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ORIGINAL ARTICLE

Polybrominated Diphenyl Ethers Orally Administration to Mice Were Tansferred to Offspring during Gestation and Lactation with Disruptions on the Immune System

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Background: The present study was undertaken to examine the immunological effects of pentabrominated diphenyl ether (penta-BDE) and decabrominated diphenyl ether (deca-BDE) on the immune system of the dams and the developmental immune system of the offsprings. **Methods:** In this study, mated female C57BL/6J mice were orally administered penta-BDE, deca-BDE or corn oil for 5 weeks, from gestational day 6 to lactational day 21. **Results:** The body weight of PND21 exposed to penta-BDE was significantly decreased relative to control mice, but that of post-natal day 63 (PND63) were recovered. Orally dosed dams with penta-BDE had significantly smaller absolute and relative spleen masses than control mice. Absolute and relative spleen and thymus masses of PND21 exposed to penta-BDE were significantly decreased over control. The exposure of dams and PND21 with penta-BDE reduced the number of splenocytes and thymocytes. As results of hematologic analysis, percentage WBC and percentage neutrophils increased in dams with deca-BDE. Splenic T cell proliferation in dams and PND21 exposed to penta-BDE was increased, and there were no significant difference in splenic B cell proliferation in all treatment groups. As results of flow cytometric analysis of splenocyte, percentage total T cell, Th cell and Tc cell in PND21 exposed to penta-BDE was slightly increased, and percentage macrophage in dams and PND21 exposed to deca-BDE was decreased. The ELISA results of antibody production show no significant difference in all treatment groups relative to controls. **Conclusion:** These results imply that PBDEs given to the dam were transferred to the offspring during gestation and lactation, and PBDEs transferred from the dam affect immune system of offspring. **[Immune Network 2010;10(2):64-74]**

INTRODUCTION

The polybrominated diphenyl ethers (PBDEs) including penta-BDE and deca-BDE (Fig. 1) are used in household plastic products, such as, children's toys, textiles, electronic, and furniture. Toxic effects of surrounding PBDEs on animals and human have been reported, and exposure to PBDEs as endocrine disruptors produces reproductive, developmental, and teratogenic toxicity, alterations in the thyroid hormone status, hepatotoxicity, teratogenicity, carcinogenicity, neurotoxicity, and immunotoxicity. In recent, several studies demonstrating the immunotoxic effects of PBDEs on the development of immune system have been conducted. The main findings published so far are changes in liver weight accompanied by histological alterations in animals given relatively large doses (1). In mice, the exposure of animals to PBDEs reduced the total number of splenocytes as well as splenic CD45R+, CD4+ and CD8+ cells. IgG production *in vitro* by splenocytes from mice exposed to PBDE was significantly lower (2). Significant suppression of the anti-sheep red blood cell response was shown only in mice exposed subchronically to PBDE and also PBDE exposure resulted in decreased thymus weight (3). In immunotoxicity of PBDEs on twenty-week-old mink, mink given 5 and 10 ppm treatments exhibited significantly increased production of antibody compared to con-

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Keywords: Penta-BDE, Deca-BDE, Developing immune system

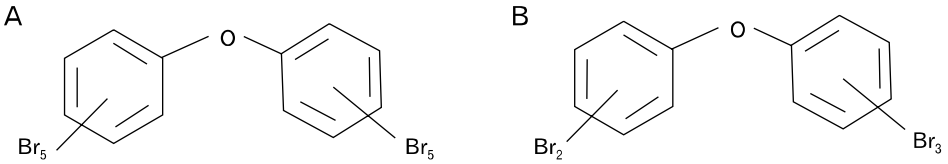

Figure 1. A general structure of polybrominated diphenyl ether (PBDEs). (A) penta-BDE, (B) deca-BDE.

trol mink. Spleens of mink exposed to 10 ppm of the pentabrominated diphenyl ether mixture, DE-71, had significantly increased germinal center development and incidence of B-cell hyperplasia. The change on hematocrit, increase of percentage neutrophils and decrease of percentage were shown (4).

These studies were undertaken to examine the immunological effects of penta-BDE and deca-BDE on the immune system of the dams. Moreover, it was addressed whether exposure to penta-BDE or deca-BDE on the dams affected on the developmental immune system of the offsprings in this study.

MATERIALS AND METHODS

Reagent

Penta-BDE was purchased from Wellington Laboratories Inc. (Guelph, ON, Canada). RPMI 1640 media was obtained from Gibco BRL (Grand Island, NY, USA). Fluorescein isothiocyanate (FITC)-conjugated anti-mouse CD3e monoclonal antibody, Phycoerythrin (PE)-conjugated anti mouse CD8a monoclonal antibody, Cy-chrome-conjugated anti-mouse CD4 monoclonal antibody used in flow cytometry were purchased from Pharmingen Inc. (San Diego, CA, USA). MTS and PMS assay kits were from Promega (Madison, WI, USA). Mouse IgG1, IgM ELISA kit were purchased from BD Bioscience (San Diego, CA, USA). Extra materials and reagents were purchased from sigma chemical Co. (St. Louis, MO, USA).

Animals and treatment

Specific pathogen-free C57BL/6J mice were provided by Central Laboratory Animal Inc. (Korea). Animals aged 8 weeks were acclimatized for 1 week before treatment. Animals were cared in accordance with the guidelines established by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). The animal room was maintained at $23\pm2^{\circ}C$ and relative humidity between $55\pm10\%$. The light/dark cycle was maintained on 12-h intervals. Virgin female mice, aged 9 weeks, were mated with male in the proportion of 2 : 1. The day sperm plug was detected by vaginal smear was decided to be day 0 of gestation. The pregnant mice were randomly divided into four groups.

Penta-BDE and deca-BDE was dissolved in corn oil and orally administrated to mice at doses of 50, 100 and 200 mg/kg/day for penta-BDE and 0.5, 2.5, 12.5 g/kg/day for deca-BDE. Mice were treated from the day 0 of gestation to postnatal day 21.

Necropsy ad histopathology

On PND 21 and PND 63, dams and offsprings were sacrificed by CO_2 inhalation. Body weights of mice were measured at the time of dosing initiation and autopsy. Organ weight including spleen, thymus, liver and kidneys was weighed and cellularity of spleen and thymus was determined by counting with a hematocytometer after red blood cell lysis.

