FULL PAPER *Toxicology*

Carcinogenicity of Biphenyl in Mice by Two Years Feeding

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ABSTRACT. Carcinogenicity and chronic toxicity of biphenyl was examined in the male and female BDF_1 mice fed a diet containing biphenyl at 667, 2,000 or 6,000 ppm for 2 years. There was no difference in survival rate between any biphenyl-containing diet-fed group of either sex and the respective control. Body weights of the males and females fed 6,000 ppm diet were significantly lower than the respective control. Incidences of hepatocellular carcinomas and hepatocellular adenomas in the females fed diets containing biphenyl were significantly increased in a dose-related manner, and exceeded a range of the historical control data in the Japan Bioassay Research Center. Incidences of basophilic cell foci in the liver were increased in the females fed 2,000 and 6,000 ppm diets. There was no increase in tumor or tumor-related lesion in the males fed diets containing biphenyl. Chronic toxicity of biphenyl was characterized by increased incidences of urothelial desquamation in the renal pelvis in males and females and mineralization in the inner stripe of renal outer medulla in females, as well as changes in serum levels of BUN, ALP and some electrolytes in males and females. In conclusion, the 2-year oral administration of biphenyl-containing diets induced pre-neoplastic and neoplastic lesions in the liver of females and non-neoplastic lesions in the kidney of males and females. Causative factors for the biphenyl-induced hepatocarcinogenicity were discussed in light of our published finding of peroxisome proliferation.

KEY WORDS: biphenyl, carcinogenicity, liver, mouse.

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Biphenyl has been widely used as a heat-transfer agent, a mordant dye, a synthetic resin, a constituent of agricultural chemicals and a post-harvest fungicide to preserve citrus fruits. Annual production of biphenyl in Japan was reported to be 5,000 tons in 2002 [4]. Biphenyl poisoning was characterized by central and peripheral nervous damage, liver injury and electroencephalographic and electromyographic abnormalities in workers [8, 24] and kidney disorder in rats [1]. The International Agency for Research on Cancer (IARC) has not evaluated the carcinogenic potential of biphenyl for humans [5]. Data of cancer epidemiology and animal carcinogenicity are important determinants for the classification and evaluation of carcinogenic potential of biphenyl. There are few animal carcinogenicity data available for the evaluation of carcinogenic potential of biphenyl by bioassay studies with rodents exposed to biphenyl for long term [10, 11, 30]. Innes *et al.* [11] reported as a preliminary note that any tumor was not induced by oral administration of biphenyl to $B6AKF_1$ and $B6C3F_1$ mice of both sexes for 18 months. Imai *et al.* [10] reported that any tumor was not induced by either oral administration of biphenyl or combined administration of biphenyl and thiabendazole in feed to female ddY mice for 2 years. However, we previously reported that 2-year oral administration of biphenyl-containing diets induced both benign and malignant bladder tumors in male F344 rats, in close association with formation of bladder calculi [30].

The present study aimed at examining the mouse carcinogenicity and chronic toxicity of biphenyl. BDF_1 mice of both sexes were fed biphenyl-containing diets at 3 different dose levels for 2 years. Causative factors for the biphenyl-induced mouse carcinogenicity were discussed in light of our published finding [29] of peroxisome proliferation.

MATERIALS AND METHODS

The present study was conducted with reference to the Organisation for Economic Co-operation and Development (OECD) Guideline for Testing of Chemicals 453 "Combined Chronic Toxicity/Carcinogenicity Studies" [19] and in conformity with the OECD Principles of Good Laboratory Practice [20].

Chemical: Biphenyl (greater than 98% purity) was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). It was confirmed by both mass spectrometry and infrared spectrometry that neither decomposition products nor impurities were detected in the test substance.

Animal: The animals were cared for in accordance with the Guide for the Care and Use of Laboratory Animals [12], and this study was approved by the ethics committee of the Japan Bioassay Research Center (JBRC). Four-week-old male and female Crj:BDF_1 mice (SPF) were purchased from Charles River Japan, Inc. (Kanagawa). The animals were quarantined and acclimated for 2 weeks, and then divided by stratified randomization into 4 weight-matched groups, each consisting of 50 mice of both sexes. The animals were housed individually in stainless-steel wire-mesh hanging cages (112 mm [W] × 212 mm [D] × 120 mm [H]) under controlled environmental conditions (a temperature of 24 ± 2°C; a relative humidity of 55 ± 10%; 15–17 room air changes/hr) in the barrier system colony room. The animals had free access to sterilized, filtered drinking water supplied

by an automatic watering system. Fluorescent lighting was controlled automatically to provide a 12-hr light/dark cycle.

