DEPOSITION OF CIGARETTE SMOKE PARTICLES IN THE RAT RESPIRATORY TRACT

Abstract — Male and female rats were exposed to mainstream cigarette smoke to determine the fractional deposition. Deposition studies were conducted by placing the rats in plethysmograph tubes for respiratory minute volume measurements and exposing them to ^{14}C-dotriacontane-labeled cigarette smoke at mass concentrations of 202 or 624 mg/m^3 for 25 min. Immediately after the exposure, the rats were sacrificed and the ^{14}C contents in various tissues and organs were analyzed. Results showed that the GI tract contained 16-31% of the total activity, indicating significant clearance from the large airways and nose to the GI tract during the exposure and during the 10-15 min between cessation of the exposure and the removal of the organs. Total deposition of the inhaled activity was 20.1 ± 1.6% for both exposure concentrations. The intrapulmonary deposition fractions (lung lobes plus airways below the lobar bronchi) were 12.4 ± 0.9% and 15.9 ± 1.4% for high and low concentrations, respectively, suggesting a slight enhancement in upper airway deposition for animals exposed to the higher smoke concentration.

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Inhalation toxicity studies using rats exposed to mainstream cigarette smoke have been conducted to simulate the exposures of human smokers.[1,2] To have a good simulation, the exposure concentration and exposure time should be designed to result in the same amount of deposition, per gram of lung, of total particulate matter (TPM) in rats as would be deposited in human smokers. The information needed for this estimation is the intrapulmonary fractional deposition (FD_{IP} = amount deposited in the intrapulmonary region/total amount inhaled) for rats and human smokers. Values for total fractional deposition (FD_T) of mainstream cigarette smoke particles in human smokers were reported to have a wide variation (22-75%), with an average of 47%.[3] There is, however, no information in the literature about the FD_T of cigarette smoke particles in rats. In this study, we determined the FD_T in Fischer-344 rats exposed to ^{14}C-labeled mainstream cigarette smoke. This information will be useful for designing chronic rat exposure studies with concentration levels and exposure times that properly simulate the inhaled doses for human smokers. In addition, deposition fractions in the upper airway and intrapulmonary regions were estimated to provide the basis for dosimetric interpretation.

METHODS

Animals

Twenty 7-mo old Fischer 344/N rats from the Institute's breeding colony were randomly assigned by weight to this study. Groups of ten (five male and five female) were exposed to cigarette smoke at each of two concentrations.

Prior to the deposition measurements, the rats were conditioned in nose-only exposure tubes, 25 min per day for two days. They were then exposed in the tubes 25 min/day for three days to unlabeled cigarette smoke at one-half of the target concentrations (target concentrations were

approximately 650 and 200 mg/m^3), and then to the full concentrations, 25 min/day for five days. The rats were placed in plethysmograph tubes once during the full concentration conditioning. During each day of exposure to radiolabeled cigarette smoke, two groups of rats (one male and one female per group) were placed in plethysmograph tubes and exposed to the high or low concentrations. The animals not due for exposure continued to be conditioned in exposure tubes.

Smoke Generation and Exposure

Figure 1 illustrates the smoke generation, delivery, and exposure systems.[4] A Walton smoking machine was modified to generate four puffs of cigarette smoke from type 1R3 research cigarettes per minute. A residence chamber was used as a buffer between the smoke generation and exposure units. This chamber (6.7 L) was selected to produce a residence time much longer than the time between puffs, but its size was small enough to rapidly fill the exposure chamber with smoke. A nose-only chamber was used for animal exposures. All three compartments contained rotating fans to create a uniform mixing of cigarette smoke. The system had a total flow rate of 5.2 L/min and provided the rats with a relatively continuous exposure to cigarette smoke, avoiding puff-by-puff variations of breathing patterns which occurred during intermittent exposures,[5] and also conserved the amount of labeled smoke which had to be generated.