Hematology was performed by automatic hematological analyzer (ADVIA120, Bayer, Germany). Thymus and spleen were fixed in neutral aqueous, phosphate-buffered 4% solution of formaldehyde, wax-embedded according to a routine processing protocol, and 5 μm sections cut and stained with hematoxylin and eosin (H&E). The slides were examined under light microscope by toxicopathologist (Korean Society of Toxicologic Pathology).

Preparation of spleenocytes and thymocytes

Spleens and thymus were removed aseptically and kept on ice in complete RPMI 1640. The organs were gently pressed through cell strainer (100 μm, Nylon, BD falcon Co., MA, USA). Both cells were washed once in RPMI 1640. The cell suspension was transferred and centrifuged at 430 g for 10 min with $4^{\circ}C$. The supernatant was discarded and the pellet was suspended with RBC lysis buffer (0.15M NH_4Cl, 1M $KHCO_3$ 0.1 mM Na_2EDTA, pH=7.4) to remove RBCs. After storage for 10 min, on ice, the suspension cells were washed twice with completer RPMI 1640 and centrifuged at 430 g for 10 min with $4^{\circ}C$. Then cell pellet was resuspended in the appropriate culture media for the assay being performed. The cell concentration was adjusted to 2×10^6 cells/ml in all studies.

Lymphoproliferation reponse of splenocytes

For the evaluation of the proliferative potency of splenocytes,

50 μl of splenocytes (2×10^6 cells/ml) were seeded in each of the 96-well flat-bottomed plates. Then, 50 μl of concanavalin A (5 μg/ml) or LPS (50 μg/ml) were added to the plates in duplicate. The plates were incubated for 72 h at 37°C in 5% CO_2. Cell proliferation was assayed using CellTiter 96® aqueous nonradioactive cell proliferation assay kit according to the manufacturer's protocol.

Flow cytometry

After centrifugation of 700 μl of splenocytes or thymocytes (2×10^6 cells/ml), splenocytes were stained with following antibodies; purified anti-mouse CD16/CD32 (blocking antibody), fluorescein isothisyanate (FITC)-conjugated anti-mouse CD3e monoclonal antibody (Pan T cell marker), phycpreythrin (PE)-conjugated anti-mouse CD8a monoclonal antibody (cytotoxic T cell marker), PE-Cy5-conjugated anti-mouse CD4 monoclonal antibody (helper T cell marker), FITC-conjugated anti-mouse CD11b monoclonal antibody (macrophage marker), PE-conjugated anti mouse CD19 monoclonal antibody (B cell marker), PE-Cy5-conjugated anti-mouse CD45R/B220 monoclonal antibody. After incubation, the cells were washed and fixed with 0.5% paraformaldehyde in PBS. Flow Cytometry analysis of splenocytes and thymocytes were performed with flow cytometer (Dual laser FACSCalibur™, Becton Dickinson, USA).

Measurement of antibody levels in sera

ELISA was used to measure total IgG1 and IgM antibody in mice sera, using ELISA kit (BD Biosciences, San Diego, CA, USA) according to the manufacturer's instructions. Briefly, 96-well plates were coating with 50 μl of anti-mouse IgG1 diluted with coating buffer (carbonated buffer, pH 9.6) for 12 hours at 4°C. After washing with buffer (0.05% Tween-PBS), the plates were blocked with 1% bovine serum albumin (1% BSA-PBS) and diluted serum samples were added to wells of the plates in duplicate. After the incubation at 37°C for one hour, the plates were washed 5 times with PBST, followed by addition of Biotin-anti-mouse IgG1 detection antibody and incubation at room temperature for one hour. Unbound detection antibody was washed from the plate after the incubation period with diluted wash buffer. Avidin-HRP was added, and incubated at room temperature for 15 min. Colorimetric detection was performed using ELISA plate reader (Molecular Device Co. USA).

Statistical analysis of data

All data were expressed as mean±standard deviation. Statistical analysis was performed using Sigma Stat Ver 2.0 (SPSS, USA). Data was analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's method as a post hoc test. At $p<0.05$ was considered significant.

Table I. Effects of penta-BDE and deca-BDE on body weight, relative organ weight and splenic and thymic cellularity in dams

Group (mg/kg)	Body weight (g)	Spleen		Thymus		Splenic cellularity ($\times10^6$/spleen)	Thymic cellularity ($\times10^6$/thymus)
		Absolute	Relative	Absolute	Relative		
Control	27.12±1.24	0.079±0.006	0.291±0.017	0.032±0.008	0.117±0.032	81.88±22.17	109.00±10.06
Penta-50 mg/kg	27.97±1.65	0.075±0.013	0.267±0.032	0.031±0.005	0.111±0.023	99.00±1.06	92.33±32.75
Penta-100 mg/kg	29.45±1.25*	0.070±0.006	0.242±0.026*	0.025±0.004	0.087±0.018	50.88±6.89	65.75±4.27
Penta-200 mg/kg	27.76±1.08	0.064±0.006*	0.234±0.026*	0.028±0.010	0.102±0.036	57.88±4.07	85.83±44.22
Deca-BDE 0.5 g/kg	26.90±1.77	0.075±0.006	0.281±0.035	0.030±0.04	0.113±0.020	76.50±51.97	128.25±0.00
Deca-BDE 2.5 g/kg	26.02±1.07	0.068±0.007*	0.262±0.022	0.039±0.007	0.148±0.023	80.00±15.91	101.88±20.68
Deca-BDE 12.5 g/kg	27.86±1.13	0.077±0.004	0.278±0.021	0.033±0.007	0.118±0.028	80.25±40.46	117.88±0.88

*Significantly different from control ($p<0.05$).

