Kor. J. Env. Hlth. Soc., Vol. 29, No. 4, pp 27~34(2003)

The Bisphenol A : A Modulator of Pregnancy in Rats

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(Received August 20, 2003/Accepted October 2, 2003)

Abstract : Bisphenol A is used in the manufacture of epoxy, polycarbonate, and corrosion-resistant unsaturated polyester-styrene resins required for food packaging materials in industrial processing. Some reports indicated the possibility of harmful effects on rats. In this study was used a method for the determination of bisphenol A in blood according to the OSHA High Performance Liquid Chromatography (HPLC) guideline. The method involved blood extraction using methylene chloride. And it was evaluated developmental and teratogenic effects in pregnant rats and second generation. The results obtained were as follows. There was a significant increase in the body weights and treated groups F1 female in liver, spleen, kidney, but according to dose-response. F1 female rat's relative body weight and absolute body weight are not different. There was a significant increase liver, spleen, kidney organ weight and reproductive organ weight epididymis, prostate gland in F1 male rats. There was a proestrous in pregnant rat, group 200 μg/kg, 2000 μg/kg, 20,000 μg/kg. The effect on rat treated with bisphenol A decrease organ weight and reproductive organ weight. Identification and quantitation were performed with using HPLC C18 column and using at retention time 5.5 min. The results of the detection of bisphenol A were at 20,000 μg/kg in average 1 μg/m*l*, 200 μg/kg average in 0.9 μg/m*l* blood samples. From those results, it could be concluded that the effects of pregnant rat and second generation(F1) by bisphenol A treatment during lactational period were estrogenic and bisphenol A was remained in serum at low level.

Keywords : bisphenol A, reproductive organ, rats, endocrine disruptor, HPLC

Introduction

It has been proposed that humans and wildlife have suffered adverse effects on reproductive health as a result of environmental exposure to chemicals that interact with the endocrine system[1,2]. Recent reports have suggested that back-ground levels of industrial chemicals and other environmental pollutants may play a role in development of breast cancer in women and decreased male reproductive success as well as the reproductive failure of some wildlife species[2-4]. During the past two decades, environmental regulations regarding the manufacutre, use and disposal of chemicals have resulted in significantly reduced emmisions of most industrial compounds and their by-products[3,4]. Levels of the more environmenally stable organochlorine pesticides and pollutants are decreasing in most ecosystems including the industrialized areas around the Great Lakes in North America[4-6]. A surge of studies to define chemical endocrine disruptors, coupled with attempts to solve problems created by preliminary studies, is leading to a confusion of terms[7-9]. Agents are already being labeled as estrogenic based on their activity in the MCF-7 assay despite the fact that a range of non-estrogenic factors can stimulate these cells to divide, and the fact, common to all of biology, that not all activities observed in vitro are realized in vivo[10-12]. There is a need for a set of toxicological definitions that will serve theis new area. There are two current definitions of an estrogen - a compound that binds to isolated estrogen receptors, and a compound that produces trophic effects on the female reproductive tract. However, what is required is definition of the toxic effects expected of exposure of a whole organism to such a chemical, a definition that may differ between species and sexes. The collective term endocrine diruptor is coming into general use, but it has yet to be defined. Other potentially relevant properties of the chemical, including any effects observed in vitro, can only

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contribute to its definition as a potential endocrine disruptor[1,8,13].

Bisphenol A (BPA) is a monomer used in the manufacture of epoxy, polycarbonate, and corrosion -resistant unsaturated polyester styrene resins used in interior coating of cans and drums, reinforced pipes, adhesives, flooring, water main filters, artificial teeth, nail polish, and food packing materials. Glydidylethers are widely used as componets of epoxy resins, which exhibits exeptional adhesive properties[3,6,12]. When these liquid resins are mixed and reacted with hardner, they crosslink and thereby eonvert into solid compounds that are applied as coating or bonding agents[14,15]. The chemical reactivity of this class of compounds is a prerequsite for their technical use, and it accounts for the sensitizing, mutagenic and in some cases carcinogenic properties of many epoxy resin monomers. Bisphenol A, also called 2,2-bis(4-hydroxyphenyl) propane or 4,4-isopropylidenediphenol, is an industrially important compound used in the production of polycarbonates and other plastics at many chemical manufacturing plants throughout the world[13,15]. The annual production of bisphenol A exceeds 930 million pounds. Manufacturing facilities generate significant quantities of waste containing bisphenol A, some of which is discharged into the terrestrial, aquatic, and marine environments[15]. The health effects from exposure to bisphenol A have been investigated elsewhere. One study used mice and guinea pigs as models ina an attempt to determine the effects of external exposure to bisphenol A and on humans[16-18]. A toxicological study on the developmental toxicity of bisphenol A in rats and mice found that organogenesis produced fetal toxicity in mice but not in rats. No alteration in the morphological development of the fetus in either species occurred[19-21]. Very little is known about the chemical fate of bisphenol A in the environment. A recent study reported that most bisphenol A in a waste stream was degraded rapidly in a chemical plant's biotreatment facility or in surface waters that received a continuous discharge of bisphenol A[3,12].

