FULL PAPER *Toxicology*

Carcinogenicity and Chronic Toxicity in Mice and Rats Exposed by Inhalation to *para*-Dichlorobenzene for Two Years

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ABSTRACT. Carcinogenicity and chronic toxicity of *para*-dichlorobenzene (*p*-DCB) were examined by exposing 50 BDF_1 mice and 50 F344 rats of both sexes by inhalation to *p*-DCB vapor at a target concentration of 0 (control), 20, 75 or 300 ppm for 6 hr/day, 5 days/week and 2 years. Incidences of hepatocellular carcinomas, hepatoblastomas and hepatic histiocytic sarcomas in the 300 ppm-exposed male mice, and hepatocellular adenomas and carcinomas and hepatoblastomas in the 300 ppm-exposed female mice were increased. An increase in the incidences of most of those liver tumors was dose-related. No increase in tumor incidence was found in any *p*-DCB-exposed rat of either sex. Centrilobular hypertrophy of hepatocytes and papillary mineralization and pelvic urothelial hyperplasia of the kidney were noted in the 300 ppm-exposed male rats. Treatment- and age-related increases in incidences of the eosinophilic globules of the respiratory and olfactory epithelia in female rats and incidences of the respiratory metaplasia of the nasal gland epithelium in mice and rats and the olfactory epithelium in mice were noted. The nasal lesion was the most sensitive endpoint of chronic inhalation toxicity. Induction of the mouse hepatocarcinogenicity and lack of the rat nephrocarcinogenicity found in the present study were compared with the mouse liver tumors and the rat renal tumors reported by the NTP gavage study, and discussed in light of the estimated *p*-DCB uptake into the body through the inhalation and the oral administration.

KEY WORDS: liver tumor, nasal lesion, *para*-Dichlorobenzene.

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para-Dichlorobenzene (*p*-DCB) has been widely used as a deodorant and a moth repellent, and as an intermediate for dyestuffs, fungicides, pharmaceuticals and industrial chemicals. Global production of *p*-DCB was 24,000 tons in 1980–1983 [18]. Because of widespread exposure to *p*-DCB used as a moth repellent and a deodorant, a mean blood *p*-DCB level of 2.1 $\mu g/l$ and a mean urinary 2,5-dichlorophenol level of 200 $\mu g/l$ were detected in the general population in the U.S.A. [12]. An average adipose tissue level of 2.3 μg *p*-DCB/g and an average blood level of 9.5 *ng p*-DCB/m*l* were found in Tokyo residents, while outdoor and indoor air concentrations of *p*-DCB were 1.5–4.2 $\mu g/m^3$ and 105–1700 $\mu g/m^3$ in Tokyo, respectively [27]. Approximately 34,000 male and 9,400 female workers in the U.S.A. were occupationally exposed to *p*-DCB in 1981–1983 according to the National Occupational Exposure Survey conducted by the National Institute for Occupational Safety and Health [30]. Hollingsworth *et al.* [14] reported painful irritation of the eyes and nose and difficult breathing among workers exposed to *p*-DCB.

Any epidemiological study has not been reported on cancer except a report that suggested a weak association between leukemia and exposure to *p*-DCB [16]. The National Toxicology Program (NTP) [31] reported that 2-year administration of *p*-DCB by gavage induced liver tumors in mice of both sexes and kidney tumors in male rats. Loeser and Litchfield [24] reported that inhalation exposures of male and female rats to *p*-DCB for 76 weeks and of female mice to *p*-DCB for 57 weeks failed to demonstrate an excess of tumors.

Since inhalation exposure to *p*-DCB is a principal route for humans exposed to *p*-DCB, carcinogenicity and chronic toxicity data from inhalation exposure of animals to *p*-DCB are more relevant for health risk assessments than those from the administration by gavage. However, there are few animal data of carcinogenicity and chronic toxicity of inhaled *p*-DCB available for assessing the health risks of humans exposed by inhalation to *p*-DCB vapor. Based on the epidemiological data of *p*-DCB carcinogenicity to humans and the carcinogenicity data of rats and mice administered *p*-DCB by gavage [31], the International Agency for Research on Cancer (IARC) made overall evaluation of *p*-DCB carcinogenicity as a possible carcinogen to humans (Group 2B) [16]. The Japan Society for Occupational Health evaluated the *p*-DCB carcinogenicity as being possibly carcinogenic to humans (Group 2B) [20].

The present study was undertaken to provide dose-response data of rodent carcinogenicity and chronic toxicity of the inhaled *p*-DCB. BDF_1 mice and F344 rats of both sexes were exposed by inhalation to *p*-DCB vapor at 0 (clean air as control), 20, 75 or 300 ppm for 2 years (104 weeks). Relationships between internal doses of *p*-DCB and induction of tumors were discussed in light of the estimated uptake of *p*-DCB into the body through the inhalation and the administration by gavage [31]. Besides, the affected organs were examined for the most sensitive endpoint of the chronic inhalation toxicity.

MATERIALS AND METHODS

The present study was conducted with reference to the Organization for the Economic Co-operation and Development (OECD) Guideline for Testing of Chemicals 453 "Combined Chronic Toxicity/Carcinogenicity Studies" [32] and in conformity with the OECD Principles of Good Laboratory Practices [33]. Mice and rats were cared in accordance with Guide for the Care and Use of Laboratory Animals [15], and the present study was approved by the ethics committee of the Japan Bioassay Research Center (JBRC).

Test substance: *p*-DCB of reagent grade (greater than 99.9% pure) was obtained from Wako Pure Chemical Industries, LTD (Tokyo, Japan) in eight different lots. The test substance was stored in a dark-colored bottle at room temperature during the study period. Each lot of *p*-DCB was analyzed for stability by gas chromatography and infrared spectrometry and for purity by mass spectrometry and infrared spectrometry before and after its use. Those analyses indicated that neither decomposition products nor impurities were detected in the test substance.

Exposure to p-DCB: Stainless-steel inhalation exposure chambers (volume: 3,700 *l* for mice and 7,600 *l* for rats) were used throughout the 2-year exposure period. *p*-DCB vapor-air mixture was generated and supplied to the exposure chambers by the method described previously [1]. Briefly, solid *p*-DCB was liquefied in a reservoir flask with a thermostatted water bath heated at 70°C. Clean air was bubbled through the liquid *p*-DCB. Airflow containing the saturated *p*-DCB vapor was conditioned at 60°C by passing through a thermostatted condenser, and then diluted with large volume of clean air, in order to prevent aerosolization of vaporized *p*-DCB in the airflow. Finally, the diluted vapor-air mixture was supplied to the inhalation exposure chambers with a dynamic air flow of 740 *liters/min* for mice and 1900 *liters/min* for rats.