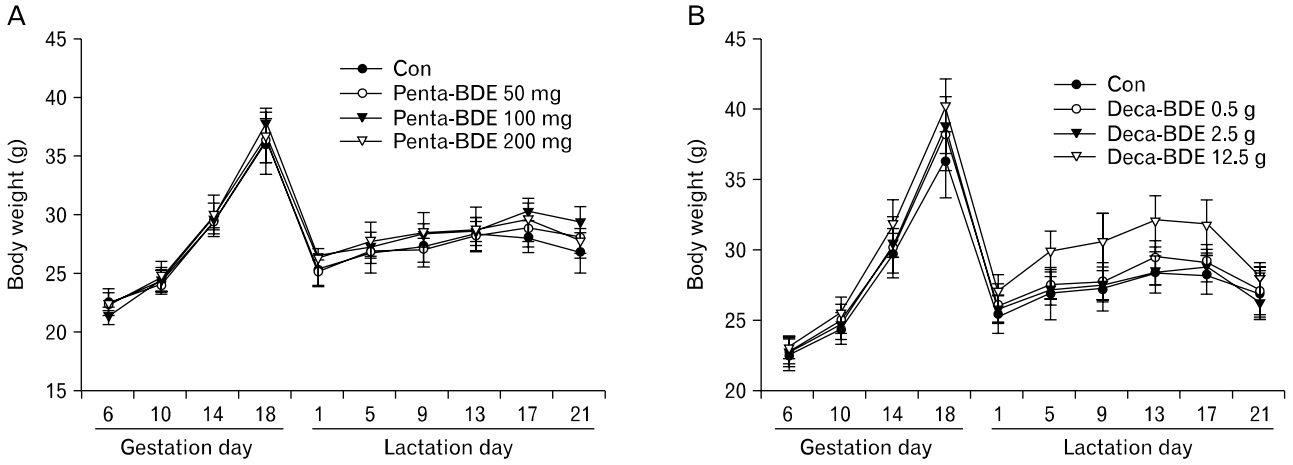

Figure 2. Body weight changes in dams.

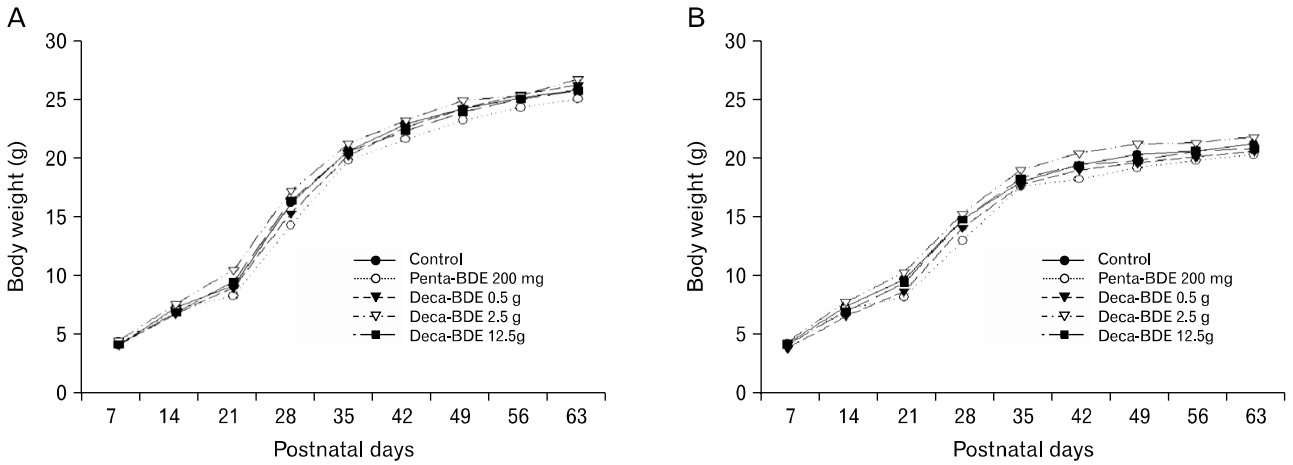

Figure 3. Body weight changes in F1.

RESULTS

Body and organ weights, cellularity

The results of body weight of dams orally administrated to penta-BDE have not shown the significant change over the control group (Table I). Body weights of PND21 exposed to deca-BDE were significantly decreased over control mice, but those of PND63 were recovered as control (Fig. 2, 3). Absolute and relative weight of spleens was decreased in dams exposed to penta-BDE, and especially significantly decreased in dams administrated to 100 mg/kg/day and 200 mg/kg/day. The thymi of penta-BDE exposed dams were weighed decreasingly (Table II, III).

In PND21, expose to penta-BDE 100 mg/kg/day and 200 mg/kg/day decreased significantly absolute and relative spleen and thymus weight. However no significant changes of spleen and thymus weight in PND63 occurred.

Hematology and histopathology

PBDE expose to dams had an effect on increase of the number of WBC and especially deca-BDE showed significantly increased the number of WBC, neutrophil and lymphocyte (Table IV). In contrast, there was slight decrease in the number of WBC, neutrophil and lymphocyte in offsprings (Table V). Histopathologically no significant changes in spleen and thymus were observed in mice treated with penta-BDE and deca-BDE, compared to control group.

Table II. Effects of penta-BDE and deca-BDE on body weight, relative organ weight and splenic and thymic cellularity in PND21

Group (mg/kg)	Body weight (g)	Spleen		Thymus		Splenic cellularity ($\times 10^6$/spleen)	Thymic cellularity ($\times 10^6$/thymus)
		Absolute	Relative	Absolute	Relative		
Control	8.94±0.77	0.057±0.014	0.634±0.147	0.0471±0.060	0.525±0.053	56.20±16.29	96.27±18.53
Penta-BDE 50 mg/kg	8.21±0.83*	0.045±0.020	0.535±0.188	0.042±0.005	0.518±0.046	46.20±15.00	80.71±18.66
Penta-BDE 100 mg/kg	7.48±0.55*	0.037±0.016*	0.478±0.175*	0.033±0.005*	0.440±0.046*	29.00±5.37*	70.85±13.94*
Penta-BDE 200 mg/kg	7.59±1.45*	0.028±0.014*	0.368±0.125*	0.035±0.008*	0.456±0.022*	35.19±3.38*	69.52±17.06*
Deca-BDE 0.5 g/kg	9.41±0.90	0.085±0.013	0.904±0.128	0.057±0.006	0.603±0.043	57.42±17.91	102.25±17.81
Deca-BDE 2.5 g/kg	10.25±0.65	0.091±0.014	0.890±0.150	0.065±0.006*	0.634±0.035*	67.46±16.31	111.67±23.08
Deca-BDE 12.5 g/kg	10.03±0.95	0.089±0.019	0.881±0.144	0.059±0.008	0.589±0.059	67.00±14.29	95.00±24.66

*Significantly different from control ($p<0.05$).

