Increase in *in Utero* Exposure to a Migrant, 4,4'-Butylidenebis(6-*t*-butyl-*m*-cresol), from Nitrile-Butadiene Rubber Gloves on Brain Aromatase Activity in Male Rats

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4,4'-Butylidenebis(6-*t*-butyl-*m*-cresol) (BBBC) can be eluted from disposable gloves made of nitrile-butadiene rubber and possibly also detected in food. It has been reported that BBBC is an androgen and estrogen antagonist *in vitro*. Previously, BBBC (1.0 mg/kg body weight (bw)/d) was subcutaneously administered to pregnant rats from gestation days 11 through 18, and the effects on male offspring (postnatal day 102) were examined. Altered levels and turnover of the monoamines dopamine, serotonin, and noradrenalin as well as their metabolites were detected. This report measured the level of serum testosterone following prenatal exposure to BBBC (0.1, 1.0, 10 mg/kg bw/d) in male rats, and measured aromatase activity of the hypothalamus-preoptic area with a close connection to the sexual differentiation and sexual behavior of BBBC-treated rat brains. The serum testosterone level rose depending on exposure, and aromatase activity of the basomedial nucleus of amygdale region was increased in the BBBC-treated group compared with the control. These results suggested that prenatal exposure to BBBC affects the central nervous system of male rat offspring, and BBBC may be an endocrine disrupting-chemical during the fetal period, and might influence the functional development of the brain.

Key words nitrile-butadiene rubber glove; brain aromatase activity; amygdale region; testosterone; 4,4'-butylidenebis(6-*t*-butyl-*m*-cresol); endocrine-disrupting chemical

Disposable gloves made of nitrile-butadiene rubber (NBR) are used for contact with foodstuffs rather than polyvinylchloride (PVC) gloves containing di(2-ethylhexyl) phthalate (DEHP), because endocrine-disruptive effects are suspected for phthalate diesters including DEHP, a widely used plasticizer, which was detected in packed lunches for sale at convenience stores and in-patients' meals in hospitals.^{1,2)} The endocrine-disrupting effects of phthalate diesters (including DEHP) and phthalic acid monoesters, a metabolite of phthalic acid,³⁾ have been studied previously along with the *in vitro* androgenic and estrogenic effects of phthalate diesters and phthalic acid mono esters. Hormonal effects of DEHP and mono(2-ethylhexyl)phthalate, a mono ester of DEHP, were not observed⁴⁾ or were very weak.⁵⁾ However, as a result of the prohibition of PVC gloves, NBR gloves have been increasingly used for preparation of food. NBR gloves were rarely used in the food industry in Japan.^{1,2)} It is thought to be necessary to clarify the effect of chemicals that elutes from substitute from the viewpoint of preventing before a problem happens. Accordingly, 4,4'-butylidenebis(6-*t*-butyl-*m*-cresol) (BBBC, CAS No. 85-60-9, Fig. 1) can be eluted from disposable gloves made of NBR, and the amount of BBBC eluted from NBR gloves was reported to be 4.22 mg/cm².^{6,7)} Disposable gloves used during cooking or packaging are sprayed with 68% (w/w) ethanol for disinfection. Alcohol sprayed onto disposable gloves increased the level of chemicals eluted into foods by 8–41-

fold (possibly 33.8–173.0 mg/cm²).²⁾ The endocrine-disrupting effects of chemicals eluted from NBR gloves were examined using *in vitro* assays, and we reported that BBBC has the strongest androgen receptor (AR) and estrogen receptor (ER) antagonist activities (in the 10⁻⁶M range of IC₅₀ values), and has the strong cell toxicity.⁸⁾ The strength of these activities was about 5 times higher than that of a known AR antagonist, 1,1'-(2,2-dichloroethylidene)bis[4-chlorobenzene] (*p,p'*-DDE), and similar to that of bisphenol A (BPA), which has strong AR antagonist and ER agonist activity.^{9,10)} Previous studies have also examined hormonal effects using *in vivo* assays^{11,12)} on some of the chemicals that showed strong positive effects using *in vitro* assays.^{4,8–10,13–16)}

