Hazard assessment of Polybrominated biphenyls

[Polybrominated biphenyls, PBB, CAS No. 67774-32-7, 59536-65-1, etc]

Some polybrominated biphenyls (PBB) have 1 - 10 bromine atoms. Totally 209 isomers are present by number and substition position of bromine atoms.

Amount of Production/ import : The manufacture and import of PBB are not conducted in Japan at present.

Usage : PBB was used as a flame retardant of ABS resin, polyurethane foam, etc.[1)].

1) HSDB, 2001.

The PBB compounds in this report are shown in the Attachment-1 at the end of this report. As an example of physicochemical properties, those of 2,4,5,2',4',5'-hexabromobiphenyl (CAS No.: 59080-40-9) are stated here.

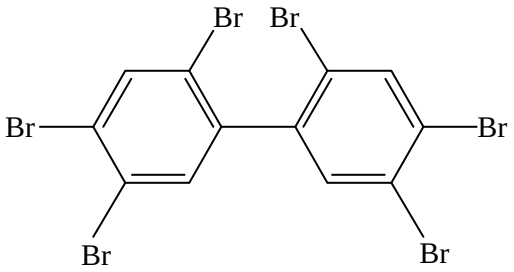
Chemical name : 2,4,5,2',4',5'-hexabromobiphenyl

Synonyms : HBB

Molecular formula : $C_{12}H_4Br_6$

Molecular weight : 627.59

Structural formula :



Appearance : White solid[1)]

Melting point : 72°C[1)]

Boiling point : No Report

Specific gravity : 2.6 g/cm^3 (25°C)[1)]

Vapor pressure	: 0.01 Pa (90°C)[1)]
Partition coefficient	: Log Pow = < 7 (calculated value)[1)]
Degradability	: Hydrolyzability: No Report
	Biodegradability: No Report
Solubility	: Water, 11 μg/*l* (25°C)[1)]
	Soluble in acetone, benzene[1)]
Applied laws and regulations	: Industrial Safety and Health Law

1. Toxicity Data

1) Information on adverse effects on human health

Polybrominated biphenyls (PBB) have a large number of structural isomers. Fire Master BP-6 (FM BP-6) and Fire Master FF-1 (FM FF-1) are mixtures of hexabromobiphenyl (HBB) and other PCB with bromine atoms of 5 - 7. Octabromobiphenyl (OBB) and decabromobiphenyl (DBB) having the bromine atoms of 8 and 10 respectively were also manufactured and distributed. The PBB compounds described in this report are shown in attachment-1, and the compositions of PBB mixtures that were tested are shown in attachment-2 if these compositions are stated in the literature.

The information on harmful nature of PBB mostly consist of the reports on PBB with the bromine atoms of 6 - 10 including FM BP-6 and FM FF-1. A huge amount of reports have been published after the pollution acident that occurred in Michigan, USA in 1973. IPCS/WHO published Environmental Health Criteria (EHC) in 1994 as an assessment of PBB.

The IPCS report (1994) deals with possible contamination with PBB in general population as well as with workers' exposure. The following aspects regarding effects of PBB are included in the Document: effect on skin, liver function, porphyria, thyroid gland, reproductive system, spermatogenesis, children's disease, neurology and psychiatric diseases, and immune system. Since no fuerther efforts of collection and evaluation of additional information have been made in the present documentation, simply summariging IPCS report, the summary of the present report is not included in this

English version. Therefore, the readers of this documents are kindly advised to refer to the EHC of IPCS (1994) for the detail of the above aspects.

Carcinogenicity

A cohort epidemiologic research was conducted in 3500 male employees who had worked in a PBB manufacturing plant from 1935 to 1976 and were possibly exposed to bromine compounds including PBB. Because of lack of quantitative data, those exposed to PBB were classified into 2 categories of "daily exposure" and "non-daily exposure". No death occurred in 91 workers in the former, but 2 of 237 classified in the latter died. It was reported that the cause of death in one of them was cancer of large intestine (IARC, 1986). Other than this report, there is no report on carcinogenicity of PBB in humans.

As a relevant report, there is a report on tumor marker determination. When the antibody titer of carcinoembryonic antigen (CEA) was compared among 611 farmers in Michigan and 138 farmers in Wisconsin, the ratio of high antibody titer cases was slightly higher in the Michigan group, but there was no statistically significant difference. Though correlation was observed between serum PBB concentration and CEA antibody titer, it is considered by the author that there were other factors to increase CEA antibody titer and that exposure to PBB was simply an additional influence (IPCS, 1994).

The carcinogenicity of PBB was classified as group 2B (possibly carcinogenic to humans) by IARC (IARC, 1987).

2) Information on endocrine system and reproductive system

(1) *in vitro* test result related to receptor binding (Attachment-3)

p,p'-Dibromobiphenyl (CAS: 92-86-4) did not demonstrate affinity to human estrogen α receptor (ERα) up to 10^{-4} M in receptor binding test (CERI, 2001). According to reporter gene assay, transciption activity mediated by ERα and dependent on estrogen responding sequence (ERE) was not observed within the range of 10^{-11} - 10^{-5} M (CERI, 2001).

(2) *in vivo* test results in mammals (Attachment-4)

(Effect on thyroid gland)

Oral administration of Fire Master FF-1 (FM FF-1) at 0, 0.3, 1.0, 3.0 and 10 mg/kg/day was conducted in male and female F344/N rats (7 - 8 weeks old) for 6 months, a dose-dependent decrease in serum triiodothyronine (T3) and thyroxine (T4) was observed. T4 value significantly decreased in males at ≥ 0.3 mg/kg/day and in females at ≥ 1 mg/kg/day. The extent of decrease in T3 level was slightly smaller than in T4 level, and statistically significant difference was noted only in females at 3 and10 mg/kg/day group. The changes were assumed to be attributable to enhanced T3 and T4 excretion (Gupta et al., 1983a).

When FM FF-1 at 0, 1, 3 and 6 mg/kg/day was administered to male SD rats for 10 days or 20 days by gavage, the time course and dose-dependent decrease in plasma T4 value was observed. After 20 days of administration, decrease in plasma T4 level as well as increase in thyroid stimulating hormone (TSH) were noted. Furthermore, ^{131}I uptake and increase in iodide were noted in thyroid gland at 6 mg/kg/day group (Allen-Rowlands et al., 1981).

Hexabromobiphenyl (HBB) at 0, 1, 5, 10 and 50 ppm was mixed in food and administered to female SD rats for 7 months, Decrease in serum T3 and T4 levels occurred due to direct action on thyroid gland (Sepkovic & Byrne, 1984: Byrne et al., 1987), but no decrease in serum T3 level was noted after administration of octabromobiphenyl (OBB) at 0 and 50 ppm mixed in food (Sepkovic & Byrne, 1984).

Single intraperitoneal administration of 3,3',4,4',5,5'-HBB was conducted at 0, 20 and 40 mg/kg/day in male juvenile Wistar rats and these rats were observed for 28 days. The serum T4 level decreased dose-dependently, but no change occurred in the T3 level (Spear et al., 1990). The decrease in blood thyroid hormone level was reported in swine as well: Decrease in serum T3 and T4 levels at 0, 10, 100, 200 ppm of Fire Master BP-6 (FM BP-6) given to dams in the later half of gestation period and lactation period (4 weeks) and also in their offsprings (Werner & Sleight, 1981).

(Effect on gonad system and adrenal cortex)

The following reports have been made on the effects of PBB on sex hormone and adrenal cortex hormone.

FM BP-6 at 0, 1, 10 and 100 ppm in diet was given to female Balb/c mice (6 weeks old) for 24 days or 30 days. A slight increase in plasma corticosterone level was observed

at 100 ppm (Fraker, 1980).

When Female SD rats were given diet containing FM BP-6 at 0 and 100 ppm from day 8 of gestation to 9 weeks old, no change was observed in plasma luteinizing hormone (LH), prolactin and corticosterone level(Johston et al., 1980).

FM FF-1 at 0, 1, 3 and 6 mg/kg/day was administered to male SD rats for 20 days. No changes were noted in plasma corticosterone and testosterone levels, but decrease in plasma prolactin level was noted at 6 mg/kg/day (Castracane et al., 1982).

FM BP-6 at low doses (1, 5, 10 and 50 ppm) was mixed in diet and given to female SD rats for 5 to 7 months. A dose-dependent decrease in serum corticosterone level as well as decrease in dehydroepiandrosterone level was noted (Byrne et al., 1988).

Female rhesus monkeys were given diet containing 0.3 ppm of FM FF-1 for 7 months (total dose of about 10 mg/monkey). Prolonged menstruation cycle and decreased serum progesterone level were reported (Allen et al., 1978: Lambrecht etr al., 1978).

(Effect on reproduction & development)

PBB mixutre (FM BP-6) at 0, 50, 100 and 1,000 ppm (equivalent to 0, 8.8, 17.5 and 175 mg/kg/day, respectively) in diet was administered to pregnant Swiss/ICR mice from day 7 to 18 of gestation, and then cesarean section was conducted on the day before delivery to examine fetuses. Decrease in body weight and malformation (ectoencephalopathy at ≥ 100 ppm, cleft palate at 1000 ppm) occurred in each dose group (Corbett et al., 1975).