Dose selection, diet preparation and feeding: Three different dose levels of 667, 2,000 and 6,000 ppm biphenyl in diets were selected, based on the results of our 13-week subchronic toxicity study [29], and with reference to the maximum tolerated dose (MTD) presented by the guidelines of the National Cancer Institute [26] and the IARC [2]. In our previous study [29], subchronic toxicity and body weight decrement were examined by feeding male and female BDF_1 mice diets containing 500, 2,000, 4,000, 8,000, 10,000 and 16,000 ppm biphenyl for 13 weeks. The dose levels of 8,000 ppm and below did not induce any of the life-threatening toxicological manifestations that were predicted to reduce the normal lifespan of mice. The dose level of 8,000 ppm was found to decrease the body weights of the males and females by 17% and 6%, respectively, apparently exceeding the MTD for the male mice. However, the dose levels of 4,000 ppm and below did not decrease the body weights, as compared with the male and female controls. Therefore, the highest dose level was determined to be 6,000 ppm for the present study of mouse carcinogenicity.

Diets containing biphenyl at 667, 2,000 and 6,000 ppm (w/w) were prepared by mixing the chemical with γ-irradiation-sterilized CRF-1 powdered diet (Oriental Yeast Co., Tokyo, Japan) in a spiral mixer for 20 min, and stored at 4°C until use. The powdered diets containing biphenyl were prepared at an interval of 3 months during the 2-year administration period. Dietary concentrations of biphenyl were confirmed by gas chromatography, and found to be kept constant within a range of 79.2–111.8% of the designated target concentrations. Groups of 50 male and 50 female mice were fed *ad libitum* a control diet or each of the biphenyl-containing diets throughout the 2-year administration period, starting at the age of 6 weeks.

Experimental design: All animals were observed daily, to assess clinical signs and mortality. Body weight and food consumption were measured once a week for the first 14 weeks of the 2-year administration period and every 4 weeks thereafter. Hematological and blood biochemical parameters were measured with Automatic Blood Cell Analyzer (TECHNICON H-1, U.S.A.) and Automatic Analyzer (HITACHI 7070, Japan), respectively, with blood samples of all surviving animals taken under etherization, after overnight fasting at the end of the 2-year administration period. Organs were removed, weighed and examined macroscopically. The tissues for microscopic examination were fixed in 10% neutral buffered formalin, and then embedded in paraffin by routine procedures. Tissue sections of 5 μm in thickness were prepared and stained with hematoxylin and eosin (H&E). Neoplastic and pre-neoplastic lesions were diagnosed with reference to the criteria presented by Frith *et al.* [6].

Statistical analysis: Incidences of non-neoplastic lesions were analyzed by Fisher's exact test. Incidences of neoplastic lesions were statistically analyzed by Peto's trend test [21] and Fisher's exact test. Body weight, food consumption, organ weight and hematological and blood biochemical parameters were analyzed by Dunnett's test. Values of body weight and blood biochemistry were expressed as mean ± standard deviation.

RESULTS

Survival, body weight, food consumptions and clinical signs: Body weights of the males and females fed 6,000 ppm diet were significantly lower than the respective control (Fig. 1). Food consumption of any biphenyl-fed group of either sex was not decreased throughout the 2-year administration period, compared with the respective control. Daily biphenyl intake, estimated by food consumption and the dietary concentration of biphenyl divided by the body weight, was found to increase proportionally with an increase in the dietary concentration of biphenyl (Table 1). There was no difference in survival rate between any biphenyl-fed group of either sex and the respective control (Fig. 2). No overt clinical signs were observed in any biphenyl-fed group.