Endocrine disruptors (EDs) show adverse effects on development and/or function of the reproductive system and nervous system, particularly when exposure occurs in the fetus or neonates. Evidence indicates that certain behaviors are especially responsive to ED exposure during gestation, and that one index of susceptibility may be reflected in the differing responses of male and female offspring, a phenomenon termed sexual dimorphism.¹⁷⁾ The action mechanism of EDs in the central nervous system is complex. Endocrine disruption caused by ingestion of trace amounts of chemicals induces abnormal thyroid metabolism and inhibits functional development of the brain in children, and this may be a cause of mental retardation and behavioral abnormalities.^{17–19)} It is believed that the entrance of harmful substances into the brain is generally prevented by the blood–brain barrier. However, the blood–brain barrier is undeveloped in fetuses, and chemical substances enter the fetal brain *via* the placenta. In addition, many environmental chemical substances are lipid-soluble, which pass more easily through the blood–brain barrier. Such chemical substances accumulate in fat-rich breast milk, resulting in exposure of the neonatal brain to a relatively high concentration of chemical substances. Moreover,

Fig. 1. The Structure of 4,4'-Butylidenebis(6-*t*-butyl-*m*-cresol)

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the ability to metabolize and excrete these chemical substances is incomplete in infants because liver and kidney functions are undeveloped. Accordingly, a trace amount of chemical substances may markedly influence the endocrine and cranial nervous systems in the fetal period and infancy, and it has been suggested that impairment of the nervous system is very likely to result in irreversible conditions.²⁰⁾ As evidence of this effect, low IQ and memory and attention deficits in children born from mothers who ate a large amount of polychlorinated biphenyl (PCB)-containing fish have been reported.²¹⁾ Abnormal behavior after growth and influences on neurotransmitters in the brain, including dopamine (DA), serotonin (5-HT), and noradrenalin (NA), due to PCB exposure during the fetal period were also clarified in experimental animals in basic studies.^{22,23)} Mizuo *et al.*¹⁸⁾ reported neurobehavioral toxicity of BPA exposure during the fetal period and infancy. Based on this viewpoint, we have reported studies^{11,12)} on the influence of exposure to trace amounts of chemical substances during the rat fetal period (11–18 d of gestation, at which stage the monoamine neurotransmission system may develop) followed by feeding with milk from the exposed mother after birth.

NOAEL (non observed adverse effect level) and LOAEL (lowest observed adverse effect level) of BBBC have not been determined because *in vivo* studies examining BBBC-mediated endocrine disruption have not been attended now. We are investigating prenatal exposure to BBBC in rats to further clarify whether BBBC is an endocrine-disrupting chemical. When the effects of BBBC on the offspring of pregnant rats were examined using *in vivo* assays,^{11,12)} the absolute weights of rat brains exposed to 1.0 mg/kg body weight (bw)/d BBBC were significantly decreased, but the relative weights were not changed, and the change in absolute and relative brain weights with 0.1 mg/kg bw BBBC-treated rats were not significant.²⁴⁾ Furthermore, the levels of the monoamines were measured as well as their metabolites in the brains of exposed rats to clarify the influence of BBBC on the central nervous system.²⁴⁾ Altered levels and turnover of these three monoamines (DA, 5-HT, and NA) as well as their metabolites were detected. These results suggested that prenatal exposure to BBBC affects the central nervous system of male rat offspring, and BBBC may be an endocrine-disrupting chemical during the fetal period.

In this report, the level of serum testosterone following prenatal exposure to BBBC was measured in male rats, and testosterone levels were found to be higher than in control rats. From this result, it was attempted to measure aromatase activity in the hypothalamus-preoptic area (HPOA), which has a close connection to sexual differentiation and sexual behavior of BBBC-treated rat brains. Aromatase (an isoform of P450; CYP 19) is a key enzyme in the conversion of androgens to estrogens and has an important role in maintaining a homeostatic balance between androgen and estrogen.

MATERIALS AND METHODS

Reagents BBBC was purchased from Tokyo Kasei Industry, Co., Ltd. (Tokyo, Japan, purity: >97% by HPLC).⁸⁾ An estrone enzyme immunoassay (EIA) kit was purchased from Cosmo Bio. Co., Ltd. (Tokyo, Japan). 4-Hydroxy-androstene-3,17-dione, phenylmethylsulfonyl fluoride (PMSF),

and β -nicotinamide-adenine dinucleotide phosphate (NADPH) were purchased from Sigma (St. Louis, MO, U.S.A.). Other reagents were of the highest grade available commercially.

