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The Effect of Inhalation Volume and Breath-Hold Duration on the Retention of Nicotine and Solanesol in the Human Respiratory Tract and on Subsequent Plasma Nicotine Concentrations During Cigarette Smoking*

by

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SUMMARY

The influence of inhalation depth and breath-hold duration on the retention of nicotine and solanesol in the human respiratory tract and on nicotine uptake was studied in ten cigarette smokers. In a first series of experiments, the subjects took seven puffs from a 10 mg 'tar' yield, test cigarette and a fixed volume of air (0, 75, 250, 500 or 1000 mL, as required by the protocol) was inhaled after each puff in order to give a controlled 'depth' of inhalation. The inhalation was drawn from a bag containing the required volume of air. Following a 2 s breath-hold, subjects exhaled normally, with the first exhalation after each puff passing through a single acidified filter pad for collection of the non-retained nicotine and solanesol. Blood samples were taken before and at intervals during and after smoking for the sessions with 0, 75 and 500 mL inhalation volumes for determination of plasma nicotine and carboxyhaemoglobin levels. Another series of experiments was conducted with a fixed inhalation volume (500 mL) and two further breath-hold durations (0 and 10 s) in addition to 2 s from above. Nicotine and solanesol retentions were measured for each breath-hold condition. The amounts of nicotine retained within the respiratory system, expressed as a percentage of the amount taken into the mouth, were consistently higher than the corresponding values for solanesol in all five inhalation conditions (0–1000 mL, 2 s breath-hold). Nicotine retention increased from 46.5% at zero inhalation to 99.5% at 1000 mL inhalation (2 s breath-hold) and from 98.0% at zero breath-hold to 99.9% at 10 s breath-hold (500 mL inhalation). Solanesol retention increased from 34.2% at zero inhalation volume to 71.9% at 1000 mL inhalation (2 s breath-hold) and from 51.8% at zero breath-hold to 87.6% at 10 s breath-hold (500 mL inhalation). Plasma nicotine decreased from pre-smoking levels after zero inhalation indicating that the nicotine retained within the mouth was poorly absorbed into the systemic circulation. After 75 mL inhalation, plasma nicotine levels were significantly greater than for zero inhalation but not significantly less than after 500 mL inhalation except at the time of maximum nicotine concentration. As in every experimental condition, a higher percentage of nicotine than solanesol was retained within the respiratory tract, it was concluded that the difference in retention of the moderately volatile nicotine and the non-volatile solanesol is consistent with the concept of nicotine evaporation from smoke particles and the subsequent efficient retention in the airways of gaseous nicotine. The retention of solanesol followed the expected pattern of particulate deposition i.e., an increase with both increasing depth of inhalation and breath-hold duration. However, nicotine retention was almost complete even at shallow inhalations and short breath-hold durations. [Beitr. Tabakforsch. Int. 21 (2004) 240–249]

ZUSAMMENFASSUNG

Der Einfluss der Inhalationstiefe und der Inhalationsdauer auf die Nikotin- und Solanesolretention im menschlichen Atmungstrakt und auf die Nikotinaufnahme wurde bei zehn

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Zigarettenrauchern untersucht. In einer ersten Reihe von Experimenten nahmen die Raucher sieben Züge aus einer Testzigarette mit einem Kondensatgehalt von 10 mg. Nach jedem Zug wurde ein festgelegtes Luftvolumen (0, 75, 250 500 oder 1000 mL wie im Versuchsprotokoll bestimmt) eingeatmet, um so eine kontrollierte „Tiefe" der Inhalation zu erhalten. Bei der Inhalation wurde die Luft aus einem Beutel eingeatmet, der das festgelegte Luftvolumen enthielt. Nach einer Inhalationsdauer von zwei Sekunden atmeten die Probanden normal aus, wobei der erste Exhalationsstrom nach jedem Zug durch einzelne mit Säure belegte Filter geleitet wurde, um das nicht retinierte Nikotin und Solanesol aufzufangen. Bei den Sitzungen, bei denen das Inhalationsvolumen 0, 75 und 500 mL betrug, wurden vor und in Abständen während und nach dem Rauchen Blutproben zur Bestimmung der Plasmanikotin- und Carboxyhaemoglobin-Konzentration genommen. Eine andere Reihe von Experimenten erfolgte mit einem festgelegten Inhalationsvolumen (500 mL) und einer zusätzlichen Inhalationsdauer (0 und 10 Sekunden) zu den beschriebenen zwei Sekunden. Die Nikotin- und Solanesolretentionen wurden für jede Inhalationsdauer gemessen. Die im Atmungstrakt retinierten Nikotinmengen, ausgedrückt als prozentualer Anteil der in den Mund aufgenommenen Mengen, waren unter allen fünf Inhalationsbedingungen (0–1000 mL, 2 Sekunden Inhalationsdauer) konsistent höher als die betreffenden Werte für Solanesol. Die Nikotinretention erhöhte sich von 46,5% bei Null-Inhalation auf 99,5% bei einem Inhalationsvolumen von 1000 mL (2 Sekunden Inhalationsdauer) und von 98,0% ohne zusätzliche Inhalation auf 99,9% bei einer zusätzlichen Inhalationsdauer von 10 Sekunden (500 mL Inhalationsvolumen). Die Solanesolretention erhöhte sich von 34,2% bei Null-Inhalation auf 71,9% bei einem Inhalationsvolumen von 1000 mL (2 Sekunden Inhalationsdauer) und von 51,8% ohne zusätzliche Inhalation auf 87,6% bei einer zusätzlichen Inhalationsdauer von 10 Sekunden (500 mL Inhalationsvolumen). Die Plasmanikotinkonzentration verringerte sich nach Null-Inhalation im Vergleich zu den vor dem Rauchen gemessenen Werten, was darauf hindeutet, dass das im Mund retinierte Nikotin nur geringfügig in den Blutkreislauf aufgenommen wurde. Nach einem Inhaltionsvolumen von 75 mL waren die Plasmanikotinwerte signifikant höher als bei Null-Inhalation aber nicht signifikant niedriger als nach einem Inhalationsvolumen von 500 mL mit Ausnahme zu der Zeit der höchsten Nikotinkonzentration.