Groups of 50 mice and 50 rats of both sexes were exposed by inhalation to *p*-DCB at a target concentration of 20, 75, or 300 ppm for 6 hr/day, 5 days/week and 2 years. A group of 50 rats or 50 mice of either sex, serving as a control, was handled in the same manner as the *p*-DCB-exposed groups, but was exposed to clean air only. Air concentrations of *p*-DCB vapor in the exposure chambers were monitored at an interval of 15 min by gas chromatography, and were maintained constant at 19.9 ± 0.4 (mean ± SD), 74.8 ± 0.8 or 298.3 ± 2.3 ppm for mice and 19.8 ± 0.4, 74.8 ± 1.0 or 298.4 ± 4.0 ppm for rats throughout the 2-year exposure period.

According to the guidelines of the National Cancer Institute (NCI) [39] and the IARC [4], the highest dose of the test agent given during the chronic study should not exceed the maximum tolerated dose (MTD) that can be predicted not to alter the animals' normal longevity from toxic effects other than carcinogenicity, or no more than a 10% weight decrement, as compared to the concurrent control groups. Therefore, the high dose level used in the present study was selected as 300 ppm on the basis of our data of both growth rate and severity of subchronic toxicity from the 13-week inhalation study [1], predicting that the inhalation exposure of rats and mice of both sexes to 300 ppm *p*-DCB vapor for 2 years does not exceed the MTD.

Animals and husbandry: Four-week-old Crj:BDF$_1$ mice and F344/DuCrj rats of both sexes were purchased from Charles River Japan, Inc. After a 2-week period of quarantine and acclimation, animals were allocated by a stratified randomization procedure into 4 body weight-matched groups, each comprising 50 mice or 50 rats of each sex, and were used in the present study at 6 weeks of age. The animals were housed individually in stainless-steel wire hanging cages (100 [W] × 116 [D] × 120 [H] mm for a mouse, 150 [W] × 216 [D] × 176 [H] mm for a rat) in the inhalation exposure chambers maintained at a temperature of 23 ± 2°C and a relative humidity of 55 ± 10% with 12 ± 1 air changes/hr. The air changes/hr was reduced to 6 ± 0.5 during the 6-hr exposure period. Fluorescent lighting was controlled automatically to give a 12-hr light/dark cycle. All mice and rats had free access to sterilized commercial pellet diet (CRF-1, Oriental Yeast Co., Ltd., Tokyo, Japan) and sterilized water supplied by an automatic watering system.

Clinical and pathological evaluation of animals: The animals were observed daily for clinical signs and mortality. Body weight and food consumption were measured once a week for the first 14 weeks and every 4 weeks thereafter. All animals underwent complete necropsy. Organs were removed, weighed and examined for macroscopic lesions at necropsy. The tissues for microscopic examination were fixed in neutral buffered 10% formalin, and embedded in paraffin. The pretreatment of the nasal cavity and the method for trimming of 3 frontal sections (Fig. 1) were described in our previous paper [29]. Tissue sections of 5 μm in thickness were prepared, and stained with hematoxylin and eosin (H & E).

Statistical analysis: Incidences of non-neoplastic lesions were analyzed using Chi-square test. Incidences of neoplastic lesions were statistically analyzed using Peto test [36] and Fisher's exact test. Body weight and food consumption were analyzed by the previously described method including Dunnett's Test [1]. Survival curves were plotted according to the method described by Kaplan and Meier [21], and the log-rank test [35] was used to test statistical significance of the difference in survival rate between any *p*-DCB-exposed group of mice or rats of either sex and the respective control.

RESULTS

Mouse study

Survival, body weight and food consumption: Survival rates of all *p*-DCB-exposed male groups tended to be decreased as compared to the male control (control: 39/49, 20 ppm: 31/49, 75 ppm: 32/50, 300 ppm: 30/49), while there was no difference in the survival rate between any *p*-DCB-exposed female group and the female control (control: 28/50, 20 ppm: 25/50, 75 ppm: 23/49, 300 ppm: 26/50) (Fig. 2

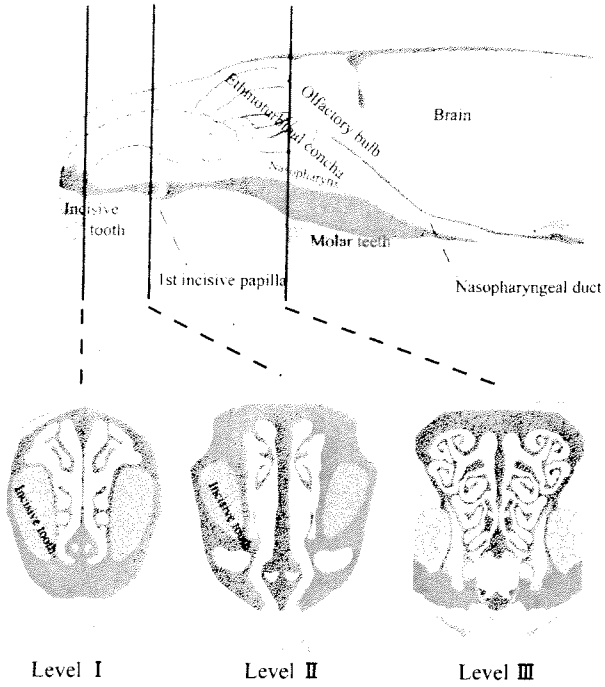

Fig. 1. Diagram of nasal passages of a mouse opened along the midline with the septum removed to show the turbinates. Three sections of the nasal cavity sectioned transversely at Levels I, II and III, i.e., at the level of the posterior edge of the upper incisive teeth (Level I), at the incisive papilla (Level II), and at the level of the anterior edge of the upper molar teeth (Level III).

A and B). Only the survival rate of 300 ppm-exposed males was significantly decreased by the Kaplan-Meier survival analysis. A total of 19 deaths consisting of 12 liver tumor deaths, 3 other tumor deaths and 4 non-neoplastic deaths occurred in the 300 ppm-exposed males, while 3 liver tumor deaths, 3 other tumor deaths and 4 non-neoplastic deaths were observed in the male control. Therefore, the significant decrease in the survival rate of 300 ppm-exposed males was attributed to an increase in the number of liver tumor deaths.