Because of the potential for exposure to BPA, in both the workplace and the home environment, the present investigation reports on the reprodutive toxicity of bisphenol A in pregnant rats exposed at organogenesis.

Materials and Methods

The experimental animals were Sprague-Dawley rats which were supplied from SamRyuk Experimental Animal Co. Female rats weighed 240-290 g on gestational day 0 (day of sperm or vaginal plug detection). Animals were individually identified by ear tags during their 7-day adaptation period. During this study, animals were housed in polycarbonate cages with stainless-steel wire lids. Feed and tapwater were available *ad libitum* throughout the study. Animal rooms were equipped with automatic light cycles (light : dark = 12 : 12 hours). Temperature and relative humidity were maintained at 21-23°C and approximately 45%. A female rat in estrus or proestrus was placed overnight in the home cage of a singlely housed male of the same the same strain. On the follwing morning, vaginal smears were examined for the presence of sperm. Each female was placed overnight in the cage of one male for mating and then examined the next morning for the presence of a vaginal plug. Sperm positive rats or plug-positive rats were group-housed (maximum of three per cage).

Bisphenol A (Cas No. 80-05-7) of greater than 99.7% purity (Aldrich Chemical Company Inc.) was suspended in commercially available food grade corn oil. The corn oil used in this experiment was analyzed for peroxidezed as an indicator of rancidity prior to first use. Dosing solutions were verified to be 95-105% of the theoretical concentration prior to and following completion of dosing.

Approximately equal numbers of sperm or plug positive female were assingned to each of five dose groups (minimum of 10 per dose group). Females of each species were assigned to dose groups by the method of stratified randomization so that body weight on GD 0 was not significantly different across dose groups.

Animals were dosed by gavage with BPA solutions or corn oil (vehicle) between 8:30 AM and 10:00 AM on DG 7 through 17. The volume adminstered (5.0 ml/kg for rats) was based on body weight recorded on each day of the dosing period. The doses selected were 0, 2, 20, 200, 2000, 20000 μg/kg/day.

Rats were weighed on GD0, 6 through 15, and 20. Dams were observed daily during treatment

for clinical signs of toxicity. All mated rats were delivered at termination of pregnancy. During the post-parturition, dams could postered the neonates and all animals were killed by cervical dislocation at post-partum day 21. Maternal liver, kidneys, spleen, ovary gravid uterine weight, and number of corpora lutea were recorded. The uteri of dams with no apparent implantations were treated with a solution of 10% ammonium sulfide in order to visualize possible implantation sites. Number of implatation sites, resorptions, dead fetuses and live fetuses were evaluated, and live fetuses were disseted from the uterus. Each live fetus was weighed and examined for external morphological abnormalities, and the viscera were examined by fresh tissue technique. Half of the fetuses were weighed organs, such as liver, kidneys, spleen, reproductive organs. Dams and fetuses blood were collected and separated to serum for analysis. Methylene chloride was added and extracted from this serum. The extracts were analyzed after added acetonitrile and filtered. High Performance Liquid Chromatography (Younglin Co. SDV 30 Plus Solvent Degassor & Value Module, M930 Solvent Delievery Pump, M720 Absorbance UV Detector) were used at this analysis. This HPLC conditions were as follows, flow rate 1.0 ml/min, retention time 15 minute, mobile Phase water : acetonitrile was 40% : 60% wave length 233 nm, He gas for carrier. Column was used C18 9.4 x 250 mm (Waters Co.). Chromatography Condition was applied from OSHA methods and chemicals were used at HPLC grade purchased from Fisher Co..