Wie unter allen experimentellen Bedingungen wurde ein höherer prozentualer Anteil an Nikotin als an Solanesol im Atmungstrakt retiniert und es wurde die Schlussfolgerung gezogen, dass die unterschiedlichen Retentionsmengen des leicht flüchtigen Nikotin im Vergleich zum nicht flüchtigen Solanesol mit der Vorstellung übereinstimmen, dass sich das Nikotin aus den Rauchpartikeln verflüchtigt und das gasförmige Nikotin nachfolgend im Atmungstrakt in hohem Maße retiniert wird. Die Retention des Solanesols folgte dem erwarteteten Muster der Partikeldeposition, und zwar einer Zunahme bei zunehmender Inhalationstiefe und längerer Inhalationsdauer. Die Nikotinretention war dagegen fast immer vollständig, sogar bei flacher Inhalation und kurzer Inhalationsdauer. [Beitr. Tabakforsch. Int. 21 (2004) 240–249]

RESUME

L'influence de la profondeur d'inhalation et de la durée d'exposition pulmonaire sur la rétention de la nicotine et du solanésol dans le système respiratoire et sur l'absorption de nicotine ont été étudiées chez dix fumeurs de cigarettes. Dans une première série d'expériences les fumeurs ont pris sept bouffées d'une cigarette d'essai à 10 mg de goudron, et un volume déterminé d'air (0, 75, 250, 500 ou 1000 mL, comme exigé selon le protocole). Ce volume d'air, tiré d'un sac contenant le volume requis, a été inhalé après chaque bouffée pour fournir une « profondeur » contrôlée d'inhalation. Après une durée d'exposition pulmonaire de 2 secondes, les fumeurs ont exhalé normalement, la première exhalation après chaque bouffée passant à travers un filtre acidifié pour la collecte de la nicotine et du solanésol non retenus. Lors des séances avec un volume d'inhalation de 0, 75 et 500 mL, des échantillons de plasma sanguin ont été pris avant, et régulièrement pendant et après le fumage, pour le dosage de la nicotine sérique et de la carboxyhémoglobine. Une autre série d'essais a été conduite avec un volume déterminé d'inhalation (500 mL) et avec deux durées supplémentaires d'exposition des poumons (0 et 10 secondes) en plus des 2 secondes déjà décrites. La rétention de la nicotine et du solanésol a été mesurée pour chaque condition d'exposition pulmonaire. La teneur en nicotine retenue dans le système respiratoire, exprimée en pourcentage de la totalité prise dans la bouche, est toujours plus élevée que les valeurs correspondantes du solanésol pour les cinq conditions d'inhalation (0–1000 mL, durée d'exposition pulmonaire de 2 secondes). La rétention de la nicotine augmente de 46,5% après zéro inhalation à 99,5% après un volume d'inhalation de 1000 mL (durée d'exposition pulmonaire de 2 secondes), et de 98,0% sans exposition pulmonaire à 99,9% après une durée d'exposition pulmonaire de 10 secondes (volume d'inhalation de 500 mL). La rétention du solanésol augmente de 34,2% après zéro inhalation à 71,9% après un volume d'inhalation de 1000 mL (durée d'exposition pulmonaire de 2 secondes), et de 51,8% sans exposition pulmonaire à 87,6% après une durée d'exposition pulmonaire de 10 secondes (volume d'inhalation de 500 mL). Après zéro inhalation, la nicotine sérique baisse par rapport à son niveau d'avant fumage, indiquant que la nicotine retenue dans la bouche est faiblement absorbée par la circulation systémique. Avec un volume d'inhalation de 75 mL, les concentrations de la nicotine sérique sont significativement plus élevées qu'après zéro inhalation mais pas significativement plus faibles qu'avec un volume d'inhalation de 500 mL, sauf au moment de la concentration maximale de nicotine. Pour chaque condition expérimentale, un pourcentage plus élevé de la nicotine que du solanésol a été retenu dans le système respiratoire. Cette différence de rétention de la nicotine, modérément volatile, par rapport au solanésol, non volatil, est en accord avec l'hypothèse de l'évaporation de la nicotine à partir des particules de la fumée, la nicotine sous forme gazeuse étant alors retenue de façon plus efficace par le système respiratoire. La rétention du solanésol suit l'hypothèse de la déposition de particule, c'est à dire une augmentation en fonction de la profondeur d'inhalation et de la durée d'exposition pulmonaire. Cependant, la réten-