Table III. Effects of penta-BDE and deca-BDE on body weight, relative organ weight and splenic andcellularity in PND63

Group (mg/kg)	Body weight (g)	Spleen		Thymus		Splenic cellularity ($\times 10^6$/spleen)	Thymic cellularity ($\times 10^6$/thymus)
		Absolute	Relative	Absolute	Relative		
Control	23.54±2.89	0.072±0.011	0.304±0.060	0.047±0.010	0.202±0.064	84.95±24.17	148.08±54.70
Penta-BDE 50 mg/kg	21.54±4.29	0.061±0.019	0.307±0.070	0.045±0.022	0.218±0.098	80.54±24.94	173.43±34.77
Penta-BDE 100 mg/kg	21.68±3.50	0.063±0.014	0.294±0.047	0.040±0.014	0.188±0.067	90.50±21.37	145.68±31.65
Penta-BDE 200 mg/kg	21.88±3.27	0.069±0.012	0.317±0.048	0.044±0.011	0.211±0.077	91.46±17.39	150.25±55.20
Deca-BDE 0.5 g/kg	23.26±3.33	0.069±0.014	0.301±0.055	0.046±0.009	0.205±0.063	107.34±26.90	153.41±62.57
Deca-BDE 2.5 g/kg	23.99±2.57	0.069±0.009	0.290±0.058	0.048±0.010	0.205±0.059	98.64±15.23	148.21±69.70
Deca-BDE 12.5 g/kg	23.26±2.85	0.066±0.011	0.293±0.057	0.043±0.010	0.191±0.061	88.61±19.97	138.77±72.93

Table IV. Hematological analysis in dams dosed orally with penta-BDE or deca-BDE ($\times 10^3$/ul)

Group (mg/kg)	WBC	Neutrophil	Lymphocyte	Monocyte	Eosinophil	Basophil
Control	1.28±0.204	0.16±0.044	1.07±0.181	0.01±0.000	0.01±0.008	0.02±0.008
Penta 50 mg/kg	1.47±0.099	0.14±0.042	1.26±0.049	0.03±0.007	0.01±0.000	0.03±0.000
Penta 100 mg/kg	0.84±0.028	0.15±0.000	0.69±0.071	0.02±0.007	0.02±0.007	0.03±0.014
Penta 200 mg/kg	2.09±0.678	0.40±0.079	1.56±0.618	0.03±0.015	0.03±0.029	0.01±0.006
Deca 0.5 g/kg	2.52±0.808*	0.58±0.356*	1.78±0.465	0.04±0.020	0.02±0.007	0.02±0.012
Deca 2.5 g/kg	2.24±0.940	0.37±0.162	1.78±0.750	0.01±0.006	0.02±0.006	0.03±0.010
Deca 12.5 g/kg	1.91±0.394	0.30±0.015	1.51±0.388	0.02±0.017	0.02±0.013	0.02±0.006

*Significantly different from control ($p<0.05$).

Table V. Hematological analysis in PND63 exposed to penta-BDE or deca-BDE ($\times 10^3$/ul)

Group (mg/kg)	WBC	Neutrophil	Lymphocyte	Monocyte	Eosinophil	Basophil
Control	1.56±0.306	0.13±0.033	1.36±0.317	0.02±0.008	0.02±0.013	0.02±0.010
Penta 50 mg/kg	1.40±0.267	0.10±0.058	1.23±0.218	0.02±0.009	0.02±0.007	0.02±0.011
Penta 100 mg/kg	1.58±0.433	0.09±0.034	1.41±0.425	0.02±0.002	0.02±0.008	0.02±0.010
Penta 200 mg/kg	1.55±0.576	0.12±0.073	1.35±0.503	0.02±0.009	0.02±0.015	0.02±0.010
Deca 0.5 g/kg	1.34±0.330	0.14±0.059	0.14±0.309	0.02±0.006	0.02±0.014	0.02±0.010
Deca 2.5 g/kg	1.89±0.551	0.15±0.042	1.64±0.546	0.02±0.008	0.02±0.008	0.02±0.008
Deca 12.5 g/kg	1.63±0.532	0.12±0.066	1.64±0.668	0.02±0.007	0.02±0.011	0.02±0.013

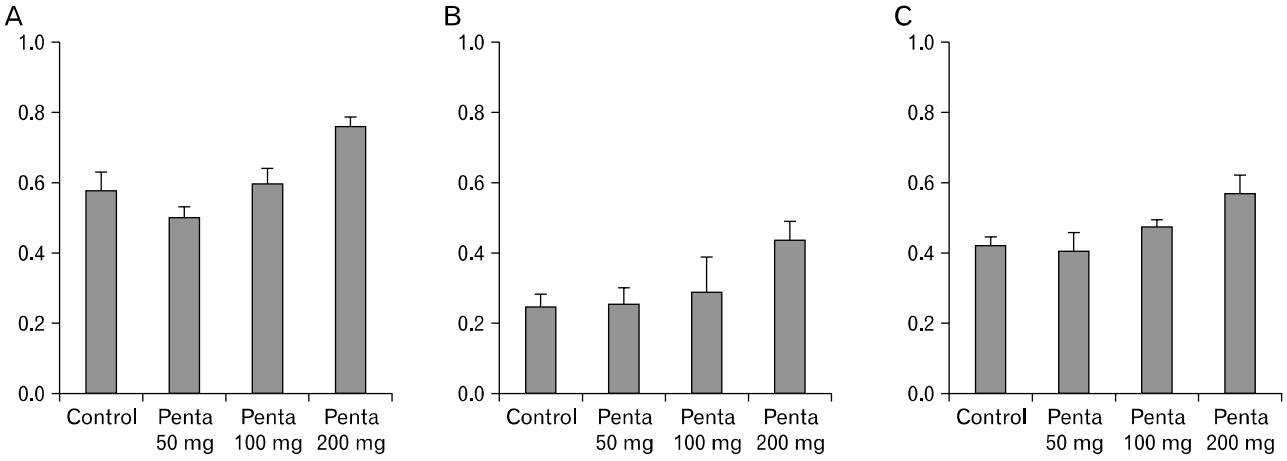

Figure 4. Effects of penta-BDE on splenic T cell proliferation responses. (A) Dam, (B) PND21, (C) PND63.