Anaylsis of data were carried out by the student T-test and ANOVA.

Results and Discussion

Body weight change of treated groups was increased according to bisphenol A treatment in general. The body weight of 200 μg/kg treated group was different from control group statistically at the day of gestation day 3, 6, 9, 12, 15 ($p<0.05$). At the 2000 μg/kg and 20000 μg/kg treated groups were showed statistical differency compared with control group at GD 21.

The body weights of female neonates were showed statistical significacy at the treated group of 2 μg/kg on post-partum day (PPD) 1, 4, 7, 14, 21. At the group of 20 μg/kg treatment showed statistical differency at the day of PPD 14, 21 and autopsy. These weight changes of different from control were showed on the treated group 200 μg/kg at PPD 7, 14, 21 and treated group 2,000 μg/kg at PPD 1, 4, 7, 14, 21, and 2,000 μg/kg treated group PPD 1, 4, 7, 14, 21 and 20000 μg/kg treated group PPD 1, 4, 7, 14.

The body weights of male neonates were showed statistical significacy at the treated group of 2 μg/kg on PPD 7, 14, 21 and 20 μg/kg treated group on PPD 4. 21 and 2000 μg/kg treated group on PPD 1, and 20000 μg/kg treated group on PPD 1, 4, 7.

The effects of bisphenol A on pregnant rats at the view of relative organ weight were as follows. The significant changes of relative organ weight at the treated groups were varied at doses, at 2 μg/kg treated group was liver, 20 μg/kg treted group was liver, 200 μg/kg treated group were right kidney, uterus, 2000 μg/kg treated group was right kidney, 20000 μg/kg treated group was liver.

Table 1. Body weight change of bisphenol A treated with pregnant rat

(mean(g) ± SD)

Group	0 day	3 days	6 days	9 days	12 days	15 days	18 days	21 days
Control	249.5±0.7	252.5±0.7	258.5±2.1	261.5±2.1	267.5±4.9	275.5±4.9	305.0±4.2	265.5±6.4
2 μg/kg	251.0±17.0	256.0±19.8	261.0±22.6	264.5±29.0	273.0±26.9	286.0±25.5	314.5±29.0	252.5±53.0
20 μg/kg	265.0±18.4	281.0±33.9	291.0±29.7	301.5±29.0	319.0±31.1	343.0±32.5	380.0±45.3	304.5±19.1
200 μg/kg	279.3±7.1*	285.0±5.2*	289.3±5.1*	294.3±4.7*	302.3±11.0*	311.3±17.1*	322.7±27.1	271.7±24.1
2,000 μg/kg	277.3±34.4	272.5±36.9	289.0±40.4	296.3±45.7	311.0±48.0	333.0±46.5	395.3±54.1	407.0±56.8*
20,000 μg/kg	255.0±32.1	260.5±33.1	265.0±32.1	267.8±32.5	284.3±33.1	298.5±33.5	331.3±31.0	379.3±32.9*

* : statistically different from control group ($p<0.05$).

Table 2. Body weight change of dams during lactation period

(mean(g) ± SD)

Group	0 day	3 days	6 days	9 days	12 days	15 days	18 days	21 days
Control	293.7±17.0*	295.8±16.4*	288.8±18.3	293.0±13.9	304.8±11.3	291.5±19.7	293.3±9.1	242.7±13.4
2 μg/kg	278.5±20.5	281.0±38.2	268.0±19.8	285.0±29.7	284.0±25.5	290.0±17.0	298.0±22.6	295.0±21.2
20 μg/kg	296.0±23.1	299.0±27.6	297.0±27.1	303.3±18.6	308.7±25.4	303.7±34.6	308.0±25.4	304.3±22.5
200 μg/kg	294.0±17.1	294.25±11.1	291.5±14.5	280.3±36.2	291.5±21.4	282.3±42.0	274.8±35.0	270.8±34.5
2,000 μg/kg	290.8±51.0	293.25±38.7	294.0±50.0	287.5±42.3	288.8±26.6	282.0±24.8	295.0±36.5	246.5±26.8
20,000 μg/kg	272.3±21.0	276.5±23.9	268.8±22.8	271.8±48.9	255.0±27.9	247.5±27.2	273.5±21.9	265.0±25.2

* : statistically different from control group ($p<0.05$).

