Pharmacokinetics of Nicotine in Rats after Single-Cigarette Smoke Inhalation

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Abstract □ The amount of nicotine absorbed following cigarette smoke inhalation was evaluated by comparing the area under the plasma concentration–time curve and urinary recovery with those observed after its intravenous injection to rats. Nicotine was absorbed rapidly, with the maximum plasma concentration occurring immediately after cessation of cigarette smoke exposure. On the average, 68% of the nicotine delivered to the inhalation chamber was absorbed. The absorption and elimination of nicotine, as well as the formation and elimination of its metabolites, followed first-order kinetics, and the derived pharmacokinetic parameters were similar to those observed after the intravenous administration of nicotine.

Keyphrases □ Nicotine—comparison of pharmacokinetic parameters following single-cigarette smoke inhalation and intravenous administration, cotinine as major nicotine metabolite, rats □ Pharmacokinetics—comparison of nicotine pharmacokinetic parameters following single-cigarette smoke inhalation and intravenous administration, cotinine as major metabolite of nicotine, rats □ Cotinine—major metabolite of nicotine, comparison of pharmacokinetic parameters following single-cigarette smoke inhalation and intravenous administration, rats

Reports of the effect of inhalation of tobacco smoke on a wide range of biological systems have increased. The principal concern has been the effect of smoke inhalation on the structure and function of the lungs (1–3), cardiovascular response (4), and teratogenicity (5). These studies have renewed interest in the metabolic fate of nicotine.

Recent studies in these laboratories investigated the pharmacokinetics of nicotine in rats following intravenous injection (6, 7). However, little is known about the rate of absorption of nicotine from cigarette smoke. Most studies on nicotine absorption reported only the blood nicotine concentrations following smoking in humans (8, 9), dogs (10), and cats (11). Armitage *et al.* (8) showed that habitual smokers retained 82–92% and that nonsmokers retained 30–66% of the inhaled nicotine, but they did not assess the absorption rate of nicotine in these subjects. McGovern *et al.* (12), using an isolated perfused rabbit lung preparation, found that nicotine absorption from tobacco smoke was rapid, with peak levels occurring almost immediately after cigarette smoke administration had ceased.

The purpose of this investigation was to characterize the rate and extent of absorption of nicotine in rats after single-cigarette smoke inhalation and to compare its disposition kinetics with those observed after intravenous administration.

EXPERIMENTAL

Chemicals—[*methyl*-^{14}C]Nicotine was obtained commercially[1] and had a specific activity of 61.6 μCi/mg. Radiochemical purity was established by TLC and radioscanning techniques. With acetone–benzene–ammonia–ethanol (40:50:5:5) as the solvent, the compound migrated as a single radioactive spot with an R_f value corresponding to authentic nicotine. Unlabeled nicotine[2] for use as a standard was obtained commercially, and unlabeled cotinine was prepared from nicotine by the method of Bowman and McKennis (13).

Cigarettes—The cigarettes[3] were stored in a desiccator that provided an atmosphere of 65% humidity. They were reported to contain 9.5 mg of nicotine/g of tobacco and to have a smoke condensate pH of 4.9. Analysis in these laboratories using a spectrophotometric assay (14) verified the nicotine content of the cigarettes.

One cigarette was spiked with ^{14}C-labeled nicotine using an infusion pump[4] equipped with a glass tube (17 cm × 8 mm i.d.) to hold the cigarette in place. Another infusion pump[5] was fitted with a 1-ml gas-tight syringe[6] containing the ^{14}C-labeled nicotine solution. The two pumps were set facing each other such that the needle delivering the radiolabeled nicotine solution mounted on one pump penetrated the cigarette mounted on the other pump. This setting allowed the delivery of the spiking solution to the entire length of the cigarette, after which the cigarette was rotated 90° and allowed to dry. This process was repeated until 225 μCi of [*methyl*-^{14}C]nicotine was delivered, yielding a final specific activity of 17.11 μCi/mg. Following spiking, the cigarette was replaced in the desiccator.

Smoke Exposure Apparatus—The intermittent smoke generation–inhalation system[7] (15–17) used was designed to simulate the smoking of a single cigarette. Briefly, a 2-sec negative pressure allowed 35 cm^3 of cigarette smoke to enter an exposure chamber where it was distributed rapidly, forming a 10% smoke aerosol. The rats were placed in restraining cylinders attached to the exposure chamber with only their noses protruding into the chamber. The smoke remained in the chamber for 20 sec, after which air was forced into the chamber to expel the smoke and to maintain an atmosphere of fresh air for 38 sec. This 1-min cycle (puff) was repeated until the cigarette burnt down to a 23-mm butt after a total of 10 puffs were delivered into the exposure chamber.