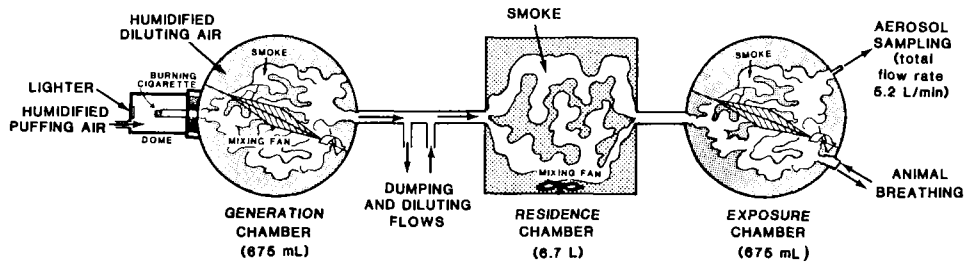

Figure 1. Schematic diagram of the cigarette smoke generation, delivery, and exposure systems. Details of the exposure system are shown in Figure 2.

The different mass concentrations were achieved by adjusting the dumping and diluting flow rates (Fig. 1). The dumping flow removed a certain amount of smoke from the system and the diluting flow provided the same amount of clean air to dilute the remaining smoke. The mean mass concentrations and particle size distributions were estimated using two glass fiber filters and a multi-jet Mercer impactor, respectively (Fig. 2). The real-time mass concentration profiles were monitored using an aerosol monitor (RAM-S).

Smoke Particle Deposition

Each cigarette was labeled with 185 kBq (5 μCi) ^{14}C-dotriacontane (^{14}C-DTC-16,17) by injecting the DTC/toluene solution with a syringe and long needle along the cigarette axis. This radiolabeled marker has been shown to directly transfer to the smoke via distillation, with about 20% in the mainstream particulate phase and a negligible amount in the gas/vapor phase.[6] During the animal exposures, the rats were placed in plethysmograph tubes with their noses exposed to the radiolabeled cigarette smoke (Fig. 2).

Rats were sacrificed 10-15 min after exposure by intraperitoneal injection of a lethal dose of sodium pentobarbital. Cardiac puncture was performed to obtain 1-2 mL of blood. The rats were then necropsied. Individual lung lobes, trachea and lobar bronchi, head (without pelt), larynx, kidneys, liver, GI tract, and depelted carcass were dissected, weighed, and then digested with tetraethylammonium hydroxide. The ^{14}C activity in the cigarette smoke particles collected on the filters, impactor substrates, and tissues were analyzed using a liquid scintillation counter.[4]

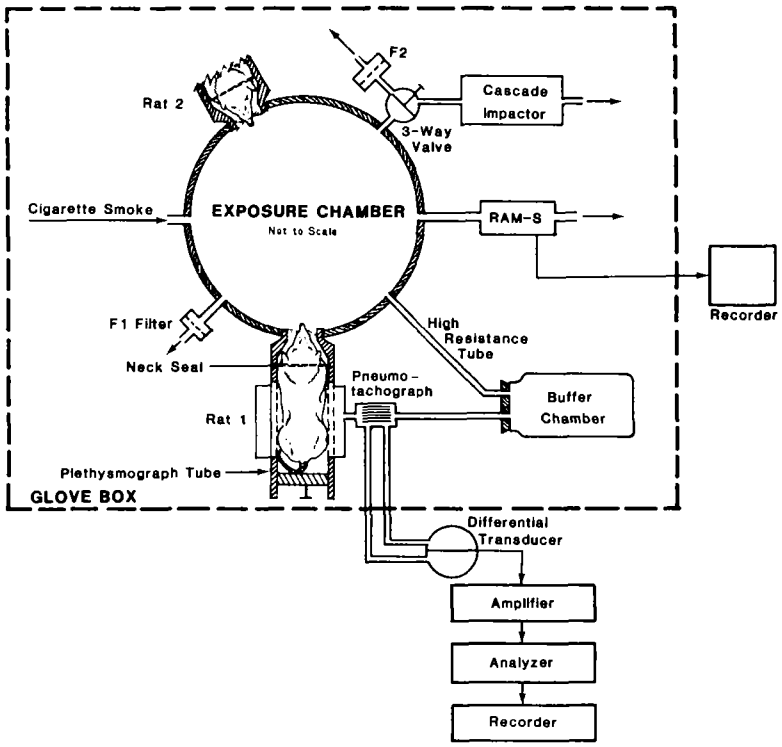

Figure 2. Schematic diagram of the exposure setup including two plethysmograph tubes, two filters, one impactor, and one RAM-S.

