separated from the mother and were placed 6 mice per cage. All the weaned mice were then tube fed in the same manner, as Group 1 (F1, control) with vehicle, group 2 (F1, BPA-TDI) with 0.05 mg/kg BW/day, and group 3 (F1, BPA-10TDI) with 0.50 mg/kg BW/day until adulthood (postnatal 9 weeks, P9W). Data were collected at each study period, i.e., at birth, P4W, and P9W. After P9W only the females of 3 experimental groups were randomly separated into 2 subgroups (6 females in each subgroup). Females of one subgroup from each experimental group was put with outsider (adult, healthy, 9 weeks old) male mice (male:female =1:3) for 24 hours. After that pregnancy was checked and mice were caged until delivery to get pups (F2) and until the pups of F2 were 7 days old to end the experiment.
The animals' body weights, AGD (anogenital distance) measured with a digital Vernier caliper from the midpoint of anus to the base of penis for male mice or anus to the midpoint of vagina for female mice, AGDI (anogenital index, AGD per body weight), aggressive behaviour*, sexual behaviour* and number of pups, live, and dead, at delivery were determined. At the end of the experiment, all mice were euthanized using pentobarbital sodium 40-50 mg/kg BW/mouse and dissected. Internal organs (brain, liver, kidneys) and reproductive organs were immediately collected, weighed and examined for gross morphological changes. Motile sperms* were also counted.

**Aggressive behaviour test*

When caged with the same sex, male or female mice were considered aggressive if other mice in the same cage (6 mice per cage) appeared injured or losing hair, but they did not. When the mice were caged 1:1 with the opposite sex for the sexuality test, they were observed for 30 min under an infrared closed-circuit television (CCTV). The female mice were considered aggressive if they attacked the male when the male came near or approached, or fought the male to deny mounting if mounted. The male mice were considered aggressive if they forced the female to mount or did not let the female go although the female fought to deny mounting.

**Sexual behaviour and sexuality test*

The experimental mice (either male or female) were tested with outsider adult healthy mice of the opposite sex. The mice (1 male and 1 female) were placed in the same cage in a dark and quiet room and were observed under infrared closed-circuit television (CCTV) for a 30 min duration. The experimental male mice were observed to count for mounting frequency and latency, intromission frequency and latency, ejaculation frequency and latency. The female mice were observed for lordosis (curvature of the back). Intromission of the male mice was considered if lordosis posture of the female was observed. Ejaculation was considered if licking of genitalia of the male after mounting was observed. The lordosis quotient (LQ) of the female was calculated from [number of lordosis of the female/ number of mountings by the male] x100.

**Motile sperm count*

Caudal epididymides were removed, weighed and torn with needles in 10 ml normal saline and stirred well. Fifteen µl of the volume was dropped on a hematocytometer to count under a microscope 40X, an average of 2 counts was presented.

Statistical tests

Student's t-test, one-way ANOVA, Fisher's LSD (when equal variance), Tamhane (when unequal variance), and Fisher's exact (for proportion) tests were used in SPSS Statistics 17.0.

Results

1. F0

The 3 experimental groups (control, BPA-TDI, BPA-

Table 1. Study results from 3 experimental groups when the mother mice were fed with vehicle (control group), Bisphenol A solution at 0.05 mg/kg body weight/day (BPA-TDI group), and Bisphenol A solution at 0.50 mg/kg body weight/day (BPA-10TDI group) started at the first gestational day until delivery.

Group	Number of mothers	Body weight of mothers F_0 (mean±SD)			Total number of pups F_1				Time to first delivery (days)
	(N)	[1]Before pregnancy	[2]During pregnancy	After delivery	Dead/Live (N/N)	Pups/ mother	%Dead	3M:F ratio	
Control	4	28.33±0.43	33.32±2.99	36.95±1.50	0/43	10.75	0	1.15	17.66±0.58
BPA-TDI	6	26.92±1.00	31.25±2.03	36.35±2.26	1/57	9.50	1.75	0.78	19.00±1.58
BPA-10TDI	6	27.87±1.52	33.18±1.75	37.36±2.90	1/56	9.33	1.78	0.81	18.75±0.50

[1]Before pregnancy=6th weeks old
[2]During pregnancy=9th gestation day
[3]M:F =male to female

10TDI) were not statistically different in mothers' body weights (F0) in all 3 intervals observed (at 6 weeks=before pregnancy, 9th day of gestation during pregnancy, after delivery), total number of pups per mother, % dead pups, time to first delivery, and male to female ratio of the pups (F1) (Table 1).