There was no significant difference in growth rate between any *p*-DCB exposed group of either sex and the respective control except for the 300 ppm-exposed males which exhibited the significantly decreased weight gain by 12% at the end of 2-year exposure period compared with the male control (Table 1, Fig. 2 C). No difference in food consumption between any *p*-DCB-exposed group of either sex and the respective control was found.

Pathology: Absolute and relative liver weights were significantly increased in the males and females exposed to 300 ppm (Table 1). Absolute and relative kidney weights were significantly increased in the females exposed to 300 ppm, while relative kidney weight was significantly increased in the males exposed to 300 ppm.

An increase in number of male and female mice bearing the liver nodules was noted at 300 ppm as a macroscopic lesion.

Incidences of hepatocellular carcinomas and hepatoblastomas were increased in the 300 ppm-exposed mice of both sexes (Table 2). The hepatocellular carcinomas exhibited a positive trend of dose-response as indicated by Peto test. Increased incidences of the histiocytic sarcomas in the 300 ppm-exposed males and the hepatocellular adenomas in the 300 ppm-exposed females were noted, both showing a positive trend of dose-response. The hepatocellular carcinoma (Fig. 3) had a wide variety of cell types from well-differentiated to extremely atypical hepatocytes. The hepatoblastoma (Fig. 4) had small, strongly basophilic and anaplastic cells with small nuclei and scant cytoplasm in thick trabecular, pseudo-glandular, or pseudo-rosette patterns. The affected tissue contained numerous vascular spaces of various sizes, and was seen within or adjacent to the hepatocellular adenoma or carcinoma. A significant positive trend of incidences of bronchiolo-alveolar carcinomas was noted in the *p*-DCB-exposed female mice (female control: 1/50, 20 ppm: 1/50, 75 ppm: 1/49, 300 ppm: 4/50). However, the incidence of the malignant lung tumor in the 300 ppm-exposed females (8%) did not significantly increase or exceed the upper limit of the JBRC historical control data for the bronchiolo-alveolar carcinoma (43 cases [2.9%] in 1498 females in 30 studies with the maximum incidence of 8% in a single study).

No significant increase in the incidence of altered cell foci in the liver was recognized in any of the *p*-DCB-exposed mouse groups of either sex. Increased incidence of centrilobular hypertrophy of hepatocytes was noted in the 300 ppm-exposed male mice (Table 2). There was no histopathological change indicating the hepatocellular injury in any of the *p*-DCB-exposed mouse groups of either sex.

In the nasal cavity, incidence of the respiratory metaplasia of the nasal gland epithelium tended to be increased in the 300 ppm-exposed females, and was significantly increased in the 75 ppm-exposed males (Table 3). Increased incidence of the respiratory metaplasia of the olfactory epithelium having slight grade of severity was noted in the females exposed to 300 ppm and in the males exposed to 75 ppm. The respiratory metaplasia was characterized by replacement of the olfactory epithelium with the respiratory-like mucosal epithelium in which those epithelial cells were similar to the ciliated cells of normal respiratory epithelium.

Rat study

Survival, body weight and food consumption: There was no significant decrease in survival rate between any exposed group of either sex and respective control except for the decreased survival rate of 300 ppm-exposed males as indicated by the Logrank analysis (male control: 33/50, 20 ppm: 34/50, 75 ppm: 29/50, 300 ppm: 18/50) (Fig. 2 D and E). A total of 32 deaths consisting of 10 leukemia, 9 other tumors of various organs, 11 chronic progressive nephropathy (CPN) and 2 unconfirmed deaths as the cause of death occurred in the 300 ppm-exposed males, while 17 deaths of the male control consisted of 3 leukemia, 8 other tumors and