Animals and Experimental Protocol Pregnant, specific-pathogen-free Sprague-Dawley rats (9 weeks old, $n=32$) purchased from Charles River Japan (Kanagawa, Japan) were received on day 7 of gestation and housed individually with woodchip bedding (Clea-chip, CLEA Japan Inc., Tokyo, Japan). All animals were maintained under controlled conditions at 22–24 °C, 45–65% humidity, 12-h lighting, and 10 ventilations per hour. The animals were given free access to general food (CE-2, Clea Japan Inc., Tokyo, Japan) and water. Observation of the general condition and measurements of body weight were performed daily. All animal studies were performed in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals.

The experimental protocol was determined in accordance with the previous paper.²⁴⁾ After an acclimatization period of 4 d, rats were divided into three groups. BBBC was dissolved in ethanol, and this solution was diluted with saline (1% ethanol solution). BBBC ($n=8$; 0.1, 1.0, and 10 mg/kg bw/d saline solution at a volume of 1.0 ml/100 g bw; respectively) or 1% ethanol-saline solution at a volume of 1.0 ml/100 g bw ($n=8$, control) was administered subcutaneously to pregnant rats once a day for 8 d from gestation days 11 through 18 (during which the monoamine nervous systems develop and mature). Four male rat offspring born from one mother were selected at random and kept with the litters until weaning (0.1, 1.0, and 10 mg/kg bw BBBC-treated rats $n=32$, respectively; control rats, $n=32$). Aromatase activity in the HPOA of the male was higher than that of the female, and because aromatase activity was too low for the female after the PND 21, it was not possible to measure it.²⁵⁾ Therefore, only the male was executed.

Male rat offspring were housed individually in stainless cages from postnatal day (PND) 25 (day of birth=PND 0). The body weight of male rat offspring was recorded at frequent intervals until the terminal day. Male rat offspring were sacrificed on PND 102 (after maturation) as described previously²⁴⁾ under ether anesthesia. After blood collection, brains were removed, weighed, and stored at –80 °C until measurement.

Measurement of Serum Testosterone Level Serum was preserved at –80 °C until measurement. The serum concentration of testosterone was measured using an EIA kit (Cayman Chemicals; Ann Arbor, MI, U.S.A.). This measurement was in accordance with the method reported previously.¹²⁾

Preparation of Homogenates The brains, stored at –80 °C, of the control and 1.0 and 10 mg/kg bw BBBC-treated groups were kept at –20 °C for about 1 h, and 800 μ m-thick coronal sections were prepared using a cryostat. Brain regions including the bed nucleus of the stria terminalis (BNST), preoptic area (PA), anterior hypothalamic nucleus (AHN), basomedial nucleus of amygdala (BNA), and ventromedial hypothalamic nucleus (VHN), were dissected out from frozen brains with a brain blocker. Dissected tissues were stored at –80 °C until use. These tissues (BNST, PA, AHN, BNA, and VHN) were homogenized in 150, 120, 120, 300, and 150 μ l of steroidogenic buffer (20 mM *N*-tris(hydroxymethyl)methyl-2-aminoethane-sulfonic acid (TES), 10 mM ethylenediaminetetraacetic acid (EDTA),

150 mM KCl, 0.1 mM PMSF, pH 7.3), respectively, and protein levels were determined according to the method of Bradford.²⁶⁾

Aromatase Assay Aromatase levels were determined in accordance with the method reported previously.²⁷⁾ Briefly, a reaction mixture contained 20 μ l of homogenate, 10 mM NADPH, 200 nM 4-hydroxy-androstene-3,17-dione, and 50 mM phosphate buffer (pH 7.5) at a final incubation volume of 120 μ l. Incubations were performed at 37 °C for 30 min, and were terminated by heating at 100 °C for 10 min.

