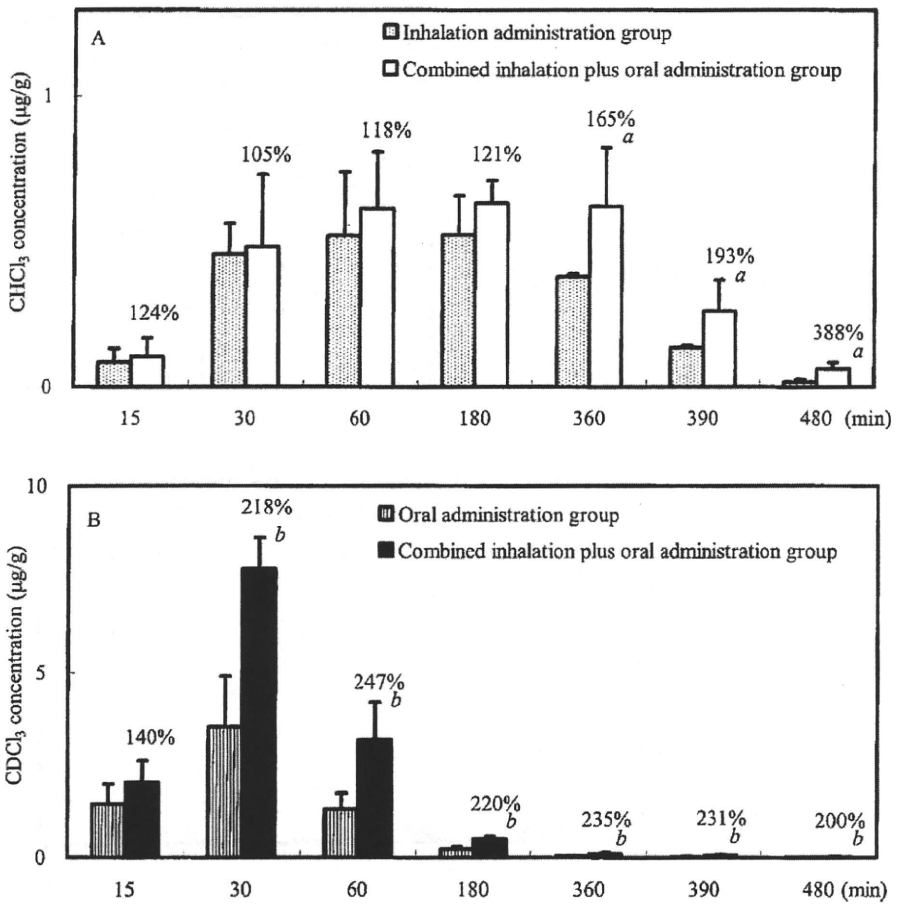

Fig. 4. $CHCl_3$ and $CDCl_3$ concentrations (mean ± SD) in the kidney at each collection time point (n=5 for each collection time/group): (A) Inhalation route. Numbers above the SD bars represent the percentage ratio of the $CHCl_3$ concentration in the kidney of the combined inhalation plus oral administration group to the $CHCl_3$ concentration in the kidney of the inhalation administration group at each collection time point. [a]Significantly different from the inhalation administration group ($P \leq 0.05$). (B) Oral administration route. Numbers above the SD bars represent the percentage ratio of the $CDCl_3$ concentration in the kidney of the combined inhalation plus oral administration group to the $CDCl_3$ concentration in the kidney of the oral administration group at each collection time. [b]Significantly different from the oral administration group ($P \leq 0.05$).

Results and discussion

Inhalation administration group

During the inhalation exposure period, $CHCl_3$ concentrations in the blood increased until 30 min after initiation of inhalation exposure and remained constant from 30 to 360 min (Fig. 2A). After the end of the exposure period, the $CHCl_3$ concentrations in the blood decreased over time. $CHCl_3$ remained detectable in the blood at 480 min (120 min after end of the exposure period). In this group the $CHCl_3$ concentration in the blood is governed by the blood-to-gas partition coefficient[20] and during the administration period the $CHCl_3$ concentration in the gas phase of the lung remained constant; therefore , the $CHCl_3$ concentration in the blood remained constant after equilibrium was reached. This pattern is very similar to the pattern of $CHCl_3$ concentration in the blood following inhalation of $CHCl_3$ vapor reported previously.[15]

The $CHCl_3$ concentrations in the liver and kidney also increased until 30 min and remained constant from 30 to 360 min (Figs. 3A and 4A). The $CHCl_3$ concentration in the liver and kidney of these rats is governed by tissue-to-blood partition coefficients[20] and elimination by metabolism[18,21–23] and excretion; the $CHCl_3$ concentration in these organs is expected to follow the observed pattern. In contrast, the $CHCl_3$ concentration in the abdominal fat increased throughout the exposure period (Fig. 5A). This is because $CHCl_3$ has high lipid solubility and the $CHCl_3$ concentration in the abdominal fat will increase until it reaches equilibrium with the blood as governed by the blood-fat partition coefficient.[18,24,25] After the end of the inhalation exposure period, the $CHCl_3$ concentrations

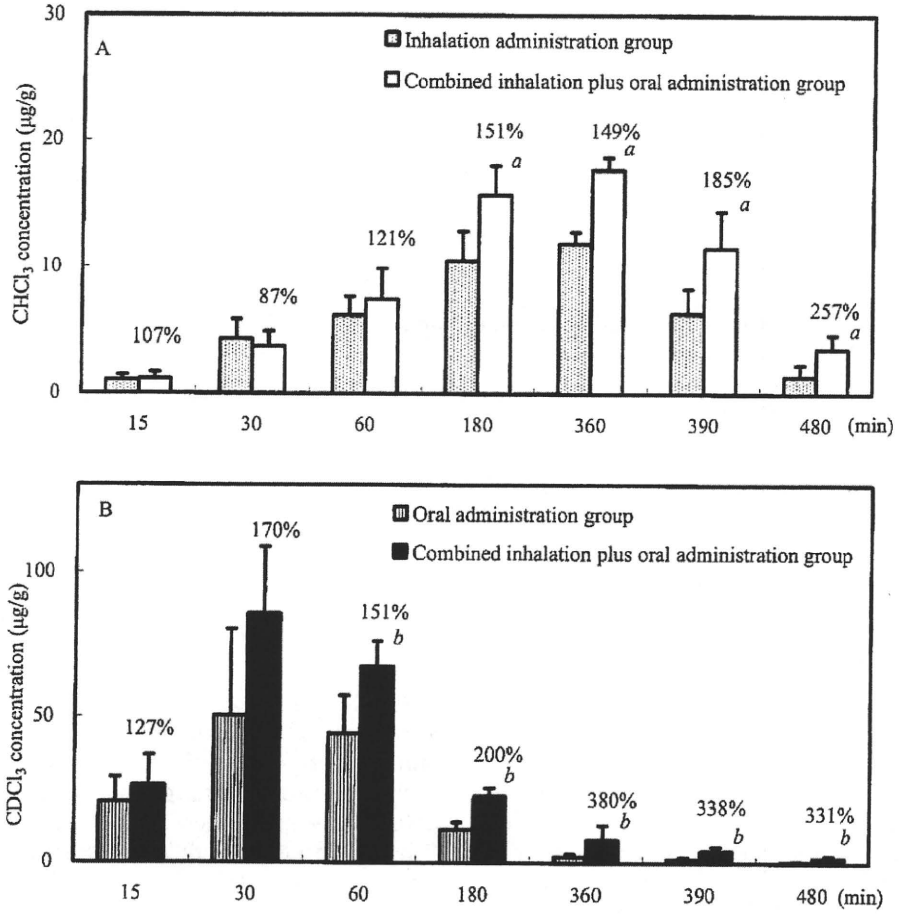

Fig. 5. $CHCl_3$ and $CDCl_3$ concentrations (mean ± SD) in the abdominal fat at each collection time point (n = 5 for each collection time/group): (A) Inhalation route. Numbers above the SD bars represent the percentage ratio of the $CHCl_3$ concentration in the abdominal fat of the combined inhalation plus oral administration group to the $CHCl_3$ concentration in the abdominal fat of the inhalation administration group at each collection time point. [a] Significantly different from the inhalation administration group ($P \leq 0.05$). (B) Oral administration route. Numbers above the SD bars represent the percentage ratio of the $CDCl_3$ concentration in the abdominal fat of the combined inhalation plus oral administration group to the $CDCl_3$ concentration in the abdominal fat of the oral administration group at each collection time. [b] Significantly different from the oral administration group ($P \leq 0.05$).

in all tissues decreased over time as $CHCl_3$ was eliminated from the body.

Oral administration group

The $CDCl_3$ concentration in the blood increased after administration, reaching a maximum concentration (Cmax) at 30 min and decreasing over time thereafter (Fig. 2B). $CDCl_3$ remained detectable in the blood at 480 min. Wang et al.[13] and Take et al.[26] reported time-course changes of $CHCl_3$ concentrations in blood following oral administration of $CHCl_3$ to rats, and the time-course changes of $CDCl_3$ concentration in the blood found in our study was very similar to their results. In addition, there are no differences in the chemical properties of $CDCl_3$ and $CHCl_3$, therefore, we conclude that $CDCl_3$ and $CHCl_3$ behave identically in our test animals.

The Cmax of $CDCl_3$ in the liver, kidney and abdominal fat was also reached at 30 min after oral administration and declined over time thereafter (Figs. 3B, 4B and 5B). $CDCl_3$ remained detectable in each tissue at 480 min. This pattern is due to the increase in $CDCl_3$ in the blood following oral administration of $CDCl_3$ and the elimination from the body of $CDCl_3$ over time. Since, the $CDCl_3$ concentrations in the tissues are dictated by the tissue-to-blood partition coefficients,[20] the general pattern of $CDCl_3$ concentration in the tissues is similar to that of the blood.