Data Analysis

The average minute volume during the exposure was calculated for each rat from the plethysmograph data. The value of FD_T for each animal was determined as follows:

$$FD_T = \frac{\sum_T a_i}{TAI}; \quad (1)$$

$$TAI = (a_f/q)\ v, \quad (2)$$

where TAI was the total activity inhaled by the animal, a_i was the activity in i-th tissue, $\sum_T a_i$ was the total activity of ^{14}C measured in all the tissues, a_f was the ^{14}C activity on the filter, q was the volumetric flow rate through the filter, and v was the average minute volume.

The fractional deposition in the intrapulmonary region (lung lobes plus airways below the lobar bronchi), FD_{IP}, was estimated in the same way, except that only the activities $\sum_{IP} a_i$ from individual lung lobes and blood-borne activity were used in the numerator of Equation 1.

The blood-borne activity was assigned to intrapulmonary deposition because of the large surface area of that region and the likelihood that most or all absorption of ^{14}C occurred there. This activity included that in the blood, kidneys, liver, depelted carcass, and a portion of the activity in the head. The fractional deposition in the upper airway, FD_{UA}, was calculated simply as $FD_T - FD_{IP}$.

RESULTS AND DISCUSSION

The rats were exposed to cigarette smoke at mean mass concentrations of 95 or 341 mg/m^3, 212 or 657 mg/m^3, and 202 or 624 mg/m^3 during the one-half and the full smoke conditioning, and ^{14}C-smoke exposures, respectively. Their mean breathing rate and mean minute volume during exposures were 86% and 72%, respectively, of those measured before exposures. Representative real-time profiles of the smoke concentration and the breathing pattern of the rats during the exposures are shown in Figure 3. These profiles indicated that the system provided a relatively uniform concentration in the exposure chamber and that the exposure method successfully avoided the marked depression of breathing reported by other researchers.[5]