2. F1

Body weights

At 4 weeks, an insignificant trend of increasing body weight with increasing exposure dose of BPA was observed in both male and female mice. The statistically significant increase of body weight, however, was observed in BPA-

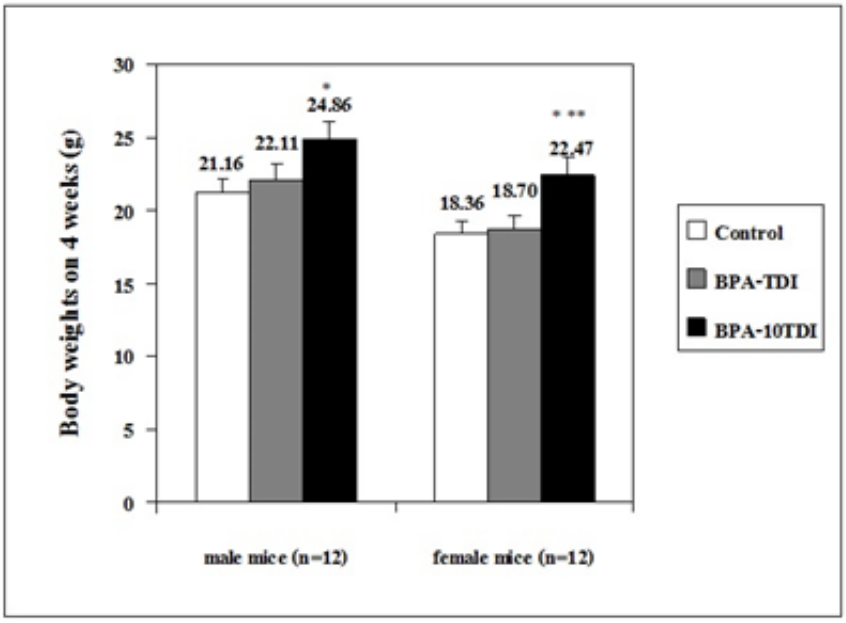

Figure 2 Body weights of the pup mice (F1) 4 weeks old after continuous exposure to vehicle (control group), BPA 0.05 mg/kg body weight/day (BPA-TDI group), and BPA 0.50 mg/kg body weight/day (BPA-10TDI group) via uterus and mother's milk until 4 weeks old. * =significant when compared to control ($p < 0.05$), **= significant when compared to BPA-10TDI ($p < 0.05$).

10TDI mice in both males and females. In females, it was significantly higher than the BPA-TDI group (p=0.002, Fisher's LSD) and control group (p=0.001, Fisher's LSD). In males, it was significantly higher than control group (p=0.041, Tamhane) (Figure 2). At 9 weeks the statistical differences of body weights among these 3 experimental groups disappeared in both males and females, although the females of the BPA-10TDI group still showed