Combined inhalation plus oral administration group

The overall pattern of $CHCl_3$ concentration (inhalation route) in this group mirrored that of the single administration group. However, the $CHCl_3$ concentrations in the

Table 1. AUC of $CHCl_3$ and $CDCl_3$ in the blood and tissues.

		AUC_{0-480}	
	Group name	*Inhalation route ($CHCl_3$)*	*Oral administration route ($CDCl_3$)*
Blood	Inhalation administration	443[a,b]	—
	Oral administration	—	748
	Combined inhalation plus oral administration	422 (0.95)[c]	986 (1.32)
Liver	Inhalation administration	271[d]	—
	Oral administration	—	488
	Combined inhalation plus oral administration	363 (1.34)	809 (1.66)
Kidney	Inhalation administration	177	—
	Oral administration	—	240
	Combined inhalation plus oral administration	236 (1.33)	534 (2.23)
Abdominal fat	Inhalation administration	3815	—
	Oral administration	—	6815
	Combined inhalation plus oral administration	5720 (1.50)	11963 (1.76)

[a] Values represent AUC_{0-480} of the mean concentration at each collection time.
[b] $\mu g/mL$ x min (Blood).
[c] Values represent ratio of AUC_{0-480} value of combined inhalation plus oral administration to inhalation administration or oral administration group.
[d] $\mu g/g$ x min (Liver, kidney and abdominal fat).

tissues of this group reached higher levels than in the single administration group. Significant differences in $CHCl_3$ concentration levels between these 2 groups were observed in the liver at 180, 390 and 480 min; the percentage ratios of the combined inhalation plus oral administration group to the inhalation administration group at these time points were 136, 189 and 272% (Fig. 3A). Significant differences were observed in the kidney at 360, 390 and 480 min; the percentage ratios of the combined inhalation plus oral administration group to the inhalation administration group at these time points were 165, 193 and 388% (Fig. 4A). Significant differences were observed in the abdominal fat at 180, 360, 390 and 480 min; the percentage ratios of the combined inhalation plus oral administration group to the inhalation administration group at these time points were 151, 149, 185 and 257% (Fig. 5A).

The $CDCl_3$ concentration (oral administration route) in this group mirrored that of the single administration group: Cmax for the blood and each tissue was reached at 30 min and concentrations decreased over time thereafter; $CDCl_3$ remained detectable in the blood and tissues at 480 min. The $CDCl_3$ concentrations in the blood and each tissue of this group reached higher levels than in the oral administration group. Significant differences in $CDCl_3$ concentration levels between these two groups were observed in the blood at 30 min; the percentage ratio of the combined inhalation plus oral administration group to the oral administration group at this time points was 122% (Fig. 2B).

Significant differences were observed in the liver at 30, 60, 180, 360, 390 and 480 min; the percentage ratios of the combined inhalation plus oral administration group to the oral administration group at these time points were 173, 154, 214, 188, 178 and 363% (Fig. 3B). Significant differences were observed in the kidney at 30, 60, 180, 360, 390 and 480 min; the percentage ratios of the combined inhalation plus oral administration group to the oral administration group at these time points were 218, 247, 220, 235, 231 and 200% (Fig. 4B). Significant differences were observed in the abdominal fat at 60, 180, 360, 390 and 480 min; the percentage ratios of the combined inhalation plus oral administration group to the inhalation administration group at these time points were 151, 200, 380, 338 and 331% (Fig. 5B).

The area-under-the-curve (AUC) values of the $CHCl_3$ and $CDCl_3$ concentrations in the blood and each tissue from 0 to 480 min (AUC_{0-480}) are shown in Table 1. The AUC_{0-480} values for the liver, kidney and abdominal fat obtained from the combined inhalation plus oral administration group were all higher than those of the single exposure route groups. The ratios of the AUC_{0-480} values for the combined inhalation plus oral administration group to the AUC_{0-480} values for the inhalation administration group were 0.95, 1.34, 1.33 and 1.50 for the blood, liver, kidney and abdominal fat. The AUC_{0-480} ratios for the combined inhalation plus oral administration group to the oral administration group were 1.32, 1.66, 2.23 and 1.76 for the blood, liver, kidney and abdominal fat. In all 3 groups (inhalation administration, oral administration and combined inhalation plus oral administration), the AUC values for the abdominal fat were higher than for the blood and other tissues.

These higher AUC values are due to the high coefficient of partition from the blood into the abdominal fat.[18,24,25] It is noteworthy that for $CDCl_3$, the AUC_{0-480} ratio for the combined inhalation plus oral administration group to the

oral administration group was 2.23 for the kidney, a higher value than the ratios for the blood (1.32), liver (1.66), and abdominal fat (1.76). This enhanced partition of chloroform to the kidney may be relevant to the results of the study by Nagano et al.,[9] which found that combined inhalation plus oral administration of chloroform markedly enhanced chloroform toxicity and tumor induction in the rat kidney.

It is possible to relate the increase in the tissue absorption of chloroform in the combined inhalation plus oral administration group to the two different exposure routes. In the oral administration route, a single large bolus of $CDCl_3$ was absorbed through the gastrointestinal mucosa and transported first to the liver by the blood and then distributed to other tissues; and thereafter, $CDCl_3$ from this bolus was being constantly eliminated from the body. In the inhalation route, on the other hand, $CHCl_3$ vapor was continuously absorbed through the lung and transported to the blood and distributed to other tissues throughout the 360-min exposure period. During this time, equilibriums were established between the $CHCl_3$ in the gas phase in the lung and the $CHCl_3$ in the blood, and between the $CHCl_3$ in the blood and the $CHCl_3$ in the tissues.

It is possible that during the period of administration of $CHCl_3$ by inhalation, removal of $CDCl_3$ via the lung was impeded resulting in slower removal of $CDCl_3$ from the tissues. The higher concentrations of $CDCl_3$ in the liver and kidney could, in turn, affect elimination of $CHCl_3$ by metabolism and excretion, resulting in higher $CHCl_3$ concentrations in these organs. Finally, impeded elimination of $CHCl_3$ could enhance partitioning of $CHCl_3$ into fatty tissues. However, the actual mechanisms resulting in increased tissue absorption of chloroform in the combined inhalation plus oral administration group remain to be elucidated; another possibility is that the increase in tissue absorption of chloroform in the combined inhalation plus oral administration group compared to the single exposure route groups may be related to the high lipid solubility of chloroform and its storage in the abdominal fat.

Conclusion

In the present study, the contribution of each of 2 separate routes of exposure to chloroform to the distribution and accumulation of chloroform in the blood and tissues of rats was delineated. This model study using $CHCl_3$ and $CDCl_3$ showed that by using MS, chloroform delivered by each of 2 different exposure routes could be monitored independently of the chloroform delivered by the other route. The effect of simultaneous exposure to chloroform by 2 separate routes on chloroform concentrations in tissues was more than additive. This result indicates that when assessing the toxicity and carcinogenicity of this environmental contaminant, exposure routes, especially multiple exposure routes, must be taken into consideration.

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Proliferative and Nonproliferative Lesions of the Rat and Mouse Respiratory Tract

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ORIGINAL ARTICLE

Inhalation carcinogenicity and toxicity of 1,2-dichloropropane in rats

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Abstract

The toxicity and carcinogenicity of 1,2-dichloropropane (DCP) were examined by inhalation exposure of male and female F344 rats to DCP for either 13 wk or 2 years. In the 13-wk study, the DCP concentrations used were 125, 250, 500, 1000, or 2000 ppm (v/v), and in the 2-year study the DCP concentrations were 80, 200, or 500 ppm (v/v). Thirteen-week exposure to DCP induced hyperplasia in the respiratory epithelium and atrophy of the olfactory epithelium at 125 ppm and above. At the higher levels of exposure, hemolytic anemia and lesions of liver and adrenal gland were observed. Two-year exposure to DCP significantly increased incidences of papilloma in the nasal cavity of male and female rats exposed to 500 ppm DCP. In addition, three cases of esthesioneuroepithelioma were observed in the DCP-exposed male rats. Total nasal tumors increased in a concentration-dependent manner. Hyperplasia of the transitional epithelium and squamous cell hyperplasia, both of which were morphologically different from the hyperplasia of the respiratory epithelium observed in the 13-wk exposure study, occurred in a concentration-dependent manner; these lesions are considered to be preneoplastic lesions. Atrophy of the olfactory epithelium, inflammation of the respiratory epithelium, and squamous cell metaplasia were also seen in the 2-year study. These results demonstrate that DCP is a nasal carcinogen in rats. Lifetime cancer risks for humans exposed to DCP in the ambient air and work environment were quantitatively estimated, using both nonthreshold and threshold approaches, with the data obtained from the 2-year study.

Keywords: *1,2-dichloropropane; inhalation; carcinogenicity; rat; mouse; chronic toxicity*

Introduction

1,2-Dichloropropane (DCP, CAS No. 78-87-5) is a colorless liquid with a molecular weight of 112.99, a boiling point of 96.4°C, and a vapor pressure of 66.2 hPa at 25°C (OECD, 2005). It is poorly soluble in water and soluble in ethanol and diethyl ether. DCP is used as a raw material in the production of many other chemicals, such as propylene, carbon tetrachloride, and tetrachloroethylene, and the total global production volume of DCP for 2001 is estimated to 350,000 tons (OECD, 2005). A National Occupational Exposure Survey conducted by the National Institute for Occupational Safety and Health (NIOSH) estimated that 2944 workers were potentially exposed to DCP in the United States from 1981 to 1983 (NIOSH, 2002). Concentrations of DCP in city air were measured at 1.2 μg/m³ in Philadelphia (Haemisegger et al., 1985) and 0.021–0.040 μg/m³ in Portland (World Health Organization [WHO], 1993). By 2009, the production of DCP has decreased considerably, but the total amounts of on-site and off-site disposal for DCP are still substantial, 442,755 (220 tons) and 150 pounds (0.075 tons), respectively (United States Environmental Protection Agency [U.S. EPA], 2009b). In Japan, the total amounts of DCP released from various sectors of industries into the ambient air and public water in 2001 were 2088 and 0.86 tons, respectively, and the mean ambient air concentration in Japan was 0.032 μg/m³ with a maximal value of 0.53 μg/m³ in 2002 (Japan Ministry of the Environment, 2004). The volume of production and import of DCP in Japan has also decreased in recent years, but remains substantial; it decreased from 12,251 tons in 1987 to 3100 tons in 2007 (Japan Ministry of the Environment, 2004, 2008).