Hematology and blood biochemistry: There was no significant difference in any hematological parameter between any biphenyl-fed group of either sex and the respective control (data not shown). BUN was increased in the males fed 2,000 and 6,000 ppm diets and in the females fed 6,000 ppm diet (Table 2). ALP was increased in the males and females fed 6,000 ppm diet. Slight increases in sodium and chloride and a decrease in potassium were observed in the males fed diets containing biphenyl, while sodium and calcium were increased in the females fed diets containing biphenyl. Although no significant change in GOT or GPT was found in any of the males fed diets containing biphenyl, dose-dependent increases in the serum levels of the transaminases were noted in the females fed 2,000 and 6,000 ppm diets. LDH was also increased in the females fed 2,000 and 6,000 ppm diets. Large individual variations of GOT, GPT and LDH, as indicated by the large standard deviations in the females fed 2,000 and 6,000 ppm diets, revealed that females bearing the malignant liver tumor had extremely high serum levels of GOT, GPT and LDH.

Organ weights and gross findings: No significant difference in organ weight between any biphenyl-fed group of either sex and the respective control was found except for relative liver weight of females. Relative liver weights of the females fed 667, 2,000 and 6,000 ppm diets were increased by 1.3-, 1.4- and 1.6-fold, respectively, compared with the female control (data not shown).

Incidences of liver nodules in the females were increased in a dose-related manner, whereas those in the males were not (Table 3). It has been recognized that those liver nodules were closely associated with pre-neoplastic and neoplastic lesions [6]. The nodules were round- or oval-shaped and cystic or solid mass, with the diameter of major axis varying from 3 to 23 mm. The present histopathological examination revealed that 5, 16 and 19 nodule-bearing females fed 667, 2,000 and 6,000 ppm diets, respectively,

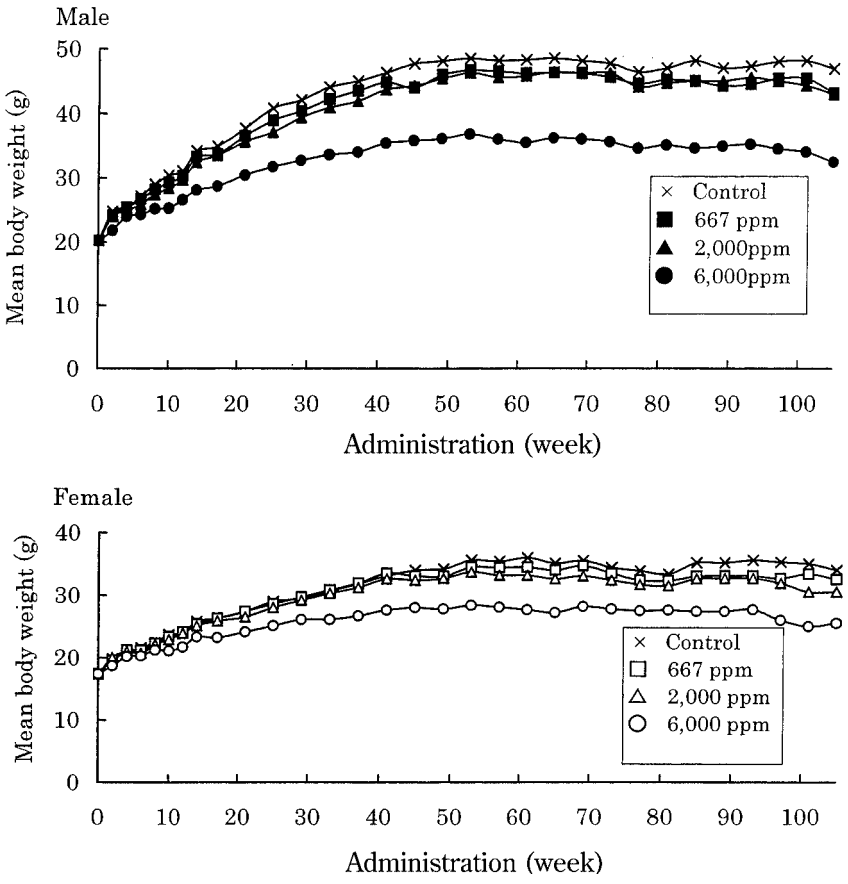

Fig. 1. Time-course changes in the mean body weights of the male or female BDF_1 mice fed diets containing biphenyl at 667, 2,000 or 6,000 ppm for two years.

had the proliferative lesions which originated from the hepatocytes.