Measurement of Aromatase Activity One hundred microliters of the reaction mixture was used as the sample for analysis using an estrone EIA kit as described previously.²⁷⁾ Briefly, goat anti-rabbit immunoglobulin G (IgG)-coated 96 well microplate was washed using a wash solution, and then a solution of biotin-labeled estrone, estrone standard or chemicals, and estrone-specific antibody were reacted at 4 °C overnight (17–19 h). After aspirating the solution and washing all wells 3 times with a wash solution, and the substrate solution was added into all wells at 25 °C for 2 h. After the fourth wash, a solution of the *o*-phenylenediamine was added at 25 °C for 20 min, and then stop solution was added into all wells. The developed color was read at 450 nm using a micro-titer spectrophotometer (SunriseRemote, TECAN, Copenhagen, Denmark), and the estrone concentration was calculated based on a standard curve (eight estrone concentrations; 0, 1.2, 4.8, 19.5, 78.1, 312.5, 1250, and 5000 pg/ml) using the manufacturer's software.

Statistical Analysis All statistical analysis and IC₅₀ values were determined using KaleidaGraph ver. 4 software (Synergy Software, PA, U.S.A.). Differences between groups were assessed for statistical significance by Student's *t*-test. Differences with $p < 0.05$ were considered significant. Data are expressed as means $\pm$ S.D.

RESULTS

General Condition There was no difference in the birth number per mother, and no effect on the male/female ratio was found for BBBC. The treatments induced no maternal toxicity. No abnormal behavior or appearance or diarrhea was noted at PND 102 in any of the control or BBBC treatment groups (0.1, 1.0 and 10 mg/kg bw), and all animals survived until completion of administration. There were no differences in liver or kidney weight or the histological changes between BBBC-treated rats and control rats at PND 102. Differences in serum levels of aspartate aminotransferase, alanine aminotransferase, cholesterol, triglyceride, and glucose *etc.* were not observed. There was no effect on 1% ethanol-saline treated rat.¹²⁾

Table 1. Body Weights in Male Rat Offspring Prenatally Exposed to BBBC

Dose of BBBC (mg/kg bw/d)	<i>n</i> ^{a)}	Body weight (g)							
		PND ^{b)} 2	PND 5	PND 10	PND 13	PND 17	PND 20	PND 24	PND 102
0	32	8.2 $\pm$ 0.7	13.9 $\pm$ 1.4	26.1 $\pm$ 2.7	34.6 $\pm$ 3.1	44.5 $\pm$ 3.4	54.9 $\pm$ 6.2	76.3 $\pm$ 7.4	531.9 $\pm$ 26.6
0.1	32	8.2 $\pm$ 0.8	14.3 $\pm$ 1.2	26.1 $\pm$ 2.1	33.9 $\pm$ 2.4	43.7 $\pm$ 2.8	56.4 $\pm$ 3.7	79.1 $\pm$ 5.3	529.9 $\pm$ 41.6
1.0	32	7.8 $\pm$ 0.7*	13.6 $\pm$ 1.0	25.5 $\pm$ 1.9	33.2 $\pm$ 2.3*	42.4 $\pm$ 3.1**	53.7 $\pm$ 4.0	74.1 $\pm$ 3.9	525.9 $\pm$ 43.2
10	32	7.7 $\pm$ 0.9*	13.2 $\pm$ 1.6	24.8 $\pm$ 2.6*	31.7 $\pm$ 2.8**	40.5 $\pm$ 3.0**	51.7 $\pm$ 3.8*	70.9 $\pm$ 4.7**	519.0 $\pm$ 44.1

BBBC (0.1, 1.0, and 10 mg/kg bw) was administered subcutaneously to pregnant SD rats as described in Materials and Methods. The control group (0 mg/kg bw) received 1% ethanol in saline. *a)* Number of animals; *b)* PND: postnatal day (day of birth=PND 0). Each value is the mean $\pm$ S.D. Significantly different (* $p < 0.05$, ** $p < 0.01$) from the control.

Body Weight Body weights were measured during the PND 2–102 period (Table 1). Significant differences in body weight in the 0, 0.1, 1.0, and 10 mg/kg bw BBBC-treated group at PND 102 were not observed. However, the body weight at lactation decreased in the 1.0 and 10 mg/kg bw BBBC-treated groups. In the 1.0 mg/kg bw BBBC-treated group significant decreases were observed at PND 2, 13, and 17 compared with the corresponding control values. In the 10 mg/kg bw BBBC-treated group, significant decreases in body weight were observed at PND 2, 10, 13, 17, 20, and 24 compared with the corresponding control values. Significant differences in body weight after delactation were not observed. Body weights in the 0.1 mg/kg bw BBBC-treated group during PND 2–102 were not changed.