PBB mixture (FM BP-6) at 0, 50, 100 and 1,000 ppm (equivalent to 0, 2.5, 5 and 50 mg/kg/day, respectively) in diet was administered to pregnant SD rats from day 7 to 20 of gestation, and cesarean section was conducted on the day before delivery to observe the fetuses. No teratogenicity was observed even though fetal body weight was lower at 100 ppm and more (Corbett et al., 1975).

Single oral administration of PBB mixture (FM BP-6) at 0, 40, 200, 400 and 800 mg/kg/day was conducted in pregnant Wistar rats one day between day 6 to 14 of gestation, and then cesarean section was conducted on day 20 of gestation to observe the effects on fetuses. No effect was noted in fetal body weight and placental weight at 40 mg/kg/day, and no malformation was observed. However, when 200 mg/kg/day or more was administered on day 6 of gestation, that is, in the process of implantation, the ratio of

embryo resorption was very high and there was no surviving fetus in the 400 mg/kg/day group. When this compound was administered after implantation, that is, day 7 onward of gestation, many embryo death and resorptions were observed at 200 mg/kg/day and above, and the lethal action on embryoes was noted in the groups that were administered this substance on any day between day 7 and 12 of gestation. Malformations including cleft palate, diaphragm hernia, etc. occurred in 1 or more fetuses per litter when 400 mg/kg/day or more was administered to the dams. The incidence of malformations was markedly high when the sbustance was administered on any day between day 11 and 13 of gestation (Beaudoin, 1977).

PBB mixture showed adverse effects on growth and liver function in subsequent generations in a 3-generation reproductive in diet study using SD rats. In this study, PBB (FM BP-6) at 0, 10 and 100 ppm was given to pregnant rats from day 8 of gestation to day 28 of delivery. After weaning, F_1 offsprings were given basic diet, but the diet containing PBB was given on day 28 of birth onward for 10 to 12 weeks. To obtain F_2 generation, sexually matured males and females of the same litter were mated. PBB was continuously administered to F_1 generation through growth, mating and gestation periods up to day 28 of delivery. The administration was conducted in F_2 generation during the same reproductive cycle as F_1 generation and the study was continued up to growth stage of F_3. The results indicated no effect on gestation period of F_0 dams and the number of offsprings in a litter. As the effects on F_1 offspring, neonatal mortality increased up to weaning in 100 ppm group. Although body weight at the time of birth was same as that in the control group, the delay was noted in growth and development indices (auricular opening, eyelid opening, vaginal opening, etc.). Increased relative weight of liver, induction of metabolic enzymes of hepatic and renal microsome (arylhydrocarbon hydroxylase, epoxidohydratase), and decreased vitamin A content in liver were observed in F_1 generation at 10 ppm and higher. There was no influence on growth and development of F_2 generation. As to effects on organs, only induction of metabolic enzymes of hepatic microsome was observed. The effect on F_1 generation in 10 ppm group including increased relative weight of liver was observed in F_2 generation in 100 ppm group, namely less than that observed in F_1 generation. No effects observed in F_1 and F_2 generations were noted in F_3 generation (McCormack et al., 1981).

When PBB mixture (FM FF-1) at dose of 0.3 ppm fed in basal diet to 7 female

rhesus monkeys for 6 months (total dose of about 10.5 mg/monkey), decreased in body weight, serum estradiol and progesterone, and prolonged menstruation cycle were observed. When these monkeys were mated with males after 6 months, all of them became pregnant but miscarriage occurred in 2 (Allen et al., 1978: Labrecht et al., 1978). The remaining 5 delivered offsprings. Though no external abnormality was noted in the neonates, body weight was lower in comparison with the neonates in the control group (Allen et al., 1979).

The following reports were available as to reproductive & developmental toxicity of octabromobiphenyl (OBB). When OBB (the compositions are shown in attached table 2) at 0, 100, 1000 and 10,000 ppm in diet was administered to pregnant SD rats (23 - 27 per group) from day 6 to 15 of gestation, and cesarean section was conducted on day 20 of gestation to observe fetuses, no fetal toxicity and deformation were noted even though body weight decreased in the dams of each OBB group (Waritz et al., 1977).

3) Information on general toxicity

(1) Acute toxicity (Table 1)

Table 1 shows the LD_{50} and LC_{50} of a PBB mixture (details of ingredients are unknown), or FM BP-6, octabromobiphenyl (OBB), nonabromobiphenyl (NBB) and decabromobiphenyl (DBB) in mice, rats and rabbits by each administraiton route (IPCS, 1994; DI Carlo et al., 1978; Aftosmis et al., 1972; Momma, 1986; Millischer et al., 1979).

Table 1 Results of acute toxicity test

	Mouse	Rat	Rabbit
Oral LD_{50}	> 15,000 mg/kg (NBB)	21,500 mg/kg (FM*) 2,000 mg/kg (OBB*) > 20,000 mg/kg (DBB*)	—
Inhalation LC_{50}	—	—	—
Percutaneous LD_{50}	—	> 5,000 mg/kg (DBB*)	> 5,000 mg/kg (FM BP-6) > 10,000 mg /kg (OBB) > 8,000 mg/kg (DBB)

* Refer to attachment-1 and -2.

(2) Repeated-dose toxicity (Attachment -5, -6)

FM FF-1 (the compositions are shown in attachment-2) at 0, 0.03, 0.3, 3 and 30 mg/kg/day was administered 22 times to male and female B6C3F_1 mice (8 weeks old) in 30 days by oral gavage, and these mice were observed for subsequent 90 days. The inhibition on body weight increase in males, and decreased Ht, Hb and RBC in females as well as decreased platelet count in both males and females were observed at 30 mg/kg/day. Also noted in the same dose gorup were increased ?-GTP in males and females, and tendency of decrease in blood glucose in females. Furthermore, increased liver weight, and swelling, focal necrosis and lipid deposition in hepatocyte in males and females as well as decreased thymus weight in males were noted at 30 mg/kg/day (Gupta et al., 1981).

FM FF-1 (the compositions are the same as those in the test conducted in mice; attachment-2) at 0, 0.03, 0.3, 3 and 30 mg/kg/day was administered to male and female F344 rats (8 weeks old) for 30 days by oral gavage, and these rats were observed for subsequent 90 days. The inhibition on body weight increase in males and females (more remarkable in females), decreased Ht, Hb and RBC in males, decreased lymphocyte count in females and decreased platelet count in males and females were observed at 30 mg/kg/day. However, these changes recovered within 1 month after completion of administraiton. Increased serum β-globulin fraction and decreased blood glucose were noted in males and females, and the former persisted until 90 days after completion of administraiton. Some of the above findings were also found in 3 mg/kg/day group. As to the effects on organs, relative liver weight increased in males and females of 3 and 30 mg/kg/day groups. Histologically, swelling of hepatocyte, necrosis accompanied by leukocyte and lymphocyte infltration, localized abscess, and neutral fat deposition in cytoplasm were detected. According to electron microscopy findings, swelling of mitochondria, increased lipid droplets and rough endoplastic reticulum and depletion of glycogen were noted in hepatocyte. Th hepatic disorders were slightly alleviated after 1 month from completion of administraiton. Other than the aboves, decrease in relative weight of thymus was noted in all dose gorups. The toxicity of FM-FF-1 in rats and mice was more marked than that of HBB (Gupta et al., 1981).

When a mixture of PBB (FM BP-6) at 0, 0.0625, 0.25, 1.0 and 4.0 mg/kg/day was orally administered to male beagle dogs (11 - 13 months old, 6/group) for 61 days, one dog in the maximum dose group seemed to have lost its eyesight after convulsion had

been induced (day 50 - 52). Another dog died on day 58. The autopsy of this dead dog indicated gastrointestinal bleeding. Increased juvenile lymphocyte in periphenral blood was found in several dogs in each dose group. The inhibition on hematopoiesis and increase in foamy large reticuloendothelial cells in bone marrow, marked decrease in lymphocyte count in T cell domain of lymph node, enhanced extramedullary hematopoiesis and decreased lymphocyte count in white pulp in spleen, and decreased plasma cell count in the pipliteal lymph node were observed in 4 mg/kg/day group. Similar changes in the bone marrow and spleen were also noted in 1 mg/kg/day, but they were milder. Other than the aboves, atrophy of thymus was noted in all the PBB groups (Ferber et al., 1978).

A PBB mixture (FM FF-1) at 0, 0.3, 1.5 and 25 ppm in diet was administered to male and female rhesus monkeys. After 1 year of administration, decreased body weight, decreased serum estradiol and progesterone as well as prolonged menstruation cycle and miscarriage were noted in females of 0.3 ppm group (2/7 monkeys). Decreased body weight and edema around orbit occurred after 5 months, and decreased serum cholesterol and fatty infiltration in liver were noted after 38 weeks in females of 1.5 ppm group. Up to 10 weeks of administraiton, decreased body weight, swollen stomach and diarrhea were noted while progressive gastroentritis and ulcer simlar to ulcerative colitis occurred after 25 weeks in males of 25 ppm group (Allen et al., 1978; Lambrecht et al., 1978; Allen & Lambrecht, 1979).

The results of repeated dose toxicity test of PBB with 8 or more of bromine atoms are shown in Attachment-6.