Medical case reports reveal that deliberate ingestion and sniffing of DCP causes hemorrhage, hemolytic anemia,

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renal and hepatic damage (Pozzi et al., 1985), and death due to cardiac or renal toxicity (Larcan et al., 1977; Thorel et al., 1986). Exposure of workers to DCP is reported to cause dermatitis (Grzywa and Rudzki, 1981; Baruffini et al., 1989) and injury to the liver and kidney (Reid, 2001). Experimental toxicology with rodents showed that DCP damaged the liver, kidneys, hematological system, and central nervous system, which in severe cases resulted in death (Pozzani et al., 1959; Smyth et al., 1969; Sidorenko et al., 1979; NTP, 1986; Bruckner et al., 1989; Imberti et al., 1990; Reid, 2001).

The evidence that DCP is carcinogenic is inconclusive. The National Toxicology Program (NTP, 1986) reported a dose-related increase of liver tumors in B6C3F$_1$ mice of both sexes administered DCP by gavage for 103 wk; however, DCP administration had no effect on liver tumors in male or female F344 rats, although a marginal increase in mammary gland tumors in female F344 rats was observed. Based on these findings, the NTP concluded that in these 2-year gavage studies, there was no evidence of carcinogenicity in male F344 rats, there was equivocal evidence of carcinogenicity in female F344 rats, and there was some evidence of carcinogenicity in male and female B6C3F$_1$ mice. Because of the lack of epidemiological data relevant to the carcinogenicity of DCP in humans and the limited evidence for carcinogenicity in experimental animals, the International Agency on Research on Cancer (IARC) classified DCP as a group 3 agent (IARC, 1999): an agent which is not classifiable as to its carcinogenicity to humans. DCP, however, is reported to be mutagenic to *Salmonella typhimurium* in the presence and absence of metabolic activation (De Lorenzo et al., 1977; Principe et al., 1981) and to induce sister chromatid exchange and chromosome aberrations in cultured Chinese hamster ovary cells and V79 cells (NTP, 1986; Galloway et al., 1987; von der Hude et al., 1987). Based on the equivocal results of carcinogenicity studies in experimental animals and the positive results of *in vitro* mutagenicity studies, the German Research Foundation (GRF) classified DCP as a Category 3B agent (Deutsche Forschungsgemeinschaft, 1998): an agent not conclusively but possibly carcinogenic to man. The GRF gives no maximum workplace concentration (maximale Arbeitsplatz-Konzentration: MAK) value. The American Conference of Governmental Industrial Hygienists (ACGIH) classified DCP as an A4 agent (ACGIH, 2006): an agent not classifiable as a human carcinogen. The ACGIH recommended a threshold limit value-time weighted average (TLV-TWA) of 10 ppm for DCP. The United States Occupational Safety and Health Administration (U.S. OSHA) assigned a Permissible Exposure Level (PEL) of 75 ppm for DCP (ACGIH, 2009).

To make quantitative health risk assessments for humans exposed by inhalation to DCP in the ambient air or in the workplace air, the present studies were designed to provide dose-response relationships of the carcinogenicity and subchronic and chronic toxicities of inhaled DCP. We undertook two experimental studies of inhalation exposure to DCP by male and female rats, one for 13 wk and the other for 2 years. We then estimated the lifetime cancer risk for DCP in the ambient air, using a nonthreshold approach, and an occupational exposure limit (OEL) for inhaled DCP in the workplace air, using both nonthreshold and threshold approaches.

Materials and methods

The present studies were conducted in accordance with the Organization for Economic Co-operation and Development (OECD) Principles of Good Laboratory Practice (OECD, 1998) and approved by the ethics committee of the Japan Bioassay Research Center (JBRC). The animals were cared for in accordance with the guide for the care and use of laboratory animals (National Research Council, 1996).

Test substance

DCP of analytical grade (>99.5% pure) was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Each lot of DCP used in the present studies was analyzed for its purity and stability by gas chromatography before and after its use. No gas chromatographic peak other than DCP was detected in the inhalation exposure chambers.

Animals

F344/DuCrj (SPF) rats of both sexes were obtained at 4 wk of age from Charles River Japan, Inc. (Kanagawa, Japan). After 2 wk quarantine and acclimation, the animals were allocated by a stratified randomization procedure into body-weight-matched, DCP-exposed, and clean air-exposed groups. The 13-wk study consisted of five DCP-exposed groups and one control group, each comprising 10 rats of each sex. The 2-year study consisted of three DCP-exposed groups and one control group, each comprising 50 rats of each sex. The animals were housed individually in stainless-steel wire hanging cages (150 mm [W] × 216 mm [D] × 176 mm [H]) in stainless-steel inhalation exposure chambers maintained at a temperature of 23 ± 2°C and at a relative humidity of 55 ± 15% with 12 ± 1 air changes/h. Fluorescent lighting was controlled automatically to give a 12-h light/dark cycle. All rats had free access to sterilized water and γ-irradiation-sterilized commercial pellet diet (CRF-1, Oriental Yeast Co., Ltd., Tokyo, Japan).

Experimental design

For the 13-wk study, groups of 10 rats of each sex were exposed to DCP at 0 (clean air control), 125, 250, 500, 1000, or 2000 ppm (v/v) for 6 h/day, 5 days/wk for 13 wk. For the 2-year study, groups of 50 rats of each sex were exposed to DCP at 0 (clean air control), 80, 200, or 500 ppm for 6 h/day, 5 days/wk for 104 wk (2 years). The highest exposure concentration of DCP for the 2-year study was chosen as 500 ppm on the basis of the nasal toxicity and growth retardation induced by the 13-wk inhalation exposure to DCP, according to the criteria of maximum tolerated dose (MTD) setup by both National Cancer Institute (NCI) and IARC guidelines (Sontag et al., 1976; Bannasch et al., 1986).

Inhalation exposure to DCP

Airflow containing DCP at designated target concentrations was prepared by a vaporization technique. The saturated vapor–air mixture was generated by bubbling clean air through the DCP liquid in a temperature-regulated glass flask (25°C), and by cooling it through a thermostatted condenser at 18°C. The airflow containing the saturated vapor was diluted with clean air, and then warmed to 23°C in a thermostatted circulator, which served to stabilize the vapor concentration by complete gasification of the DCP. The flow rate of the vapor–air mixture was regulated with a flowmeter, further diluted with humidity- and temperature-controlled clean air in a spiraling line mixer, and then supplied to an inhalation exposure chamber. Six inhalation exposure chambers of 1060 l, each accommodating 20 individual cages for 10 male and 10 female rats, were used for the 13-wk study. Four inhalation exposure chambers of 4300 l, each accommodating 100 individual cages for 50 male and 50 female rats, were used for the 2-year study. Chamber concentrations of DCP were monitored by gas chromatography every 15 min throughout the entire exposure periods, and maintained at 125.3 ± 0.7 (mean ± SD), 250.8 ± 1.0, 500.5 ± 2.6, 1000.4 ± 3.4, and 2001.3 ± 5.9 ppm for the 13-wk study and 80.2 ± 0.5, 200.5 ± 1.3, and 500.2 ± 2.4 ppm for the 2-year study.

Clinical observations and analysis, and pathological examinations

The animals were observed daily for clinical signs and mortality. Body weight and food consumption were measured once a wk in the 13-wk study, and once a week for the first 14 wk and once every 4 wk thereafter in the 2-year study. All rats, including those found dead or moribund, received complete necropsy. For hematology and blood biochemistry, blood was collected under etherization at terminal necropsy after overnight fasting. The blood sample was analyzed with an automatic blood cell analyzer (ADVIA120, Bayer Co. NY, USA) and an automatic analyzer (Hitachi 7080, Hitachi, Ltd., Ibaraki, Japan) for blood biochemistry.

Organs were removed, weighed, and examined for macroscopic lesions at necropsy. All organs and tissues and the entire respiratory tract including nasal cavity, pharynx, and larynx were examined for histopathology in all the animals. The organs and tissues were fixed in 10% neutral buffered formalin. The nasal cavity was decalcified in formic acid-formalin solution before trimming, and was transversely trimmed at three levels according to the procedure described in our previous paper (Nagano et al., 1997): at the level of the posterior edge of the upper incisor teeth (Level 1), at the incisive papilla (Level 2), and at the level of the anterior edge of the upper molar teeth (Level 3). The tissues were embedded in paraffin, and 5 μm-thick sections were prepared and stained with hematoxylin and eosin (H&E). Nasal lesions were diagnosed with reference to the criteria of the International Classification of Rodent Tumours (IARC, 1992) and the International Harmonization of Nomenclature and Diagnostic Criteria for Lesions in Rats and Mice (Renne et al., 2009).

Statistics and data analysis

Peto's test (1980) was used to evaluate statistically significant relationships between incidences of neoplastic lesions and the level of exposure to DCP. Fisher's exact test was used to evaluate the statistical significance of the differences in the incidences of neoplastic lesions between DCP-exposed groups and the clean air-exposed control group. The Chi-square test was used to evaluate the statistical significance of the differences in the incidences of pre- and nonneoplastic lesions between DCP-exposed groups and the clean air-exposed control group. Survival curves were plotted according to the method of Kaplan and Meier (1958), and the log-rank test (Peto et al., 1977) and Fisher's exact test were used to test for statistically significant differences in survival rates between any DCP-exposed rat group of either sex and the clean air-exposed control group. Body and organ weights were analyzed by Dunnett's test. Two-tailed tests were used for all statistics except for Peto's test. In all cases, a P-value of 0.05 was used as the level of significance. In analyzing results for uncommon tumors in the DCP-exposed groups that were not statistically significant, the tumor incidence was tested for biological significance using a range of minimum and maximum tumor incidences in the JBRC's historical control data, which were compiled from 2-year inhalation studies of rodent carcinogenicity conducted by the JBRC during the 23-year period from 1987 to 2009.