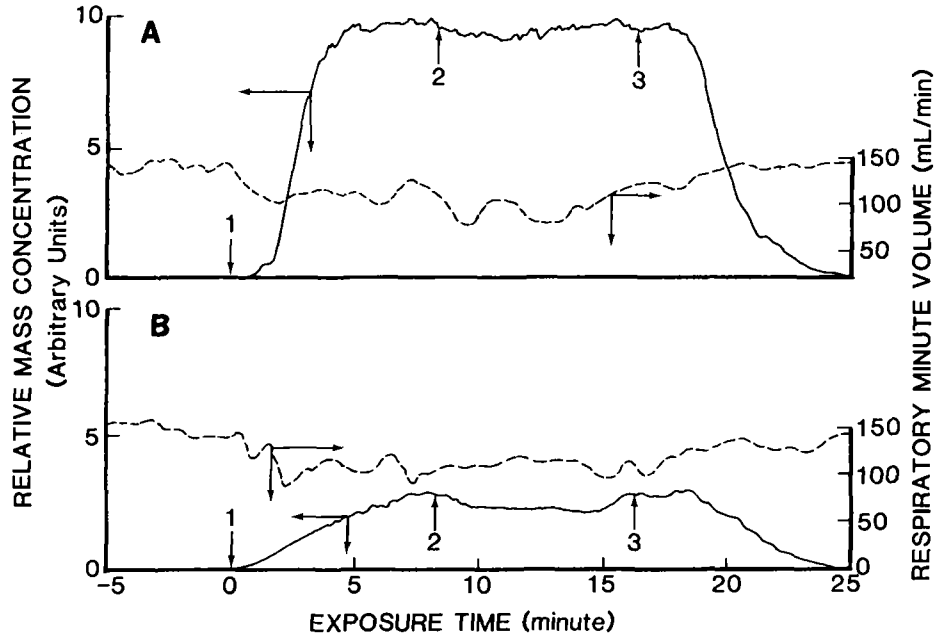

Figure 3. The relative mass concentration profiles in the exposure atmosphere and the corresponding respiratory minute volume traces of the rats [(a) female and (b) male] before and during the exposures to (a) high concentration, and (b) low concentration cigarette smoke; 1, 2, and 3 represent the times at which the exposure started, the first cigarette was replaced with the second one, and the second cigarette was taken out, respectively.

The median aerodynamic diameters of cigarette smoke particles used in this study were 0.40 and 0.47 μm for the low and high concentrations, respectively, regardless of whether the measurements were based on mass or activity. The geometric standard deviation ranged from 1.4 to 1.6. This size distribution was very similar to that reported previously using 2R1 research cigarettes.[7] Although the median sizes were different for the two concentrations, the deposition efficiencies of particles of 0.4-0.5 μm are relatively similar and the small size differences in this study would not have had significant effects on the fractional deposition.[8]

The distribution of ^{14}C activity within the rats is given in Table 1. The lung lobes contained the majority of the activity, indicating that a large portion of the TPM was deposited in the lower respiratory tract. The presence of ^{14}C activity in the GI tract indicates that mucociliary clearance of smoke particles from the large airways and nose occurred during the 25 min of exposure and during the 10-15 min that elapsed between cessation of exposure and removal of organs. Good agreement was found between our data and those from Kendrick *et al.*[5] in the

Table 1

^{14}C Radioactivity in Various Organs of Rats Following Inhalation of ^{14}C-Dotriacontane-Labeled Cigarette Smoke[a]

Tissue	Activity Distribution (%)	
	High[b]	Low[b]
Larynx	0.37 (0.12)[c]	0.31 (0.07)
Trachea and lobar bronchi[d]	0.34 (0.04)	0.66 (0.05)
Lungs (total)	(61.08)	(71.53)
Right apical	8.29 (0.57)	9.01 (0.52)
Right cardiac	8.60 (0.40)	9.47 (0.84)
Right diaphragmatic	14.47 (1.34)	17.74 (0.67)
Right intermediate	6.94 (0.48)	8.70 (0.62)
Left diaphragmatic	22.78 (1.62)	26.61 (1.69)
Blood[e]	0.08 (0.04)	0.24 (0.14)
Head	4.75 (0.36)	6.26 (0.65)
GI tract	31.35 (3.48)	15.78 (1.89)
Kidneys	0.06 (0.03)	0.20 (0.06)
Liver	0.24 (0.08)	0.83 (0.30)
Depelted carcass	2.09 (0.54)	7.08 (2.03)

[a]No significant difference (Student's t-test at $p > 0.05$) was found between data from male and female rats.

[b]High and low represent high and low cigarette smoke concentrations, respectively.

[c]Mean (standard error) ($n = 10$).

[d]Airways from the trachea to entry into lobes.

[e]Blood sample (1-2 mL).

percentage of activity in the lungs (65%) and in the GI tract (30%) immediately after inhalation exposures. A more detailed analysis of the regional distribution within the lung indicated that the values of ^{14}C activity/tissue weight in the lung lobes were relatively uniform for both concentrations, with the least activity in the right diaphragmatic lobe, similar to the results of Kendrick et al.[5]

Table 2 lists the mean total, intrapulmonary, and upper airway fractional depositions. Variations among animals were small. The total fractional deposition (FD_T) was 20%, and was independent of exposure concentration and sex. The FD_T values were in good agreement with those reported by Raabe et al.,[7] in which FD_T were 14.0 and 32.3% for aluminosilicate particles of aerodynamic diameter of 0.52 and 0.2 μm, respectively. The upper airway fraction (FD_{UA}) and the intrapulmonary fraction (FD_{IP}) in the rats appeared to be influenced by the exposure concentration of cigarette smoke. For the higher concentration, a higher percentage deposition of activity was found in the upper airway region than in the intrapulmonary region (Table 2). The differences were significant (two-sample, unpaired Student's t-test) at $p < 0.05$ and could not be explained based on the data obtained. They could have been the result of slight differences in the particle size of smoke in the exposure chamber, in the altered breathing pattern of exposed rats, and in the mucociliary clearance of smoke particles in the rats between the high and low exposure concentrations.