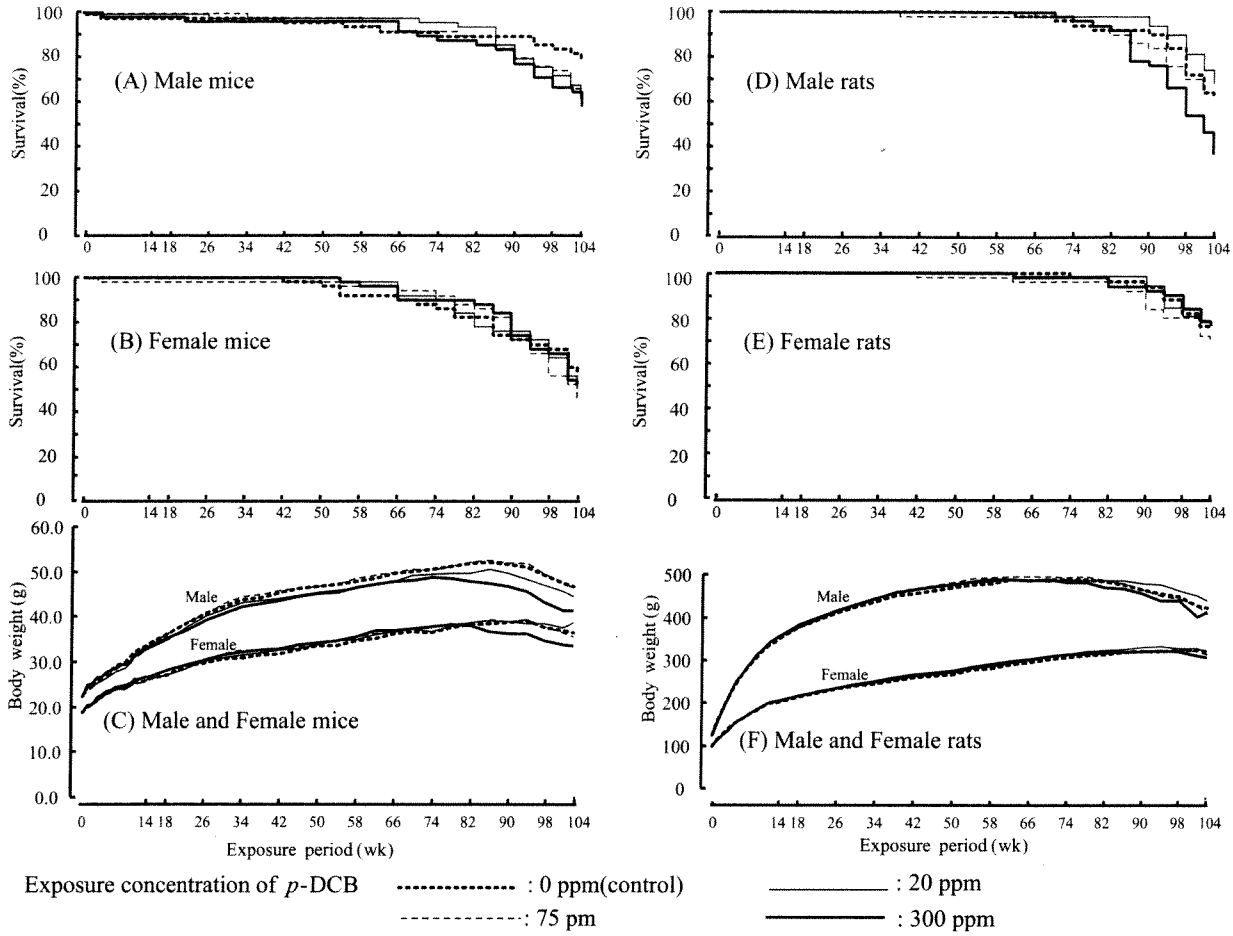

Fig. 2. Survival curves (A: male mice, B: female mice, D: male rats and E: female rats) and growth curves (C: male and female mice and F: male and female rats) of mice and rats of both sexes exposed by inhalation to *p*-DCB and clean air as controls for 2 years.

6 CPN. Therefore, the significant decrease in the survival rate of 300 ppm-exposed males was attributed to an increase in number of leukemia and CPN deaths.

There was no difference in growth rate or food consumption between any *p*-DCB-exposed group of either sex and the respective control throughout the 2-year exposure period (Table 1, Fig. 2 F).

Pathology: Significant increases in absolute and relative organ weights were observed in the liver of male and female rats exposed to 300 ppm and in the kidneys of males exposed to 300 ppm (Table 1). No macroscopic lesion was observed in the organs of any *p*-DCB-exposed rat of either sex.

No significant increase in the incidences of neoplastic nor tumor-related lesions was recognized in any organ of the *p*-DCB-exposed rat groups of either sex. Notably, any renal tumor was not induced in the *p*-DCB-exposed rats of either sex. In addition, although leukemia deaths were increased in the 300 ppm-exposed male rats, the incidences of leukemia were not dose-related (male control: 9/50, 20 ppm: 14/50, 75 ppm: 10/50, 300 ppm: 13/50).

Incidence of centrilobular hypertrophy of hepatocytes was increased in the 300 ppm-exposed male rats (Table 2). Increased incidences of papillary mineralization and hyperplasia of the pelvic urothelium in the kidney were noted in the 300 ppm-exposed male rats. High incidences of CPN which is known as a spontaneous disease of aged rats [22] were observed in all the exposed and control groups of both sexes. The incidences and severities of CPN were not related to the *p*-DCB exposure, but the average grade of severity was more profound in males than in females (Table 2). Any histopathological change indicating excessive accumulation of α_{2u}-globulin in the epithelial cells of renal proximal tubules was not found in this 2-year inhalation study, although both the hyaline droplets and the granular casts in the proximal tubules of male rats were detected in our 13-week inhalation study [1].

In the nasal cavity, incidences of the eosinophilic globules were increased in the olfactory epithelium (Fig. 5) having marked grade of severity in the females exposed to 75 and 300 ppm and in the respiratory epithelium (Fig. 6) having slight grade of severity in the 300 ppm-exposed females

Table 1. Body and organ weights of mice and rats exposed by inhalation to *p*-DCB for 2 years

p-DCB concentrations		Male				Female			
		0 ppm	20 ppm	75 ppm	300 ppm	0 ppm	20 ppm	75 ppm	300 ppm
<Mice>									
Number of animals examined[a]		39	31	32	30	28	25	23	26
Body weight	g[b]	42.5±8.1	40.3±8.4	41.6±6	37.6±3.9**	32.4±5.4	34.3±6.1	31.2±5.3	29.6±2.3
Liver	g[b]	1.700 ± 0.45	1.908 ± 0.616	1.984 ± 0.756	3.159 ** ± 0.163	1.583 ± 0.429	1.857 ± 1.283	1.571 ± 0.433	5.354** ± 2.955
	%[c]	4.212 ± 1.776	5.062 ± 2.395	4.868 ± 2.1	8.606** ± 4.766	5.010 ± 1.703	5.620 ± 4.412	5.020 ± 0.791	17.947** ± 8.83
Kidneys	g[b]	0.674 ± 0.266	0.637 ± 0.071	0.655 ± 0.064	0.722 ± 0.346	0.467 ± 0.093	0.469 ± 0.045	0.457 ± 0.062	0.514 * ± 0.117
	%[c]	1.686 ± 1.082	1.655 ± 0.438	1.605 ± 0.283	1.955 ** ± 1.1	1.474 ± 0.378	1.396 ± 0.22	1.480 ± 0.156	1.749** ± 0.433
<Rats>									
Number of animals examined[a]		33	34	29	18	38	34	38	36
Body weight	g[b]	394 ± 40	406 ± 45	382 ± 42	382 ± 43	300 ± 31	304 ± 40	298 ± 45	295 ± 42
Liver	g[b]	13.007 ± 1.669	14.309 ± 4.983	13.773 ± 2.357	14.957** ± 1.883	7.864 ± 0.899	8.032 ± 1.187	8.193 ± 1.378	9.055** ± 1.681
	%[c]	3.331 ± 0.546	3.583 ± 1.375	3.634 ± 0.69	3.950** ± 0.562	2.644 ± 0.379	2.657 ± 0.33	2.774 ± 0.394	3.159** ± 0.901
Kidneys	g[b]	3.040 ± 0.261	3.183 ± 0.59	3.193 ± 0.352	3.636** ± 0.493	2.088 ± 0.178	2.042 ± 0.175	2.081 ± 0.182	2.172 ± 0.23
	%[c]	0.779 ± 0.108	0.800 ± 0.204	0.849 ± 0.165	0.967** ± 0.194	0.703 ± 0.09	0.680 ± 0.093	0.715 ± 0.134	0.758 ± 0.186

a) The number of animals at terminal necropsy. b) Absolute organ weights are expressed as means ± SD.
c) Relative organ weights, the percentage of absolute organ weight to body weight are expressed as means ± SD.
* and **: significantly different from control at $p \leq 0.05$ and $p \leq 0.01$ by Dunnett's test, respectively.

(Table 3). The increased incidences of the eosinophilic globules were closely associated with a marked decrease in the number of olfactory cells in the olfactory epithelium of 300 ppm-exposed females (Fig. 5). Incidence of the respiratory metaplasia of the nasal gland epithelium was increased in the females exposed to 300 ppm. The eosinophilic globules were abundantly present in both the supporting cells of the olfactory epithelium and the ciliated and non-ciliated cells of the respiratory epithelium.