The exposure unit was modified by attaching a cambridge filter[8] to the exposure chamber. This filter is capable of trapping 99.9% of the total particulate matter, including nicotine, from the smoke forced out of the exposure chamber (18). This modification allowed for an account of the material balance and, hence, calculation of the amount of nicotine delivered to the inhalation chamber and the amount absorbed by rats.

The restraining cylinders also were modified by drilling an orifice at the proximal part to the exposure chamber through which the animal's previously implanted cannula was brought out. Thus, blood samples could be collected during the smoke inhalation period.

Animals—Male Fischer-344 rats[9], 226–263 g, were housed in stainless steel cages and had free access to food[10] and water. At least 48 hr prior to the study, each animal was placed under light ether anesthesia and a chronic cannula was implanted into the abdominal aorta (19). The study was initiated with eight rats, but only four rats completed the study due to a malfunction of the cannula.

Protocol—Rats were placed in the restraining cylinders 30 min prior to lighting the cigarette to condition them to the smoke exposure–inhalation system (20). The cigarette was consumed in 10 min (10 puffs), after which the rats were transferred to plastic metabolism cages for the remainder of the experiment. Blood samples (0.2 ml) were collected into heparinized syringes at 4, 8 (smoking phase), 15, 30, 45, 60, and 90 min and 2, 3, 5, 8, 14, 16.5, 24, and 30 hr (postsmoking phase). The plasma was

[1] ICN, Irving, CA 92664.

[2] Eastman Kodak Co., Rochester, NY 14650.
[3] Code No. 31, provided by Enviro Control, Rockville, MD 20852.
[4] Sage model 341, Orion Research Inc., Cambridge, MA 02139.
[5] Sage model 351, Orion Research Inc., Cambridge, MA 02139.
[6] No. 1001, Hamilton Co., Reno, NV 89510.
[7] Maddox-ORNL, Oak Ridge National Research Laboratory, Oak Ridge, TN 37830.
[8] Phipps and Bird, Richmond, VA 23261.
[9] Microbiological Associates, Walkersville, MD 21793.
[10] Purina Laboratory Chow, Ralston-Purina Co., St. Louis, MO 53188.

0022-3549/80/0900-1087$01.00/0

Table I—Nicotine Absorbed following Cigarette Smoke Inhalation by Rats

Rat[a] (weight, kg)	Amount Absorbed Based on *AUC*[b]		Amount Absorbed Based on Urinary Recovery[b]	
	dpm/kg	mg/kg	dpm/kg	mg/kg
1 (0.240)	5.061×10^6	0.04	5.258×10^6	0.04
2 (0.235)	7.752×10^6	0.06	5.234×10^6	0.04
3 (0.226)	7.911×10^6	0.06	8.472×10^6	0.06
4 (0.263)	8.199×10^6	0.06	5.765×10^6	0.04
Mean (0.241)	7.231×10^6 [c]	0.06	6.182×10^6[c]	0.05
SEM (0.008)	0.729×10^6	0.01	0.773×10^6	0.01

[a] Eight rats were placed in the inhalation chamber, but four did not complete the study because of malfunction of the indwelling cannula. [b] No significant differences were observed at the $p = 0.05$ level between the amount calculated from plasma concentrations *versus* time and the urinary recovery data. [c] If it is assumed that the same amount of nicotine also was absorbed by the four rats that did not complete the study, the total amount absorbed represents 69.8 (*AUC* method) and 66.3% (urinary recovery method) of the amount of nicotine delivered to the inhalation chamber, with a mean of 68%.

separated from whole blood by centrifugation and stored at −20° until the time of analysis. Urine was collected in containers immersed in ice for up to 48 hr and frozen until the time of analysis. The separation and quantitation of nicotine and its metabolites were described earlier (6, 7).

Pharmacokinetic Analysis—The concentration–time profiles of nicotine, cotinine, and other metabolites for each rat were plotted on semilogarithmic graph paper, which provided an insight to the operative pharmacokinetic models. These plots also were employed to obtain initial estimates for curve fitting of the data using the SAAM 26 digital computer program (21). Each data point was assigned a statistical weight proportional to the reciprocal of the square of a standard deviation representing 10% of the data point.