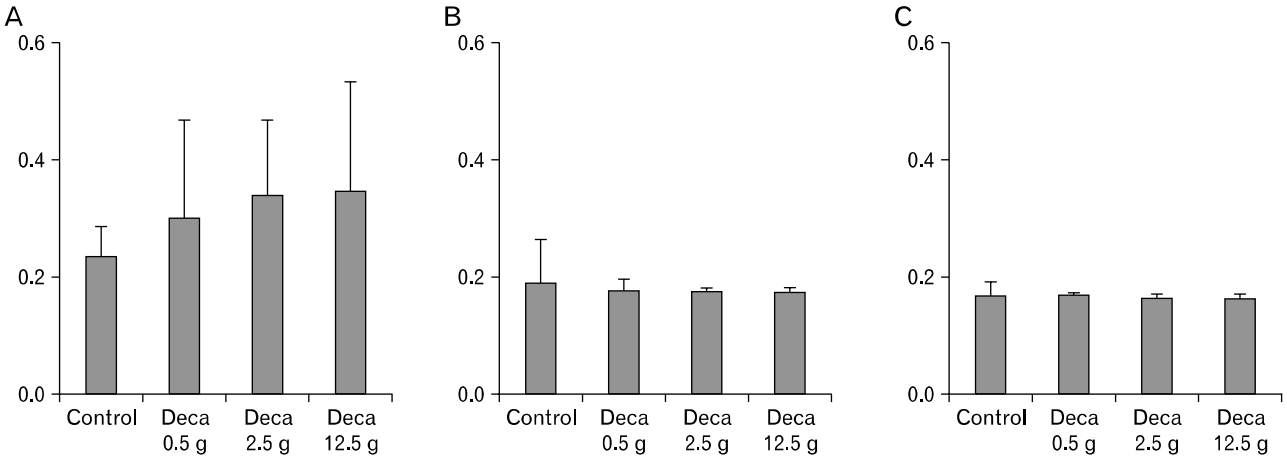

Figure 5. Effects of deca-BDE on splenic T cell proliferation responses. (A) Dam, (B) PND21, (C) PND63.

Lymphoproliferative response in splenocyte cultures

When spleen cells from penta-BDE exposed to mice were stimulated with ConA as a T lymphocyte mitogen, the proliferation of these cells was dose-dependent (Fig. 4). Penta-BDE high dose dams and PND63 showed significant T cell proliferation ($p<0.05$). But T cell proliferation in deca-BDE

treatment dams showed slight increase (Fig. 5).

Unlike T cell proliferation by ConA, there were no significantly changes of B cell proliferation by LPS between PBDEs treated dams, PND21 and PND63 (Fig. 6, 7).

Flow cytometry

Flow cytometric analysis of both splenocytes and thymocytes subpopulations was carried out to explore further the nature of the change in lymphoid organ weights in C57Bl/6J mice exposed to PBDEs from gestational day 6 to lactational day 21. Unlike dams and PND63, PND21 exposed to penta-BDE showed increase of total T cell population, CD3+/CD4+ T cell, and CD3+/CD8+ T cell and PND21 exposed to penta-BDE 100 mg/kg/day and 200 mg/kg/day were significant (Fig. 8~10). There were no significant differences between deca-BDE treatment groups.

B cell population was decreased in dams exposed to penta-BDE, but deca-BDE induced B cell population in spleen. PND21 showed B cell population to be decreased by PBDEs, but PND63 showed no significantly differences (Fig. 11). Deca-BDE showed significant decrease of CD11b+ macrophage population in PND21, but penta-BDE did not seem to be effective to dams and offsprings (Fig. 12).

Serum levels of antibodies

Total IgG1 was detected increasingly in dams administrated with PBDEs by deca-BDE rather than penta-BDE. However, PND21 exposed to penta-BDE showed decrease of IgG1 pro-

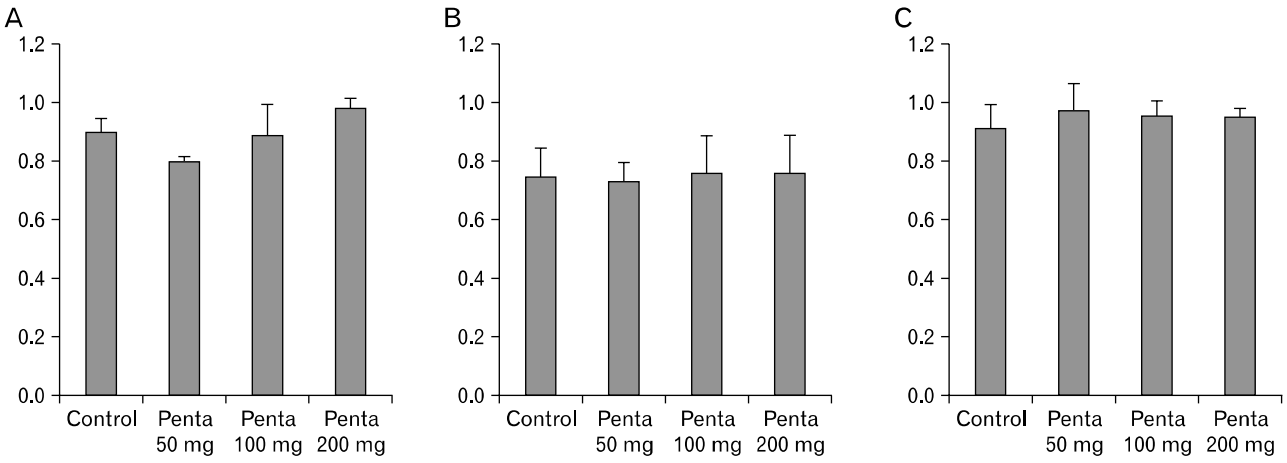

Figure 6. Effects of penta-BDE on splenic B cell proliferation responses. (A) Dam, (B) PND21, (C) PND63.