Brain Weight Significant decreases ($p = 0.01$) in brain absolute weights from 2.25 g (control, $n = 32$) to 2.19 g (exposed to 1.0 mg/kg bw BBBC, $n = 32$) were observed in rat offspring prenatally exposed to BBBC (data not shown). However, the relative weights per 100 g bw were not changed (1.0 mg/kg bw BBBC-treated: 419 mg, control: 423 mg). Differences in the brain absolute and relative weights between the 0.1 and 10 mg/kg bw BBBC-treated groups were not observed.

Serum Testosterone Levels The serum testosterone levels in male rats were measured at PND 102 (Fig. 2). The serum testosterone level was significantly higher in the 1.0 mg/kg bw (6.08 $\pm$ 2.96 ng/ml) and 10 mg/kg bw (7.09 $\pm$ 4.72 ng/ml) BBBC-treated groups than in the control group (2.79 $\pm$ 1.31 ng/ml). No significant differences between the 0.1 mg/kg bw BBBC-treated group (3.49 $\pm$ 1.26 ng/ml) and the control group were noted.

Fig. 2. Effects of BBBC Treatment on Serum Testosterone Levels in Male Rats

BBBC (0.1, 1.0, and 10 mg/kg bw) was administered subcutaneously to pregnant SD rats as described in Materials and Methods. The control group received 1% ethanol in saline. Bars denote S.D. $n = 12$. * Significantly different ($p < 0.01$) from the control.

Table 2. Effects of BBBC Treatment on Aromatase Activity in Male Rat Brain

Brain region	Aromatase activity (pg/mg protein)			
	Control (n^a)=6)	1.0 mg BBBC (n =6)	Control (n =9)	10 mg BBBC (n =12)
Bed nucleus of stria terminalis	23.40±14.51	22.27±9.53	22.28±11.05	29.15±12.60
Preoptic area	51.55±29.77	61.41±26.61	57.04±16.99	46.04±15.28
Anterior hypothalamic nucleus	76.26±57.04	82.26±48.41	68.23±16.67	55.27±12.05
Basomedial nucleus of amygdale	47.00±26.57	106.18±47.83*	50.03±10.70	73.81±22.26*
Ventromedial hypothalamic nucleus	20.71±9.83	19.31±7.26	19.35±6.26	20.18±7.68

BBBC (1.0 and 10 mg/kg bw) was administered subcutaneously to pregnant SD rats as described in Materials and Methods. The control group received 1% ethanol in saline. a) Number of animals. Each value is the mean±S.D. * Significantly different ($p<0.01$) from the control.

Effects of BBBC on Brain Aromatase Activity The brain aromatase activity was measured at the same time by EIA and radioisotope (RI) methods, and the values obtained were compared between each other as well as with rat ovarian microsomes. The values obtained using the EIA and RI methods were positively correlated, with a Pearson's correlation coefficient of 0.91 ($p<0.01$) (data not shown). Aromatase activities were measured in the BNST, PA, AHN, BNA, and VHN regions of rat brain of the 1.0 and 10 mg/kg bw BBBC-treated groups (Table 2). In the BNA region, aromatase activities were significantly increased in the 1.0 and 10 mg/kg bw BBBC-treated groups compared with the control group. In the BNST, PA, AHN, and VHN regions, no differences were observed between the two groups.

DISCUSSION

It was reported previously that BBBC exhibited a stronger anti-androgen effect than p,p' -DDE and an anti-estrogen effect comparative to that of BPA in an *in vitro* study.⁸⁾ Because the actions of chemicals suspected be EDs are not limited to the reproductive system, and hormone receptors are abundantly localized in the cranial nervous system, serious influences of environmental chemical substances on the functional development of the brain, sexual differentiation, and behavioral development are of concern.¹⁹⁾ We have reported studies^{11,12)} on the influence of exposure to trace amounts of chemical substances during the rat fetal period followed by feeding with milk from the exposed mother after birth. Rat fetuses were exposed to BBBC (1.0 mg/kg bw) using this *in vivo* system, and decreased brain absolute weight was found at PND 102, as well as altered levels of neurotransmitters, their metabolites, and their metabolic rate in the brain.²⁴⁾