Histopathological examinations: Table 3 shows incidences of neoplastic and pre- and non-neoplastic lesions in the liver and kidney of the males and females fed biphenyl-containing diets for 2 years. The organs affected were primarily the liver and kidney. Incidences of hepatocellular adenomas and combined incidences of hepatocellular adenomas and carcinomas were significantly increased in the females fed 2,000 and 6,000 ppm diets, and those tumor incidences were dose-related, as indicated by a significant positive trend by Peto's test. Incidence of hepatocellular carcinomas was also significantly increased in the females fed 2,000 ppm diet. Although 5 cases of hepatocellular carcinoma in the females fed 667 or 6,000 ppm diet were not statistically significant, the tumor incidence (10%) exceeded a range of the JBRC historical control data for hepatocellular carcinoma (26 cases [2.5%] in 1,048 female mice in 21 carcinogenicity studies, with the maximum incidence of 8%). Notably, incidences of hepatocellular adenomas and carcinomas were not increased in any of the males fed diets containing biphenyl.

The hepatocellular adenoma was characterized by a well-circumscribed lesion compressing adjacent parenchyma and by the absence of normal lobular architecture, and was composed of well-differentiated hepatocytes. The hepatocellular carcinoma was characterized by a trabecular or pseudoglandular structure accompanied by sinusoidal dilatation (Fig. 3). The hepatocellular carcinoma had an irregular border between the tumor and the adjacent parenchyma, resulting primarily from local invasion and compression of the adjacent parenchyma by the affected tissue. Focal necrosis was observed around or within the malignant liver tumors. No hepatocellular carcinoma metastasized to other organs.

Three types of altered cell foci in the liver were found in the mice fed diets containing biphenyl (Table 3). Incidence of basophilic cell foci was significantly increased in the females fed 2,000 and 6,000 ppm diets. Although the incidences of basophilic cell foci (12%) and clear cell foci (12%) were significantly increased in the males fed 667 ppm diet, the incidences of those pre-neoplastic lesions were not dose-related. Those altered cell foci were found as a discrete area in the liver parenchyma having a normal lobular architecture. The basophilic cell foci were composed of the affected hepatocytes being stained homogeneously, deeply basophilic in the cytoplasm. The nuclei of the hepa-

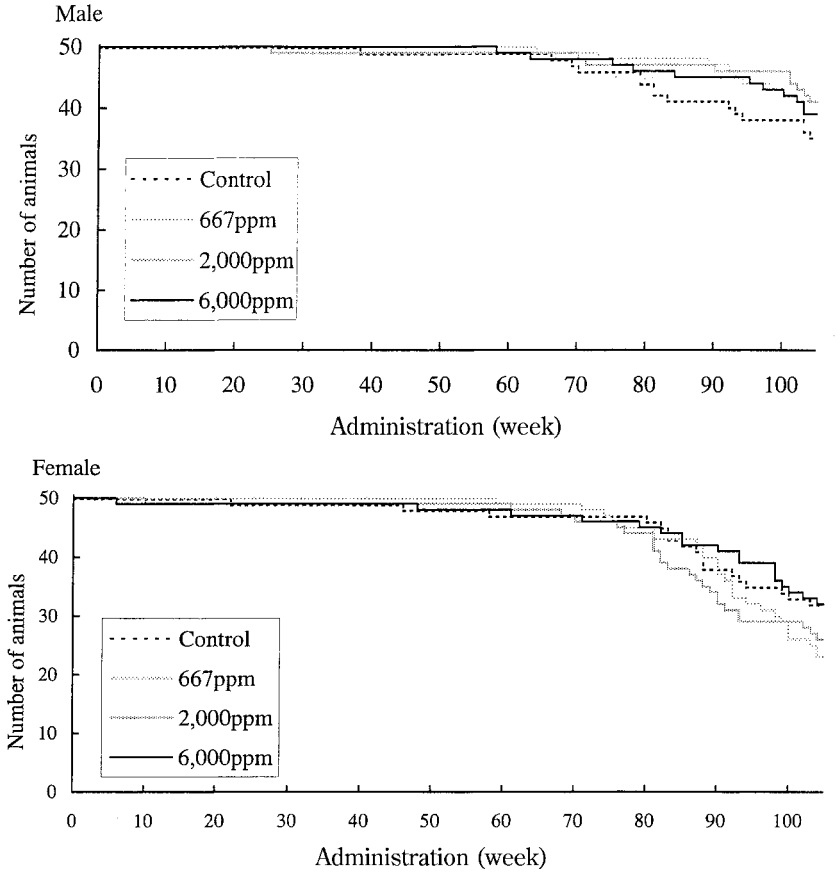

Fig. 2. Time-course changes in the survival rates of the male or female BDF_1 mice fed diets containing biphenyl at 667, 2,000 or 6,000 ppm for two years.