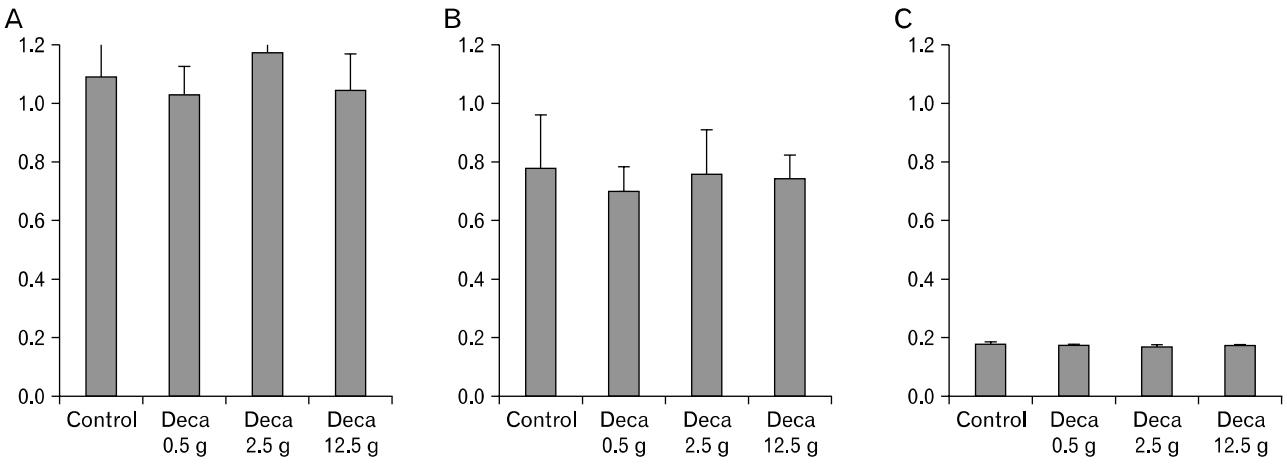

Figure 7. Effects of deca-BDE on splenic B cell proliferation responses. (A) Dam, (B) PND21, (C) PND63.

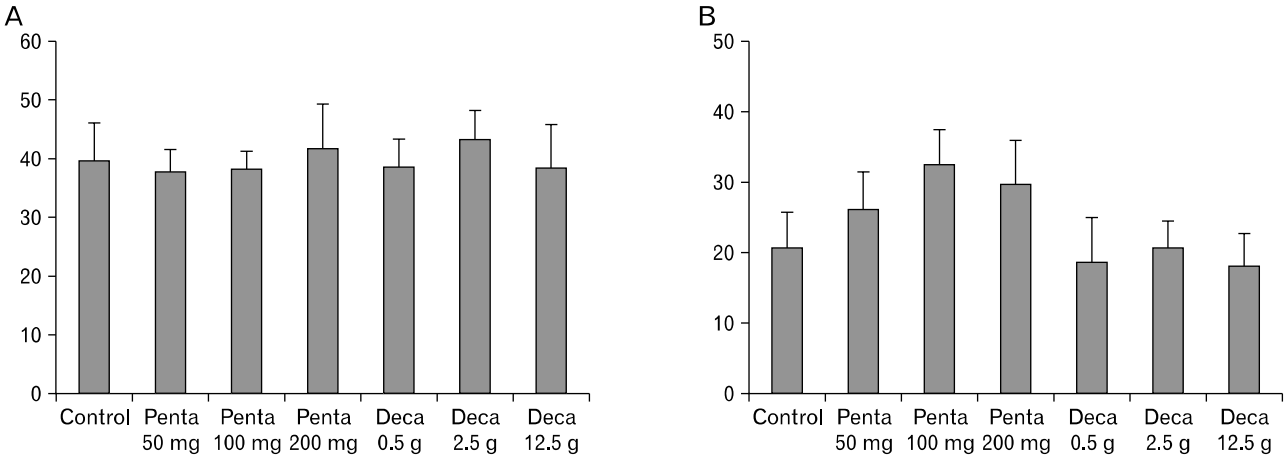

Figure 8. Effects on penta-BDE or deca-BDE on relative distribution of total T cell in spleen. (A) Dam, (B) PND21.

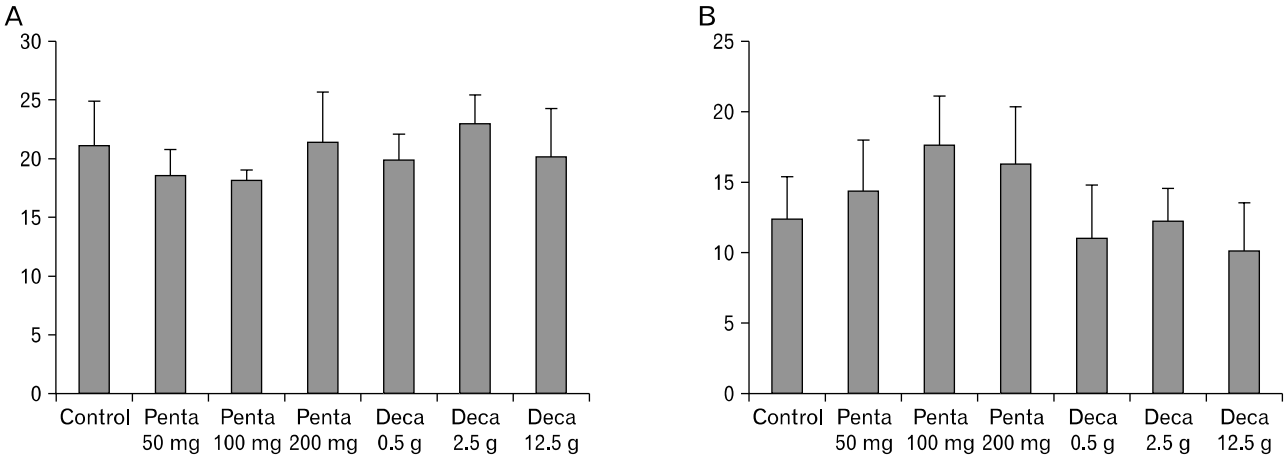

Figure 9. Effects on penta-BDE or deca-BDE on relative distribution of helper T cell subsets in spleen. (A) Dam, (B) PND21.