When OBB (the compositions are shown in attachment-2) at 0, 1, 10, 100 and 1,000 ppm in diet given to male SD rats (11 weeks old) for 28 days, increase in relative weight of liver was noted in 100 ppm group and above. Histopathologically, hypertrophy of hepatocyte, concentration of basophilic granule in periphery of cells in cytoplasm as well as depletion of hepatic glycogen were detected. No adverse effects were observed except that on the liver in this study and no observed effect level (NOEL) was estimated to be 10 ppm (Waritz et al., 1977).

When OBB (the compositions are shown in attachment-2) at 0, 100, 1000 and 10,000 ppm (corresponding to 0, 8, 80 and 800 mg/kg/day) in diet were given to male SD rats for 30 days, swelling of liver, centrilobular hypertrophy of hepatocyte and vaculation,

hyaline-like degeneration in renal cytoplasm and hyperplasia of thyroid gland were observed in all dose groups. In addition, kidney weight increased in 1,000 ppm group and RBC and Ht decreased in 10,000 ppm group (Norris et al, 1975).

When nonabromobiphenyl (NBB) at 0, 100 and 300 ppm in diet were given to male and female B6C3F_1 mice (5 weeks old) up to 15 months, body weight increase and food consumption decreased in each dose gorup. In addition, death occurred in 1 male in 300 ppm group after 51 weeks and all the males in this group died in 57th week. After 15 months, decreased Ht (males and females), decreased WBC and increased platelet (males), increased serum glutamic pyruvic transaminase (GPT) activity and decreased triglyceride (males and females), decreased albumin/globulin (A/G) ratio (males), decreased non-esterified fatty acid (NEFA) (females) as well as increased liver weight and hypertrophy of hepatocyte (males and females) were observed in 100 ppm group. Similar changes were observed in females of 300 ppm group (Momma, 1986). There is no report on short term repeated toxicity test of NBB.

When decabromobiphenyl (DBB) at 0, 100, 500 and 2,000 ppm in diet were given to male and female SD rats for 13 weeks (90 days), no toxic changes were observed in 100 and 500 ppm groups. However, increased relative liver weight as well as histologically hypertrophy of hepatocyte, vacuolation and marked decrease in glycogen were noted in 2,000 ppm group (Millischer et al., 1979). As such, the toxicity of PBB becomes weaker along with increase in bromine number.

(3) Neuro-ethological toxicity

A PBB mixture (FM FF-1) at 0, 3 and 10 mg/kg/day was administered to male and female F344/N rats (5 - 6 weeks old) 5 times a week for 26 weeks (total 130 times) by oral gavage to investigate neuro-ethological effects. The suppressed behavior and decreased muscle force, inhibition of spontaneous movement (more markedly noted in females than in males), decreased grip strength (more markedly noted in males than in females), decreased hind leg stretch reflex (similarly noted in the males and females) and decreased surprise response (more markedly noted in females than in males) were noted mainly in 10 mg/kg/day group, but no change attributable to FF-1 administration was observed in learning ability of males to avoid electric stimulation. In a group administered FF-1 at 30 mg/kg/day for total 30 days, body weight increase was suppressed, and reduced

behavior and decreased muscle force were marked. Excluding higher neural activity such as avoidance behavior, all the indices of neuro-ethological toxicity were inhibited, but these findings suggested that the inhibition was attributable to toxic expression of PBB that decreased skeletal muscle function rather than action of FM FF-1 on nerve system (Tilson & Cabe, 1979). On the other hand, when the effects of FM FF-1 administration for 20 days (doses: 0, 1.0, 3.0 and 6.0 mg/kg/day) on learning ability and discrimination learning was investigated in male SD rats (≥ 3 months old) by operant conditioned learning behavior method using sound and light stimulation, no influence on learning ability and discrimination learning was observed at the doses of 6 mg/kg/day or less. However, when excessive response frequency demonstrated in the absence of conditioning was investigated, the frequecy increased in 1 mg/kg/day group while it decreased in 6 mg/kg/day group. When the amount of PBB contained in brain tissue was determined, the PBB corresponding to the dose was detected. The effect on learning ability was not observed because sensitivity of the test method was low or because nerve system development was sufficient so that the effect of PBB mixture was not fully manifested. On the other hand, the changes in excessive response seemed to indicate that the PBB mixture accumulated in the brain could excite the animals at low dose and inhibit them at high dose (Geller et al., 1979).

There is a report on the effect of PBB on physical growth and neuro-ethology of offspring born from the dams (rats) administered PBB. When PBB mixture (FM BP-6) at 0, 0.2 and 2 mg/kg/day was administered to pregnant SD rats (7 - 9 weeks old) from day 6 of gestation to 24 days after delivery by oral gavage to investigate any toxic symptom in the dams and the effect on physical growth and neuro-etohology of offspring, no toxic sign was detected in the dams, and no influence on mortality of offspring were noted in any of the dose groups. As to the male neonate on day 0 of nursing period, body weight was slightly lower in 2 mg/kg/day, and shorter head-to-gluteal length was noted in 0.2 and 2 mg/kg/day groups. Compared with the control group, body weight increase shifted at lower level in males and females of 2 mg/kg/day group up to 4 weeks from the birth. However, no effect on the number of days to eyelid opening, testis descent and vaginal opening was noted in F_1 neonate. On the other hand, the results of behaviological test of neonate indicated delay in duration (number of days) to become capable of forward movement with the body and head erect and the duration for cage emergence behavior

(determined when the animals were 60 days old) in males and females of 2 mg/kg/day group. In addition, inhibition of the behavior was observed in an open field test conducted on day 12 - 24 of birth. In other words, delayed growth and neuro-ethological toxicity were noted in the offspring born from the dams administered PBB (Henck et al., 1994; IPCS, 1994).

4) Information on mutagenicity/genotxicity and carcinogenicity

(1) Mutagenicity/genotoxicity (Table 2, 3)

The result of the mutagenicity of PBB are shown by bromine atoms, that is, the data obtained with PBB with 4 or less of bromine atoms are shown in Table 2, and 5 or more in Table 3. Few reports have been available on PBB with 5 or less of bromine atoms. Though there is a report that 4-bromobiphenyl was positive in metabolic activation system using rat liver microsome of *Salmonella typhimurium* (TA1538 strain) (Kohli et al., 1978), the result was negative in a subsequent test (Haworth et al., 1983).

Concerning *in vitro* mutagenicity of PBB with 5 or more of bromine atoms, FM FF-1, FM BP-6 and HBB were used in reverse mutation test using bacteria (Tennat et al., 1966; Haworth et al., 1983), and mammalian cells (Kavanagh et al., 1985; Williams et al., 194), DNA repair synthesis test (Williams et al., 1984) and DNA adduct formation test (Dannan et al., 1978), and micronucleus test (Shelby et al., 1993), chromosome aberration test (Wertz & Fisco, 1978; Ficsor & Wertz, 1976; Garthoff et al., 1977) and sex-linked recessive lethality test (Fouremen et al., 1994) were conducted as *in vivo* tests. The reuslts of these tests indicated that mutagenicity of PBB was negative as a whole.

Table 2 Results of mutagenicity, genetic toxicity tests

PBB with 4 or less of bromine atoms

Test method		Test conditions	Result *	References
in vitro	Reverse mutation test	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537, S9(+/-), 2-bromobiphenyl (no description of concentraiton)	−	Haworth et al., 1983
		Salmonella typhimurium TA98, TA100, TA1535, TA1537, S9(+/-), 3- bromobiphenyl (no description of concentraiton)	−	Haworth et al., 1983
		Salmonella typhimurium TA98, TA100, TA1535, TA1537, S9(+/-), 4- bromobiphenyl (no description of concentraiton)	−	Haworth et al., 1983
		Salmonella typhimurium TA1538, S9(-/+), 4- bromobiphenyl, 0, 50, 100, 150μg/plate (Positive in S9+)	+	Kohli et al., 1978
	Mutagenicity test	Chainese hamster V79 cell (HGPRT locus), S15(+/-) 3,3',4,4'-tetrabromobiphenbyl 0, 1, 2.5, 10μg/mL	−	Kavanagh et al., 1985

Table 3 Results of mutagenicity, genetic toxicity tests

PBB with 5 or more of bromine atoms (including FM FF-1 and FM BP-6)

Test method		Test conditions	Result **	References
in vitro	Reverse mutation test	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537, S9(+/-), FM FF-1 (no description of concentraiton)	−	Tennant et al, 1986
		Salmonella typhimurium TA98, TA100, TA1535, TA1537, S9(+/-), HBB* (no description of concentraiton)	−	Haworth et al, 1983
	Mutagenicity test	Chainese hamster V79 cell (HGPRT and Na-K ATPase loci), S15(+/-) FM BP-6* 0, 1, 10, 40μg/ml,	−	Kavanagh et al., 1985
		Co-incubation system using rat liver epithelial cells (HGPRT locus) and human fibroblast cell D-550 · rat hepatocyte, FM FF-1* 0, 10, 100, 1000 μM	−	Williams et al., 1984
		Chainese hamster V79 cell and WB rat hepatocyte (HGPRT locus), 2,2',4,4',5,5'-HBB* 0, 20, 40, 50μg/ml	−	Kavanagh et al., 1985
		Chainese hamster V79 cell and WB rat hepatocyte (HGPRT locus), 3,3'4,4',5,5'-HBB* 0, 7, 10, 12μg/ml	−	Kavanagh et al., 1985
	DNA repair synthesis test	Primary culture of hepatocyte of mouse, rat and hamster, FM FF-1* 0, 10, 100, 1000 μM	−	Williams et al., 1984