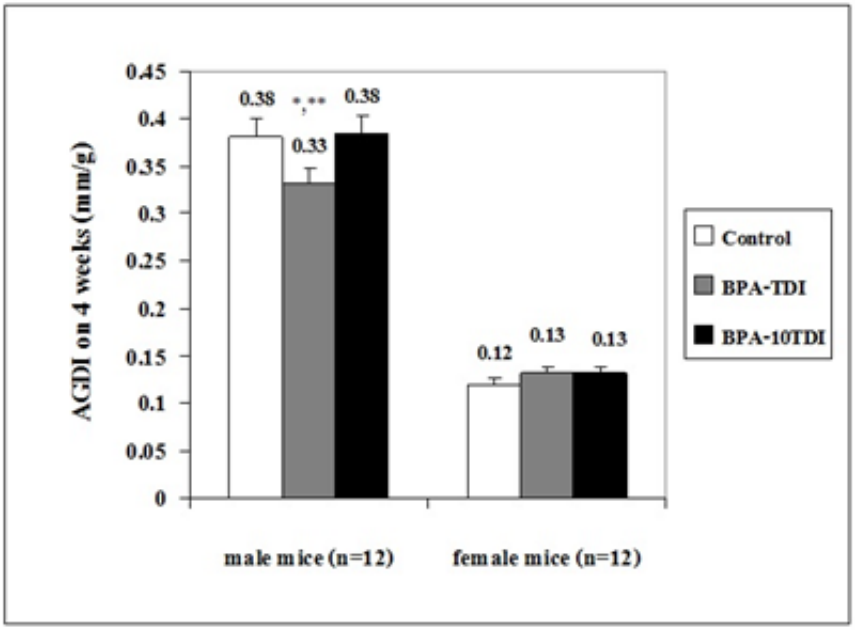

Figure 3 Anogenital distance index (AGDI) of the mice (F1) at 4 weeks old after continuous exposure to vehicle (control group), BPA 0.05 mg/kg body weight/day (BPA-TDI group), and BPA 0.50 mg/kg body weight /day (BPA-10TDI group) via uterus and mother's milk until 4 weeks old. * =significant when compared to control ($p < 0.05$), **= significant when compared to BPA-10TDI ($p < 0.05$).

insignificantly higher weights among the 3 experimental groups (data not shown).

Anogenital Distance Index (AGDI)

At 4 weeks, AGDIs of females among the 3 groups were not different. AGDIs of the BPA-TDI males were significantly the shortest among the 3 experimental groups. They were lower than the control (p=0.031, Fisher's LSD) and the BPA-10TDI males (p=0.022, Fisher's LSD) (Figure 3).

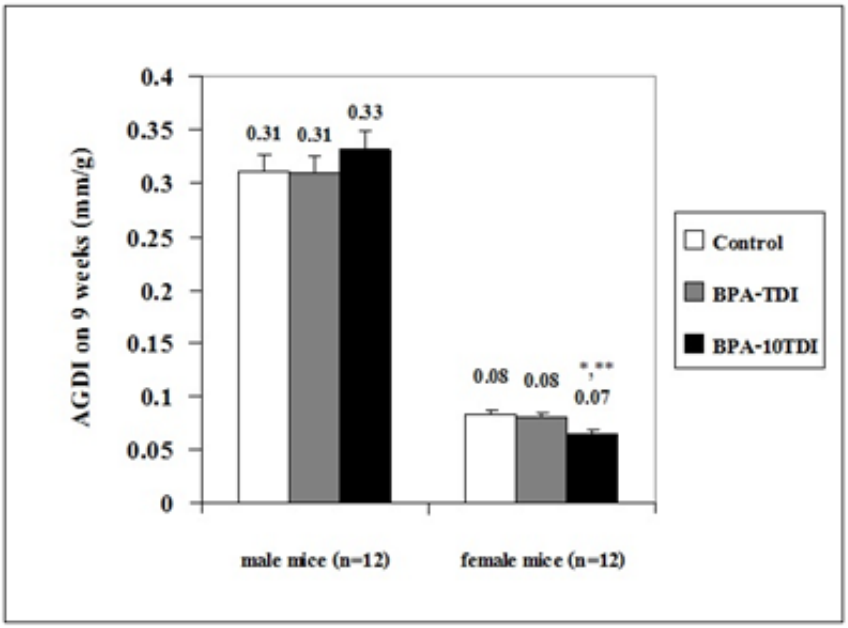

Figure 4 Anogenital distance index (AGDI) of the mice (F1) at 9 weeks old after continuous exposure to vehicle (control group), BPA 0.05 mg/kg body weight/day (BPA-TDI group), and BPA 0.50 mg/kg body weight/day (BPA-10TDI group) via uterus and mother's milk until 4 weeks old, then orally fed via a feeding tube until 9 weeks old. * =significant when compared to control ($p < 0.05$), **= significant when compared to BPA-TDI ($p < 0.05$).

At 9 weeks, AGDIs of BPA-10TDI females were significantly the shortest among the 3 experimental groups. They were lower than the control (p=0.011, Fisher's LSD) and the BPA-TDI females (p=0.031, Fisher's LSD). Whereas, among the males, the AGDIs of the 3 experimental groups were insignificantly different (Figure 4).