Benchmark concentration

Based on the dose-response relationships between exposure concentrations and incidences of tumors or preneoplastic lesions obtained in this study, a 95% lower confidence limit of the benchmark concentration associated with 10% risk over background ($BMCL_{10}$) was calculated using the linearized multistage model for total nasal tumors and the Weibull model for the total preneoplastic lesions of nasal tumors with U.S. EPA's benchmark dose software (ver. 2.1.1) (U.S. EPA, 2009a).

Results

Thirteen-week study

Survival, body weights, and food consumption

No deaths occurred in any of the DCP-exposed male rat groups. One female exposed to 2000 ppm died during the 12th wk of the exposure period. There were no DCP-related clinical signs in any of the DCP-exposed groups of either sex. Growth rates were clearly suppressed in both male and female rats exposed to 1000 and 2000 ppm. The body weights of 0 (clean air control), 125, 250, 500, 1000, and 2000 ppm-exposed groups measured at the end of the 13-wk exposure period were 307 ± 16 (mean ± SD) g, 286 ± 10 g, 292 ± 14 g, 281 ± 12 g, 257 ± 19, and 223 ± 21 g for male rats and 173 ± 9 g, 167 ± 7 g, 166 ± 9 g, 164 ± 4 g, 157 ± 3 g, and 142 ± 12 g for female rats. Food consumption was lowered in both male and female rats exposed to 2000 ppm (data not shown).

Hematology and clinical chemistry

Hemolytic anemia occurred in both male and female rats exposed to 500 ppm and above, as indicated by decreases in some of the erythrocyte parameters including red blood cell count together with concomitant increases in reticulocytes and platelets (Table 1). Total bilirubin and γ-GTP activity significantly increased in the male rats exposed to 2000 ppm and in the female rats exposed to 1000 and 2000 ppm.

Pathology

The relative weights of the spleens significantly increased in both male and female rats exposed to 2000 ppm. The absolute and relative weights of the livers significantly increased in female rats exposed to 500 ppm and above (data not shown).

Incidences of selected microscopic lesions in the DCP-exposed rats surviving to the end of the 13-wk exposure period are presented in Table 2. Thirteen weeks of inhalation exposure to DCP affected the nasal cavity, liver, hematopoietic system, and adrenal gland. In the nasal cavity, hyperplasia of the respiratory epithelium and atrophy of the olfactory epithelium occurred in both male and female rats exposed to 125 ppm and above, and the averaged severity scores of these two lesions increased in a concentration-related manner. Hyperplasia of the respiratory epithelium (Figure 1A and 1B) was characterized by an increased number of ciliated columnar epithelial cells and accompanied by goblet cell hyperplasia. The hyperplasia was located diffusely in the dorsal or septum region of Level 1. Atrophy of the olfactory epithelium (Figure 1C and 1D) was characterized by decreases in epithelial thickness and the number of olfactory sensory cells and often accompanied by necrosis of the olfactory sensory cells and respiratory metaplasia of the olfactory epithelium. Atrophy was located in the dorsal region of Levels 2 and 3. Inflammation of the respiratory epithelium significantly increased only in the male rats exposed to 1000 and 2000 ppm. In the liver, swelling of centrilobular hepatocytes was observed in both male and female rats exposed to 2000 ppm. Increased hematopoietic activity in the spleen and bone marrow, a compensatory response to hematotoxicity, was noted in the 1000 and 2000 ppm-exposed rats of both sexes. Increased hemosiderin deposition resulting from hemolysis of erythrocytes was observed in the spleen of male rats exposed to 1000 and 2000 ppm and female rats exposed to 500 ppm and above. Fatty change in the adrenal gland was significant in the female rats exposed to 2000 ppm. No exposure-related lesions were observed in any other organs in the DCP-exposed rats of either sex.

Two-year study

Survival, body weight, food consumption, and clinical observations and analyses

There was no significant difference in the final survival rate between any DCP-exposed group of either sex and the control. At the end of the 2-year exposure period, the survival rates of the 0 (clean air control), 80, 200, and 500 ppm-exposed groups were 80, 78, 82, and 72% for male rats and 74, 82, 76, and 64% for female rats. The growth rates of the DCP-exposed groups of male rats were slightly suppressed in a concentration-related manner. The body weights of the 0, 80, 200, and 500 ppm-exposed groups measured at the end of the 2-year exposure period were 409±26 (mean ± SD),

Table 1. Hematological and blood biochemical parameters of the rats exposed by inhalation to DCP or clean air for 13 wk.

Group (ppm)	0 (Control)	125	250	500	1000	2000
Male						
No. of animals examined	10	10	10	10	9[a]	10
Red blood cell (10^6/μL)	9.31±0.21	9.36±0.19	9.33±0.16	8.95±0.17**	8.00±0.22**	7.58±0.36**
Hemoglobin (g/dL)	15.9±0.4	16.0±0.4	15.8±0.4	15.4±0.3*	14.7±0.2**	14.6±0.5**
Hematocrit (%)	45.6±1.2	46.1±1.1	46.0±0.7	45.2±0.8	43.4±0.8**	43.7±1.2**
Platelet (10^3/μL)	780±57	804±39	809±53	816±67	925±59**	959±64**
Reticulocyte (%)	1.9±0.1	1.8±0.2	1.9±0.2	2.3±0.2	5.5±0.6**	10.5±3.0**
No. of animals examined	10	10	10	10	9[a]	10
γ-GTP (IU/L)	2±1	4±5	3±1	2±1	2±1	6±10*
Total bilirubin (mg/dL)	0.13±0.02	0.13±0.01	0.13±0.01	0.13±0.01	0.14±0.01	0.18±0.02**
Female						
No. of animals examined	9[a]	10	10	10	10	9[b]
Red blood cell (10^6/μL)	8.60±0.21	8.59±0.20	8.44±0.24	8.13±0.27**	7.77±0.24**	7.18±0.39**
Hemoglobin (g/dL)	15.9±0.5	15.8±0.4	15.7±0.4	15.4±0.6	15.1±0.4**	14.3±0.8**
Hematocrit (%)	44.3±0.9	44.4±1.0	44.2±1.0	43.7±1.2	43.7±1.1	42.5±1.4**
Platelet (10^3/μL)	817±64	783±56	825±58	863±78	874±54	932±114**
Reticulocyte (%)	1.9±0.2	1.9±0.3	2.5±0.3	3.5±0.4*	6.4±2.7**	11.5±4.5**
No. of animals examined	9[a]	10	10	10	10	9[b]
γ-GTP (IU/L)	3±1	2±1	3±1	3±1	5±2**	10±2**
Total bilirubin (mg/dL)	0.16±0.02	0.16±0.03	0.15±0.03	0.16±0.02	0.20±0.03*	0.25±0.06**

Note: Values were expressed as means ± standard deviation.

[a,b]Number of rats examined were 9 instead of 10, because blood sampling failed for one rat[a], and because another rat died before the end of the 13-wk exposure period[b]. Significant difference: *$p<0.05$; **$p<0.01$ by Dunnett's test.

γ-GTP, γ -glutamyl transpeptidase.

396±47, 382±32, and 362±41 g for male rats and 267±37, 252±19, 266±22, and 246±34 g for female rats. The decrease of 11% in male rats and 8% in female rats exposed to 500 ppm DCP was significant. Neither overt clinical signs nor suppression of food consumption was observed in any DCP-exposed group of either sex (data not shown).

In hematological examination, an anemic tendency was evident in the female rats exposed to 500 ppm, as indicated by a slight decrease (−4%) in red blood cell count. γ-GTP levels in the blood significantly increased only in female rats exposed to 500 ppm DCP (data not shown).

Pathology

Microscopic examination revealed that 2-year inhalation exposure to DCP induced lesions in the nasal cavity (Table 3). Incidences of papillomas increased in both male and female rats in a concentration-dependent manner, and the increased incidence was statistically significant in both male and female rats exposed to 500 ppm. The papillomas (Figure 2A) were characterized by expansile, nodular masses which protruded into the nasal cavity and were located in the dorsal region at Levels 1 and 2. Most papillomas were composed primarily of transitional epithelium-like tissue and contained squamous epithelium-like or glandular tissue. A few papillomas were composed primarily of a glandular structure consisting of nonciliated, cuboidal to low columnar cells. A total of three cases of esthesioneuroepitheliomas were observed in the nasal cavity of male rats exposed to 80 (two cases) and 200 (one case) ppm DCP. Since JBRC's historical control data showed no cases of esthesioneuroepithelioma in 2399 male F344 rats in 48 two-year carcinogenicity studies, it was concluded that the esthesioneuroepithelioma was induced by inhalation exposure to DCP. The esthesioneuroepithelioma was characterized by a rosette-like structure and located in the dorsal region of Levels 2 and 3. Incidences of hyperplasia of the transitional epithelium were significantly increased in all DCP-exposed groups of both sexes, and incidences of squamous cell hyperplasia were significantly increased in male rats exposed to 200 and 500 ppm and in female rats exposed to 500 ppm DCP. The severities of these two types of hyperplasias were increased in an exposure concentration-related manner. Hyperplasia of the transitional epithelium (Figure 2B) was characterized by an increased number of nonciliated cuboidal, epithelial cells in a focal area. Squamous cell hyperplasia (Figure 2C) was characterized by a thickening of five or more epithelial layers. These two hyperplasias, which were assessed as being preneoplastic, were similar to the papillomas but were not clearly expanding into the surrounding tissue and were accompanied by hyperplasia of the submucosal gland. Incidences of nonneoplastic

Table 2. Number of rats bearing the selected histopathological lesions and their severities in the rats exposed by inhalation to DCP or clean air for 13 wk.