In this study, fetuses were exposed to 10 mg/kg bw BBBC, 10 times higher than the dose that influenced brain weight, using the *in vivo* system, similarly to that in the previous report.²⁴⁾ No abnormality during pregnancy or changes in the weights of main organs, including the brain, macroscopic observations, or serology/biochemistry were noted, but the body weight during the nursing period was significantly lower in animals exposed to 1.0 mg/kg BBBC than those in the control group. Measurement of the serum testosterone level showed a significant increase in a manner dependent on the dose of BBBC. Based on these findings, it was assumed that aromatase, an enzyme converting androgen to estrogen already expressed in the fetal brain, was affected. Because androgen promotes aromatase expression in the brain, when the blood androgen level rises, aromatization occurs in

brain cells and increases the estrogen level (aromatase hypothesis).^{28,29)} BNST, PA, AHN, BNA, and VHN of the HPOA are reportedly closely related to sexual differentiation and behavior, and the express aromatase at a high level.^{30,31)} The aromatase activity level in these regions was measured in the groups treated with 1.0 and 10 mg/kg bw BBBC, in which the serum testosterone level was increased significantly. A sensitive EIA²⁷⁾ for aromatase activity measurement was employed, rather than the conventional method using RI,³²⁾ by which a very low amount in individual brain regions, not the entire HPOA, could be measured.

Considering that letrozole (aromatase inhibitor) has been reported to significantly elevate the serum testosterone level in healthy adult men,³³⁾ the finding that the serum testosterone level rose in male rats in a manner dependent on the fetal BBBC exposure level suggests that BBBC acted as an aromatase inhibitor *in vivo*. In addition, the brain aromatase activity level rose significantly as expected based on the aromatase hypothesis. Furthermore, the fact that the activity level rose in the BNA indicates that the amygdale³⁴⁾ is not only involved in the sexual differentiation of the brain and reproductive behavior but also responsible for the regulation of endocrine function, memory, cognition, and emotional behavior, and all kinds of information, such as auditory and visual sensations, are collected in this region. Considering the previously reported changes in neurotransmitters in the brain, BBBC exposure during the fetal period may impair the nervous system similarly to PCB and BPA. The incidence of developmental disorders of the brain in children, such as abnormal behavior, attention-deficit hyperactivity disorder, and autism, has increased recently, and environmental chemical substances are considered to influence intelligence and behavior in children, *i.e.*, functional development of the brain may be affected.¹⁹⁾ Although it was unclear why the absolute brain weight was altered only at a BBBC exposure dose of 1.0 mg/kg, it was suggested that BBBC exposure during the fetal period may influence functional development of the brain and subsequently behavior. Thus, it may be necessary to investigate behavior at PND 102, aromatase activity and testosterone level of the brain during the fetal period, and integrative function of the brain during the nursing period, in which the body weight was significantly lower. Furthermore, based on a report in which the level of thyroid hormone, which influences intellectual development of the brain, was altered in animals with reduced behavior and memorizing ability following PCB exposure during the fetal period,³⁵⁾ measurements of thyroid-related hormones in sera of animals exposed to BBBC during the fetal period are currently being

performed.