Aggressive behaviour

At 8-9 weeks, when caged with the same sex, BPA-10TDI females were significantly the highest aggressive scores among the 3 experimental groups, higher than both control and BPA-TDI (both p=0.025, Fisher's LSD). Males of both BPA-10TDI and BPA-TDI had significantly higher aggressive scores than controls (p=0.009 and

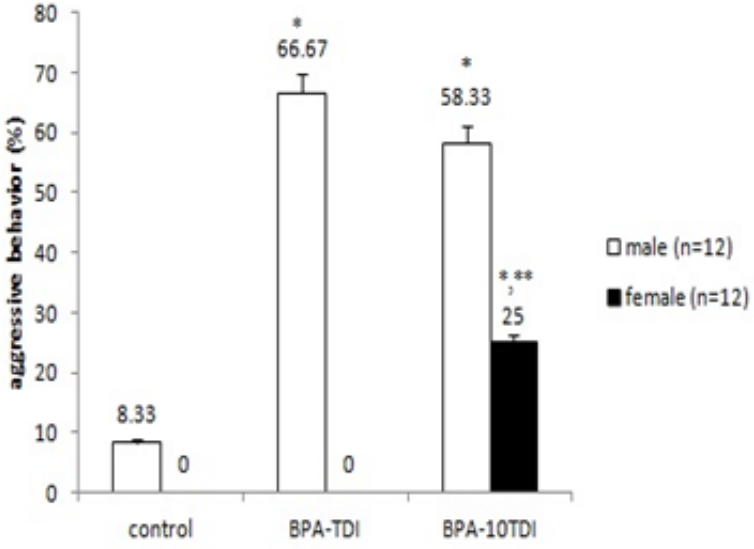

Figure 4 Aggressive behaviour detected in the mice (F1) at 8-9 weeks old when caged with the same sex mice (6 mice per cage) after continuous exposure to vehicle (control group), BPA 0.05 mg/kg body weight/day (BPA-TDI group), and BPA 0.50 mg/kg body weight/day (BPA-10TDI group) via uterus and mother's milk until 4 weeks old, then orally fed via a feeding tube until 9 weeks old. * =significant when compared to control ($p < 0.05$), **= significant when compared to BPA-TDI ($p < 0.05$).

p=0.003, Fisher's LSD), but no difference between BPA-TDI and BPA-10TDI groups (Figure 5). When caged with the opposite sex for mating purposes with adult, healthy mice outside the experiment, however, no statistical differences in aggressive scores were found among the 3 groups in both male and female mice, although females of both BPA-TDI and BPA-10TDI groups had insignificantly higher scores than controls, and males of the BPA-TDI group had insignificantly higher scores than BPA-10TDI and control groups (data not shown).

Sexuality

At 9 weeks during mating, BPA-TDI females had significantly higher lordosis quotients (LQ) than BPA-10TDI and control (p=0.031 and p=0.017, Fisher's LSD). In males, BPA-10TDI had significantly lower intromission latencies than controls (p=0.031, Fisher's LSD) and BPA-TDI had significantly higher ejaculation latencies than BPA-10TDI (p=0.008, Fisher's LSD) by which ejaculation was unsuccessful in all the BPA-10TDI males. Both BPA-TDI and BPA-10TDI males had significantly lower motile sperm counts than control males (p=0.022 and p=0.007, Fisher's LSD) (Table 3).

Table 2. Sexuality detected in the mice (F1) at 9 weeks old observed during mating after continuous exposure to vehicle (control group), BPA 0.05 mg/kg body weight/day (BPA-TDI group), and BPA 0.50 mg/kg body weight/day (BPA-10TDI group) via uterus and mother's milk until 4 weeks old, then orally fed via a feeding tube until 9 weeks old.

Sexual behaviour	**Mice (mean ± S.D.)**		
	Control (n=12)	BPA-TDI (n=12)	BPA-10TDI (n=12)
Male			
Mounting frequency (N)	2.67 ± 3.55	2.33 ± 2.67	1.50 ± 2.93
Mounting latency (min)	7.56 ± 8.93	5.56 ± 6.87	3.29 ± 4.84
Intromission frequency (N)	2 ± 3.38	1.42 ± 1.62	0.33 ± 0.89
Intromission latency (min)	2 ± 3.38	6.22 ± 7.48	1.55 ± 3.64*
Ejaculation frequency (N)	0.92 ± 2.61	0.67 ± 0.99	0.00±0.00
Ejaculation latency (min)	2.92 ± 7.03	9.27 ± 11.92**	NS
Female			
Lordosis quotient (LQ)	1.71 ± 4.10	25.64 ± 37.44*,**	NS