DISCUSSION

Carcinogenicity: It was found in the present study that the 2-year inhalation exposure to *p*-DCB vapor produced a dose-related increase in the incidences of benign and malignant liver tumors in mice but not in rats. The high dose level of 300 ppm is considered to be approximately equal to the MTD or at least not to exceed it, because both the 10% decrease in the growth rates of the 300 ppm-exposed animals and the decreased survival rates of the 300 ppm-exposed animals resulting from an increase in the number of tumor deaths were found to meet the MTD criteria set by IARC [4] and NCI [39]. The MTD for rats was also based on our previously reported results [1] that inhalation exposure of male rats to 600 ppm for 13 weeks increased both the blood urea nitrogen and the incidence of renal lesion. Loeser and Litchfield's result [24] that inhalation exposure of rats to 500 ppm for 76 weeks increased both the urinary protein and the coproporphyrin excretion was also taken into consideration of MTD for rats.

The *p*-DCB-induced hepatocarcinogenicity found in the present study can be contrasted with the results of both Loeser and Litchfield [24] and the NTP gavage study [31]. Loeser and Litchfield [24] reported that repeated inhalation exposure to 75 and 500 ppm *p*-DCB for 76 weeks (rats) and 57 weeks (mice) failed to demonstrate an excess of tumors. However, the exposure periods used in their study [24] seem to be too short to evaluate the rodent carcinogenicity of *p*-DCB. The present finding of the hepatocarcinogenicity was consistent with that of the NTP study that 2-year administration of *p*-DCB by gavage increased the incidences of hepatocellular adenomas and carcinomas and hepatoblastomas in $B6C3F_1$ mice of both sexes dosed at 600 mg/kg/day, while a dose of 300 mg/kg/day increased the incidence of hepatocellular adenomas only in the male mice [31]. Consistent with very low incidence of hepatoblastomas (0/1091) in male $B6C3F_1$ mice of vehicle control in the NTP historical control data [31], occurrence of the spontaneous hepatoblastomas was found to be rare (10/1496 for male BDF_1 mice and 0/1498 for female BDF_1 mice in the JBRC historical control data). Although the spontaneous hepatoblastoma was rare in both the JBRC and NTP historical control data [31], the inhalation exposure to 300 ppm induced the hepatoblastoma not only in male but also in female BDF_1 mice, but the

Table 2. Incidences of selected lesions in the livers and kidneys of mice and rats exposed by inhalation to *p*-DCB for 2 years

p-DCB concentrations	Male					Female				
	0 ppm	20 ppm	75 ppm	300 ppm	Peto test	0 ppm	20 ppm	75 ppm	300 ppm	Peto test
< Mice >										
Number of animals examined	49	49	50	49		50	50	49	50	
Liver										
Hepatocellular adenoma	13	9	7	13		2	10	6	20 **	↑↑
Hepatocellular carcinoma	12	17	16	38 **	↑	2	4	2	41 **	↑↑
Hepatoblastoma	0	2	0	8 **		0	0	0	6 *	
Histiocytic sarcoma	0	3	1	6 *	↑	2	1	1	0	
Hepatocellular hypertrophy: centrilobular[a)]	0	0	0	34 ##		0	0	0	2	
< Rats >										
Number of animals examined	50	50	50	50		50	50	50	50	
Liver										
Hepatocellular hypertrophy: centrilobular[b)]	0	0	0	5 #		0	0	0	3	
Kidney										
Mineralization: papilla[c)]	0	1	0	41 ##		1	0	0	0	
Urothelial hyperplasia: pelvis[b)]	7	8	13	32 ##		0	0	0	0	
Chronic progressive nephropathy[d)]	50 (3.34)	49 (3.27)	49 (3.35)	50 (3.48)		43 (2.28)	46 (2.15)	45 (2.24)	48 (2.29)	

* and **: Significantly different from control at p≤0.05 and p≤0.01 by Fisher's exact test. ↑ and ↑↑: Significantly different from control at p≤0.05 and p≤0.01 by Peto test.

and ##: Significantly different from control at p≤0.05 and p≤0.01 by Chi-square test.

a) 25 and 9 males had slight and moderate grades of severity, respectively, in the 300 ppm-exposed male group. One female had slight and the other had moderate grade in the 300 ppm-exposed female group.

b) All cases had slight grade of severity. c) All cases had slight grade of severity except the one having the moderate grade in the 300 ppm-exposed male group.

d) The values in parenthesis indicate the average of severity grade index of the lesion in affected animals. The average of severity grade index are calculated with a following equation. [Σ (grade × number of animals with grade)]÷number of examined animals. Grade: 1=slight, 2=moderate,

administration of *p*-DCB by gavage induced the hepatoblastoma only in male but not in female B6C3F$_1$ mice [31]. The similarity of mouse hapatocarcinogenicity between the present study and the NTP study [31] seems to be attributable to an approximately equal amount of *p*-DCB uptake into the mouse body, although there was a difference in the exposure route. The administration of *p*-DCB to mice by gavage at the doses of 600 and 300 mg/kg/day was estimated to correspond to the uptakes of 426 and 213 mg/kg/day into the body, respectively, since the gastro-intestinal absorption rate of *p*-DCB was 71% for mice [9]. On the other hand, the daily uptakes of *p*-DCB into the body through the 6-hr inhalation of 300 and 75 ppm *p*-DCB vapor were estimated to correspond to 474 and 119 mg/kg body weight, respectively, assuming that the air containing 300 ppm *p*-DCB vapor was inhaled for 6 hr/day by a mouse having a minute volume of 1239 ml/min/kg body weight [11] with a lung absorption rate of 59% for *p*-DCB [9]. Therefore, these two estimates of *p*-DCB uptake can be taken to indicate that a *p*-DCB uptake of greater than 200 mg/kg/day increased the incidence of liver tumors in mice, although administration of *p*-DCB by gavage is considered to rapidly increase blood and organ levels of *p*-DCB, possibly causing severer target organ toxicity than inhalation exposure to *p*-DCB vapor.