Table 3. Body weight change of F1 female rat during treated with bisphenol A

(mean(g) ± SD)

Group	1 day	4 days	7 days	14 days	21 days	at Sacrifice(22)
Control	5.6±0.19	8.2±0.35	10.3±0.50	19.6±0.97	26.9±1.00	25.8±1.12
2 μg/kg	6.4±0.63*	9.5±0.74*	16.3±3.33*	28.0±2.13*	43.2±4.36*	37.2±2.90
20 μg/kg	5.7±0.69	7.9±1.24	11.0±2.07	25.0±3.45*	39.2±5.10*	42.6±2.42*
200 μg/kg	6.5±0.63	7.6±1.83	13.6±1.85*	26.2±4.81*	41.3±6.73*	41.7±8.07*
2000 μg/kg	6.6±0.65*	9.5±1.18*	13.5±2.12*	24.4±3.23*	33.4±5.47*	30.8±6.54
20000 μg/kg	6.8±0.41*	9.4±0.77*	13.5±1.20*	21.9±2.00*	28.9±3.86	30.1±2.40

* : statistically different from control group ($p<0.05$).

Table 4. Body weight change of F1 male rat treated with bisphenol A

(mean(g) ± SD)

Group	1day	4days	7days	14days	21days	at Sacrifice(22)
Control	6.4±1.04	9.1±1.44	13.0±2.20	24.0±5.26	33.7±6.47	33.9±7.18
2 μg/kg	6.5±0.59	9.6±0.34	17.6±3.77*	30.2±2.60*	47.2±8.20*	44.8±12.26*
20 μg/kg	5.9±0.58	7.9±1.51*	11.5±3.10	24.6±3.22	39.5±6.24*	40.6±8.94
200 μg/kg	6.6±0.82	8.5±1.58	14.6±3.30	24.6±4.37	37.4±7.54	32.7±4.38
2,000 μg/kg	7.3±0.50*	9.4±2.49	13.9±1.63	25.0±2.43	34.8±4.80	32.9±4.67
20,000 μg/kg	7.3±0.78*	10.3±1.01*	14.7±1.25*	24.7±2.45	32.4±3.82	36.5±4.85

* : statistically different from control group ($p<0.05$).

Table 5. Relative organ weight of pregnant rat treated with bisphenol A

(mean(%) ± SD)

Group	Liver	Spleen	Kidney(L)	Kidney(R)	Ovary(L)	Ovary(R)	Uterine
Control	36.33±4.11	1.92±0.40	4.03±0.31	4.03±0.25	0.115±0.03	0.132±0.04	0.99±0.13
2 μg/kg	43.37±4.08*	2.02±0.18	3.85±0.57	4.01±0.55	0.150±0.02	0.152±0.05	0.99±0.35
20 μg/kg	45.61±4.45*	2.17±0.22	4.09±0.33	4.18±0.13	0.126±0.01	0.137±0.02	1.32±0.56
200 μg/kg	38.30±5.46	2.31±0.81	3.86±0.49	2.36±1.82*	0.145±0.03	0.138±0.02	1.49±0.40*
2,000 μg/kg	38.01±2.43	1.71±0.23	4.41±0.37	4.42±0.11*	0.105±0.03	0.118±0.03	0.99±0.13
20,000 μg/kg	49.11±9.21*	2.52±0.64	3.95±0.37	4.08±0.49	0.118±0.02	0.101±0.03	1.08±0.23

* : statistically different from control group ($p<0.05$).

The effects of bisphenol A on female neonates were borned from treated dams at the view of relative organ weight were as follows. The significant changes of relative organ weight at the treated groups were varied at doses, at 2 μg/kg treated group was left ovary, 20 μg/kg treted group were liver, spleen, ovaries, 200 μg/kg treated group were liver, spleen, right kidney.

On male neonates, some significant changes of relative organ weight at the treated groups were varied at doses, at 2 μg/kg treated group was left kidney, 20 μg/kg treated group were liver, spleen, kidneys, testes, left seminal vesicle, prostate, 200 μg/kg treated group were liver, spleen, right kidney, 2,000 μg/kg treated group was right testis, 20,000 μg/kg treated group were liver, right kidney, right epididymis.