*** =significant when compared to control (p < 0.05), **= significant when compared to BPA-TDI (p < 0.05).**
NS=not successful

Table 3. Motile sperm counted in the male mice (F1) at 9 weeks old after continuous exposure to vehicle (control group), BPA 0.05 mg/kg body weight/day (BPA-TDI group), and BPA 0.50 mg/kg body weight/day (BPA-10TDI group) via uterus and mother's milk until 4 weeks old, then orally fed via a feeding tube until 9 weeks old.

Study group	**Motile sperm count (mean ± S.D.), x 106 ml**
Control (n=12)	14.618 ± 4.490
BPA-TDI (n=12)	9.039 ± 3.814*
BPA-10TDI (n=12)	10.000 ± 3.541*

*** =significant when compared to control (p < 0.05)**

Internal organs and reproductive organs weight and morphology

At 9 weeks, after euthanasia and dissection, the internal organs and reproductive organ weights (liver, kidney, brain, ovary) of the unmated females of the 3 groups were not significantly different (data not shown). At 13 weeks, in the mated females, after delivery of pups, the BPA-10TDI group had significantly heavier weights of livers and kidneys than the BPA-TDI group ($p<0.05$ and $p=0.05$, Fisher's LSD) (Table 4). In males, the BPA-TDI had significantly heavier weights of cauda epididymides than BPA-10TDI and controls.

Table 4. Internal organ weights of the mated female mice (F1) at 13 weeks old after delivery of the pups and the pups were 7 days old. The mice received continuous exposure to vehicle (control group), BPA 0.05 mg/kg body weight/day (BPA-TDI group), and BPA 0.50 mg/kg body weight/day (BPA-10TDI group) via uterus and mother's milk until 4 weeks old, then orally fed via a feeding tube until 13 weeks old. They were mated at 9 weeks old.

Organs weight (g)	**Mated Female (13 weeks) (mean ± S.D.)**		
	Control (n=6)	BPA-TDI (n=6)	BPA-10TDI (n=6)
Liver	2.05 ± 0.69	1.73 ± 0.10	2.51 ± 0.85*
Kidney (left)	0.40 ± 0.02	0.36 ± 0.02	0.41 ± 0.05
Kidney (right)	0.41 ± 0.04	0.38 ± 0.03	0.43 ± 0.05*
Brain	0.73 ± 0.04	0.75 ± 0.05	0.75 ± 0.07
Ovary (left)	0.08 ± 0.04	0.06 ± 0.03	0.06 ± 0.03
Ovary (right)	0.13 ± 0.13	0.06 ± 0.03	0.06 ± 0.03

*** =significant when compared to BPA-TDI ($p < 0.05$)**

Table 5. Internal organ weights of the male mice (F1) at 9 weeks old after continuous exposure to vehicle (control group), to BPA 0.05 mg/kg body weight/day (BPA-TDI group), and to BPA 0.50 mg/kg body weight/day (BPA-10TDI group) via uterus and mother's milk until 4 weeks old, then orally fed via a feeding tube.

Organs weight (g)	**Male (mean ± S.D.)**		
	Control (n=12)	BPA-TDI (n=6)	BPA-10TDI (n=6)
Liver	2.247 ± 0.121	2.417 ± 0.262	2.373 ± 0.208
Kidney (left)	0.479 ± 0.032	0.509 ± 0.070	0.507 ± 0.049
Kidney (right)	0.494 ± 0.368	0.526 ± 0.081	0.517 ± 0.045
Brain	0.711 ± 0.026	0.701 ± 0.026	0.727 ± 0.044
Testis (left)	0.278 ± 0.019	0.266 ± 0.027	0.283 ± 0.020
Testis (right)	0.285 ± 0.026	0.278 ± 0.029	0.283 ± 0.022
Cauda epididymis (left)	0.092 ± 0.031	0.117 ± 0.023*,**	0.081 ± 0.022
Cauda epididymis (right)	0.093 ± 0.034	0.112 ± 0.024**	0.085 ± 0.020

*** =significant when compared to control ($p < 0.05$), **= significant when compared to BPA-10TDI ($p < 0.05$).**

The weights and morphology of other internal organs of males among the 3 groups were not significantly different (Table 5).