Table 2

Total (FD_T), Intrapulmonary (FD_{IP}), and Upper Airway (FD_{UA}) Deposition Fractions of ^{14}C-labeled Cigarette Smoke Particles in Rats

	Fractional Deposition (%)		
	FD_T	FD_{IP}	FD_{UA}
High[a]	19.8 (1.6)[b]	12.4 (0.9)	7.4 (1.2)
Low[a]	20.3 (1.6)	15.9 (1.4)	4.4 (0.5)

[a]High and low represent high and low cigarette smoke concentrations, respectively.

[b]Mean (standard error) (n = 10).

SUMMARY

This study demonstrates that if a cigarette smoke aerosol of uniform concentration is used throughout an exposure, the drastic change in breathing pattern reported by others can be avoided and the fractional deposition of smoke particles in rats can be measured. With ^{14}C-dotriacontane (DTC) as a marker for smoke particles, the mean total deposition in the respiratory tract of rats was 20%, regardless of the sex and exposure concentration. The upper airway fractions were 7.4% and 4.4% and the intrapulmonary fractions were 12.4% and 15.9% for the high and low concentrations, respectively. These results indicated a slight shift toward greater upper airway deposition at the higher concentration of cigarette smoke.

REFERENCES

1. Dalbey, W. E., P. Nettesheim, R. Griesemer, J. E. Caton, and M. R. Guerin. Chronic Inhalation of Cigarette Smoke by F344 Rats, J. Natl. Cancer Inst. 64: 383-390, 1980.

2. Loscutoff, S. M., R. A. Jaffe, D. I. Hilton, D. W. Phelps, D. B. Carr, and A. P. Wehner. Dosimetry and Cardiopulmonary Function in Rats Chronically Exposed to Cigarette Smoke, Toxicol. Appl. Pharmacol. 64: 335-352, 1982.

3. Hinds, W. W., M. W. First, G. L. Huber, and J. W. Shea. A Method for Measuring Respiratory Deposition of Cigarette Smoke During Smoking, Am. Ind. Hyg. Assoc. J. 44: 113-118, 1983.

4. Chen, B. T., R. E. Weber, H. C. Yeh, D. L. Lundgren, M. B. Snipes, and J. L. Mauderly. Deposition of Cigarette Smoke Particles in the Rat, Fundam. Appl. Toxicol., submitted.

5. Kendrick, J., P. Nettesheim, M. R. Guerin, J. E. Caton, W. E. Dalbey, R. Griesemer, I. Rubin, and W. Maddox. Tobacco Smoke Inhalation Studies in Rats, Toxicol. Appl. Pharmacol. 37: 557-569, 1976.

6. Jenkins, R. W., R. H. Newman, R. D. Carpenter, and T. R. Osdene. Cigarette Smoke Formation Studies, Beitr. zur Tabakforsch. 5: 295-298, 1970.

7. Chen, B. T., J. Namenyi, H. C. Yeh, J. L. Mauderly, and R. G. Cuddihy. Physical Characterization of Cigarette Smoke Aerosol Generated from a Walton Smoke Machine, Aerosol Sci. Technol., in press, 1988.

8. Raabe, O. G., H. C. Yeh, G. J. Newton, R. F. Phalen, and D. J. Valesquez. Deposition of Inhaled Monodisperse Aerosols in Small Rodents, in Inhaled Particles IV (W. H. Walton, ed.), Pergamon Press, NY, pp. 3-21, 1977.