A Passive Tobacco Smoke Exposure System for Laboratory Animals

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Abstract : A simple passive tobacco smoke exposure system was developed for laboratory animals. The system consisted of a tobacco smoke generator and an exposure chamber. Performance testing was conducted using Fischer 344 rats. The rats were exposed to passive tobacco smoke generated by 20 cigarettes per day for 3 months. The concentration of the cigarette smoke aerosol (particle phase) in the chamber can be kept almost constant during exposure. The daily average mass concentration was 10.0 ± 4.3 mg/m³ and the carbon monoxide was 79 ± 22 ppm during exposure.

Key words : inhalation chamber, passive tobacco smoke, smoke generator.

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Introduction

Many tobacco smoke generators have been used in exposure experiments using small rodents (Asano, 1979). However, these generators have no stability for long-term inhalation. The primary reason for this is that some chemical compounds in tobacco smoke are cohesive and adhesive. Such chemicals adhere to the walls along the stream of tobacco smoke and cause interruption of the smooth flow of smoke in the exposure system. To avoid this phenomenon, the smoke generator should be a simple mechanism and easy to clean when it is to be used for long-term inhalation toxicity studies. We have developed a simple tobacco smoke generator that meets these criteria. The performance of the system was tested by exposing rats to passive tobacco smoke for 3 months.

Methods

Experimental apparatus

Figure 1 illustrates the smoke generator and the exposure chamber. The chamber was of a whole-body exposure type. The chamber volume was 100 ℓ. The filtered air was introduced into the top of the chamber for dilution and the flow directed downward.

Fig. 1. Schematic diagram of a passive tobacco smoke exposure system for laboratory animals.

The flow rate in the chamber was 50 l/min (30 complete air changes per hour). The temperature and the relative humidity in the exposure chamber was kept at $25 \pm 2^\circ\text{C}$ and $50 \pm 10\%$, respectively.

The smoke generator consisted of a glass tube with an aspirator made of rubber attached to one end for air inflow. The cigarette was inserted into the other end of the glass tube. To prevent cigarette ashes from falling on the rodents, a conical beaker was placed immediately below the cigarette as shown in Fig. 1.

The mass concentration of the passive tobacco smoke aerosol during exposure was determined by using glass fiber filter samples. The grab concentration of carbon monoxide in the chamber was measured by a direct reading gas detector tube (Kitagawa Co. Japan).

Experimental procedure

After lighting the cigarette manually, the smoke generator was immediately inserted through the top of the exposure chamber. As the cigarette burned, the smoke descended slowly with the air flow and exited from the bottom of the chamber. One cigarette produced smoke for 12 min. After the cigarette burned down to the filter, the smoke generator was withdrawn from the chamber and a new cigarette attached. This method was performed continuously until all 20 cigarettes were used up.

Cigarettes

The cigarettes used in this experiment were purchased from the Japan Tobacco Inc.

Japan. Their trade name is "Peace (Long Size)" and contain 2.0 mg of nicotine and 23 mg of tar per cigarette.

Animals

The rodents used were Fischer 344 male rats (6 weeks old). The rats were randomly divided into one control (8 rats) and two test groups (6 and 7 rats). Each group was housed together in a stainless steel wired cage. Animals had unrestricted access to commercially available certified feed pellets (CE-2, Clea Japan Inc.) and tap water. Feed and water were not available during the inhalation exposure.

Exposure

The Fischer male rats were exposed to the passive tobacco smoke of 20 cigarettes/day (4 hr/day), 5 days per week for 3 months. Immediately after each daily exposure, the smoke generator, the exposure chamber and the cage were cleaned.

Results and Discussion

Usually a burning cigarette extinguishes without a flow of air. With this experimental method, the cigarette was located near the filtered air inlet at the top of the chamber. The cigarette continues to burn due to the air passing through the filtered air inlet (superficial velocity: 17 cm/sec).

All the parts in the exposure system that came into contact with the tobacco smoke after daily exposure were cleaned for 1 hr. This cleaning is one of the most important factors to keep the mass concentration of the smoke constant in the chamber over a long period of time. All parts of the exposure system were designed for easy cleaning. The experimental conditions are summarized in Table 1.