3. F2

The F1 mothers of the 3 experimental groups (the mated subgroups) were not significantly different in the body weights at all 3 time intervals detected (at 6

weeks-before pregnancy, 9th days of gestation, and after delivery), and time to first delivery. The total numbers of pups per mother (14 per 6 in control group. 0 per 6 in BPA-TDI group, 18 per 6 in BPA-10TDI) were significantly different between the 3 groups (p=0.003). The fertility rates among the 3 groups (1 of 6 in control group, 0 of 6 in BPA-TDI group, 3 of 6 in BPA-10TDI group were pregnant) were not statistically different. Other data of the F2 females such as, % dead pups, time to first delivery, male to female ratio, AGDI at 7 days of age among the same sex pups, and body weights among the same sex pups comparing between control and BPA-10TDI groups were insignificantly different (data not shown).

Discussion and conclusion

BPA dosages used in this study were the tolerable daily intake (BPA-TDI) and 10 times the tolerable daily intake (BPA-10TDI) doses since this TDI dose was expected to be safe dose of exposure for humans.[7] The effects of BPA on the reproductive system were clearly demonstrated in this study. In male mice, AGDI was decreased in the BPA-TDI group at 4 weeks old. The female mice decreased AGDI in the BPA-10TDI group at 9 weeks old. It was reported that AGD is controlled by dihydrotestosterone, a metabolite of testosterone, the androgen hormone.[9] Whereas BPA was reported to have complex effects, it contains an anti-androgen effect,[10,11] estrogen effect,[12,13] and it is the endocrine disrupting chemical with a non-monotonic dose-response relationship.[14] Therefore, the effects of BPA on AGDI may not be easily explained and the sensitivities of response to BPA in males and females could be different. The effects of BPA on AGD were also reported in Sprague-Dawley female rats by Kobayashi et al.[2] and in male children by Miao et al.[3] From the current results, it would seem that the AGD of the male mice were more sensitive to the low dose of BPA, but not the high dose, and the effect was overcome when the male mice grew older. The female mice were more tolerable to BPA, thus the effects did not show at low dose and it took a longer time to appear.

In the present study, the male mice of both BPA-TDI and BPA-10TDI groups showed a significantly lower motile sperm count. Intromission latency and ejaculation latency were also significantly affected. Jasarevic et al.[15] had reported that male deer mice receiving 50 mg/kg BW/day via uterine exposure had lost sexual attraction as the mating choice to females. Jones et al.[16] also reported that male Long-Evans rats receiving 50µg/kg BW/day via uterine exposure had significantly abnormal sexual behaviour. In addition, in humans, Li et al.[17] also reported that male workers in the epoxy resin industry had significantly reduced sexual interest, ejaculation potency, and sexual satisfaction. These findings all support the effects of BPA on the male reproductive system. In the current study the female mice showed significantly higher lordosis quotients in the BPA-TDI group, , shortened AGDI and higher number of pubs per mother in the BPA-10TDI group. It seemed that BPA decreased the male fertility but increased the female fertility in the mice. However, reproductive morphology was also affected. These findings have demonstrated that continuous exposure to BPA may have different effects on reproductive systems of male and female mice and a non-monotonic dose-response relationship may have existed as commented by Welshon, et al.[14]

In the present study BPA exposure also increased aggressiveness in both male and female mice and was clearly observed when caged with the same sex. This effect was also reported by Kawai et al.[18] and Wolstenholme et al.[10] Wolstenholme et al.[12] explained that exposure to BPA during gestation interfered with neocortical development and affected synapse and dendrite production of the nervous system. This may have explained the increased aggressiveness found in the current study from BPA exposure. When caged with the opposite sex it could be that the drive for mating overcame the BPA effects, thus, no differences between groups were found.