The puffing profile of the smoke inhalation machine was incorporated into the pharmacokinetic model used to fit the data both during and after cigarette smoke inhalation. The amount of nicotine absorbed by inhalation (A_{ih}) was computed from (22):

$$A_{ih} = \text{dose}_{iv}(AUC_{ih}/AUC_{iv}) \qquad \text{(Eq. 1)}$$

where dose_{iv} is the dose injected by intravenous administration and AUC_{ih} and AUC_{iv} are the areas under the plasma concentration–time curve ($0 \rightarrow \infty$) following cigarette smoke inhalation and intravenous administration, respectively. The dose and *AUC* from the 0.08-mg/kg iv study reported earlier (7) were used in Eq. 1. An alternative method for calculating A_{ih} is provided by (22):

$$A_{ih} = \text{dose}_{iv}\, Nu_{\infty,ih} f_{iv}/Nu_{\infty,iv} f_{ih} \qquad \text{(Eq. 2)}$$

where Nu_∞ is the amount of nicotine ultimately excreted in urine at time infinity and f_{ih} and f_{iv} represent the fractions of total radioactivity excreted as nicotine following cigarette smoke inhalation and intravenous administration, respectively. The dose and $Nu_{\infty,iv}$ from the 0.08-mg/kg dose reported earlier (7) were used in these calculations.

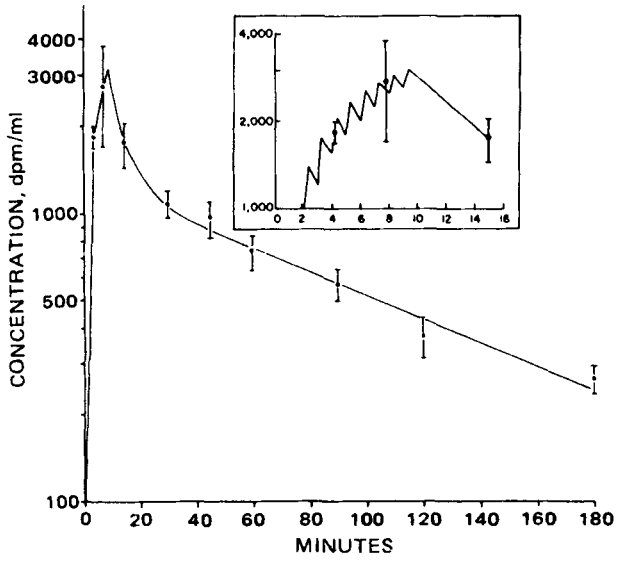

Figure 1—*Semilogarithmic plot of plasma nicotine concentration (mean ± SEM) as a function of time following cigarette smoke absorption of nicotine. Inset shows nicotine absorption during the smoke exposure period.*

The plasma nicotine concentration (C_n) *versus* time data for each rat were fitted to a two-compartment open model with a first-order rate constant of absorption described by (22):

$$C_n = Ae^{-k_a t} + Be^{-\alpha t} + Ce^{-\beta t} \qquad \text{(Eq. 3)}$$

where *A*, *B*, and *C* are constants, k_a is the absorption rate constant, and α and β are hybrid rate constants describing the rapidly and slowly declining phases of the plasma concentration, respectively. The dose absorbed by each rat (calculated using Eqs. 1 and 2) was divided by 10 (each representing one puff) and was included in the data-fitting procedure.

The first-order rate constant of elimination (K_e) and the ratio of the fraction (F) of nicotine converted to cotinine to the volume of distribution of the latter compound (V_d) were obtained by curve fitting of the plasma cotinine concentration–time data to the one-compartment open model with a first-order rate constant of formation (K_f) as reported earlier (6, 7). Also, the pharmacokinetic parameters of the origin activities were estimated as described previously (6, 7).

Renal clearance (*Cl*) was calculated for nicotine and its metabolites employing (22):

$$Cl_{r,i} = Xu_{i,t_1 \rightarrow t_2}/AUC_{i,t_1 \rightarrow t_2} \qquad \text{(Eq. 4)}$$

where i represents nicotine, cotinine, or origin activity, Xu is the amount excreted between t_1 and t_2, and *AUC* is the area under the plasma concentration–time curve over the same time interval.

Statistical Analysis—The t test was used to evaluate differences in the pharmacokinetic parameters of nicotine and its metabolites between the cigarette smoke inhalation and the intravenous administration studies at the $p < 0.05$ and < 0.01 levels (23).