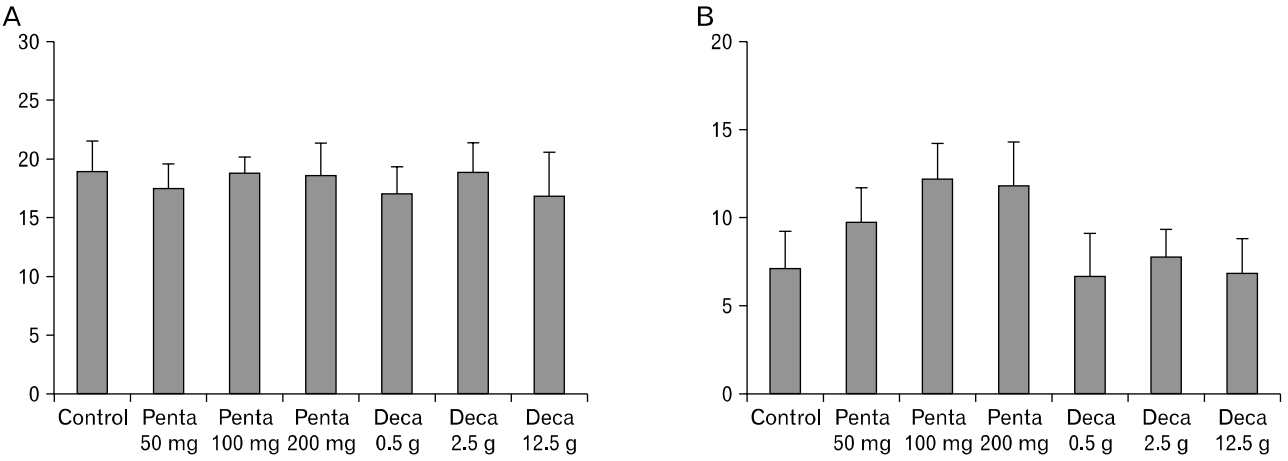

Figure 10. Effects on penta-BDE or deca-BDE on relative distribution of cytotoxic T cell subsets in spleen. (A) Dam, (B) PND21.

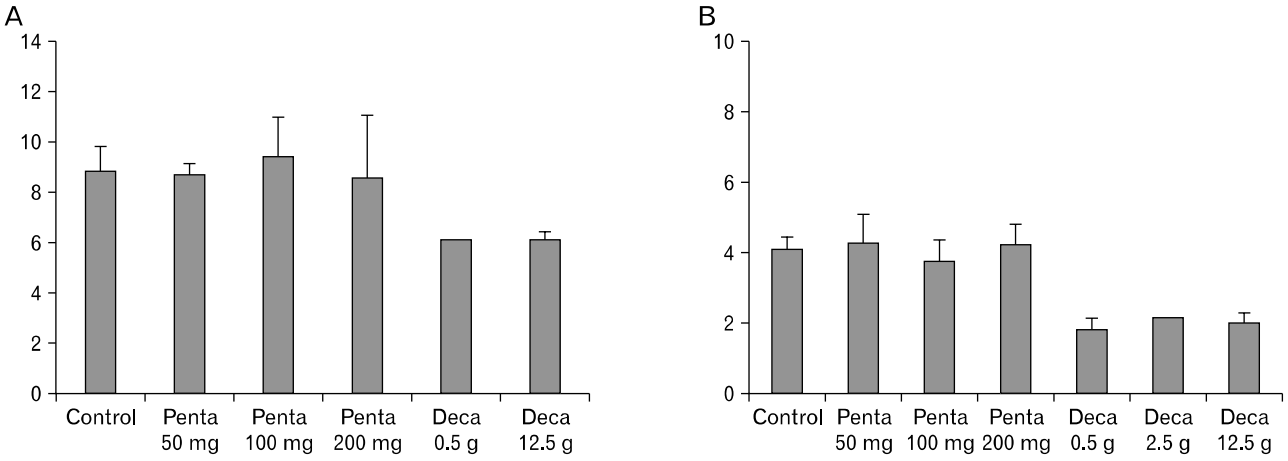

Figure 11. Effects on penta-BDE or deca-BDE on relative distribution of B cell in spleen. (A) Dam, (B) PND21.

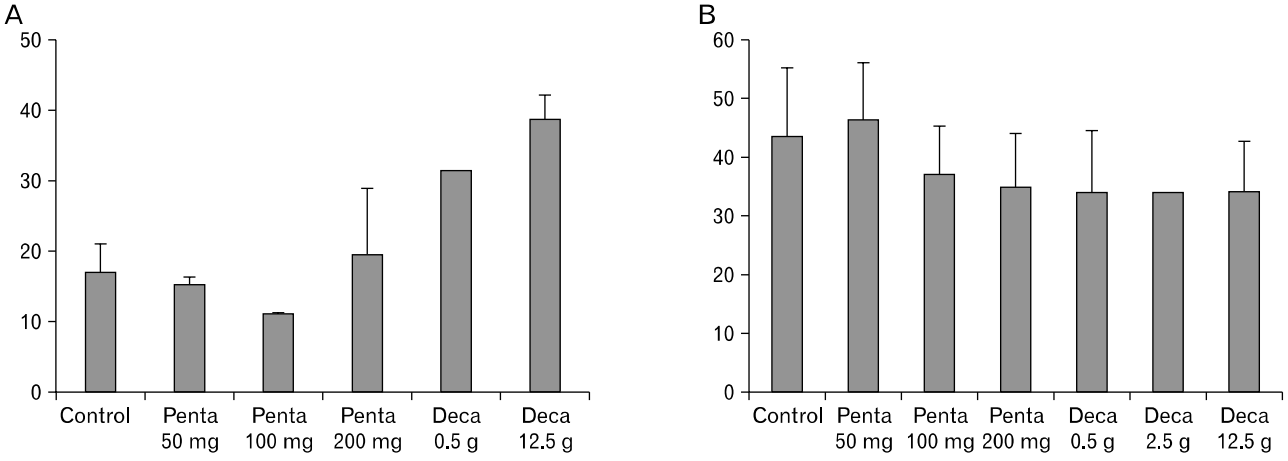

Figure 12. Effects on penta-BDE or deca-BDE on relative distribution of macrophage in spleen. (A) Dam, (B) PND21.

duction, and PND63 produced IgG1 similar to control (Fig. 13).

As the results of IgM level, all groups exposed to penta-BDE showed decrease of IgM production, but dams by deca-BDE produced more IgM than control (Fig. 14).

DISCUSSION

Polybrominated diphenyl ethers (PBDEs) have been used as a flame retardant. PBDEs have similar chemical structure to PCBs and dioxins with many similar chemical characteristics, and so have been suspected of being hazardous to humans, endocrine disruptors, etc (5-9). While penta-BDE has been banned in E.U. nations and in California because of greatest bioavailable concentrations in the environment (10-12), deca-BDE mixture makes up over 80% of the global PBDE market (13). Deca-BDE has extremely poor water solubility, and so precipitates from the water column and accumulates in sediments. Several studies of PBDEs monitoring in mammal and humans are being reported, and human breast milk samples extracted from women near Austin, Texas, had summed PBDE concentrations higher than 400 ng/g-lipid (14).

We evaluated the developmental immunotoxicity of penta-BDE and deca-DBDE in offspring mice indirectly exposed to PBDEs. Pregnant female C57BL/6J mice were orally administered penta-BDE, deca-BDE or corn oil for 5 weeks, from gestational day 6 to lactational day 21 to evaluation the developmental immunotoxicity of the offsprings.