The daily mean $\pm$ SD mass concentrations in the chamber was 10.0 ± 4.3 mg/m³ as

Table 1. Inhalation conditions

Test aerosol	: Passive tobacco smoke
Test species	: Fischer 344 male rats
Number of rats per group	: 6 and 7 rats
Exposure time	: 360 hr (4 hr/d, 5 d/wk, for 3 mo) (20 cigarettes/d=4 hr/d)
Concentration of cigarette smoke	: 10.0 ± 4.3 mg/m ³
CO concentration	: 79 ± 22 ppm
Chamber volume	: 100 ℓ
Air flow rate	: 50 ℓ /min
Temperature & humidity in chamber	: $25 \pm 2^\circ\text{C}$ & $50 \pm 10\%$

The exposure system is cleaned every exposure day.

measured from the glass fiber filter samples. There was no significant difference between the two exposure chambers used. This suggests that the smoke concentration was kept almost constant during exposure.

The size distribution of the tobacco smoke particles was not measured in this experiment. Higashi *et al.* (1985) reported that the count median diameter (CMD) and the geometric standard deviation (GSD) of the side stream of tobacco smoke measured by a scanning electron microscope were $0.43\ \mu\text{m}$ and 1.33, respectively. The relationship between mass median diameter (MMD) and count median diameter is shown in the following equation (Tanaka, 1987):

$$\ln\text{MMD} = \ln\text{CMD} + 3 (\ln\text{GSD})^2.$$

The MMD is $0.55\ \mu\text{m}$ as calculated from the CMD of $0.43\ \mu\text{m}$. Chen *et al.* (1987) reported that the mass median aerodynamic diameter (MMAD) measured by a multi-jet impactor increased linearly from 0.40 to $0.65\ \mu\text{m}$ in a concentration range of $100 - 800\ \text{mg}/\text{m}^3$. These reports indicate that passive tobacco smoke contains respirable particles.

The concentration of carbon monoxide (CO) in the chamber was $79 \pm 22\ \text{ppm}$ measured by the direct reading gas detector tube. Chen *et al.* (1987) showed that the relationship between the CO concentration and the mass concentration (MASS) is as follows:

$$\text{CO (ppm)} = 0.52 \text{ MASS (mg/m}^3\text{)} + 16.9; \quad 100 < \text{MASS} < 800\ \text{mg/m}^3.$$

Fig. 2. Comparison of growth (body weight) between tobacco smoke exposed and control rats. Asterisk * indicates a significant difference between the exposed and control rats, $P < 0.01$ by Student's *t* test.
 ● Exposure, ○ Control

By substituting 10.0 mg/m³ from our experiment into the equation, the estimated CO concentration would become 22 ppm, that is, 5 times smaller than the values measured in our experiment. The cause of this is the difference in the conditions of burning (temperature, velocity, flow rate of the clean air, etc.).

During tobacco smoke exposure, we found that urine volume increased in the exposed group, but quantitative measurements were not made. Figure 2 shows the comparison of body weight between the exposed and control rats. There were significant differences ($P < 0.01$) in body weight between the exposed and the control groups after 4 weeks of exposure. Exposed rats had smaller weight gains than the controls.

The biochemical and histopathological changes of passive tobacco smoke exposed rats will be reported separately.

We believe the exposure system developed is valid for investigating the chronic health effects of passive inhalation of tobacco smoke.

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タバコ副流煙吸入暴露装置の試作

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要 旨： タバコ受動喫煙による生体影響を調べるための小動物用吸入暴露装置を試作し、その性能を Fischer 344 雄性ラットを用いてテストした。試作された装置は、タバコ副流煙発生装置と吸入暴露チャンバーとから構成されている。性能テストのための吸入暴露条件は 1 日 20 本 (4 時間)、1 週 5 日で 3 ヶ月間行われた。この間の日平均粒子濃度は 10.0 ± 4.3 mg/m³、一酸化炭素濃度は 79 ± 22 ppm であった。暴露期間中はほぼ一定の濃度を保って暴露できることが確認されたことから、試作された本装置は、タバコ受動喫煙による生体影響を調べる装置として有用であると考えられる。

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