A species difference that the liver tumors were induced in mice but not in rats was noted in the present study. Since chemically induced cell death followed by regenerative cell proliferation is recognized to be an important mechanistic consideration for both genotoxic and non-genotoxic hepatocarcinogenicity [5, 8, 43], this species difference may be causally related to severer hepatotoxicity of *p*-DCB in mice than in rats, which was evidenced by necrosis, increased serum levels of liver transaminases and hepatocellular hypertrophy with varying nuclear size and shape in the mice exposed to *p*-DCB for 13 weeks but not in the rats [1]. Severer hepatotoxicity of *p*-DCB-exposed mice may be closely associated with greater metabolic capacity of the hepatic P450 enzymes in mice than in rats, which caused larger production of the covalently bound, reactive metabolites of *p*-DCB such as 2,5-dichlorohydroquinone and 2,5-dichlorobenzoquinone in mice [13, 23]. In addition, recent studies of *in vitro* assays [2, 3, 6, 19, 34, 37] seem to support genotoxicity of *p*-DCB, although mutagenicity of *p*-DCB was once recognized to be negative with the conventional *in vitro* assays [17]. Therefore, the hepatocellular injury and regenerative cell proliferation as well as the genotoxicity are thought to be involved in the *p*-DCB-induced hepatocarcinogenicity in mice.

On the other hand, there was a notable difference in the *p*-DCB-induced nephrocarcinogenicity between the NTP study [31] and the present study. A marginally but significantly increased incidence (14%) of renal tubular cell adenocarcinomas was observed in F344 male rats administered *p*-DCB by gavage for 2 years at a dose of 300 mg/kg/day but not at 150 mg/kg/day [31], whereas 2-year inhalation expo-

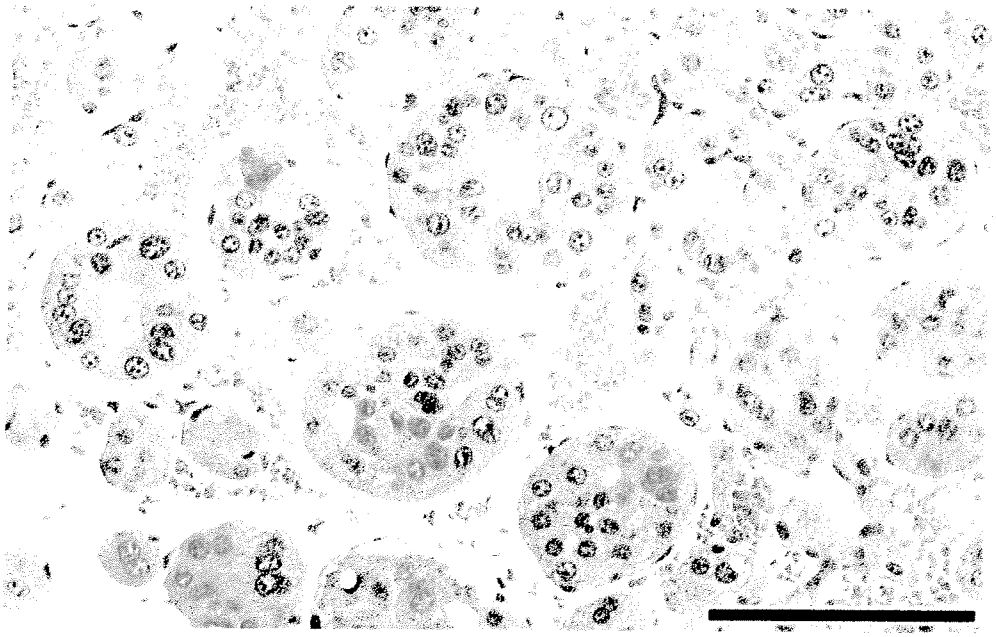

Fig. 3. Hepatocellular carcinoma in the liver of a male mouse exposed to 300 ppm *p*-DCB for 2 years. Bar indicates 100 μm. H & E stain.

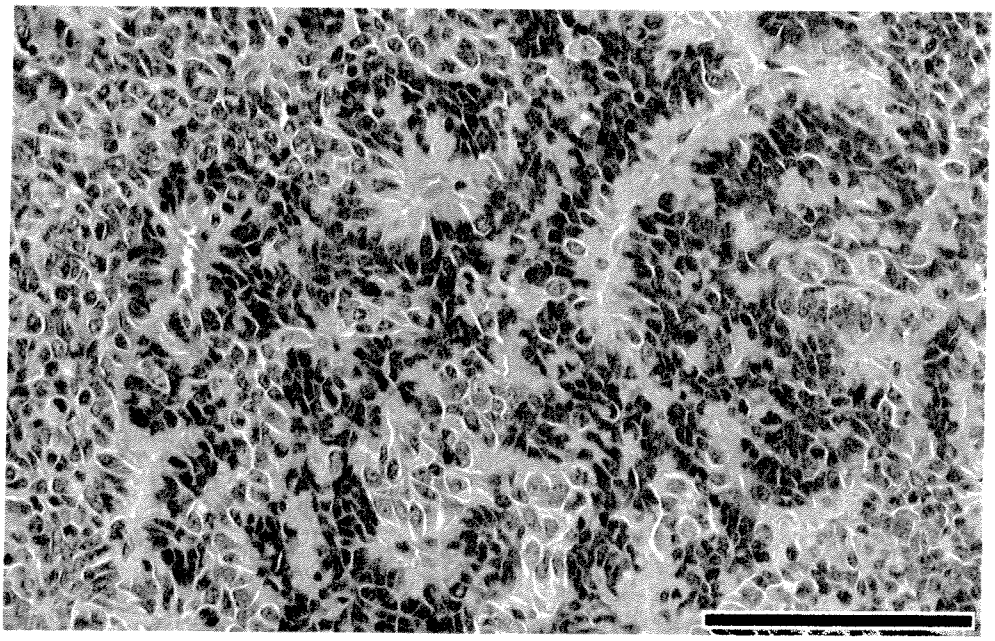

Fig. 4. Hepatoblastoma in the liver of a female mouse exposed to 300 ppm *p*-DCB for 2 years. The psudo-rossette was noted. Bar indicates 100 μm. H & E stain.

sure of the F344 male rats to 300 ppm *p*-DCB vapor did not induce any renal cell adenoma or carcinoma. This difference can be accounted for by greater *p*-DCB uptake into the rat body through the administration by gavage than through the 6-hr inhalation. An uptake of *p*-DCB for the administration of 300 and 150 mg/kg/day to rats by gavage was estimated to correspond to 186 and 93 mg/kg/day, respectively, since a gastro-intestinal absorption rate of *p*-DCB was 62% for rats [9]. On the other hand, an uptake of *p*-DCB through the 6-hr inhalation exposure of rats to 300 ppm vapor was estimated to correspond to 90 mg/kg/day on the basis of the area-under-curve method (AUC: a measure of total exposure) method [44] and to 54 mg/kg/day on the assumption of a male rat having a minute volume of 254 ml//min/kg body