Test method		Test conditions	Result **	References
	DNA adduct test	Covalent binding of 2, 2',4,4',5,5'-HBB* and 2, 2'3,4,4'5,5'-heptabiphenyl with ^{14}C-labeled mixture (^{14}C-PBB) to salmon DNA, addition of rat liver microsome, 50μg ^{14}C-PBB/20 mg DNA	—	Dannan et al., 1978
in vivo	Micronucleus test	Continuous intraperitoneal administraiton of PBB (details are unknown) at 0, 500, 1,000 and 2000 mg/kg/day to male B6C3F$_1$ mice every day for 3 days. RBC in the bone marrow was determined after 48 hours	—	Shelby et al, 1993
	Chromosome aberration test	Single oral administration of FM (details are unknown) was conducted at 0, 50 and 500 mg/kg/day to male Swiss mice (35±2 g), after which bone marrow cells were prepared at 12, 24 and 48 hours starting from 5 hours after colchicine processing	—	Wertz and Ficsor, 1978
		PBB (details are unknown) at 0 and 100 mg/kg/day was administered 6 times every other day starting from day 6 of gestation to pregnant rats by oral gavage. On day 19 of gestation. Bone marrow cells were prepared at 5 hours after colchicine processing.	—***	Ficsor and Wertz, 1976
		FM BP6* at 0, 5, 50 and 500 pmin diet was given to rats (250 g) for 5 weeks, after which bone marrow and spermatogonia were investigated	—	Garthoff et al., 1977
	Sex-linked recessive lethal test	in *Drosophia*; PBB used was a mixture with the bromine numbers of 5 - 7, 1,000 ppm mixed in diet	—	Foureman et al., 1994

* Refer to attaches tables 1 and 2 **—: Negative
***: Though arrest of cell division was observed in bone marrow, chromosome aberration was not definite.

(2) Carcinogenicity (Table 4)

Concerning carcinogenicity of PBB mixture in rodents, there is a report on a carcinogenicity test conducted by NTP. After oral administration of PBB mixture (FM BP-6 containing 2.0% calcium trisilicate) at 0, 0.1, 0.3, 1.0, 3.0 and 10.0 mg/kg/day to male and female B6C3F1 mice (7 - 8 weeks old) for 6 months (5 times/week), the animals were reared for 24 months. The incidence of hepatocarcinoma was 95% in males and 88% in females in the 10.0 mg/kg PBB group, definitely showing an increase in the incidence in comparison with 48% and 0% respectively in the control group (NTP, 1983; Gupta et al., 1983b).

After oral administration of a PBB mixture (the same as above) at 0, 0.1, 0.3, 1.0, 3.0 and 10.0 mg/kg/day to male and female F344 rats (7 - 8 weeks old) for 6 months

(5 times/week), the animals were reared for 23 months. The incidence of hepatocellular carcinoma and bile duct cancer in males and females as well as that of tumorous nodes in liver in females showed marked increase. In each case, a statistically significant increase was noted in each incidence in comparison with that in the control group (NTP, 1983; Gupta et al., 1983b).

When a PBB mixture whose major ingredient was nonabromobiphenyl (NBB) at 0, 100 and 300 ppm in diet was given to male and female B6C3F_1 mice (5 weeks old) for 18 months, the incidence of hepatoccellular carcinoma was 14% in males and 0% in females in the control group. On the other hand, the incidence in males was 77.6% and that in females was 17.4% in 100 ppm group, and 28.3% and 75.5% respectively in 300 ppm group, indicating a significant increase in the treatment groups (without no explanation of reduced incidence in males at higher dosage). A significant increase in the incidence of tumorous nodes in liver was observed in males and females, and that of hepatoblastoma in males at 100 ppm or more. On the other hand, hypertrophy of thyroid gland was observed in most of the females in 300 ppm group, histologically demonstrating hypertrophy and papillary hyperplasia of follicles (Momma, 1986).

F_1 rodents exposed to PBB at 0, 3, 10 and 30 ppm for 8 weeks continuously from fetal period and for 2 years subsequently were compared with those exposed to the same for 2 years since when they were 8 weeks old to investigate any difference in tumor incidence between these animals. According to the results obtained from male and female B6C3F_1 mice, the incidence of hepatic tumor (hepatocellular carcinoma or adenoma) reached 84 - 98% both in males and females administered 10 ppm or more and it was not possible to conclude presence or absence of any increase in the incidence attributable to the exposure. On the other hand, incidence of hepatic tumor (hepatocellular carcinoma or adenoma) significantly increased in females in group exposed to PBB continuously since their fetal period by administration of 0, 1, 3 and 10 ppm to the dams during fetal and neonatal periods and through ingestion of diet mixed with PBB at 3, 10 and 30 ppm after weaning, as compared with females in the group in which the treatment started at the age of 8 weeks old. The results indicate that carcinogenicity was increased by exposure from fetal period. On the other hand, no increase in the incidence was observed in males by exposure since fetal period (Chhabra et al., 1993).

PBB with bromine atoms of 6 - 10 is classified as 2B (possible carcinogenicity in human) by IARC and b (substance rationally expected to have carcinogenicity in human) by NTP (IARC, 1987; NTP, 2000).

Table 4 Carcinogenicity assessment by national and international organizations

Organizations	Classification	Significance	References
EPA	Group D*	Substance that cannot classified as to carcinogenicity in human.	JETOC, 1999
EU	—	Carcinogenicity was not assessed.	JETOC, 2000
NTP	**b****	Substance rationally expected to have carcinogenicity in human.	NTP, 2000
IARC	Group 2B**	Substance with possible carcinogenicity in human.	IARC, 2001
ACGIH	—	Carcinogenicity was not assessed.	ACGIH, 2000
Japan Society for Occupational Health	—	Carcinogenicity was not assessed.	Japan Society for Occupational Health, 2001

* : As hexabromobiphenylether
** : As polybromobiphenyl (Fire Master BP-6 and FF-1, PBB with the bromine numbers of 6, 8 and 10)

5) Information on immune system (Attachment-7)

The influence of PBB on human immune system has been described in detail in IPCS (1994). The effect on immune system of experimental animals, has been descripted under 3)-(2) Repeated dose toxicity. Atrophic changes in thymus occurred in mice, rats and dogs. Decreased lymphocyte count in spleen (white pulp), bone marrow and lymph nodes was noted in dogs as well as decreased peripheral blood lymphocyte. In this chapter, immunological toxicity other than those mentioned above is described and the results are shown in attached Table 7.

When PBB mixture (FM FF-1) at 30 mg/kg was administered to mice for a short term (30 days) or a long term (6 months) 5 times a week by gastric gavage, low levels of serum IgG and IgM were observed (Luster et al., 1978; Loose et al., 1981). The suppression of antibody reaction to sheep erythrocyte or bovine ?-globulin was observed in experiment in which FM was administered to mice for 30 days (Luster et al., 1978, Fraker, 1980; Loose et al., 1981) and in another in which FM was administered to rats for 6 months (Luster et al., 1980). The mortality in mice infected with Listeria was not influenced by long term administration of FM FF-1, but the duration up to death was curtailed, suggesting increased sensitivity to Listeria infection (Luster et al., 1980). After

FM FF-1 at 5 and 167 ppm in diet was given to mice for 3 - 6 weeks, and then they were infected with *Plasmodium berghei* (malarial parasite in rodents). No influence was observed on the infection resistance and mean survival time (Mudzinski et al., 1979; Loose et al., 1981). On the other hand, when FM FF-1 was administered to mice for 3 - 6 weeks (Mudzinski et al., 1979; Loose et al., 1981) and when the mice were exposed to FM FF-1 after birth (Luster et al., 1980), an increase in endotoxin sensitivity was observed. However, no increase in endotoxin sensitivity was observed after administration to mice for 6 months (Luster et al., 1980).

When single intraperitoneal administration of 150 μmol/kg of 3,3',4,4'-tetrabromobiphenyl was conducted in rats, the endotoxin sensitivity increased after 1 or 2 days, but no increase was noted after 20 days (Shedolfsky et al., 1991).

FM FF-1 inhibited division and growth reaction of T and B cells induced by mitogen (cell division promoter) stimulation in mice, rats (Luster et al., 1978; Luster et al., 1980) and pigs (Howard et al., 1980). The delayed hypersensitive response (DHR) was inhibited in rats when PBB at 10 mg/kg/day was administered for 6 months but no DHR change occurred in mice (Luster et al., 1980).

6) Fate and Metabolism

As exposure routes in human, inhalation and skin contact are generally assumed to have occurred in the workers of chemical plant. However, general inhabitants in Michigan State orally ingested PBB dissolved in the fat of meat and milk when PBB had been accidentally mixed in livestock food (IPCS, 1994).