Table 1. Survival rate, body weight, food consumption and daily biphenyl intake of the mice fed diets containing biphenyl for two years

Biphenyl concentration in diet (ppm)	Survival rate at the end of 2-yr administration period	Body weight at the end of 2-yr administration period[a)] (g)	Averaged food consumption[b)] (g/day)	Daily biphenyl intake[b)] (mg/kg)
Male				
Control	35/50	46.9 ± 4.9	5.6	0
667	41/50	43.1 ± 7.9	5.5	97
2,000	41/50	42.9 ± 6.0*	5.5	291
6,000	39/50	32.4 ± 3.6**	5.4	1,050
Female				
Control	31/50	34.0 ± 4.0	5.9	0
667	22/50	32.5 ± 3.3	5.8	134
2,000	25/50	30.5 ± 3.1**	5.9	414
6,000	32/49	25.5 ± 3.0**	5.9	1,420

* and **: Significantly different at $p<0.05$ and $p<0.01$, respectively ,by Dunnett's test.
a) Values of body weight were expressed as mean ± standard deviation.
b) Food consumption and biphenyl intake were averaged over the 2-yr administration period.

tocytes with the basophilic cell foci were enlarged and hyperchromatic with prominent nucleoli (Fig. 4).

Incidences of desquamation of the urothelium in the renal pelvis were significantly increased in the males and females fed 6,000 ppm diet. The necrotic urothelium accompanied by mineralization was found in the renal pelvic cavity

Table 2. Blood biochemistry in the male and female mice fed diets containing biphenyl for two years

	Male				Female			
Group Name	Control	667 ppm	2,000 ppm	6,000 ppm	Control	667 ppm	2,000 ppm	6,000 ppm
No. of Animals	34	39	37	37	28	20	22	31
GOT (IU/*l*)	85 ± 92	58 ± 38	69 ± 60	88 ± 151	75 ± 27	120 ± 110	211 ± 373**	325 ± 448**
GPT (IU/*l*)	73 ± 113	34 ± 31	36 ± 49	43 ± 80	32 ± 18	56 ± 46	134 ± 231**	206 ± 280**
ALP (IU/*l*)	178 ± 111	155 ± 30	169 ± 36	261 ± 102**	242 ± 90	256 ± 121	428 ± 499	556 ± 228**
LDH (IU/*l*)	321 ± 230	252 ± 126	432 ± 868	283 ± 200	268 ± 98	461 ± 452	838 ± 2,000	1,416 ± 4,161*
BUN (mg/d*l*)	20.2 ± 3.6	22.0 ± 4.0	23.2 ± 4.4*	22.9 ± 2.7**	14.9 ± 2.0	14.8 ± 3.4	21.0 ± 20.5	23.8 ± 11.7**
Calcium (mg/d*l*)	9.2 ± 0.6	9.0 ± 0.5	9.1 ± 0.5	9.2 ± 0.3	9.0 ± 0.2	9.1 ± 0.4	9.5 ± 0.7**	9.6 ± 1.1**
Sodium (mEq/*l*)	152 ± 1	153 ± 2	153 ± 2	155 ± 2**	152 ± 2	152 ± 2	152 ± 3	155 ± 4**
Potassium (mEq/*l*)	4.4 ± 0.4	4.2 ± 0.4	4.2 ± 0.4	4.1 ± 0.3**	4.1 ± 0.3	4.3 ± 0.4	4.1 ± 0.7	4.0 ± 0.5
Chloride (mEq/*l*)	122 ± 3	124 ± 3	124 ± 2*	125 ± 3**	125 ± 3	124 ± 3	122 ± 6	124 ± 5

Values were expressed as mean ± standard deviation.
* and **: Significantly difference at P<0.05 and P<0.01, respectively, by Dunnett's test.
GOT: glutamic oxaloacetic transaminase, GPT: glutamic pyruvic transaminase, ALP: alkaline phosphatase, LDH: lactate dehydrogenase, BUN: blood urea nitrogen.