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REFERENCES

- 1) Tsumura Y., Ishimitsu S., Nakamura Y., Yoshii K., Kaihara A., Tono-gai Y., *Shokuhin Eiseigaku Zasshi*, **42**, 128—132 (2001).
- 2) Tsumura Y., Ishimitsu S., Kaihara A., Yoshii K., Nakamura Y., Tono-gai Y., *Food Addit. Contam.*, **18**, 569—579 (2001).
- 3) Eigenberg D. A., Bozigian H. P., Carter D. E., Sipes I. G., *J. Toxicol. Environ. Health*, **17**, 445—456 (1986).
- 4) Satoh K., Nonaka R., Ikeda M., Satoh T., Kamimura H., Nagai F., *Ann. Rep. Tokyo Metro. Inst. Pub. Health*, **55**, 307—314 (2004).
- 5) Takeuchi S., Iida M., Kobayashi S., Matsuda T., Kojima H., *Toxicology*, **210**, 223—233 (2005).
- 6) Mutsuga M., Wakui C., Kawamura Y., Maitani T., *Food Addit. Con-tam.*, **19**, 1097—1103 (2002).
- 7) Mutsuga M., Kawamura Y., Waku C., Maitani T., *Shokuhin Eiseigaku Zasshi*, **44**, 103—109 (2003).
- 8) Satoh K., Nonaka R., Ohyama K., Nagai F., Ogata A., Iida M., *Biol. Pharm. Bull.*, **31**, 375—379 (2008).
- 9) Satoh K., Ohyama K., Aoki N., Iida M., Nagai F., *Food Chem. Toxicol.*, **42**, 983—993 (2004).
- 10) Satoh K., Nonaka R., Ohyama K., Nagai F., *J. Health Sci.*, **51**, 557—568 (2005).
- 11) Sakamoto Y., Satoh K., Ando H., Kubo Y., Nagasawa A., Yano N., Yuzawa K., Takahashi H., Ogata A., Kamimura H., *Ann. Rep. Tokyo Metro. Inst. Pub. Health*, **56**, 343—346 (2005).
- 12) Ohyama K., Satoh K., Sakamoto Y., Ogata A., Nagai F., *Exp. Biol. Med.*, **232**, 301—308 (2007).
- 13) Satoh K., Nagai F., Aoki N., Nishijima M., *J. Pharm. Soc. Japan*, **120**, 1429—1433 (2000).
- 14) Satoh K., Nagai F., Aoki N., *J. Health Sci.*, **47**, 495—501 (2001).
- 15) Satoh K., Nagai F., *Ann. Rep. Tokyo Metro. Inst. Pub. Health*, **53**, 265—267 (2002).
- 16) Satoh K., *FFI. J.*, **212**, 398—414 (2007).
- 17) Weiss B., *Environ. Health Perspect.*, **110** (Suppl.), 387—391 (2002).
- 18) Mizuo K., Narita M., Suzuki T., *Brain Sci.*, **25**, 1133—1140 (2003).
- 19) Kurado J., *Sci. J. Kagaku*, **73**, 1234—1243 (2003).
- 20) Kawahara M., Kuroda Y., *Igaku No Ayumi*, **201**, 159—161 (2002).
- 21) Jacobson J. L., Jacobson S. W., *N. Engl. J. Med.*, **335**, 783—789 (1996).
- 22) Schantz S. L., Seo B. W., Wong P. W., Pessah I. N., *Neurotoxicology*, **18**, 457—467 (1997).
- 23) Seegal R. F., Bush B., Brosch K. O., *Toxicology*, **86**, 71—87 (1994).
- 24) Satoh K., Nonaka R., Ohashi N., Shimizu M., Oshio S., Takeda K., *Biol. Pharm. Bull.*, **31**, 2211—2215 (2008).
- 25) Takahashi Y., *Kitakanto-Igaku*, **38**, 449—464 (1988).
- 26) Bradford M. M., *Anal. Biochem.*, **72**, 248—254 (1976).
- 27) Satoh K., Nonaka R., Ishikawa F., Ogata A., Nagai F., *Biol. Pharm. Bull.*, **31**, 357—362 (2008).
- 28) Roselli C., Klosterman S. A., *Endocrinology*, **139**, 3193—3201 (1998).
- 29) Baum M. J., Tobet S. A., *Physiol. Behav.*, **37**, 111—1118 (1986).
- 30) Balthazart J., Foidart A., Harada N., *Brain Res.*, **122**, 137—148 (1990).
- 31) Balthazart J., Foidart A., Surlemont C., Harada N., *Cell Tissue Res.*, **263**, 71—79 (1991).
- 32) Satoh K., Sakamoto Y., Ogata A., Nagai F., Mikuriya H., Numazawa M., Yamada K., Aoki N., *Food Chem. Toxicol.*, **40**, 925—933 (2002).
- 33) Raven G., Jong F. H., Kaufman J. M., Ronde W., *J. Clin. Endocrinol. Metab.*, **91**, 3324—3328 (2006).
- 34) Sakuma Y., *Clin. Neurosci.*, **5**, 166—167 (1987).
- 35) Shishiba Y., *Igaku No Ayumi*, **201**, 127—132 (2002).