PBB is known to be absorbed from digestive tract in a large number of animal species including human, cow, pig, dog, mink, guinea pig, rat, mouse, cotunix quail, hen, fish, etc. However, there is no report in humans and experimental animals that occurred on transcutaneous absorption or through inhalation. No data are available on quantitative analysis of PBB absorption in humans (IPCS, 1994).

After administration of ^{14}C-labeled 2,2',4,4',5,5'-HBB at 1, 2.5 and 7.5 mg/kg/day to male SD rats (200 - 250 g) by oral gavage, 90% or more of radioactivity was absorbed (Di Carlo et al., 1978; Matthews et al., 1977). The BP-6 concentration was 2 μg/ml in the blood of children and adults living in livestock farms that were quarantined against PBB pollution. On the other hand, the blood concentration was much higher at 24 - 47 μg/m*l*

in cows after single oral administration of 3 g capsule (Di Carlo et al., 1978). When single oral administration of ^{14}C-OBB at 1.0 mg (in OBB/kg) was conducted in male and female SD rats, 62% of total radioactivity administered was excreted into feces within 24 hours, indicating that oral absorption of OBB was low both in males and females (Norris et al., 1975). No information is available on absorption of NBB and DBB.

Intravenous administration of ^{14}C-2,2',4,4',5,5'-HBB was conducted in male SD rats for 6 weeks to investigate radioactivity distribution in skin, muscle, fat tissue and liver. After one day from administration, 40% and 10% of the total radioactivity were detected in muscle and liver respectively, but the ratios decreased to 5% and 2% respectively after 7 days. Most of the radioactivity that disappeared from these tissues was re-distributed in fat tissue. The radioactivity found in fat tissue amounted to 25% and 60% after 1 and 7 days respectively from administration, indicating an increase within 1 week. The radioactivity in muscle and liver gradually decreased in the subsequent 5 weeks while the radioactivity level in fat tissue continuously increased. The radioactivity distribution in skin was constant within the range of 15 - 20% throughout the 6 weeks (Matthews et al., 1977).

When oral administration of ^{14}C-2,2',4,4',5,5'-HBB was conducted in male SD rats to investigate the radioactivity distribution in organs, the initial distribution in organs and re-distribution in fat tissue were observed, indicating that similar phenomenon occurred after oral administration (Matthews et al., 1977). After 16 days from single oral administration of ^{14}C-OBB , the residual radioactivity rates in fat tissue, adrenal, heart and skin (0.14 - 0.25% of radioactivity administered) were higher than those in liver, pancreas and spleen (0.01 - 0.06%) (Norris et al., 1975). Non-radioactive OBB in diet was given to male SD rats for 6 months (0.1 mg/kg/day), after which the bromine concentrations in fat tissue and liver were determined. The result indicated that the bromine concentrations increased for 6 months in each tissue without reaching a plateau, but it was not detected in kidney, skeletal muscle and testis (Di Carlo et al., 1978; Norris et al., 1975).

In an experiment with Holstein milk cows, PBB concentrations in muscle, fat, liver and kidney increased dose-dependently. Especially at 10 - 100 mg/kg/day, PBB definitely accumulated in these tissues. Furthermore, it was reported that PBB dose-dependently distributed in bone marrow in milk cows and that PBB was detected in adrenal, brain, heart, kidney, liver, mammary gland, muscle, spleen and thyroid gland of

cows (and also in sheep) as well as in bile, lung, lymph node, ovary, gastric wall, spinal cord, cerebrospinal fluid, thymus, tongue and uterus of cows, indicating that PBB was detected in almost all the tissues (Di Carlo et al., 1978; IPCS, 1994).

The researches on PBB distribution in human are limited to the cases of direct or indirect exposure to Fire Master. According to the result of investigation in the 15 death cases after exposure to high concentration FM in Michigan State, PBB (determined as 2,2',4,4',5,5'-HBB concentration) distributed to almost all the organs of human body, and the concentrations were especially high in fat and tissues containing abundant fat. On an average 475 ng/g of HBB was detected in fat around kidney. The HBB concentrations in adrenal, atheromatous aorta and thymus were about half of fat around kidney, while about 1/10 concentrations were noted in the remaining tisssues (IPCS, 1994). In most of the researches conducted on PBB distribution in surviving cases, the samples were collected from sera, subcutaneous fat and mother's milk. The extent of increase in HBB concentration in subcutaneous fat over blood concentration differed between subject populations. The level was the lowest in breast-feeding women and pregnant women. While the mean concentration in subcutaneous fat in general inhabitants in Michigan was 340 times higher than that in serum, the concentrations in the breast-feeding women and pregnant women were only 140 - 180 times and 100 times higher respectively (IPCS, 1994). On the other hand, the HBB concentration in milk of breast-feeding women was about 100 times higher than that in serum, and the ratio of HBB in fat of mother's milk to that in somatic fat was 0.7 - 09:1. It was reported that the HBB levels in placental tissue, umbilical cord and fetal serum were 1/6 - 1/10 of mother's serum HBB concentration (IPCS, 1994).

When PBB was added to blood in an *in vitro* experiment, it was distributed in plasma, erythrocyte and leukocyte by distribution rate of 89:9:2 or less. However, when compared with the amount of PBB per cell, the amount contained in leukocyte is calculated to be 100 times more than that in erythrocyte. In terms of PBB amount per mg of protein in blood of humans exposed to PBB, the concentration is higher in lymphocyte than in erythrocyte. These results suggest that PBB was distributed at a comparatively higher concentration in leukocyte and that about 80% of PBB was bound to apolipoproteins B and A at a ratio of 3:1 while about 20% of it remained free and not bound to protein (IPCS, 1994).

In vitro and *in vivo* experimental results were reported as to metabolism of PBB. In an *in vitro* experiment in which SD rat liver microsome enzymes induced by phenobarbital (PB) or PBB was used, only 2 of 12 PBB ingredients of PBB contained in Fire Master, that is, 2,4,5,2',5'-pentabromobiphenyl and 2,3,6,2',4',5'-HBB, were metabolized. When unprocessed microsome and microsome induced by 3-methylcocholanthrene (3-MC) was added, no metabolism occurred (Dannan et al., 1978; IPCS, 1994).

The following structure activity correlation is assumed regarding metabolism of PBB ingredients other than Fire Master. PBB ingredients in which no bromine is attached to meta- and para-carbon that are adjacent to biphenyl crosslinkage on at least one benzene ring are metabolized by microsome induced by phenobarbital. For metabolism of PBB with bromine atoms of 4 or less, bromine has to be absent in ortho- and meta-position on at least one benzene ring. However, no metabolism of PBB with bromine atoms of 5 or 6 occurs even when this condition is satisfied. As the major metabolites of PBB with small bromine atoms, mono- or dihydroxide was identified (IPCS, 1994).

A polar fat soluble metabolite though a trace amount, was detected in an *in vitro* experiment when liver microsome unprocessed or induced by PBB was added to ^{3}H-2,2',4,4'5,5'-HBB (purity of 98% or more). The metabolite of PBB with bromine atoms of 4 or less detected under *in vitro* conditions was mono- or dihydroxide. Though some of the PBB ingredients promoted metabolism of themselves or other PBB ingredients, the PBB ingredients that induced microsome enzyme were not necessarily metabolized (IPCS, 1994).

When PBB with bromine atoms of 4 or less was administered to rats, rabbits on pigs in *in vivo* experiment, mono- or dihydroxide was detected as an urinary metabolite. However, when 2,2',4,4',5,5'-HBB with bromine atoms of 6 was administered to rats in *in vivo* experiment, no metabolite was identified in urine, feces and bile. After single oral administration of 3 g of FM BP-6 to cows, no hydroxide was detected in urine and milk. After oral administration of FM BP-6 to dogs, 6-hydroxy-2,2',4,4'5,5'-HBB was identified as a metabolite in feces but no hydroxide of HBB was detected in liver. Intestinal flora was reportedly responsible for this metabolism. Accordingly, it was reported that PBB was not metabolized, but PCB in which chlorine was substituted in the 2,2',4,4',5,5'

position was metabolized in dogs (IPCS, 1994).

The excretion of PBB from the body of mammals was investigated using HBB and DBB. PBB was mainly excreted into feces through bile duct and intestine, and excretion occurs gradually, taking a time. As PBB is insoluble, hardly any is excreted into urine, but for PBB with smaller bromine number its metabolitse are excreted into urine, though the amount is small (IPCS, 1994).

The excretion of ^{14}C-2,2',4,4',5,5'-HBB was very slow in rats. After 6 weeks of single intravenous administration, only 6.6% and 0.1% or less of the total radioactivity administered were excreted into urine. By mathematical extrapolation of the data into infinite time, it was assumed that 9.5% of the total PBB dose was excreted into feces (Di Carlo et al., 1978). Compared with HBB, the excretion of OBB in rats occurred more quickly. After single oral administration of ^{14}C-OBB, 65% of the total radioactivity administered was excreted into feces within 1 day and 73% within 16 days. A large portion of excreta within 24 hours was considered to be attributable to insufficient absorption (Di Carlo et al., 1978). In the case of pigs, only 1% of FM BP-6 was excreted into urine and feces within 7 days after single intraperitoneal administration. In the case of cows, more PBB was excreted into feces than into urine, and 50% or more of PBB administered was excreted into feces within 7 days while 24% was excreted into the milk within 95 days (Di Carlo et al., 1978).