	Male						Female					
Group (ppm)	0	125	250	500	1000	2000	0	125	250	500	1000	2000
Number of animals examined	10	10	10	10	10	10	10	10	10	10	10	9[a]
Nasal cavity												
Hyperplasia: respiratory epithelium	0	10** [1.0]	10** [1.3]	10** [1.3]	10** [2.0]	10** [2.0]	0	7** [1.0]	10** [1.0]	9** [1.0]	10** [1.2]	9** [1.1]
Inflammation: respiratory epithelium	0	0	2	4	8**	8**	0	0	0	0	3	4
Atrophy: olfactory epithelium	0	10** [1.0]	10** [1.2]	10** [1.5]	10** [2.2]	10** [2.7]	0	10** [1.0]	10** [1.0]	10** [1.1]	10** [1.0]	9** [2.1]
Liver												
Swelling: centrilobular	0	0	0	0	0	9** [1.0]	0	0	0	0	1	6** [1.8]
Spleen												
Deposition of hemosiderin	0	0	0	1	10**	10**	0	0	4	10**	10**	9**
Increased extramedullary hematopoiesis	0	0	0	0	10**	10**	0	0	0	1	8**	9**
Bone marrow												
Increased hematopoiesis	0	0	0	1	10**	10**	0	0	0	0	10**	9**
Adrenal gland												
Fatty change	0	0	0	0	0	1	0	0	0	0	2	9**

Note: The values in brackets indicate the averaged severity grade index of the lesion in affected animals, according to the following equation. [Σ(grade × number of animals with grade)]/number of affected animals. Grade: "slight" scored as 1, "moderate" as 2, "marked" as 3, and "severe" as 4.
[a]Number of female rats examined were 9 instead of 10, because one rat died before the end of the 13-wk exposure period. Significant difference: $*p \leq 0.05$; $**p \leq 0.01$ by χ^2-test.

lesions, squamous cell metaplasia, and inflammation in the respiratory epithelium, were significantly increased in all DCP-exposed groups of both sexes. Atrophy of the olfactory epithelium was often accompanied by necrosis of the olfactory sensory cells and respiratory metaplasia of the olfactory epithelium and located in the dorsal region of Levels 2 and 3, and its severity scores increased in a concentration-related manner. No exposure-related lesions were observed in any other organs in the DCP-exposed rat groups of either sex.

$BMCL_{10}$

$BMCL_{10}$ values were calculated for relationships between exposure concentrations and incidences of total nasal tumors or total preneoplastic lesions summed over male and female rats exposed to clean air or DCP for 2 years. The $BMCL_{10}$ value for the DCP concentration associated with total nasal tumors, calculated using a linearized multistage model, was 234 ppm (Figure 3A). The $BMCL_{10}$ value for the DCP concentration

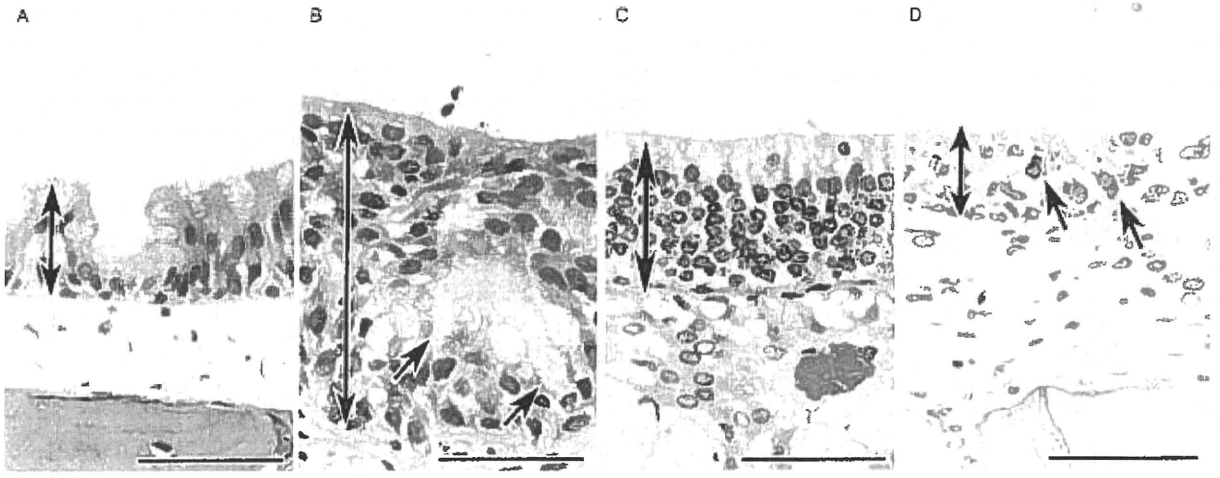

Figure 1. (A) Normal respiratory epithelium in the nasal cavity in the dorsal region of Level 1 in a male rat of the control group. (B) Hyperplasia of the respiratory epithelium in the nasal cavity in the dorsal region of Level 1 in a male rat exposed to 2000 ppm DCP for 13 wk. An increase in the number of epithelial cells and an increase in the thickness of the respiratory epithelium accompanied by goblet cell hyperplasia (arrows) can be seen. (C) Normal olfactory epithelium in the nasal cavity in the dorsal region of Level 3 in a male rat of the control group. (D) Atrophy of the olfactory epithelium in the nasal cavity in the dorsal region of Level 3 in a male rat exposed to 2000 ppm DCP for 13 wk. A decrease in epithelial thickness (double-headed arrow) and number of olfactory sensory cells, and necrosis of the olfactory sensory cells (arrows) can be seen. Bars indicate 50 μm. H&E stain.

Table 3. Number of rats bearing the selected histopathological lesions of the nasal cavity in the rats exposed by inhalation to DCP or clean air for 2 years.

	Male				Peto	Female				Peto
Group (ppm)	0	80	200	500	test	0	80	200	500	test
Number of animals examined	50	50	50	50		50	50	50	50	
Neoplastic lesions										
Papilloma	0	0	3	15″	↑↑	0	0	0	9″	↑↑
Esthesioneuroepithelioma	0	2	1	0		0	0	0	0	
Total nasal tumors	0	2	4	15″	↑↑	0	0	0	9″	↑↑
Pre-neoplastic lesions										
Hyperplasia:	0	31**	39**	48**		2	21**	39**	48**	
transitional epithelium		[1.1]	[1.1]	[1.8]		[1.0]	[1.2]	[1.1]	[1.5]	
Squamous cell hyperplasia	0	2	6*	27**		0	0	3	20**	
		[1.0]	[1.0]	[1.1]				[1.0]	[1.3]	
Total pre-neoplastic lesions	0	31**	39**	50**		2	21**	39**	48**	
Non-neoplastic lesions										
Squamous cell metaplasia:	5	31**	41**	49**		3	15**	37**	46**	
respiratory epithelium	[1.0]	[1.0]	[1.0]	[1.2]		[1.0]	[1.0]	[1.2]	[1.5]	
Inflammation:	20	35**	47**	47**		10	30**	39**	40**	
respiratory epithelium	[1.0]	[1.0]	[1.0]	[1.2]		[1.0]	[1.0]	[1.0]	[1.1]	
Atrophy:	0	48**	50**	49**		0	50**	50**	50**	
olfactory epithelium		[1.1]	[1.9]	[2.0]			[1.0]	[1.9]	[2.0]	

Note: The values in brackets indicate the averaged severity grade index of the lesion in affected animals, according to the following equation. [Σ(grade × number of animals with grade)]/number of affected animals. Grade: "slight" scored as 1, "moderate" as 2, "marked" as 3, and "severe" as 4. Significant difference: $*p \leq 0.05$; $**p \leq 0.01$ by χ^2-test, $'p \leq 0.05$; $''p \leq 0.01$ by Fisher's Exact test ↑: $p \leq 0.05$, ↑↑: $p \leq 0.01$ by Peto's test.

associated with total preneoplastic lesions, calculated using a Weibull model, was 11.5 ppm (Figure 3B).

Discussion

In these studies, 13-wk inhalation exposure of rats to DCP was found to induce nasal lesions, hemolytic anemia, and lesions of liver and adrenal gland. Atrophy of the olfactory epithelium and hyperplasia of the respiratory epithelium were the lesions most sensitive to DCP, occurring at 125 ppm and above, whereas hemolytic anemia and lesions of liver and adrenal gland appeared at higher exposure concentrations. Two-year inhalation exposure to DCP produced a dose-dependent and statistically significant increase in the incidence of papillomas in the nasal cavity of both male and female rats as well as a biologically significant increase in esthesioneuroepitheliomas in the olfactory epithelial region of male rats. Combined incidences of papillomas and esthesioneuroepitheliomas increased in a concentration-dependent manner. Two-year inhalation exposure to DCP also resulted in significant increases in the incidences of hyperplasias of the transitional epithelium and squamous cell hyperplasia. Induction of these lesions occurred at lower exposure concentrations than those, which induced nasal tumors. These two types of hyperplasias were morphologically different from the hyperplasia of the respiratory epithelium that appeared during the 13-wk exposure to DCP. On the one hand, the hyperplasia of the respiratory epithelium induced by short-term exposure to DCP was accompanied by goblet cell hyperplasia and was regarded as an adaptive response to the irritant DCP vapor (Monticello et al., 1990;

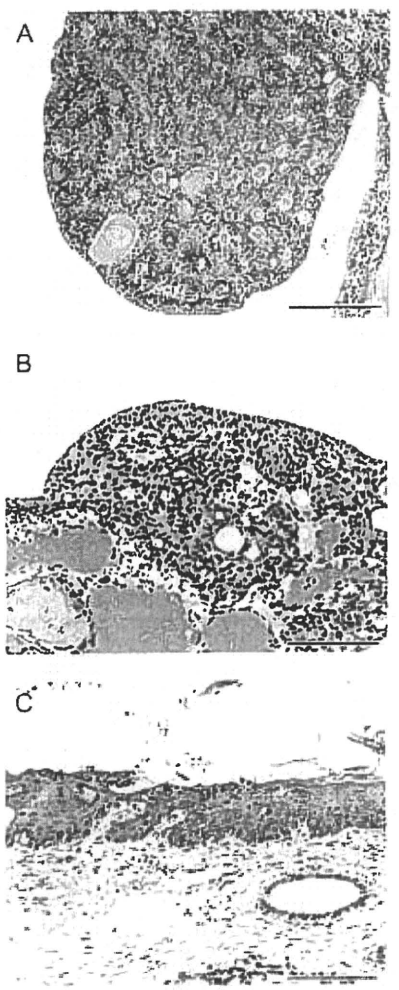

Figure 2. (A) A papilloma in the nasal cavity of a male rat exposed to 500 ppm DCP for 2 years. The papilloma is composed of epithelial and glandular structures consisting of cuboidal cells and obviously protrudes into the nasal cavity. Bar indicates 200 μm. (B) Hyperplasia of the transitional epithelium in the nasal cavity of a male rat exposed to 500 ppm DCP for 2 years. An increase in the number of nonciliated cuboidal cells and in the thickness of the transitional epithelium at the focal area can be seen. Bar indicates 100 μm. (C) Squamous cell hyperplasia in the nasal cavity of a male rat exposed to 500 ppm DCP for 2 years. An increase in the number of epithelial cells and in the thickness of the keratinized squamous epithelium can be seen. Bar indicates 100 μm. H&E stain.