Estrus cycle was monitored to all dams before sacrification. Control, 2 μg/kg treated group and 20 μg/kg treated dams showed contineous diestrus before sacrification. 200 μg/kg treated group, 2000 μg/kg treated group, and 20000 μg/kg treated dams showed proestrus or estrus before sacrification. This means that high dose groups induced early estrus cycle in dams(Table 8).

Standard curve of bisphenol A plotted by HPLC was shown in figure. The function of this curve was $Y = 19.198x - 37.59$ and $R^2 = 0.9995$. The result of the serum contents of bisphenol A was calculated.

Table 6. Relative organ weight of F1 female exposure to bisphenol A

(mean(%) ± SD)

Group	Liver	Spleen	Kidney(L)	Kidney(R)	Ovary(L)	Ovary(R)	Uterine
Control	32.79±1.78	4.25±0.74	6.01±1.60	6.49±0.19	0.156±0.043	0.060±0.038	0.773±0.127
2 μg/kg	33.50±2.13	4.24±0.81	5.84±0.24	6.13±0.10*	0.082±0.036	0.173±0.045	0.711±0.032
20 μg/kg	39.50±1.37*	6.32±1.03*	6.43±0.31	6.50±0.09	0.159±0.033	0.151±0.045	0.679±0.129
200 μg/kg	35.79±2.13*	5.73±0.82*	5.97±0.70	6.15±0.77	0.151±0.023	0.143±0.036	0.779±0.104
2,000 μg/kg	35.81±3.86*	4.12±1.75	6.44±0.48	6.55±0.33	0.164±0.051	0.152±0.052	0.815±0.168
20,000 μg/kg	42.82±2.14*	4.52±1.24	6.77±0.39	7.14±0.34*	0.190±0.062	0.179±0.047	0.838±0.313

* : statistically different from control group ($p<0.05$).

Table 7. Relative organ weight of F1 male exposure to bisphenol A

(mean(%) ± SD)

Group	Liver	Spleen	Kidney (L)	Kidney (R)	Testis (L)	Testis (R)	Epididymis (L)	Epididymis (R)	Seminal vesicle (L)	Seminal vesicle (R)	Prostate gland
Control	32.06± 2.27	4.28± 0.77	6.23± 0.36	6.08± 0.39	2.66± 0.24	2.74± 0.29	0.440± 0.125	0.409± 0.115	0.146± 0.033	0.123± 0.036	0.506± 0.076
2 μg/kg	35.05± 2.96	4.73± 1.79	5.67± 0.26*	5.96± 0.17	2.79± 0.29	2.95± 0.37	0.432± 0.055	0.418± 0.036	0.107± 0.034	0.130± 0.028	0.530± 0.044
20 μg/kg	36.33± 4.00*	5.85± 0.84*	6.06± 0.27	6.28± 0.25	2.17± 0.46*	2.18± 0.52*	0.341± 0.091	0.386± 0.088	0.093± 0.017*	0.110± 0.008	0.387± 0.070*
200 μg/kg	36.26± 1.19*	6.08± 1.67*	6.01± 0.35	6.62± 0.28*	2.48± 0.11	2.51± 0.27	0.448± 0.032	0.414± 0.063	0.136± 0.039	0.115± 0.030	0.450± 0.072
2000 μg/kg	33.73± 2.73	3.77± 0.81	6.13± 0.71	6.37± 0.60	2.93± 0.41*	2.98± 0.43	0.459± 0.056	0.459± 0.076	0.125± 0.027	0.130± 0.045	0.450± 0.100
20000 μg/kg	41.50± 2.12*	4.53± 1.19	6.20± 0.45	6.61± 0.32*	2.65± 0.17	2.69± 0.17	0.371± 0.038	0.985± 1.113*	0.124± 0.021	0.092± 0.028	0.581± 0.152

* : statistically different from control group ($p<0.05$).