PBB was excreted into bile in rats, rhesus monkeys and cows. In the case of rats, 0.68% of HBB was collected in the bile within 4 hours after intravenous administration. Considering the extremely small amount, there is a report suggesting that the important excretion route could be enterohepatic circulation in the case of rats. On the other hand, HBB concentration 2 or 3 times higher than that in the plasma was detected in bile of cow (IPCS, 1994).

The result of HBB determination in those working in livestock farms and chemical plants in Michigan suggested that PBB concentrations in human bile and stools were very low in comparison with the exposure amount. The HBB concentrations in bile and stools were about 1/2 - 7/10 of that in serum and were estimated to be about 0.5% of that in fat tissue (IPCS, 1994).

PBB is well-known to migrate into human milk, but no data on its excretion into urine and stools are available. Though excretion of PBB from skin is conceivable, no

data in this regard are available (Di Carlo et al., 1978).

2. Hazard assessment at present

The information on harmful nature of PBB currently available is mostly on the mixtures with bromine atoms of 6 or more. Typical compounds are FM BP-6 and FM FF-1 that are PBB mixtures with bromine atoms of 5 - 8. All the information on humans obtained was on the exposure of consumers to PBB that was accidentally mixed in the livestock food in USA.

As effects on human endocrine system, increased follicle stimulating hormone (FSH) concentration, decreased thyroidal function and occurrence of nodes in thyroid gland correlated with length of employment period were observed in workers of DBB or DBB oxide manufacturing plant. On the other hand, nothing abnormal was reported in the number, motility and morphology of sperms in general public exposed to PBB. Though the influence on reproductivity and on subsequent generations was investigated in humans, the relation to PBB exposure was not clarified.

As to animal experiments, PBB mixture (Fire Master) was administered mainly to rats. As a result, decreased thyroid hormone (T3, T4) and increased TSH were observed. Also noted in some experiments was the onset of goiter. These results corresponded with the effects observed in humans. When FM was administered to female monkeys for a long term, prolonged menstruation cycle, spontaneous abortion, decreased serum progesterone level, etc. were reported.

It is known that fetal toxicity and neonate deformation occur at a low dose of several mg/kg/day upward when mice and rats are exposed to PBB mixtures (mainly FM) during the gestation period. In a 3-generation reproductivity test using rats, it was reported that PBB mixtures influenced growth and hepatic function of next generation, but that the influence decreased in subsequent generation. However, the result of an experiment in pregnant rats indicated that no fetal toxicity and deformation were induced when the dose was increased up to the toxic level in the dams.

Decreased T cell and B cell functions, fluctuation in lymphocyte ratio, immunoglobulinemia (IgG), enhanced sensitivity to Streptococcus, etc. were reported as the findings obtained from humans exposed to PBB. The effect on skin (halogen acne) was also observed.

The acute toxicity of PBB with bromine atoms of 6 - 10 in animal experiments was weaker in all cases. As to repeated dose toxicity, FM administration to mice and rats demonstrated effects on liver (weight increase, hypertrophy of hepatocyte, focal necrosis and lipid deposition, etc.) and on hematopoietic system (decreased RBC, Hb, Ht and platelet). In addition, thymus weight decreased. As to the experiments conducted in dogs, inhibition of hematopoiesis in bone marrow, enhanced extramedullary hematopoiesis in spleen and decreased lymphocyte count in white pulp and various lymph nodes were observed. These changes were noted from the dose of several mg/kg/day upward in rodents and dogs. Similar changes were also noted in the experiments of OBB and NBB in rats or mice. However, no toxic changes were found up to 500 ppm when DBB was mixed in food and given to the animals for 13 weeks, indicating definitely lower toxicity in comparison with other types of PBB.

In neuro-ethological toxicity experiment in rats, FM at 1 -6 mg/kg/day was orally administered for 20 days, but no influence was noted in learning function of rats. However, there is a report that excitement at a low dose and a tendency of inhibition at a high dose occurred in time of response to a given condition. When FM at a low dose (0.2 - 2 mg/kg/day) was administered to pregnant rats, growth of F_1 was delayed and behavior test indicated inhibition of their behaviors even though no toxic signs were noted in the dams. These animal experiments were conducted because PBB was anticipated to decrease memory and concentration as well as to induce depression in humans, and to decrease intellect and induce growth insufficiency in children. However, the result of extensive clinical studies indicated that nothing abnormal was detected in the objective examination of human nerve system and psychiatric function, and the relation to PBB exposure was not statistically evidenced.

As to mutagenicity and genetic toxicity, most of the results obtained from *in vitro* and *in vivo* experiments using PBB mixture FM were negative. As to carcinogenicity, a significant increase in tumor incidence in liver was observed with PBB mixture (FM) in mice and rats, and with NBB in mice. According to the carcinogenicity assessment PBB with the bromine atoms of 6 - 10 is classified as 2B (substance with possible carcinogenicity in human) by IARC and b (substance rationally expected to have carcinogenicity in human) by NTP.

The influence on immune system was also observed in experimental animals.

Decreased lymphocyte count and decreased function (decreased response of T and B cells to mitogen stimulation, decreased antibody generation) were observed in various lymphatic organs including peripheral blood, bone marrow, spleen, etc. In some experiments, enhanced sensitivity to endotoxin derived from bacteria and increased mortality after Listeria infection (enhanced sensitivity), inhibition of delayed hypersensitive reaction, etc. were noted, indicating that these immunosuppressive findings endorsed influence of this substance on humans.

3. Risk assessment and other necessary future measures

There is no finding to demonstrate effects of PBB on endocrine system including receptor binding. Though much has to be elucidated for effects on humans, there is enough evidence obtained from animal experiments to show that PBB influences the thyroid gland and reproductive organs, and gives adverse effects on subsequent generations. Abundant reports have been availabale on the effects of PBB on liver and immune system. The harmful nature of PBB with 6 bromine atoms or more has been fully proven. However, no sufficient information is available on PBB with bromine atoms of 5 and information on hazard of PBB with bromine atoms of 4 or less, is rarely available at present. Nevertheless, considering that PBB is no longer manufactured in Japan or imported to Japan, there seems to be no need to take an urgent measure against PBB. PBB's with bromine atoms of 6 or more are new chemical substances based on Law Concerning the Examination and Regulation of Manufacture, etc. of Chemical Substances, and have to be notified for manufacturing or importing. On the other hand, PBBs with bromine atoms of 5 or less are the exsiting chemical substances based on the Law, but there is no evidence of production, import and usage in Japan at present.

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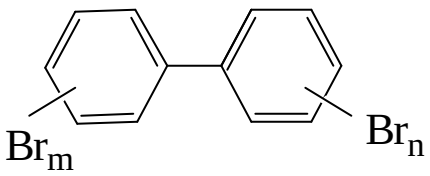
Attachment-1 PBB compounds investigated in this assessment

Type of PBB	PBB investigated	CAS	Remark
PBB mixture	FireMaster BP-6 (FM BP-6)	59536-65-1	PBB mixture with bromine stoms of 5 - 7
PBB mixture	FireMaster FF-1 (FM FF-1)	67774-32-7	2% calcium poly-silicate was added to FM BP-6 (anti-caking)
Monobromobiphenyl ($C_{12}H_9Br$)	2-bromobiphenyl 3-bromobiphenyl 4-bromobiphenyl	2052-07-7 2113-57-7 92-66-0	Number of isomers: 3
Dibromobiphenyl ($C_{12}H_8Br_2$)			Number of isomers: 12
Tribromobiphenyl ($C_{12}H_7Br_3$)			Number of isomers: 24
Tetrabrmobiphenyl ($C_{12}H_6Br_4$)	3,3´,4,4´-bromobiphenyl	77102-82-0	Number of isomers: 42
Pentabromobiphenyl ($C_{12}H_5Br_5$)	2,2´,4,5,5´-pentabromobipheny	67888-96-4	Number of isomers: 46
Hexabromobiphenyl ($C_{12}H_4Br_6$)	2,2´,4,4´,5,5´-HBB 2,2´,3,4´,5´,6-HBB 3,3´,4,4´,5,5´-HBB	59080-40-9 69278-59-7 60044-26-0	Number of isomers: 42
Heptabromobiphenyl ($C_{12}H_3Br_7$)	2,2´,3,4,4´,5,5´-heptabromobiphenyl	67733-52-2	Number of isomers: 24
Octabromobiphenyl ($C_{12}H_2Br_8$)	Commercialized OBB is a mixture with bromine atoms of 8 - 9	61288-13-9	Number of isomers: 12
Nonabromobiphenyl ($C_{12}HBr_9$)	Commercialized NBB is a mixture (details unknown)	27753-52-2	Number of isomers: 3
Devabromobiphenyl ($C_{12}Br_{10}$)	DBB: 2,2',3,3',4,4',5,5',6,6'-DBB Commercialized DBB contains NBB	13654-09-6	Number of isomers: 1



m = 0,1,2,3,4,5; n = 0,1,2,3,4,5; m = n ¹ 0

Attachment-2 Compositions of PBB used in the study (those listed in the References)