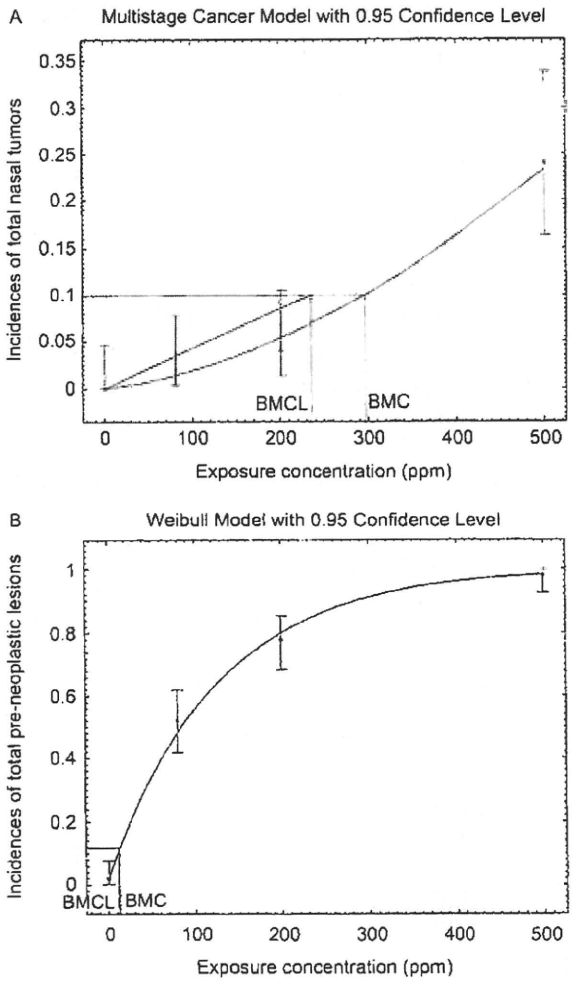

Figure 3. Curves fitted with linearized multistage and Weibull models showing the relationships between the exposure concentrations and the incidences of total nasal tumors (A) and total nasal preneoplastic lesions (B), respectively, in rats exposed to DCP by inhalation for 2 years. The $BMCL_{10}$ values for the DCP concentration associated with total nasal tumors and total preneoplastic lesions were calculated using the linearized multistage and Weibull models.

Renne et al., 2009). On the other hand, both hyperplasia of the transitional epithelium and squamous cell hyperplasia were highly similar to the preneoplastic lesions described by Schuller et al. (1990) and Morgan and Harkema (1996). DCP also induced nonneoplastic lesions of the nasal cavity: atrophy of the olfactory epithelium, inflammation of the respiratory epithelium and squamous cell metaplasia of the respiratory epithelium. Atrophy of the olfactory epithelium and inflammation of the respiratory epithelium were persistent throughout the 2-year exposure period, while squamous cell metaplasia of the respiratory epithelium was observed at the end of the 2-year exposure period. These lesions occurred at the lowest DCP exposure concentrations.

In this 2-year study, the terminal body weights of male and female rats exposed to 500 ppm were decreased by only 11 and 8%, and no significant difference in the terminal survival rate was found between any DCP-exposed group of either sex and the control. Therefore, the highest exposure concentration of 500 ppm fulfills the criteria of MTD setup by both the NCI and the IARC guidelines for the 2-year rodent carcinogenicity studies (Sontag et al., 1976; Bannasch et al., 1986). Fulfillment of the MTD criteria indicates that a relevant extrapolation can be made of tumor incidences observed at relatively high exposure concentrations to those which are expected to be encountered at environmentally observed, low levels of DCP in the ambient air and work environment. In addition, DCP-induced papillomas and esthesioneuroepitheliomas are likely to occur in humans as well as in rats: Nasal tumors including squamous carcinoma, transitional cell carcinoma, adenocarcinoma, and esthesioneuroepithelioma (aesthesioneuroblastoma) are reported to occur in humans exposed by inhalation to occupational agents in a nickel refinery (Torjussen et al., 1979) and in workers in the furniture, boot and shoe industries (Acheson et al., 1968; Acheson, 1976; Buiatti et al., 1983; Magnavita et al., 2003).

This study's findings that inhalation exposure to DCP induces nasal tumors are in sharp contrast with NTP's findings (1986) that oral administration of DCP by gavage for 103 wk at daily doses of 125 and 250 mg/kg body weight (male and female mice and female rats) or 62 and 125 mg/kg body weight (male rats) produced a dose-related increase in hepatocellular tumors in both male and female $B6C3F_1$ mice, but did not induce any tumors in male F344 rats and only the higher levels of exposure, which decreased survival, induced a marginal increase in adenocarcinoma of the mammary gland, but no other tumors, in female F344 rats (NTP, 1986). A difference in carcinogenicity between these two studies could be attributed to the difference in routes of exposure, oral administration in the NTP study and inhalation in our study. It can be inferred, therefore, that the nasal carcinogenicity of DCP results from direct exposure of the nasal tissue to inhaled DCP entering through the nasal cavity, whereas induction of the hepatocellular tumors in the mice given DCP by oral gavage can be accounted for, in part, by the effect of orally administered DCP entering the liver after gastrointestinal absorption.

The question as to whether a genotoxic or nongenotoxic mechanism operates in DCP-induced carcinogenicity is an important determinant for carcinogen risk assessment. The results of mutagenicity studies on *S. typhimurium* TA100 and TA1535 in the presence and absence of metabolic activation were positive by the plate incorporation method, while negative results were reported with TA98, TA1537, TA1538, and TA1978 (DeLorenzo et al., 1977; Principe et al., 1981). On the one hand, DCP induced sister chromatid exchange and chromosome aberrations in cultured Chinese hamster ovary cells and V79 cells in the presence and absence of metabolic activation (NTP, 1986; Galloway et al., 1987; von der Hude et al., 1987). Therefore, it is possible that a genotoxic mode of action operates in DCP-induced carcinogenesis. On the other hand, the long-term stimulation of the nasal epithelium with the irritant DCP vapor caused persistent atrophy of the olfactory epithelium accompanied by necrosis and inflammation of the respiratory epithelium, resulting in enhanced regeneration of epithelial cells. Assuming as a working hypothesis that there is a threshold below which necrosis and subsequent regeneration do not result in development of preneoplastic hyperplasia, a nongenotoxic mechanism of carcinogenicity having a threshold level of DCP exposure required for tumor induction may also contribute to DCP-induced carcinogenesis, as suggested by Butterworth et al. (1992).

Quantitative carcinogenic risk assessment of humans exposed to DCP in the ambient air can be estimated, assuming DCP as a genotoxic carcinogen, using a nonthreshold approach of linear extrapolation with a $BMCL_{10}$ value of 234 ppm for total nasal tumors. The 6-h/day and 5-day/wk exposure concentration was adjusted to a 24-h and 7-day exposure, as follows:

$$BMCL_{10[ADJ]} = Cobs\,(mg/m^3) \times D\,(6\,h/24\,h) \times W\,(5\,day/7\,days) \quad (1)$$

where Cobs is the observed exposure concentration, and D and W are correction factors for whole day and whole week exposure. A human equivalent concentration (HEC) can be derived from the adjusted rat $BMCL_{10}$ value by multiplying by a regional gas dose ratio for the extrathoracic region [RGDR (ET)], according to the following equation (U.S.EPA, 1994):

$$RGDR\,(ET) = \frac{MVa}{S(ET)a} / \frac{MVh}{S(ET)h} \quad (2)$$

where MVa, minute volume in the rat (0.30 m^3/day), was calculated by an allometric scaling equation given by the U.S. EPA (1994) using an averaged body weight of 300 g throughout this study; S(ET)a is the surface area of the extrathoracic region in the rat (15 cm^2); MVh is the minute volume in the human (20 m^3/day); and S(ET)h is the surface area of the extrathoracic region in the human (200 cm^2). The calculated $BMCL_{10}$ (HEC) for urban residents breathing ambient air for 24 h and 7 days/wk is 8.4 ppm. Use of the nonthreshold approach allows estimation of a reference

ambient air concentration for DCP, which is associated with an upper bound lifetime cancer risk level of 1 in 100,000 of 0.84 ppb (3.9 μg/m³) or for a risk level of 1 in 1,000,000 of 0.084 ppb (0.39 μg/m³). It is interesting to note that the ambient air concentration of 3.9 μg/m³ for DCP which is associated with a cancer risk level of 1 in 100,000 is comparable with the inhalation reference concentration estimated by the Japan Ministry of the Environment (2006) of 1.6 μg/m³ for a homolog of DCP, 1,2-dichloroethane, which is associated with a cancer risk level of 1 in 100,000. And, this value is 10 times higher than the 0.4 μg/m³ value for 1,2-dichloroethane associated with a cancer risk level of 1 in 100,000 derived by the U.S. EPA (2008).