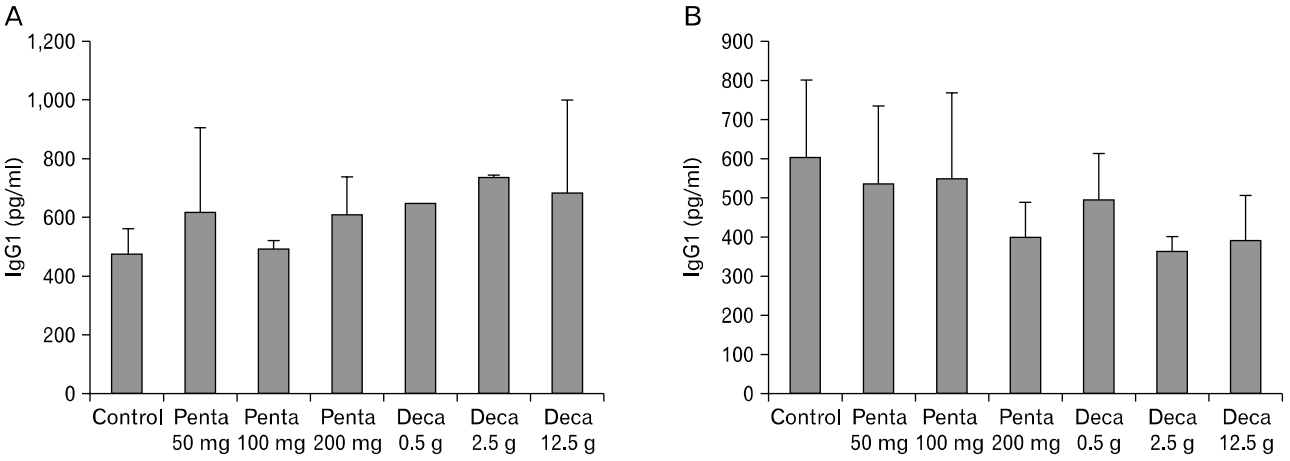

Figure 13. The change of serum IgG1 level in mice exposed to penta-BDE or deca-BDE. (A) Dam, (B) PND21.

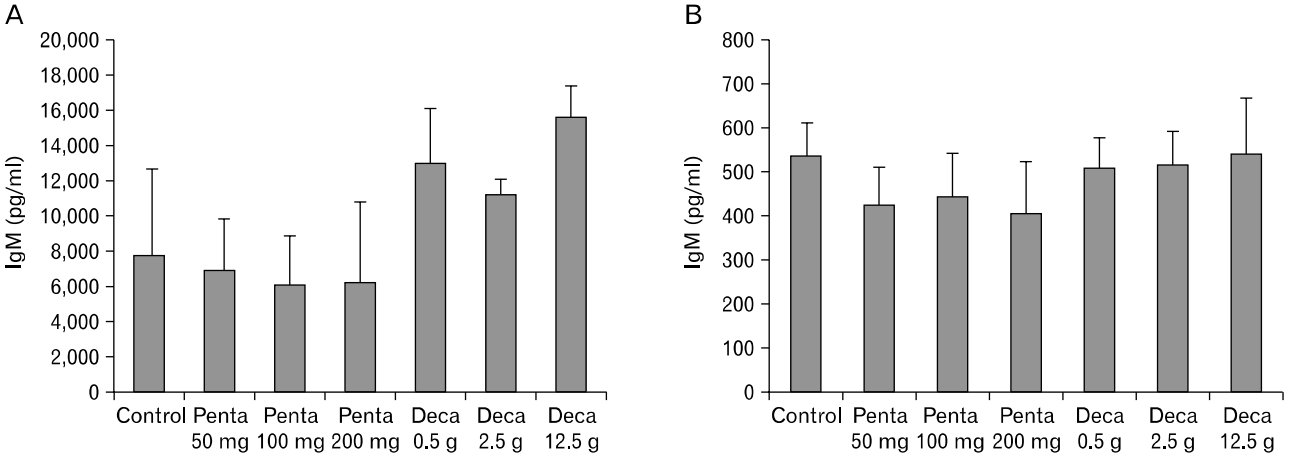

Figure 14. The change of serum IgM level in mice exposed to penta-BDE or deca-BDE. (A) Dam, (B) PND21.

An *in vivo* study using in C57BL/6J found only a moderate immune suppression at the longest duration of sub-chronic exposure of DE-71, a commercial penta-BDE mixture (15). In this study, dams and PND21 with penta-BDE had significantly smaller absolute and relative spleen and thymus masses than control mice. The relatively smaller spleens of mice exposed to penta-BDE may be due to compromised immune development. Yet no significant differences were found in dams and PND21 exposed to deca-BDE. The thymic atrophy and altered thymocyte cellularity are characteristic effects of halogenated aromatic hydrocarbons toxicity via the aryl hydrocarbon receptor. AhR seemed to be probably only minimally bound by PBDEs (16). In another immunotoxicity study, dosing human lymphocytes *in vitro* with two PBDE congeners, a tetra-BDE (BDE-47) and a penta-BDE (BDE-85), no significant differences were found that mitogen-induced proliferation and IgG synthesis were not affected by the PBDE (1). This study reveals that splenic T cell proliferation in dams and PND21 exposed to penta-BDE was increased, and there were no significant difference in splenic B cell proliferation in all treatment groups. The antibody (total IgG1 and IgM) production show no significant difference in all treatment groups relative to controls. In flow cytometric analyses of splenic lymphocytes, the PBDE exposure resulted in significant reductions in CD4+ and CD8+ T-cells. B-cells also showed similar reductions in the mouse tetra-BDE dose group only. PBDE congener equally suppressed murine splenic T- and B-cells (2). In this study, total T cell, Th cell and Tc cell,

only in PND21 exposed to penta-BDE, was slightly increased.

This study presented slightly the changes of immune systems in dams and F1 offsprings by PBDEs exposure, but significantly in PND21 exposed to penta-BDE. As the results, PBDE exposure at high enough levels may lead to immune impairment, especially in young age, but immune system of offsprings may be recovered as they grew. These results imply that PBDEs given to the dam were transferred to the offspring during gestation and lactation, and PBDEs transferred from the dam affect immune system of offspring.

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CONFLICTS OF INTEREST

The authors declare no financial or commercial conflicts of interest.

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