Type of PBB	Compositions	References
FM BP-6	Hexabromobiphenyl, 62.6% (of which 2,2',4,4',5,5'-HBB, heptabromobiphenyl and pentabromobiphenyl account for 56.0%, 33.4% and 4.0% respectively)	Gupta et al., 1981
	Hexabromobiphenyl content is >60% - 90%. Other than that, contains hepta-, penta-biphenyl	Di Carlo et al., 1978
FM FF-1	2% calcium polysilicate was added to FM BP-6	Gupta et al., 1981
OBB (Octabromobiphenyl)	Octabromobiphenyl, 33.0% Nonabromobiphenyl, 60.0% Decabromobiphenyl, 6.0% Heptabromobiphenyl, 1.0%	Waritz et al., 1977
	Octabromobiphenyl, 45.2% Nonabromobiphenyl, 47.4% Decabromobiphenyl, 5.7% Heptabromobiphenyl, 1.8%	Norris et al., 1975
DBB(Decabromobiphenyl)	Decabromobiphenyl, 96.8% Nonabromobiphenyl, 2.9% Octabromobiphenyl, 0.3%	Di Carlo et al., 1978

Attachment-3 Results of *in vitro* test on receptor binding

Item	Test methods and conditions	Result	Remark	References
Binding test to estrogen receptor (ER)	Method: Binding test to human ERα (recombinant human ER α ligand domain)	IC50: p,p'-dibromobiphenyl: $>10^{-4}$ M (E2: 1.2 X 10^{-9} M)	Demonstrates no binding	CERI, 2001
Reporter gene assay using recombinant incubated cell	Cell: HeLa cell transfected with human ER α expression gene and ER responding sequence Incubation concentration: 10^{-11} - 10^{-5} M	p,p'-dibromobiphenyl: Agonist activity was negative within the range of 10^{-11} - 10^{-5} (E2: PC50: $<10^{-11}$ M)	No gene transcription activity	CERI, 2001

ER: Estrogen receptor; E2: 17β-estradiol;

IC50: 50% inhibition concentration; PC50: Concentration corresponding to 50% of the maximum activity by E2

Attachment-4 Results of tests on endocrine system and reproductive system of mammals

Animal species	Administration method	Administration period	Dose	Target organs	References
Rat (F344/N, males and females, no description of age and body weight)	gavage p.o.	6 months (Administration starting time is unknown)	FM FF-1* 0, 0.3, 1.0, 3.0, 10 mg/kg/day	Males at ≥ 0.3 mg/kg/day, females at ≥ 1.0 mg/kg/day: Dose-dependent decrease in serum T4. Females at ≥ 3 mg/kg/day: Decrease in serum T3	Gupta et al., 1983a
Rat (SD, male, 300 - 400 g)	gavage p.o.	10 - 20 days (Administration starting time is unknown)	FM FF-1* 0, 1, 3, 6 mg/kg/day	≥ 1 mg/kg/day: Decreased plasma T4, increased TSH (day 20) 6 mg/kg/day: Increased iodide content and 131I uptake in thyroid gland	Allen-Rowla-nds et al., 1981
Rat (SD, female, 225 g)	By feeding	7 months (Administration starting time is unknown)	HBB* (commercialized product) 0, 1, 5, 10, 50 ppm	Decreased serum T3 an dT4 (dose-dependent) 5 and 10 ppm: Reduced response of serum T3 and T4 to exogenous TSH (5 - 7 months)	Sepkovic and Byrne, 1984 Byrne et al., 1987
Rat (SD, female, 225 g)	By feeding	7 months (Administration starting time is unknown)	OBB* (commercialized product) 0, 50 ppm	No effect on serum T3	Sepkovic and Byrne, 1984
Rat (Wistar, male, juvenile)	i.p.	Single administration, blood collection up to day 28 (Administration starting time is unknown)	3,3',4,4',5,5'-HBB* 0, 20, 40 mg/kg	20 & 40 mg/kg: Decreased serum T4,; No change in serum T3	Spear et al., 1990
Pig (female, 200 kg)	By feeding	During the latter half of gestation period and nursing period	FM BP-6* 0, 10, 100, 200 ppm	Dams: Decreased serum T3 and T4 at 200 ppm Piglets (at birth): Decreased serum T3 and T4 at 200 ppm Piglets (4 weeks after birth): Decreased serum T3 and T4 at ≥ 100 ppm	Werner & Sleight, 1981
Mouse (Balb/c, female, 6 weeks old)	By feeding	24 - 30 days	FM BP-6* 0, 100 ppm	Slight increase in plasma corticosterone	Fraker, 1980

Animal species	Administration method	Administration period	Dose	Target organs	References
Rat (SD, pregnant females and F_1; no description of age and body weight)	By feeding	From day 8 of gestation up to 9 weeks after birth of F_1 After weaning of F_1 when they were 4 weeks old, FM BP-6 was mixed in food and given to them	FM BP-6* 0, 100 ppm	F_1 Both males and females at 100 ppm: No effect on plasma corticosterone, prolactin and LH	Johnston et al., 1980
Rat (SD, female, 300-500 g)	Gavage p.o. 5 days/ weeks	Administration 20 times, sacrificed on day 26	FM FF-1* 0, 1, 3, 6 mg/kg/day	6 mg/kg/day: Decreased plasma prolactin but no change in plasma corticosterone and testosterone. No change in adrenal and testis weights	Castracane et al., 1982
Rat (SD, female, 225-250 g)	By feeding	5 - 7 months	FM BP-6* 0, 1, 5, 10, 50 ppm	Decreased plasma corticosterone and dehydroepiandrosterone (dose-dependent), decreased adrenal weight	Byrne et al., 1988
Monkeys (rhesus monkeys, female, 6.0 kg)	By feeding	7 months	FM FF-1* 0, 0.3ppm (about 10.5 mg/monkey as the total dose)	Prolonged menstruation cycle (4/7 monkeys; 28 days in control group, Vs 31 days in dose groups), decreased serum progesterone	Allen et al., 1978 Lambrecht et al., 1978
Mouse (Swiss/ICR, pregnant female; no description of age and body weight)	By feeding	Pregnant 7-18 days	FM BP-6* 0, 50, 100, 1,000 ppm	≥ 50 ppm: Decreased fetal body weight ≥ 100 ppm: Ectoencephalopathy 1,000 ppm: Cleft palate	Corbett et al, 1975
Rat (SD, pregnant female; no description of age and body weight)	By feeding	Pregnant 7-20 days	FM BP-6* 0, 50, 100, 1,000 ppm	≥ 100 ppm: Decreased fetal body weight No external deformation	Corbett et al, 1975

Animal species	Administration method	Administration period	Dose	Target organs	References
Rat (Wistar, pregnant female; no description of age and body weight)	Gavage p.o.	One day (once) between day 6 and 14 of gestation Autopsy on day 20 of gestation	FM BP-6* 0, 40, 200, 400, 800 mg/kg	40 mg/kg: Fetal body weight, no deformation 200 mg/kg: Increased embryo resorptions by administration once between day 6 and 12 of gestation 400 mg/kg: Death and resorption of all the embryoes or most of embryoes by administration once between day 6 and 12 of gestation; Administration after day 11 of gestation resulted in deformations in fetuses such as cleft palate and diaphragm hernia	Beaudoin, 1977
Rat (SD, pregnant female; no description of age and body weight) Multi-generation reproductivity test	By feeding F_0: From day 8 of gestation to 28 days after delivery F_1: From day 28 of birth through mating and pregnancy up to 28 days after delivery of F_2 F_2: Administration up to 28 days after delivery of F_3 as in the case of F		FM BP-6* 0, 10, 100 ppm	F_0: No effect on gestation period, number of offspring in a litter, body weight of F_1 offsprings at birth Increased mortality up to weaning of F_1 offspring at 100 ppm F_1: ≥ 10 ppm: Increased relative weight of liver, induction of hepatic and renal microsome metabolizing enzymes, decreased vitamin A content in liver 100 ppm: Delayed growth and physical development indices F_2: ≥ 10 ppm: Induction of hepatic microsome metabolic enzymes 100 ppm: Increased relative weight of liver, induction of hepatic and renal microsome metabolizing enzymes, decreased vitamin A content in liver F_3: No influence	McCormack et al., 1981

* Refer to attachment-1, -2

Attachment-5 Repeated dose toxicity test results

HBB* (including FM FF-1*, FM BP-6*)

Animal species	Administration method	Administration period	Dose	Result	References
Mouse ($B6C3F_1$/N, female and male, 8 weeks old)	Gavage p.o.	30 days	FM FF-1 0, 0.03, 0.3, 3, 30 mg/kg/day	30 mg/kg/day: Inhibition on body weight increase (male), tendency of decrease in Hb, Hb and RBC (males), decreased platelet count (males & females), increased r-GTP (males & females), increased liver weight (males & females), decreased thymus weight (males), histological changes in liver (swollen hepatocyte, focal necrosis, lipid deposition)	Gupta et al., 1981
Rat (F344, female and male, 8 weeks old)	gavage p.o.	30 days	FM FF-1 0, 0.03, 0.3, 3, 30 mg/kg/day	30 mg/kg/day: Inhibition on body weight increase (males & females), decreased Hb, Hb and RBC (males), decreased lymphocyte count (females), decreased platelet count (males & females), increased serum β-globulin fraction and decreased blood glucose (males & females), increased liver weight and decreased thymus weight (males & females) Light microscopy findings of liver (swollen hepatocyte, focal necrosis, lipid deposition) Electron microscopy findings of liver (swollen mitochondria, increased lipid droplets in cytoplasm, rough endoplastic reticlum, depleted glycogen)	Gupta et al., 1981