Table 3. Incidences of gross and histopathological findings in the male and female mice fed diet containing biphenyl for two years

Sex of animal	Male				Peto's	Female				Peto's
Group (ppm)	Control	667	2,000	6,000	test	Control	667	2,000	6,000	test
Number of mice examined	50	49	50	50		50	50	50	49	
Gross finding										
Liver										
Nodule	20	16	14	11		7 (5)[c)]	13 (5)	24 (16)	26 (19)	
Histopathological findings										
Liver										
Hepatocellular adenoma	8	6	7	3		2	3	12*	10*	↑
Hepatocellular carcinoma	8	8	5	4		1	5	7*	5	
Hepatocellular adenoma + carcinoma[a)]	16	12	9	7		3	8	16**	14*	↑↑
Basophilic cell foci[b)]	0	6**	1	2		1	1	12**	6*	
Clear cell foci[b)]	0	6**	2	0		2	1	3	2	
Eosinophilic cell foci[b)]	0	0	0	0		0	1	0	0	
Kidney										
Desquamation: pelvis[b)]	0	0	0	10**		4	0	0	15**	
Mineralization in the inner stripe-outer medulla[b)]	9	8	14	14		3	5	12*	26**	

* and **: Significantly different at p<0.05 and p<0.01, respectively, by Fisher's test.
↑ and ↑↑: Significantly different at p<0.05 and p<0.01, respectively, by Peto's test.
a) Combined incidence of hepatocellular adenoma and carcinoma.
b) Number of the histopathological finding with a different grade (slight, moderate, marked or severe) was summed.
c) The parenthesized value indicates the number of the animals bearing the liver nodule in which the proliferative lesion was histopathologically observed.

(Fig. 5). Incidences of the mineralization in the inner stripe of outer medulla of the kidney were significantly increased in the females fed 2,000 and 6,000 ppm diets.

DISCUSSION

It was found in the present study that the 2-year oral administration of biphenyl-containing diets to female BDF_1 mice significantly increased the incidences of hepatocellular adenomas and the combined incidences of hepatocellular adenomas and carcinomas at 2,000 and 6,000 ppm and the incidence of hepatocellular carcinomas at 2,000 ppm. The incidences of hepatocellular carcinomas in the females fed 667 and 6,000 ppm diets were also found to exceed a range of the JBRC historical control data. A dose-related increase in the incidences of hepatocellular adenomas and the combined incidences of the benign and malignant hepatocellular tumors was noted. The incidences of basophilic cell foci, known as a pre-neoplastic lesion [6], were significantly increased in the females fed 2,000 and 6,000 ppm diets, compared with that in the control females. In contrast, no dose-related increase in the incidence of pre-neoplastic or neoplastic lesion was found in the liver of males. Imai *et al.* [10] reported that 2-year administration of biphenyl in feed did not increase any tumor incidence in female ddY mice, and that no case of the liver tumors was observed. The dif-

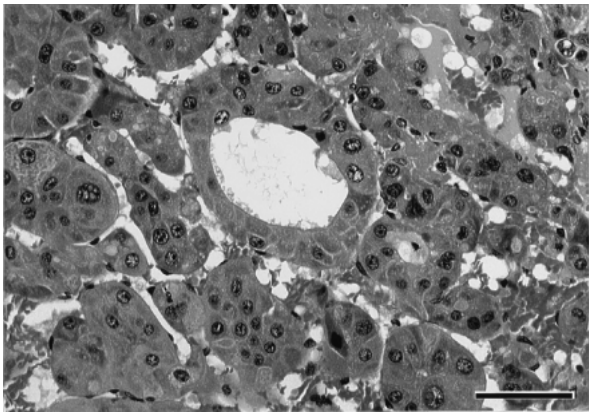

Fig. 3. The hepatocellular carcinoma from a female BDF_1 mouse fed 6,000 ppm diet. The trabecula was two or more cell layers thick and separated by dilated vascular space. The pseudo-glandular pattern or island-like structure was noted. H & E stain, Bar: 100 μm.