RESULTS AND DISCUSSION

Nicotine—About 4% (2.0×10^7 dpm) of the nicotine present in the mainstream of cigarette smoke (4.9×10^8 dpm) was delivered to the inhalation chamber. This amount represents the sum of the nicotine absorbed by the rats, retained on the filter (4.7×10^6 dpm), and present in the washing of the chamber[11] (1.3×10^6 dpm). The amount of nicotine absorbed calculated from Eqs. 1 and 2 is shown in Table I. Since there were no significant differences between the two methods of computation, the amount of nicotine absorbed can be obtained by either the plasma concentration *versus* time plots or the urinary recovery data. The amount of nicotine absorbed ranged from 0.04 to 0.06 mg/kg[12], representing an average of 68% of the amount of nicotine delivered. This value is similar to that reported by Armitage *et al.* (8), who showed that nonsmokers absorbed between 30 and 60% of the nicotine delivered to their mouths.

The mean plasma nicotine concentration–time profile shown in Fig. 1 indicates that nicotine is absorbed rapidly by inhalation. During the 10 puffs needed to consume the cigarette, the plasma nicotine levels paralleled the intermittent pattern of the smoke exposure machine (shown in the inset of the figure). A maximum of 3000 dpm/ml (79.2 ng/ml) was achieved at the cessation of the cigarette smoke exposure. Following this rapid absorption, which appeared to be described ade-

[11] After delivery of each puff into the airtight exposure chamber, the smoke remained in the chamber for 20 sec. Fresh air then was forced in the chamber, which caused the unabsorbed nicotine (as well as other particulate matter) to be retained on the filter or absorbed onto the inside walls of the chamber, both of which were assayed for radioactivity.

[12] The similarity in the dose and pharmacokinetic parameters obtained in this study and those observed in the 0.08-mg/kg iv study reported earlier (7) warranted the use of the intravenous data in Eqs. 1 and 2.

Table II—Pharmacokinetic Parameters of Nicotine following Cigarette Smoke Inhalation by Rats

Rat	k_a, min^{-1}	α, min^{-1}	β, min^{-1}	$AUC_{0\to\infty}$, (dpm hr)/(ml kg)	Cl_r, ml/hr/kg
1	0.084	—[a]	0.0090	7.64×10^3	402.0
2	0.089	0.0753	0.0105	11.70×10^3	221.0
3	0.404	0.1380	0.0062	11.94×10^3	160.0
4	0.082	0.6470	0.0076	12.37×10^3	119.0
Mean	0.165	0.2870	0.0083	10.91×10^3	226.0
SEM	0.080	0.1810	0.0009	1.10×10^3	62.0

[a] Although the α-phase in this rat was discernible by examination of the log concentration–time plot, it did not include sufficient data points to enable computer estimation of this parameter.

Table III—Pharmacokinetic Parameters of Cotinine following Cigarette Smoke Inhalation of Nicotine by Rats

Rat	t_{max}, hr	K_f, hr^{-1}	K_e, hr^{-1}	$AUC_{0\to\infty}$[a], (dpm hr)/(ml kg)	Cl_r, ml/hr/kg	$\frac{AUC_{cot}}{AUC_{nic}}$	F/V_d, kg/liter[b] From *AUC*	F/V_d, kg/liter[b] From Urinary Excretion
1	1.50	1.544	0.118	56.35×10^3	14.71	7.88	0.303	0.292
2	1.50	1.205	0.103	104.58×10^3	11.32	8.80	0.314	0.466
3	2.00	1.351	0.077	104.86×10^3	17.48	10.64	0.229	0.214
4	2.00	1.366	0.096	94.10×10^3	8.90	7.51	0.273	0.389
Mean	1.75	1.366	0.099	89.97×10^3	13.10	8.71	0.280	0.340
SEM	0.14	0.069	0.009	11.48×10^3	1.88	0.70	0.019	0.055

[a] Obtained by extrapolation of the plasma cotinine concentration data to infinity. [b] There were no significant differences between the F/V_d values calculated from the *AUC* and those from the urinary excretion data.

quately by first-order kinetics, plasma nicotine concentrations declined biexponentially in a fashion similar to that of intravenous administration.

The pharmacokinetic parameters derived from these data are reported in Table II. The mean half-life of absorption ($t_{1/2,k_a}$) was 6.6 min; the mean half-life values of the rapidly and slowly declined phases ($t_{1/2,\alpha}$ and $t_{1/2,\beta}$) were 5.1 and 86.5 min, respectively. There were no significant differences in α and β between the present cigarette smoke inhalation study and the intravenous studies reported previously (6, 7).