Table 3. Nasal lesions in mice and rats of both sexes exposed by inhalation to *p*-DCB for 2 years

p-DCB concentrations		Male				Female			
		0 ppm	20 ppm	75 ppm	300 ppm	0 ppm	20 ppm	75 ppm	300 ppm
< Mice>									
No. of animals examined		49	49	50	49	50	50	49	50
Respiratory metaplasia									
Nasal gland	T[a)]	37	42	47 *	41	9	6	8	19
	(1+)	(28)	(29)	(27)	(30)	(9)	(6)	(8)	(18)
	(2+)	(9)	(12)	(18)	(11)	(0)	(0)	(0)	(1)
	(3+)	(0)	(1)	(2)	(0)	(0)	(0)	(0)	(0)
Olfactory epithelium	T[a)]	23	30	38 **	24	7	6	2	20 **
	(1+)	(23)	(30)	(37)	(22)	(7)	(6)	(2)	(20)
	(2+)	(0)	(0)	(1)	(2)	(0)	(0)	(0)	(0)
< Rats>									
No. of animals examined		50	50	50	50	50	50	50	50
Eosinophilic globules									
Respiratory epithelium	T[a)]	4	1	5	4	11	10	14	38 **
	(1+)	(4)	(1)	(5)	(4)	(11)	(10)	(14)	(38)
Olfactory epithelium	T[a)]	33	22	21	26	49	46	46 **	50 **
	(1+)	(32)	(20)	(19)	(19)	(22)	(17)	(7)	(3)
	(2+)	(1)	(1)	(1)	(7)	(21)	(27)	(16)	(27)
	(3+)		(1)	(1)	(0)	(6)	(2)	(23)	(20)
Respiratory metaplasia									
Nasal gland	T[a)]	3	0	0	0	5	4	4	33 **
	(1+)	(3)	(0)	(0)	(0)	(5)	(4)	(4)	(33)

a) Total number of animals bearing the nasal lesion.
* and **: Significantly different from control at $p \leq 0.05$ and $p \leq 0.01$ by Chi Square test.

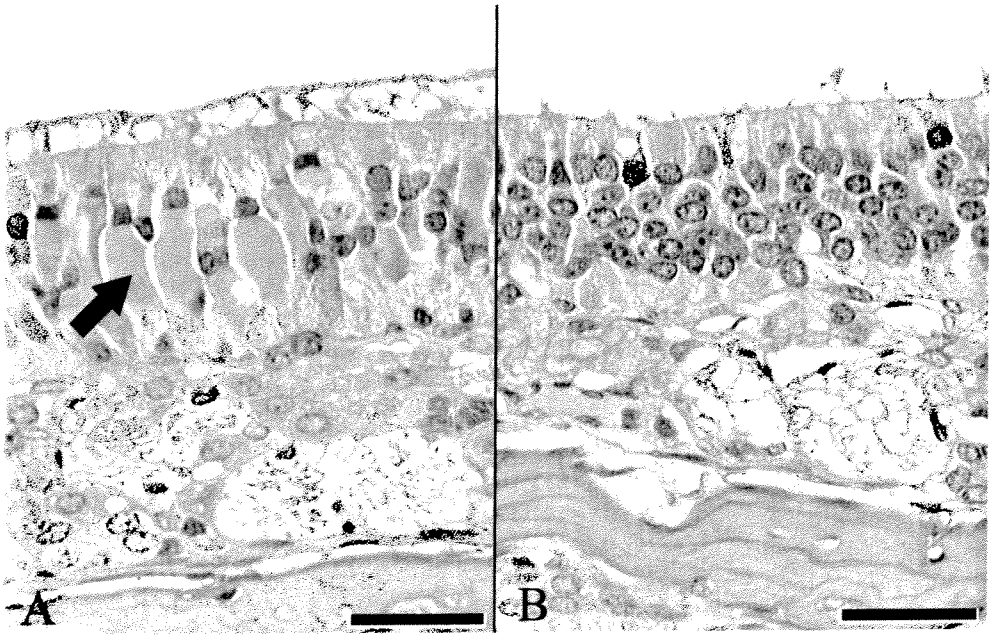

Fig. 5. (A): Eosinophilic globules (arrow) of the olfactory epithelium in a male rat exposed to 300 ppm *p*-DCB for 2 years. (B): The normal olfactory epithelium in a male rat exposed to clean air for 2 years. Note markedly decreased number of olfactory cell nuclei in the olfactory epithelium as compared to control (B). The ethmoturbinate was sectioned at Level III. Bar indicates 25 μm. H & E stain.

weight [25] and a lung absorption rate of 33% for *p*-DCB [9]. Therefore, it can be estimated that the daily amount of *p*-DCB uptake that caused the marginally increased incidence of renal tumors in the NTP gavage study [31] was greater than that inducing no renal tumors in our 2-year inhalation study.

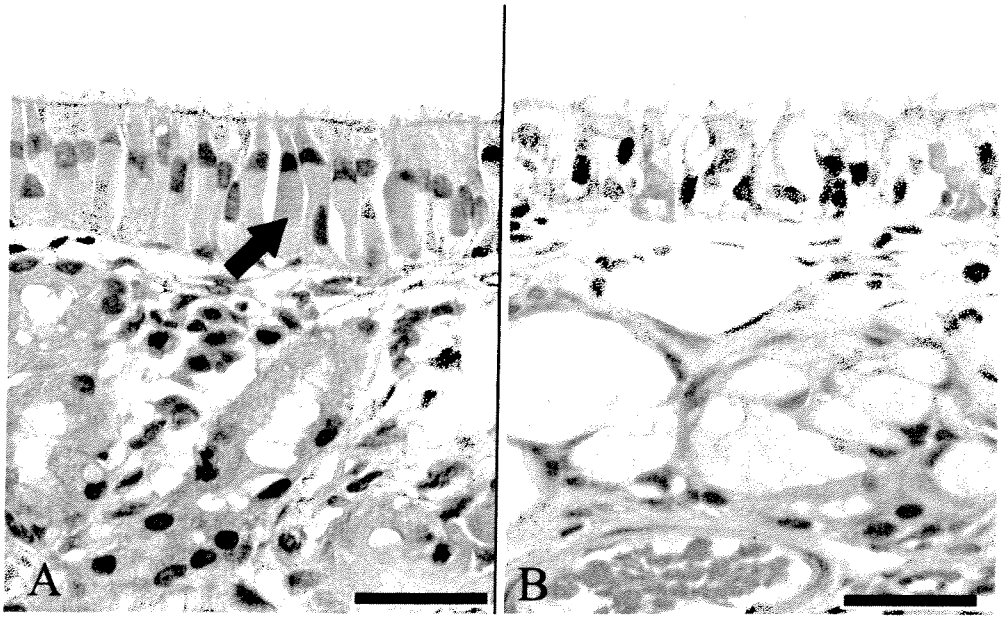

Fig. 6. (A): Eosinophilic globules (arrow) of the respiratory epithelium in a female rat exposed to 300 ppm *p*-DCB for 2 years. (B): The normal respiratory epithelium in a female rat exposed to clean air for 2 years. The nasal septum was sectioned at Level II. Bar indicates 25 μm. H & E stain.