It was found that BPA-10TDI increased the body weights of both male and female mice which was observed in 4 week old mice and the effects disappeared when the mice grew older. This may due to the estrogenic effects of BPA[13] which increased the water retention in the body and was reflected as body weight gain.[19] When the mice grew older, it could be that the body was better in regulation of the body water volumes. Therefore, the water retention effect disappeared. The internal organ weights (liver and kidney) were significantly higher in the mated female mice in BPA-10TDI. This may due to the interactive effects between the higher dose of BPA (10TDI) and mouse pregnancy hormones which overcame the regulation of body water. This study also showed that exposure to BPA for a short period during adulthood had no effects since there were no differences among the 3 experimental groups of F_0 mice in many parameters measured. This may reflect weak BPA effects on a mature body system.

Our study had followed the effects of exposure to BPA in many different conditions, i.e., first exposure during adulthood as in F0, continuous exposure via uterus until adulthood as in F1, and exposure via uterus from mothers who had been exposed continuously until reproductive age as in F2, and various parameters previously debated were reported. Our results had confirmed that the expectedly safe dose –TDI, is not confidently safe.

The U.S. CDC has found BPA in the urine of 93% of surveyed Americans over the age of 6. There was a report saying that, “If you don’t have BPA in your body, you’re not living in the modern world”.[20] A study in 2011 investigating the number of chemicals pregnant women were exposed to in the U.S. found BPA in 96% of women.[21] These reports confirmed the abundance of BPA for exposure in people of all age groups. Therefore, continuous exposure of BPA is likely to occur in humans from uterine exposure via the mother to adulthood as designed in this experiment. Safety of BPA has been under debate for many decades. This may have been due to political issues[22] despite hundreds of studies

indicating the risk of hazards on many organs such as prostrate, breast, testis, mammary gland, body size, brain structure and chemistry and behavior of laboratory animals, and other endocrine systems such as altered insulin sensitivity and others.[13] There are reports that rodents have significant age-dependent differences in metabolic capabilities, while the newborn and young primates metabolize BPA at or very near the level of adult metabolism.[23] As BPA is an endocrine disruptor with potent estrogenic effects it is worth paying attention to and using a precautionary approach to exposure to BPA. BPA can be leached from bottles easily[4,24] and very young children are more at risk to higher exposure to BPA due to smaller body volumes per exposure and immature development of the body's metabolizing systems. It is sound enough for EFSA to reduce TDI from the current 50µg/kg BW/day to 4 µg/kg BW/day, although the clear mechanisms of actions require further study to explain.

In summary, the experiments herein showed that continuous exposure (prenatal to adulthood) to BPA even at the expectedly safe dose (TDI at 50µg/kg BW/day) had adverse effects on reproductive and related systems in both male and female mice.