Animal species	Administration method	Administration period	Dose	Result	References
				3 mg/kg/day: Decreased lymphocyte count (females), increased serum β-globulin fractions (males & females), increased liver weight, decreased thymus weight (males & females) Light microscopy findings & electron microscopy findings of liver NOAEL= 0.3 mg/kg/day	
Dog (beagle, 11 - 13 months old)	p.o.	61 days	FM FF-1 0, 0.0625, 0.25, 1.0, 4.0 mg/kg/day	4.0 mg/kg/day: Death (1/6: digestive tract bleeding), loss of eyesight (1/6) 0.25 - 4.0 mg/kg/day: Blast lymphocyte in peripheral blood, inhibition of hematopoiesis in bone marrow, increased foamy large reticuloendothelial cells in cytoplasm, enhanced extramedullary hematopoiesis and decreased lymphocyte count in white pulp in spleen, depleted lymphocyte count in T cell domain of lymph node, atrophy of thymus 0.0625 mg/kg/day: Blast lymphocyte in peripheral blood, atrophy of thymus	Farber et al., 1978

Animal species	Administration method	Administration period	Dose	Result	References
Monkey (rhesus monkey, female 6 kg, male 9 kg)	By feeding	10 weeks - 1 year	FM FF-1 0, 0.3, 1.5, 25 ppm (Corresponding to 0, 0.01, 0.07, 0.16 mg/kg/day)	0.3 ppm (administration for 1 year): Decreased body weight, serum estradiol and progesterone, prolonged menstruation cycle, abortion (2/7) 1.5 ppm (administration for ≥ 5 months): Decreased body weight, edema around orbit, decreased serum cholesterol, fat deposition in liver 25 ppm (administration for 10 weeks): Decreased body weight, swollen stomach, diarrhea, progressive gastroenteritis and ulcerous lesion in large intestine (2/7)	Allen & Lambrecht, 1979 Allen et al., 1978

* Refer to attachment-1, -2

Attachment-6 Repeated dose toxicity test results

OBB*, NBB* and DBB*

Animal species	Administration method	Administration period	Dose	Result	References
Rat (SD, female, 11 weeks old)	By feeding	28 days Recovery period: 18 weeks	OBB, 0, 1, 10, 100, 1,000 ppm (Corresponding to 0, 0.06, 0.6, 6, 67 mg/kg)	≥ 100 ppm: Increased relative weight of liver, hypertrophy of hepatocyte, localized concentration of basophilic granule in cytoplasm, depleted glycogen NOEL=10 ppm (Corresponding to 0.6 mg/kg)	Waritz et al, 1977
Rat (SD, male, no description of age and body weight)	By feeding	30 days	OBB 0, 0.01, 0.1, 1% (Corresponding to 0, 8, 80, 800 mg/kg)	≥ 0.01%: Swollen liver, hypertrophy and vacuolation of hepatocyte, bleeding in kidney, hyaline degeneration in renal cytoplasm, hyperplasia of thyroid gland ≥ 0.1%: Increased kidney weight 1%: Decreased RBC and Ht	Norris et al, 1975

Animal species	Administration method	Administration period	Dose	Result	References
Mouse ($B6C3F_1$, female and male, 5 weeks old)	By feeding	15 months	NBB 0, 100, 300 ppm	≥ 100 ppm: Inhibition of body weight increase, slight decrease in food consumption 300 ppm (males): Death (in all animals within 57 weeks) 100 ppm: Decreased Ht (males & females), decreased WBC and increased platelet (males), increased MCHC** (females), increased GPT and decreased TG (males & females),increased ALP and GOT, decreased A/G ratio (males), decreased NEFA (females), increased liver weight and hypertrophy of hepatocyte (males & females) 300 ppm (females only): Increased MCHC and GPT, decreased NEFA and TG, increased liver weight, hypertrophy of hepatocyte, increased relative weight of heart	Momma, 1986
Rat (SD, males & females, age and body weight are unknown)	By feeding	13 weeks	DBB 0, 100, 500, 2,000 ppm	100 & 500 ppm groups: No toxic changes 2,000 ppm group: Increased relative weight of liver, hypertrophy and vacuolation of hepatocyte, depleted glycogen	Millischer et al., 1979

* Refer to attachment-1, -2

** MCHC: Mean hemoglobin concentration; GPT: Glutamic pyruvic transaminase; TG: Triglyceride; ALP: Alkaliphosphatase; GOT: Glutamic oxaloacetic transaminase; A/G: Albumin/globulin; NEFA: Non-esterified fatty acid

Attachment-7 Repeated dose toxicity test results

Animal species	Administration method	Administration period	Dose	Target organs	References
Mouse ($B6C3F_1$, female, no description of age and body weight)	gavage p.o.	30 days, 5 times/ weeks × 22 times	FM FF-1 0, 0.03, 0.3, 3, 30mg/kg/day	3.0 mg/kg/day: Decreased response to T cell mitogen (PHA, COnA) stimulation in splenic lymphocyte 30 mg/kg/day: Decreased response to T cell mitogen (PHA, COnA) stimulation and decreased response to B cell mitogen (LPS of E. coli) stimulation in splenic lymphocyte, decreased antibody production to sheep erythrocyte, decreased serum IgM and IgG2 level	Luster et al., 1978
Mouse ($B6C3F_1$, female and male, no description of age and body weight)	gavage p.o.	6 months, 5 times/ weeks × 122 times	FM FF-1 0, 0.1, 0.3, 1, 3, 10mg/kg/day	0.3 mg/kg/day: Increased serum IgG 1.0 mg/kg/day: Increased serum IgG ≥ 1.0 mg/kg/day: Increased CFU count of bone marrow cell (females only) 10 mg/kg/day: Decreased serum IgG, IgM and IgA, decreased response to T and B cell mitogen stimulation (PHA, ConA, LPS of *E. coli*), increased sensitivity to Listeria infection	Luster et al., 1980
Mouse (Balb/c, female, 7weeks old)	By feeding	30 days	FM BP-6 0, 1, 10, 100, 1000 ppm	≥ 10 ppm: Decreased antibody production (IgM and IgG) to sheep erythrocyte ≤ 100 ppm: No changes in delayed hypersensitivity reaction	Fraker., 1980

Animal species	Administration method	Administration period	Dose	Target organs	References
Mouse (Balb/c, male, female, 18 - 20 g)	By feeding	3 weeks or 6 weeks	FM FF-1 0, 5, 167 ppm	5 ppm: Decreased serum IgM (week 3 and 6) 167 ppm: Increased sensitivity to endotoxin (LPS of *Salmonella*), decreased primary antibody production to sheep erythrocyte (wee 3 only). No influence on mean duration from Plasmodium berghei (malarial parasite) infection up to death.	Loose et al., 1981
Pregnant mice ($B6C3F_1$, males, no description of age and body weight)	gavage p.o..	From day 0 of gestation up to weaning of F_1, 5 days/week	FM FF-1 0, 0.3, 1, 3, 10 mg/kg/day	1 mg/kg/day: Increased CFU count in bone marrow cell	Luster et al., 1980
Rat (Fisher, male, no description of age and body weight)	gavage p.o.	30 days, 5 times/ weeks × 22 times	FM FF-1 0, 0.03, 0.3, 3, 30mg/kg/day	3.0 mg/kg/day: Decreased response to T cell mitogen (PHA) stimulation in splenic lymphocyte 30 mg/kg/day: Decreased response to T cell mitogen (PHA, ConA) stimulation	Luster et al., 1978
Rat (Fisher, female, no description of age and body weight)	gavage p.o..	6 months 5 days/ weeks × 122 times	FM FF-1 0, 0.1, 0.3, 1, 3, 10 mg/kg/day	≥ 1 mg/kg/day: Decreased response to T cell mitogen (PHA, ConA) stimulation ≥ 3 mg/kg/day: Inhibition of delayed hypersensitivity reaction 10 mg/kg/day: Decreased response to B cell mitogen (PWM) stimulation, inhibition of antibody response to bovine r-globulin	Luster et al., 1980

Animal species	Administration method	Administration period	Dose	Target organs	References
Pregnant pigs (female, no description of month age and body weight)	By feeding	12 weeks including latter half of gestation period (8 weeks) and nursing period (4 weeks)	FM BP-6 0, 100, 200 ppm	200 ppm: Decreased response to mitogen (PHA, PWM) in peripheral lymphocyte	Howard et al., 1980
Piglet	By feeding Administration to dams	Latter half of gestation period (8 weeks) and nursing period (4 weeks)	FM BP-6 0, 100, 200 ppm Doses administered to dams	At birth: No influence on response to mitogen (PHA, PWM) in peripheral lymphocyte. 4 weeks old: Decreased response to mitogen (PWM) in 200 ppm group	Howard et al., 1980

CFU: colony forming unit, Colony forming unit; ConA: Cancanavalin A; LPS: Lipopolysaccharide;
PHA: phytohemagglutinin antigen (vegetable hamagglutinin; PWM: Pork weed mitogen