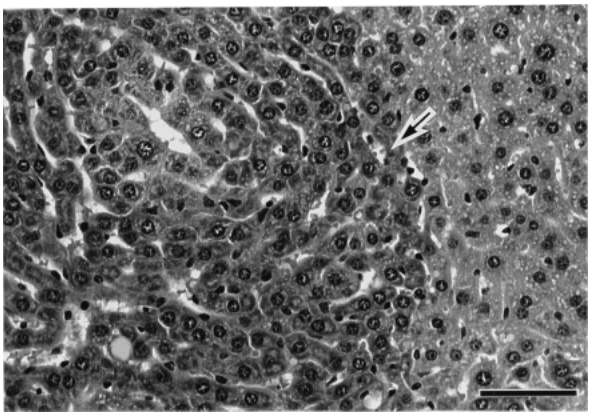

Fig. 4. The basophilic cell foci (arrow) in the liver of a female BDF_1 mouse fed 6,000 ppm diet. The lesion was composed of the affected hepatocytes being stained homogeneously, deeply basophilic in the cytoplasm. H & E stain, Bar: 100 μm.

ference in the biphenyl-induced hepatocarcinogenicity between the present study and the study of Imai *et al.* [10] might be attributed to a difference in the mouse strain used (BDF_1 mice versus ddY mice).

As to the non-neoplastic lesion found in this study, chronic toxicity of biphenyl was characterized by histopathological and biochemical changes in the kidney of males and females. This was evidenced by the necrotic desquamation of urothelium in the renal pelvis in the males and females and the mineralization in the inner stripe of outer medulla in the females, as well as the increased serum levels of BUN and the changes in some electrolytes in the males and females fed diets containing biphenyl. The increased ALP in the males and females fed 6,000 ppm diet was also suggestive of the kidney damage [27].

One of the causative factors for the biphenyl-induced hepatocarcinogenicity in the female mice was explored by

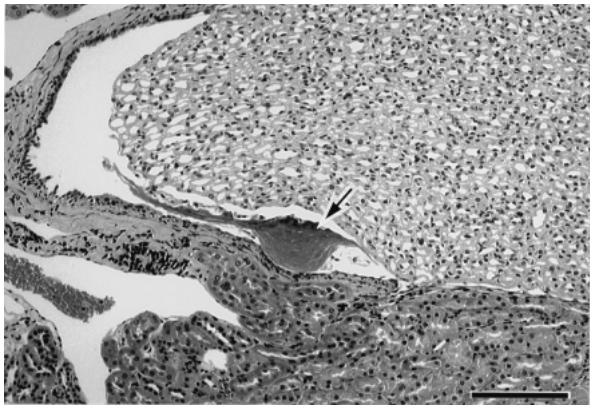

Fig. 5. The desquamation of urothelium of pelvis in the kidney of a male BDF_1 mouse fed 6,000 ppm diet. The necrotic urothelium was accompanied by the mineralization in the renal pelvic cavity (arrow). H & E stain, Bar: 200 μm.

the electron-microscopic study of 13-week oral administration of biphenyl-containing diets to male and female BDF_1 mice [29]. The liver of the females fed diet containing biphenyl was histopathologically characterized by clearly enlarged hepatocytes filled with eosinophilic fine granules which were found to correspond to the increased number of peroxisomes by electron microscopy. The eosinophilic fine granules were not found in the liver of the male mice fed diets containing biphenyl. The present electron-microscopic finding of the biphenyl-induced peroxisome proliferation in the liver of the females was consistent with the result of Sunouchi *et al.* [28] that oral administration of biphenyl to female BDF_1 mice increased the peroxisomal fatty acid β-oxidation activity, while the β-oxidation activity was not increased in the male mice. The findings of the morphological [29] and biochemical examinations [28] suggest that the biphenyl-induced peroxisome proliferation occurred only in the liver of female mice but not in that of male mice. Furthermore, 2,5-dihydroxy-biphenyl (2,5-DHBP), a biphenyl metabolite, was structurally similar to CI-924 that was reported to be a peroxisome proliferator [31]. It was noteworthy that CI-924 induced both the peroxisomal fatty acid β-oxidation activity and the morphologic features of the peroxisomes more profoundly in the female mice than in the male mice [31]. Long-term administration of many peroxisome proliferators to rodents has been known to cause the liver tumors [3, 15]. Therefore, it is thought that the peroxisome proliferation in the liver was involved in the biphenyl-induced hepatocarcinogenicity in female mice.