Acknowledgement

References

1. National Toxicology Program, U.S. Department of Health and Human Services. (2008). NTP-CERHR monograph on the potential human reproductive and developmental effects of Bisphenol A. Retrieved November 22, 2015, from https://ntp.niehs.nih.gov/ntp/ohat/bisphenol/bisphenol.pdf
2. Kobayashi K, Kubota H, Ohtani K, Hojo R, Miyakawa M. (2012). Lack of effects for dietary exposure of bisphenol a during in utero and lactational periods on reproductive development in rat offspring. J Toxicol Sci 37(3): 565-573.
3. Miao M, Yuan W, He Y, Zhou Z, Wang J, Gao E, Li G, Li DK. In utero exposure to bisphenol-a and anogenital distance of male offspring. Birth Defects Res A Clin Mol Teratol 2011; 91(10): 867-872.
4. Toxics Link. (2014). Toxic BPA chemical found in baby feeding bottles. Retrieved November 22, 2015, from http://toxicslink.org/?q=article/toxic-bpa-chemical-found-baby-feeding-bottles
5. U.S. Food and Drug Administration. (2014). Bisphenol A (BPA): Use in Food Contact Application. Retrieved November 22, 2015, from http://www.fda.gov/Food/IngredientsPackagingLabeling/FoodAdditivesIngredients/ucm064437.htm
6. European Food Safety Authority (EFSA). (2015). Scientific opinion on BPA. Retrieved November 22, 2015 from http://www.efsa.europa.eu/sites/default/files/corporate_publications/files/factsheetbpa150121.pdf
7. European Food Safety Authority (EFSA). (2011). Statement on the ANSES reports on Bisphenol A. EFSA Journal 2011; 9(12): 2475.
8. Sattayasai J, Srisuwan S, Arkaravichien T, Aromdee C. Effects of andrographolide on sexual functions, vascular reactivity and serum testosterone level in rodents. Food Chemical Toxicol 2010; 48: 1934-1938.
9. Clark RL, Antonello JM, Grossman SJ, Wise LD, Anderson C, Bagdon WJ, Prahalada S, MacDonald JS, Robertson RT. External genitalia abnormalities in male rats exposed in utero to finasteride, a 5 alpha - reductase inhibitor. Teratology 1990; 42: 91-100.
10. Xu LC, Sun H, Chen JF, Bian Q, Qian J, Song L, Wang XR (2005). Evaluation of androgen receptor transcriptional activities of bisphenol a, octylphenol and nonylphenol in vitro. Toxicology 216(2-3): 197-203.
11. Lee HJ, Chattopadhyay S, Gong EY, Ahn RS, Lee KS. Antiandrogenic effects of bisphenol a and nonylphenol on the function of androgen receptor. Toxicol Sci 2003; 75: 40-46.
12. Wolstenholme JT, Rissman EF, Connelly JJ. (2011). The role of bisphenol a in shaping the brain, epigenome and behavior. Horm Behav 59: 296-305.
13. Alonso-Magdalena, P; Ropero, AB; Soriano, S; García-Arévalo, M; Ripoll, C; Fuentes, E; Quesada, I; Nadal, Á. "Bisphenol-A acts as a potent estrogen via non-classical estrogen triggered pathways.". Mol Cell Endocrinol 2012; 355 (2): 201–7. doi:10.1016/j.mce.2011.12.012. PMID 22227557.
14. Welshons WV, Thayer KA, Judy BM, Taylor JA, Curran EM, vom Saal FS. (2003). Large effects from small exposures. I. Mechanisms for endocrine-disrupting chemicals with estrogenic activity. Environ Health Perspect 111(8): 994-1006.
15. Jasarevic E, Sieli PT, Twellman EE, Welsh Jr TH, Schachtman TR, Roberts RM, Geary DC, Rosenfeld CS. (2011). Disruption of adult expression of sexually selected traits by developmental exposure to Bisphenol A. Proc Natl Acad Sci U S A. 2011; 108 (28): 11715-11720.
16. Jones BA, Shimell JJ, Watson NV. (2011). Pre- and postnatal bisphenol a treatment results in persistent deficits in the sexual behavior of male rats, but not female rats, in adulthood. Horm Behav 59: 246-251
17. Li DK, Zhou Z, Miao M, He Y, Qing D, Wu T, Wang J, Weng X, Ferber J, Herrinton LJ, Zhu Q, Gao E, Yuan W. Relationship between urine bisphenol-a level and declining male sexual function. J Androl 2010; 31(5): 500-506.
18. Kawai K, Nozaki T, Nishikata H, Aou S, Takii M, Kubo C. Aggressive behavior and serum testosterone concentration during the maturation process of male mice: the effects of fetal exposure to bisphenol a. Environ Health Perspect 2003; 111: 175-178.
19. Stachenfeld, NS. Sex hormone effects on body fluid regulation. Exerc Sport Sci Rev 2008; 36(3): 152-159.
20. Walsh, B. (2010). The Perils of Plastic – Environmental Toxins – Toxic plastic. Time (April 01, 2010). Retrieved November 22, 2015, from http://content.time.com/time/specials/packages/article/0,28804,1976909_1976908_1976938-2,00.html
21. Woodruff, T J. (2011). 99% of pregnant women in US test positive for multiple chemicals including banned ones, study suggests. Environ Health Perspect 119 (6). ScienceDaily. pp. 878–885. doi:10.1289/ehp.1002727. Retrieved 1 February 2012.
22. Vogel, S A. The Politics of Plastics: The Making and Unmaking of Bisphenol A "Safety". Am J Public Health 2009; 99(S3): S559–S566.
23. Birnbaum LS, Aungst J, Schug TT, Goodman JL Working together: Research- and scientific-based regulation of BPA. Environ Health Perspect 2013; 121 (7): A206–A207. doi:10.1289/ehp.1306963. PMID 23817036. Retrieved 2014-02-01.
24. Chatsantiprapa, K., Srihan, S., Navakoksri, R., Kunthaprom, N. (2013). Risk assessment of Bisphenol A migration from baby feeding bottle. KKU Res. J. 2013; 18(6):1021-1025.