Armitage *et al.* (8) estimated that the half-life of nicotine following cigarette smoke inhalation in humans, assuming a one-compartment open model, ranged from 24 to 84 min, with a mean of 40 min. Unfortunately, these investigators carried out their experiment for only 1 hr; nicotine distribution may not have been completed at this time and their reported half-lives may have been underestimated. The results in Table II also indicate that the renal clearance of nicotine following cigarette smoke inhalation ranged from 119 to 402 (mean 226) ml/hr/kg. This renal clearance accounted for ~9% of the total plasma clearance of nicotine observed at the same dose following intravenous administration (7).

Cotinine—Figure 2 shows the plasma concentration–time profile of this major metabolite of nicotine (mean ± *SEM*). The formation of cotinine is rapid. Approximately 20% of the maximum cotinine concentration was achieved within the first 3 min of cigarette smoke inhalation. The pharmacokinetic parameters of cotinine are reported in Table III. The time to reach the maximum plasma concentration (t_{max}) ranged from 1.5 to 2.0 hr. When statistical analysis was performed on t_{max}, there were no significant differences between intravenous administration and cigarette smoke inhalation of nicotine.

The mean half-life of formation of cotinine was 30.6 min and that of its elimination ranged from 5.9 to 9.0 hr. There were no significant differences among the means between cigarette smoke inhalation and intravenous administration with respect to the rate constants of formation and elimination (Table III). The renal clearance of cotinine ranged from 8.9 to 17.48 ml/hr/kg. This range is ~6% of the renal clearance of nicotine, indicating that cotinine is cleared by the kidney at a much slower rate than nicotine. There were no significant differences between the means of renal clearance following cigarette smoke inhalation and intravenous administration of nicotine (Table III).

To determine if changes occurred in the formation of cotinine from nicotine following cigarette smoke inhalation compared to the intravenous study, the ratio AUC_{cot}/AUC_{nic} was examined. The ratio ranged from 7.5 to 10.6 (Table III) and appeared to be slightly lower ($p < 0.01$) than that observed after the intravenous administration of nicotine at a comparable dosage (7). It is not clear whether this difference is biologically significant or is indicative of a first-pass metabolism of nicotine in the lungs. McGovern *et al.* (12) found no significant first-pass effect of nicotine in the isolated rabbit lung preparation.

Changes in the fraction of nicotine converted to cotinine (F), as well as in the apparent volume of distribution of cotinine, also were evaluated by examining the F/V_d ratio. This ratio (Table III) ranged from 0.21 to 0.47 kg/liter. There were no significant differences in the F/V_d ratios between those calculated from the plasma and urine data. However, these ratios were somewhat lower ($p < 0.05$) than those observed following the intravenous administration of similar doses of nicotine (7). This observation implies an increase in the volume of distribution or a decrease in the fraction of the dose converted to cotinine.

Table IV—Pharmacokinetic Parameters of Origin Activity following Cigarette Smoke Inhalation by Rats

Rat	t_{max}, hr	$t_{1/2}$, hr	$AUC_{0\to\infty}$, (dpm hr)/(ml kg)	Cl_r, ml/hr/kg	$\frac{AUC_{orig\ act}}{AUC_{nic}}$
1	1.50	24.75	65.38×10^3	117.29	9.14
2	0.75	28.88	112.66×10^3	75.96	9.48
3	0.50	24.79	68.96×10^3	160.04	7.00
4	0.75	26.67	157.53×10^3	38.14	12.58
Mean	0.88	26.27	101.13×10^3	97.86	9.55
SEM	0.22	0.98	21.65×10^3	26.28	1.15

Other Metabolites—Some of the nicotine metabolites remained at the origin of the thin-layer plate, and their possible compositions were discussed previously (6, 7). It is evident from the time course of these metabolites (Fig. 3) that ~25% of their maximum concentration (c_{max}) was formed within the first 3 min following the inhalation of cigarette smoke. Table IV reports the pharmacokinetic parameters derived from these data. There were no significant differences in t_{max} between intravenous administration and cigarette smoke inhalation. This observation suggests that the rate constants of formation and elimination have not