A genotoxic mode of action might be initiated through the damage on DNA by biphenyl or its metabolites in the biphenyl-induced hapatocarcinogenicity, as suggested by the following findings reported in literature [7, 9, 13, 16, 23, 25]. Bacterial mutagenicity of biphenyl was negative with and without S9 activation, but the negative mutagenicity of biphenyl with bacteria might be attributed to antibacterial

action of biphenyl [7, 13]. Although mammalian cell clastogenicity of biphenyl was negative without S9 activation, it was positive in Chinese hamster V79 cell with the liver homogenate from rats [7] and in Chinese hamster CHL/IU cell with the S9 activation of mice [25]. Biphenyl was positive in a micronucleus test with Chinese hamster cells in the S9 activation, suggesting positive clastogenicity by biphenyl metabolites (Personal communication: the JBRC study of micronucleus test by T. Sasaki *et al.*). 2-Phenyl-1,4-benzoquinone (2-PBQ), an active metabolite of 2-hydroxylbiphenyl (2-HBP), was reported to cross-link to DNA [9] and to damage DNA in the epithelial cells of the rat urinary bladder [16] and in the mouse liver, bladder and other organs [23]. Therefore, it is inferred on the basis of the above literature that the genotoxic mode of action operates in the biphenyl-induced hepatocarcinogenicity, mediating through the possible DNA damage by active biphenyl metabolites.

We previously reported that the 2-year oral administration of a biphenyl-containing diet at 4,500 ppm induced bladder transitional cell papillomas and carcinomas in male F344 rats, in close association with formation of bladder calculi [30]. In addition, any histopathological changes in the liver were not observed in the male and female rats fed 4,500 ppm diet for 2 years [30]. Therefore, the present and previous studies demonstrated that biphenyl produced both benign and malignant tumors in a dose-related manner in the bladder of male rats and in the liver of female mice. Notably, species and sex differences were recognized in development of the biphenyl-induced carcinogenicity found in the present and previous studies.

A clue to understand the species and sex differences in the biphenyl-induced carcinogenicity seems to be in differences in metabolic pathway of biphenyl. Biphenyl was reported to be hydrolyzed in the liver at the first phase, conjugated with sulfate or glucuronide at the second phase, and to be finally excreted into urine [14, 17, 18, 32]. At the first phase, biphenyl was biotransformed to 4-hydroxy-biphenyl (4-HBP) as a major metabolic pathway in rats and mice [17, 32], whereas the quantity of 2-HBP metabolized from biphenyl as the minor pathway was smaller in rats than in mice [32]. It is, therefore, suggested that the lack of the biphenyl-induced hepatocarcinogenicity in rats was attributable to the major metabolic pathway of biphenyl to 4-HBP but not to 2-HBP in mature rats [17], whereas 2-HBP was further metabolized to 2,5-DHBP, a possible peroxisome proliferator, and 2-PBQ, a genotoxicant [16, 22]. It was reported that the second phase metabolic pathway of 4-HBP to its sulfate conjugate was causally related to the calculus formation in the bladder of the male rats fed a diet containing biphenyl, leading to the development of bladder tumors [17, 18]. This is in sharp contrast to the present finding of the lack of calculus formation and bladder tumor in the mice fed diets containing biphenyl. It is inferred, therefore, that the lack of the bladder tumor in the mice fed diets containing biphenyl is causally related to the lack of bladder calculi. However, the mouse biphenyl metabolism relating to the lack of calculus formation in the bladder remains to be solved for elucidation of the species difference in the bladder tumor.

Further investigation will be needed to explore causative factors underlying the biphenyl-induced hepatocellular tumors in the female mice with emphasis on the species and sex differences in addition to mode of actions in the carcinogenesis.

In conclusion, the present study demonstrated that the 2-year oral administration of a biphenyl-containing diet produced dose-related increases in both benign and malignant hepatocellular tumors and the pre-neoplastic liver lesion in the female mice, together with non-neoplastic kidney lesions in both male and female mice.

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