Calculation of a margin of exposure (MOE) is considered to be a practical approach to characterize a population's risk of developing an adverse response to an environmental toxin. The MOE is determined by dividing the BMCL (HEC), referred to as the human equivalent concentration corresponding to a point of departure [POD (HEC)], by the concentration of the agent to which the population is exposed. Here, we use the rat $BMCL_{10}$ value of 11.5 ppm for the POD (HEC), and this value is the $BMCL_{10}$ obtained from the preneoplastic endpoint of the transitional epithelium and squamous cell hyperplasia. The endpoint of the preneoplastic hyperplasia was chosen for the POD, because it is recommended that MOE analysis be based on a precursor response rather than tumor incidence due to the greater sensitivity of precursor endpoints (U.S.EPA, 1999); in this study, the $BMCL_{10}$ for the preneoplastic endpoint is 20 times lower than the $BMCL_{10}$ for the nasal tumor endpoint. The $BMCL_{10}$(HEC) for the preneoplastic endpoint is 0.41 ppm (1.9 mg/m³) DCP for the POD(HEC). The concentration of DCP used here for the calculation of the MOE is the reported maximum concentration of human exposure to DCP 1.2 μg/m³ in the ambient air of Philadelphia (Haemisegger et al., 1985), resulting in a calculated MOE of approximately 1600. Comparison of the MOE value with expected total uncertainty factors including human variation in susceptibility, types of responses examined, and dose-response curves suggests that an ambient air concentration of DCP of 0.84 ppb (3.9 μg/m³), which is associated with a lifetime risk level of 1 in 100,000 of developing a nasal tumor, is adequately protective of public health.

Estimation of an OEL for DCP using a nonthreshold approach

The $BMCL_{10}$ value for the induction of nasal tumors of 234 ppm obtained in this study, in which rats were exposed for 6 h/day, is adjusted to 8 h (× 6/8) and further adjusted to human equivalency with RGDR (ET) according to Equation 2. The human equivalent, 8-h adjusted $BMCL_{10}$ value for the induction of nasal tumors is 35.1 ppm. Using the nonthreshold approach, the workplace air concentration of DCP at an upper-bound lifetime excess cancer risk level of 1 in 1000 is estimated to be approximately 0.35 ppm.

Estimation of an OEL for DCP using a threshold approach

A threshold approach makes use of a working hypothesis for the existence of a no-observed-adverse-effect level (NOAEL) at and below which preneoplastic lesions leading to development of nasal tumors would not be elicited in any of the rats exposed to DCP for 2 years. Gaylor and Kodell (2002) reported that $BMCL_{10}$ values are approximately equal to experimentally derived NOAELs. The human equivalent, 8-h adjusted $BMCL_{10}$ value is calculated using the $BMCL_{10}$ value for the induction of preneoplastic lesions in rats (11.5 ppm); the value for humans is 1.7 ppm. Therefore, a human NOAEL for a preneoplastic endpoint is approximately 1.7 ppm. The OEL for DCP using a threshold approach is estimated by dividing the human NOAEL by the uncertainty factors. Total uncertainty factors of 5 can be used: Workers' individual variation of susceptibility can be assigned a value of 1, because workers are healthy, and a preneoplastic endpoint can be assigned a value of 5, because an uncertainty factor for cancer was assigned a value of 10 by WHO (WHO, 1994). Using these values, the OEL for DCP in the workplace air is estimated to be approximately 0.34 ppm.

Table 4 shows the current OEL values for DCP by U.S. OSHA and ACGIH, and the $BMCL_{10}$ values and the estimated OEL derived from the present animal data. The OEL value of 0.35 ppm derived from the nonthreshold approach using the nasal tumor endpoint was found to agree well

Table 4. The current OEL values for DCP by U.S. OSHA and ACGIH, the $BMCL_{10}$ values and the estimated OEL derived from the present animal data.

Estimation		Comments
U.S. OSHA (PEL)	75 ppm	
ACGIH (TLV-TWA)	10 ppm	Based on body weight reduction and respiratory system (nasal) irritation of the exposed rats in the 13 wk inhalation study.
Estimates from the present animal data		
Nonthreshold approach		
$BMCL_{10}$ (tumor)	234 ppm	A linearized multistage model for the incidences of total nasal tumors in the 2-yr inhalation study.
Estimated OEL	0.35 ppm	An upper-bound lifetime excess cancer risk level of 1 in 1000, and adjusted to 8 hours and to human equivalency with RGDR.
Threshold approach		
$BMCL_{10}$ (preneoplastic lesion)	11.5 ppm	A Weibull model for the incidences of total nasal preneoplastic lesions in the 2-yr inhalation study.
Estimated OEL	0.34 ppm	NOAEL for the nasal preneoplastic endpoint and uncertainty factors of 5, and adjusted to 8 hours and to human equivalency with RGDR.

with that of 0.34 ppm derived from the threshold approach using the preneoplastic endpoint. Those OELs estimated from the present animal data are 30 times and 200 times lower than the TLV-TWA value of ACGIH and the PEL of U.S. OSHA (ACGIH, 2009), respectively. The TLV-TWA value of 10 ppm recommended by ACGIH (2006) was assigned on the basis of a repeated rat inhalation study (6 h/day, 5 days/wk and 13 wk) that found body-weight reductions and respiratory system (nasal) irritation at exposure levels greater than 15 ppm (Nischke et al., 1988). A possible explanation for this discrepancy is that we used the human equivalent, 8-h adjusted values for both the $BMCL_{10}$ for the nasal tumor endpoint and the NOAEL for the nasal preneoplastic endpoint, both of which were derived from a 2-year rat inhalation study. Therefore, we believe that our estimates of the OEL for DCP represent a health-based level that would protect workers from nasal tumors over a working lifetime.

Conclusions

Thirteen-week inhalation exposure of male and female rats to DCP at five different concentrations up to 2000 ppm induced cytotoxic lesions in the nasal cavity, hemolytic anemia, and lesions of liver and adrenal gland. Two-year inhalation exposure of male and female rats to DCP at three different concentrations up to 500 ppm was found to significantly and dose-dependently increase incidences of total nasal tumor including papillomas and esthesioneuroepitheliomas and nasal preneoplastic lesions. These results demonstrate that DCP is a nasal carcinogen in rats. Lifetime cancer risks for humans exposed to DCP in the ambient air, using a nonthreshold approach, and in the work environment, using both nonthreshold and threshold approaches, were quantitatively estimated with the data obtained from the present 2-year inhalation study. This paper provides novel information about inhalation carcinogenicity and toxicity of DCP and their dose-response relationships for reconsideration of the current OEL.

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Declaration of interest

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Proliferative and Nonproliferative Lesions of the Rat and Mouse Respiratory Tract

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ABSTRACT FOR PROLIFERATIVE AND NONPROLIFERATIVE LESIONS OF THE RAT AND MOUSE RESPIRATORY TRACT

The INHAND Project (International Harmonization of Nomenclature and Diagnostic Criteria for Lesions in Rats and Mice) is a joint initiative of the Societies of Toxicologic Pathology from Europe (ESTP), Great Britain (BSTP), Japan (JSTP) and North America (STP) to develop an internationally-accepted nomenclature for proliferative and non-proliferative lesions in laboratory animals. The purpose of this publication is to provide a standardized nomenclature for classifying microscopic lesions observed in the respiratory tract of laboratory rats and mice, with color photomicrographs illustrating examples of some lesions. The standardized nomenclature presented in this document is also available electronically on the internet (http://www.goreni.org/). Sources of material included histopathology databases from government, academia, and industrial laboratories throughout the world. Content includes spontaneous developmental and aging lesions as well as lesions induced by exposure to test materials. A widely accepted and utilized international harmonization of nomenclature for respiratory tract lesions in laboratory animals will decrease confusion among regulatory and scientific research organizations in different countries and provide a common language to increase and enrich international exchanges of information among toxicologists and pathologists.

Keywords: respiratory system; lesions of the rat respiratory system; lesions of the mouse respiratory system.

INTRODUCTION

The INHAND Project (International Harmonization of Nomenclature and Diagnostic Criteria for Lesions in Rats and Mice) is a joint initiative of the Societies of Toxicologic Pathology from Europe (ESTP), Great Britain (BSTP), Japan (JSTP), and North America (STP) to develop an internationally accepted nomenclature for proliferative and nonproliferative lesions in laboratory animals. The purpose of this publication is to provide a standardized nomenclature for classifying proliferative and nonproliferative lesions observed in the respiratory tract of laboratory rats and mice. Standardized nomenclature of proliferative (Schwartz et al. 1994) and nonproliferative (Renne et al. 2003) respiratory tract lesions in rats were previously published by the STP. The standardized nomenclature of respiratory tract lesions presented in this document is also available electronically at the goRENI Web site (http://www.goreni.org/). This document follows a similar anatomical approach. Consequently, nasal cavity, larynx and trachea, major airways, and lung parenchyma have been separated in this nomenclature scheme, even though there may be considerable redundancy regarding responses at various levels.

Inhalation studies in laboratory rodents are used throughout the world to test drugs, chemicals, and environmental pollutants for potential toxicity and carcinogenicity. Induced respiratory tract lesions in rodents form the basis for a number of current inhalation exposure regulatory guidelines (Morris 2006). Respiratory tract cancer and chronic obstructive pulmonary disease (COPD), two of the leading causes of human mortality and morbidity worldwide, are major research efforts that use rodent models (Wakamatsu et al. 2007; Wright, Cosio, and Churg 2008). A widely accepted and used international harmonization of

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nomenclature for respiratory tract lesions in laboratory animals will decrease confusion among regulatory and scientific research organizations in different countries and provide a common language to increase and enrich international exchanges of information among toxicologists and pathologists.