Notably, the α_{2u}-globulin nephropathy as evidenced by excessive accumulation of α_{2u}-globulin in the renal tubule epithelium of the male rats exposed to *p*-DCB found in the previous 13-week inhalation study [1] was in good agreement with the renal histopathology in the NTP 13-week gavage study [31]. It has been recognized that a variety of non-genotoxic chemicals induce the α_{2u}-globulin nephropathy in male rats, which was reported to ultimately progress to the renal tumors [40, 41]. The α_{2u}-globulin nephropathy-mediated nephrocarcinogenicity was explained in the term that long-term exposure of male rats to *p*-DCB caused cytotoxicity resulting from excessive accumulation of α_{2u}-globulin and subsequent cell proliferation in the proximal tubule epithelium of kidneys [7, 43], which promoted the initiated cells to form atypical tubule hyperplasia and renal cell tumors [40, 41]. It was found, however, in the present and previous studies [1] that the α_{2u}-globulin nephropathy in male F344 rats exposed by inhalation to 270 and 600 ppm *p*-DCB for 13 weeks did not promote any renal tumor after the 2-year inhalation exposure of the male rats to *p*-DCB vapor at the MTD level of 300 ppm. The present and previous findings would provide novel information about applicability of a non-genotoxic-mode-of-action hypothesis of the α_{2u}-globulin nephropathy-mediated nephrocarcinogenicity to the *p*-DCB-induced nephrocarcinogenicity in the male rats exposed by inhalation to *p*-DCB vapor at the MTD level. The U.S. Environmental Protection Agency [45] noted in 1991 that renal tumors in male rats that are produced by exposure to substances that produce α_{2u}-globulin and are non-mutagenic need not be considered in assessing potential neoplastic health risks to humans. However, non-genotoxicity of *p*-DCB may be questioned, as discussed before in the section of the mouse hepatocarcinogenicity. Sustainability of the cytotoxicity and subsequent cell proliferation in the renal tubule epithelium of *p*-DCB-gavage-dosed male rats during a time period that allows promotion of the initiated cells to form atypical tubule hyperplasia and renal cell tumors remains to be solved, because the expression of α_{2u}-globulin was strongly age-dependent as shown by a gradual decline and ultimate loss of the hepatic synthesis of α_{2u}-globulin and its mRNA in male rats during aging and senescence [38], and because oral administration of hydrocarbon to 26-month-old male rats failed to cause the α_{2u}-globulin nephropathy or to increase the hepatic and renal contents of α_{2u}-globulin [28]. The *p*-DCB-induced nephrocarcinogenicity and genotoxicity of *p*-DCB will warrant further investigation for regulatory purposes.

Chronic toxicity: The p-DCB-induced chronic inhalation toxicity appeared in the liver, kidneys and nasal cavity. Centrilobular hypertrophy of hepatocytes was observed in the male mice and male rats exposed to 300 ppm. Mineralization of the renal papilla and urothelial hyperplasia of the renal pelvis in the 300 ppm-exposed male rats were noted. Incidences of CPN were higher in male rats than in females, but were found not to be dose-related. The nasal lesions appeared at a lower exposure level than any lesions of other affected organs, because the incidences of eosinophilic globules of the olfactory epithelium having marked grade of severity were increased in the female rats exposed to 75 and 300 ppm. Therefore, the present finding revealed that the nasal lesion appeared at the exposure levels of higher than 20 ppm as the most sensitive endpoint of chronic inhalation

toxicity.

The *p*-DCB-induced nasal lesions were characterized by the respiratory metaplasia of both the nasal gland epithelium in mice and rats and the olfactory epithelium in mice and by the eosinophilic globules of the respiratory and olfactory epithelia in female rats. Those nasal lesions were reported to occur in aged mice and rats of both sexes [29, 42]. In the previous 13-week inhalation study [1], however, no nasal lesion was detected in any of the 19-week-old, *p*-DCB-exposed or control mice or rats of either sex (data not shown there because those nasal lesions were absent). Therefore, the nasal lesions appearing only after the 2-year inhalation exposure to *p*-DCB were found to be age-related and treatment-related. The eosinophilic globules may reflect a morphological correlate for accumulation of proteinaceous materials in supporting cells of the olfactory epithelium and in ciliated and non-ciliated cells of the respiratory epithelium, since those epithelial cells were reported to release proteinaceous materials [26]. The respiratory metaplasia of the olfactory epithelium is considered to represent replacement of the affected epithelium with the respiratory-like epithelium in which those epithelial cells showed characteristics of the ciliated and non-ciliated cells of adjacent normal respiratory epithelium. The present microscopic observations of the *p*-DCB-induced nasal lesions led us to conclude on the basis of the previously reported findings on the nasal cavity of aged animals [29, 42] and chemically exposed animals [10, 26] that long-term inhalation exposure to *p*-DCB accelerates the age-related changes in degenerative responses of the nasal cavity to the inhaled toxicant, including the loss of olfactory cells.

Conclusion: Two-year inhalation exposure to *p*-DCB was found to produce clear evidence of carcinogenicity for both the male and female BDF_1 mice, as shown in the increased incidences of hepatocellular carcinomas and hepatoblastomas and histiocytic sarcomas in the 300 ppm-exposed male mice, and in the increased incidences of hepatocellular adenomas and carcinomas and hepatoblastomas in the 300 ppm-exposed female mice. No tumor was induced in any *p*-DCB-exposed rat of either sex. The most sensitive endpoint of the chronic inhalation toxicity was the nasal lesion characterized by treatment- and age-related increases in the incidences of the eosinophilic globules of the respiratory and olfactory epithelia in female rats and the incidence of the respiratory metaplasia of the nasal gland epithelium in female rats and the olfactory epithelium in mice. Induction of the mouse hepatocarcinogenicity and lack of the rat nephrocarcinogenicity were discussed in light of the estimated *p*-DCB uptake through the inhalation and the oral administration.

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