Table 8. Estrus cycle of dams before the autopsy

Group	Proestrus	Estrus	Diestrus	Total
Control	-	-	8	8
2 μg/kg	-	-	8	8
20 μg/kg	-	-	6	6
200 μg/kg	1	1	5	7
2,000 μg/kg	-	1	5	6
20,000 μg/kg	-	2	6	8

Table 9. Concentration of bisphenol A in dams serum sample

Group (μg/kg)	2	20	200	2,000	20,000
Concentration	-	-	0.900	0.987	1.00

The residue of bisphenol A which was detected in serum 21 days after the llast shot were as follows. The residue of 20,000 μg/kg treated dams was 1 μg/m*l*, and 2,000 μg/kg treated dams had 0.987 μg/m*l* of bisphenol A, 200 μg/kg treated dams showed 0.9 μg/m*l* of serum level of bisphenol A, 2 and 20 μg/kg treated groups had not detected in the serum.

The bisphenol A residue in male and female neonates were as follows. The female serum had 0.82 μg/m*l* of bisphenol A in the 20,000 μg/kg treated dams and male neonates had 0.74 μg/m*l* of bisphenol A. 2,000 μg/kg treated group showd 0.71 μg/m*l* of bisphenol A in female neonates and 0.69 μg/m*l* in male neonates.

Susan *et al.* reported[27] (1997) that the weight of prostate was increased when the bisphenol A 20 and 2 μg/kg the equivalent dose of 150 in adult (75 kg) and 50 μg in child (25 kg) were dosed to pregnant mice. Vom Saal *et al.*[28] reported that 2 ppb of bisphenol A induced heavy weight of preputial gland and low wegiht of epididymis, and

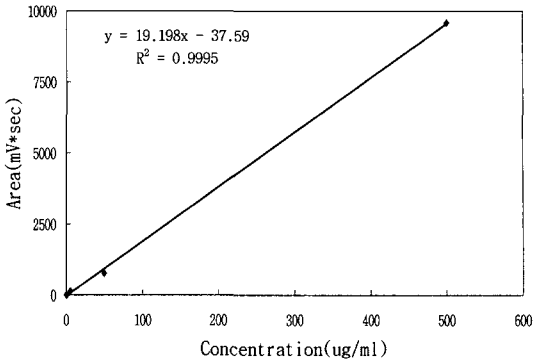

Fig. 1. Standard Curve of Bisphenol A.

that experiment designed to found out the effects of bisphenol A to the sperm activity and accessory reproductive organ functions in the neonates from treated dams during organogenesis. But this results didn't matched to our results from the epididymis weight increasing.

National Toxicology Program of USA reported (1982) two important things, the one was that low or high dose of bisphenol A induced polyneucleated megalo-hepatocytosis in mice, the other one was that bisphenol A exposure was related to the hematopoietic cancer in F344 rats and B6C3F1 mice[22,23]. A investigation group reported series of experiment from 1980 to 1995, and this results pointed that the mice and rat showed negative effects in reproduction due to the exposure of bisphenol A[24-26,28].

The effects of bisphenol A to the estrus of dams in 200 μg/kg treated dams, 2,000 μg/kg treated and 20,000 μg/kg treated dams showed 2, 1, 2 rats early advent of estrus, respectively, that means bisphenol A could induced effect to the estrus of rat.

Conclusion

In this study was used a method for the deter-

Table 10. Concentration of bisphenol A in f1 rats serum sample

Group	2		20		200		2,000		20,000	
(μg/kg)	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Concentration (μg/m*l*)	-	-	-	-	-	-	0.69	0.71	0.74	0.82

mination of bisphenol A in blood according to the OSHA High Performance Liquid Chromatography (HPLC) guideline. The method involved blood extraction using methylene chloride. And it was evaluated developmental and teratogenic effects in pregnant rats and second generation. The results obtained were as follows. There was a significant increase in the body weights and treated groups F1 female in liver, spleen, kidney, but according to dose-response. F1 female rat's relative body weight and absolute body weight are not different. There was a significant increase liver, spleen, kidney organ weight and reproductive organ weight epididymis, prostate gland in F1 male rats. There was a proestrous in pregnant rat, group 200 μg/kg, 2000 μg/kg, 20,000/kg. The effect on rat treated with bisphenol A decrease organ weight and reproductive organ weight. Identification and quantitation were performed with using HPLC C18 column and using at retention time 5.5 min. The results of the detection of bisphenol A were at 20,000 μg/kg in average 1 μg/m*l*, 200 μg/kg average in 0.9 μg/m*l* blood samples. From those results, it could be concluded that the effects of pregnant rat and second generation(F1) by bisphenol A treatment during lactational period were estrogenic and bisphenol A was remained in serum at low level.

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