Nasal and pulmonary epithelia have extensive capability for metabolism of xenobiotics and are frequently affected by inhaled chemicals. A variety of metabolic and biotransformation enzymes and enzyme systems have been identified in the nasal and pulmonary epithelia of rats and mice (Philpot et al. 1971; Bogdanffy 1990; Harkema and Morgan 1996; Smith and Bryan 1991; Pino et al. 1999; Thornton-Manning and Dahl 1997). The distribution of respiratory tract lesions is generally dependent on the regional deposition, uptake, physical and chemical properties, concentration and duration of exposure of the inhaled xenobiotic, local cell susceptibility, or a combination of these factors (Philpot et al. 1971; Morgan and Monticello 1990; Morris 2006). Airflow patterns also play an important role in the distribution of nasal and laryngeal lesions.

Proliferative lesions in laboratory rodents may arise from infectious agents or as part of the aging process, but the most toxicologically important proliferative respiratory tract lesions result from exposure (usually repeated inhalation exposure) to potentially toxic test materials. Cellular damage from repeated exposure to toxicants induces a repair process in which the damaged tissue may proliferate (hyperplasia) and/or undergo metaplasia to a different, more resistant cell type if return to normal morphology is not complete. The site of these changes is heavily dependent upon the nature of the toxicant and the type of tissue exposed. Ciliated columnar and olfactory epithelia are the most fragile respiratory epithelia and thus the most susceptible to damage from inhaled toxicants. Cuboidal epithelium is more resistant, and squamous epithelium is the most resistant to damage from direct contact with toxic or irritant materials.

Nonproliferative lesions in general are also associated with experimental perturbation or are a result of degenerative changes frequently associated with aging. Modern laboratory animal management practices within rodent facilities are such that spontaneous infectious processes should be infrequently encountered; thus, the lesions related to infectious respiratory tract diseases are not described in detail in this document. Excellent reviews have been published on the effects of infectious disease, diet, and environmental factors on the rat respiratory tract (Everitt and Richter 1990; Castleman 1992; Baker 1998).

MORPHOLOGY

I. Nasal Cavity

The nasal cavity is structurally complex, reflecting the diverse physiological functions associated with this anatomical site. St. Clair and Morgan (1992) provide an overview of normal development, growth, and age-related changes in rat nasal passages. Several articles describe the anatomy (Young 1981; Uraih and Maronpot 1990; Harkema 1991; Harkema, Carey, and Wagner 2006) and physiology (Proctor and Chang 1983; Barrow et al. 1986; F. Miller 1995) of rodent nasal cavity tissues. Identification of metabolic functions of nasal epithelia has sparked considerable interest in the morphology of the nasal passages, and consequently, the recognition of chemically induced lesions has become more commonplace in recent years (Bond 1986; Dahl 1986; Reed 1993; C. Keenan, Kelly, and Bogdanffy 1990; Adams et al. 1991; Jeffrey, Iatropoulos, and Williams 2006). In addition, there has been considerable recent interest in transport of toxicants into the brain via the olfactory nerves (Dorman et al. 2002; Harkema, Carey, and Wagner 2006). Location of lesions is likely related to several factors, including dose to affected area, site-specific susceptibility, local metabolism, species and sex (Kai et al. 2006), or a combination of these factors. Localization and documentation of lesions are quite dependent upon consistency of sampling and preparation of nasal tissues (Morgan 1991; Hardisty et al. 1999; Boorman et al. 1990; Mery et al. 1994; Herbert and Leininger 1999; Kittel et al. 2004). Diagrams for recording distribution of nasal lesions have been published (Morgan 1991; Mery et al. 1994; Robinson et al. 2003). Careful description of lesion distribution is important to help understand the nature of the toxic compound (Mery et al. 1994; Hardisty et al. 1999).

The areas of rodent nasal mucosa that most frequently develop nonproliferative and proliferative lesions in response to inhaled toxicants are the transitional and respiratory epithelia lining the distal third of the nasal and maxillary turbinates in the anterior nasal cavity, and the rostral extension of olfactory epithelium lining the dorsal medial meatus (Harkema et al. 2006; Renne et al. 2007). However, the location of induced lesions varies with the chemical and physical nature of the toxicant, and induced lesions have been described throughout the nasal mucosa, including severe lesions in the squamous epithelium lining the atrioturbinates, an area not routinely examined microscopically. The morphologic sequelae to injury of nasal epithelium depend on extent and duration of the injury as well as the time post-injury that tissues are sampled and preserved. Sequelae range from regeneration of epithelium identical to the original epithelium, to atrophy, metaplasia to a different epithelial type, proliferation in the form of hypertrophy, hyperplasia, and/or neoplasia. Loss of surface respiratory or transitional epithelium with retention of an intact basement membrane is followed by formation of a layer of fibrin and inflammatory cells covering the affected area (Jiang, Morgan, and Beauchamp 1986). Adjacent undamaged epithelium undergoes rapid proliferation in response and, depending on the size of the lesion, migrates to form a layer of slightly flattened cuboidal epithelium completely or partially covering the defect (Haschek-Hock and Witschi 1991). If the injury is not repeated, the affected area will be repaired with epithelium of the same type as the original. Epithelial degeneration, necrosis, and regeneration are often present together in nasal tissues repeatedly exposed to injurious chemicals, resulting in a disorganized morphologic picture (Gaskell 1990; Hardisty et al. 1999).

Repeated loss of epithelium leads to transformation (metaplasia) to a more resistant cell type: squamous or mucous cell metaplasia in transitional or respiratory epithelia and squamous or

respiratory metaplasia in olfactory epithelium (Harkema, Carey, and Wagner 2006; Jiang et al. 1986; Kumar, Morgan, and Beauchamp 2004). Metaplasia results from reprogramming and differentiation along a new maturation pathway of stem cells normally present in the affected epithelium (Kumar, Abbas, and Fausto 2004). It is important to distinguish true squamous metaplasia from the comparatively thin layer of flattened epithelium present in early regeneration of ulcerated nasal epithelium. Schlage et al. (Schlage, Bulles, Friedrichs, Kuhn, and Teredesai 1998; Schlage, Bulles, Friedrichs, Kuhn, Teredesai, and Terpstra 1998) published information on changes in cytokeratin expression patterns in the various nasal epithelia, which was useful in demonstrating subtle effects of inhaled xenobiotics on these tissues.

Metaplasia to squamous epithelium may provide a barrier sufficient to prevent further epithelial loss from exposure to toxicants, but frequently, squamous metaplasia and inflammation in response to repeated exposure are accompanied by some loss of surface epithelium, resulting in an increased rate of cell turnover and eventually, hyperplasia of affected mucosal epithelium. Hyperplasia of transitional and respiratory epithelium lining the distal turbinates in the anterior nasal section is one of the most frequently observed lesions in rodents exposed to irritant compounds. Atypical hyperplasia of respiratory and olfactory epithelium and associated glandular tissue has been described (Boorman, Morgan, and Uraih 1990; Greaves 1996). Progression of hyperplastic respiratory nasal epithelium to neoplasia has been reported in the literature but is much less frequent than might be expected by the reported incidence of hyperplasia and squamous metaplasia. Hyperplasia and neoplasia of respiratory and olfactory epithelium are frequently accompanied by hyperplasia of the associated glandular tissue.

A. Congenital Lesions

Cleft Palate: Nasal Cavity

Pathogenesis/cell of origin: Longitudinal defect in bone and mucosa of the midline of the hard palate resulting from failure of fusion of the lateral palatine shelves from the maxillary processes (Jones, Hunt, and King 1997).

Diagnostic features:

- Midline space defect in oral mucosa and hard palate
- Visible grossly or microscopically
- No evidence of trauma

Differential diagnoses:

- Trauma: Evidence of necrosis, fracture, or inflammatory response
- Malocclusion: Gross evidence of overgrowth of incisor teeth; microscopic evidence of necrosis and inflammation of palate

Comment: Cleft palate has been induced in fetal rats exposed to triamcinolone (Walker 1971) or vitamin A palmitate (Hayes et al. 1981).

Deviation of the Nasal Septum: Nasal Cavity

Pathogenesis/cell of origin: Median nasal septum.

Diagnostic Feature: Deviation of the septum visible on nasal sections.

Differential diagnoses:

- Trauma: Visible inflammation, hemorrhage, or other evidence of trauma
- Artifact: Evidence of distorted or missing tissue; lack of evidence of tissue response

B. Epithelial Changes (squamous, transitional, respiratory, olfactory, glandular)

Septal Perforation (Figure 1): Nasal Cavity

Pathogenesis/cell of origin: Median nasal septum.

Diagnostic features:

- Complete loss of all tissue layers of the nasal septum
- Usually found in the ventral portion of the nasal septum
- Sequel to severe necrosis/ulceration with cartilage loss
- Usually associated with moderate to severe inflammation in adjacent nasal tissues

Differential diagnoses:

- Trauma: Evidence of damage to adjacent bone, cartilage, or soft tissue
- Artifact: Evidence of distorted or missing tissue, lack of evidence of tissue response

Atrophy (Figures 2–4): Nasal Cavity

Pathogenesis/cell of origin: Squamous, transitional, respiratory, olfactory, or glandular epithelium.

Diagnostic features:

- Thinning of the affected mucosa
- Decrease in cell numbers and/or decrease in cell height
- Atrophy of olfactory epithelium is often accompanied by loss of axon bundles in subjacent lamina propria
- Underlying turbinate bone may also be atrophied

Differential diagnoses:

- Postmortem autolysis: Uniform dissolution of entire tissue section with no change in organization or depth of cell layers
- Metaplasia: Change in epithelial cell types present, usually with mixture of cell types in areas of transition
- Degeneration: Loss of cilia and cellular organization but no decrease in thickness of epithelial layer
- Tangential section through epithelium: Microscopic evidence of tangential cut in other tissue structures

Comment: Atrophy is a condition commonly observed as a sequel to degeneration of nasal epithelium. Atrophy of nasal olfactory epithelium secondary to degeneration of sensory or