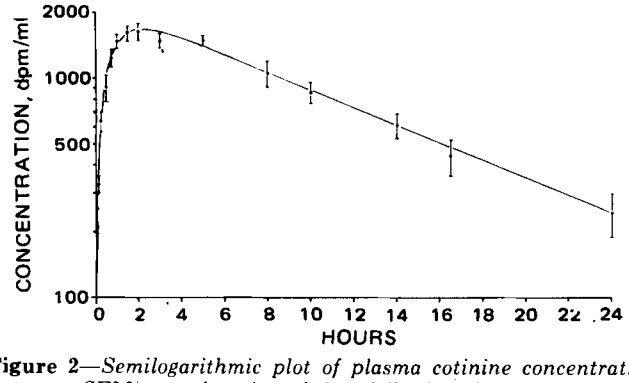

Figure 2—*Semilogarithmic plot of plasma cotinine concentration (mean ± SEM) as a function of time following cigarette smoke absorption of nicotine.*

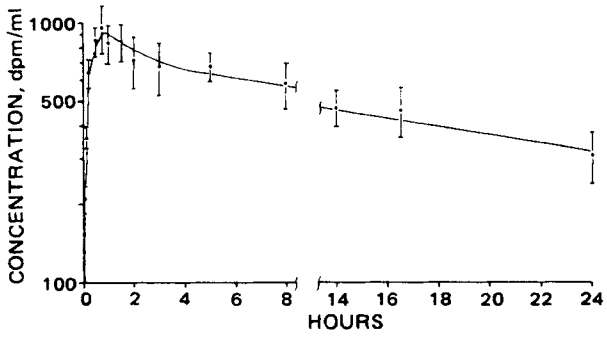

Figure 3—*Semilogarithmic plot of plasma origin activity concentration (mean ± SEM) as a function of time following cigarette smoke absorption of nicotine.*

changed between cigarette smoke inhalation and intravenous administration. Although the origin activity is composed of more than one metabolite, an overall half-life of elimination was determined for comparative purposes. The overall half-life of elimination mean (±*SEM*) was 26.27 (±0.98) hr. There were no significant differences in this parameter between the intravenous administration of nicotine (7) and the present study.

The amount of origin activity formed following cigarette smoke inhalation was compared to that observed after intravenous nicotine administration (7) by assessing the ratio of $AUC_{\text{orig act}}$ to AUC_{nic} in each study. The ratios (range of 7.0–12.6 with an overall mean of 9.5) were not significantly different from those obtained in the intravenous study (7). Therefore, the formation of the origin activity appears to be independent of the route of administration of nicotine. The renal clearance of the origin activity also appears to be independent of the route of administration, since there were no significant differences in this parameter between cigarette smoke inhalation and intravenous administration studies (7).

Urinary Excretion of Nicotine and Metabolites—The 48-hr excretion of nicotine and its metabolites following cigarette smoke inhalation is shown in Table V. There were no significant differences among the mean recoveries of radiolabeled substances in the urine between intravenous administration and cigarette smoke inhalation. The average recovery was ~66% of the amount absorbed. Approximately 16 and 13% of the total radioactivity excreted in urine consisted of nicotine and cotinine, respectively, while 33% was composed of the origin activity. No significant differences were found when these values were compared to the intravenous results. Consistent with the plasma concentration data, the ratios of cotinine or the origin activity to that of nicotine excreted in the urine in the present study also were not significantly different from those found in the intravenous study (7).

CONCLUSION

This study indicates that the absorption of nicotine present in the mainstream of cigarette smoke is rapid and does not seem to undergo first-pass metabolism through the lungs. In addition, the pharmacokinetics of nicotine and its metabolites were essentially similar to those observed previously following the intravenous injection of a single dose of nicotine (6, 7). The effect of multiple-cigarette smoke inhalation on the pharmacokinetics of these compounds is being investigated.

Table V—Urinary Excretion of Nicotine and Its Metabolites in Rats 48 hr after Cigarette Smoke Inhalation

Rat	Percent of Absorbed Dose Excreted in Urine as Total Radioactivity	Percent of Total Radioactivity Excreted in Urine as		
		Nicotine	Cotinine	Origin Activity
1	78.5	23.4	13.1	37.0
2	51.0	18.0	14.0	37.3
3	80.9	9.3	9.2	17.9
4	53.1	11.5	15.0	40.6
Mean	65.9	15.6	12.8	33.2
SEM	8.0	3.2	1.3	5.2

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ACKNOWLEDGMENTS

Abstracted in part from a dissertation submitted by K. S. Rotenberg to the University of Maryland in partial fulfillment of the Doctor of Philosophy degree requirements.

Supported in part by National Cancer Institute Contract N01-CP-43312.