Table 1. Blend composition, casing ingredients and physical specifications of the test cigarette

Tobacco blend (% blend component)	Casing ingredients (% ingredient in final blend)	Cigarette design specifications
Flue-cured (31%)	Glycerin (1.4%)	Cigarette length: 83.5 mm
Burley (20.5%)	Propylene glycol (2.7%)	Circumference: 24.7 mm
Oriental (12%)	Sugar (1.1%)	Tobacco rod length: 56.5 mm
Expanded tobacco (11.5%)	Cocoa (0.4%)	Tobacco rod weight: 661 mg
Shredded stem (5.0%)	Chocolate (0.2%)	Tobacco rod density: 241 mg/cc
RTS (20%) (RTS comprised of 91% tobacco, 6% wood pulp and 3% glycerin)	Honey (1.4%)	Paper permeability: 27 Coresta units
	Licorice (0.4%)	Burn additive: 0.55% citrate
		Filter type: cellulose acetate
		Plasticiser: 7% triacetin
		Filter pressure drop: 76 mmWG
		Filter ventilation: 24%
		Total pressure drop: 122 mmWG
		Filter length: 27 mm

tion de la nicotine est presque complète même après un faible degré d'inhalation et une brève durée d'exposition pulmonaire. [Beitr. Tabakforsch. Int. 21 (2004) 240–249]

INTRODUCTION

Nicotine is predominantly present in the particulate phase of mainstream cigarette smoke as it exits the cigarette (1,2,3). Mainstream cigarette smoke consists of particles with mass mean aerodynamic diameters <0.5 µm (4) which, theoretically, are expected to penetrate the small airways and alveolar region of the lungs when inhaled. It has generally been assumed that nicotine is absorbed from these regions of the lungs during smoke inhalation and it has been suggested that this results in a rapid transport (<10 s) of a high concentration, arterial bolus of nicotine from the lung to the brain following the inhalation of each puff (5). Recent publications have questioned these assumptions regarding the mechanisms of smoke deposition and nicotine absorption from the lung. FROST *et al*. (6) measured the amounts of nicotine retained within the respiratory tracts of four smokers and observed approximately 90% nicotine retention following even a very shallow inhalation (< 100 mL). The authors concluded from the different retentions of nicotine and 'tar' at various depths of inhalation that nicotine evaporates from the smoke particle during inhalation and is absorbed in the upper airways as a vapour.

ROSE *et al.* (7) reported that the delivery of nicotine into arterial blood following cigarette smoke inhalation was substantially less and slower than had previously been assumed. The authors postulated that nicotine initially distributes into the respiratory tract tissue thus slowing its entry into the arterial circulation.

The current study was designed to determine the contribution of particulate deposition and nicotine evaporation to the respiratory retention of nicotine by examining the influence of changing inhalation patterns on the retention of nicotine and a non-volatile particulate phase marker, solanesol, in the mouth and respiratory tract. Additionally, the study attempted to determine the influence of the inhalation patterns and sites of nicotine deposition on the uptake of nicotine into the systemic circulation.

SUBJECTS, MATERIALS AND METHODS

Subjects

Ten male smokers, employees of Covance Laboratories Ltd., were provided with details of all the procedures to be used in the study prior to their giving consent to participate. They were given a financial payment for participation. The Covance ethical committee approved the study procedures and rates of reimbursement. Subjects were between 21 and 40 years old and claimed to be inhaling smokers of fifteen to twenty 8–12 mg 'tar' yield cigarettes per day. Regular smoking status was confirmed by saliva cotinine measurements exceeding 100 ng/mL (range 223–564 ng/mL) in accordance with STERLING *et al.* (8). The subjects were selected to be within normal limits for body mass index and respiratory function. Test cigarettes were provided to the subjects two days prior to experimental sessions to allow acclimatisation to the tobacco blend style, which was different from their normal cigarettes. Subjects were asked to smoke only the test cigarette during this period and to keep a record of their smoking activity. The use of nicotine gum and/or nicotine patches was prohibited. Subjects were asked to refrain from smoking for a minimum of one hour before each test smoking session. When blood sampling was to be performed, smoking cessation for at least four hours was required.

Test cigarette

The test cigarette used in this study was a 10 mg 'tar' product with a tobacco blend typical of the US market. Details of the test cigarette specifications are contained in Table 1.

The mainstream smoke yields for machine smoking of this cigarette under ISO conditions (one puff/min, 2 s puff duration, 35 mL puff volume, 35 mm butt length) were: 'tar' 9.6 mg; nicotine 0.67 mg; and CO 10.1 mg (9–11). The cigarettes were selected for weight and pressure drop (mean ± 5%) to reduce variability.

Experimental procedure

For each experimental smoking session, the test cigarette was smoked through a cigarette holder attached to a smoking

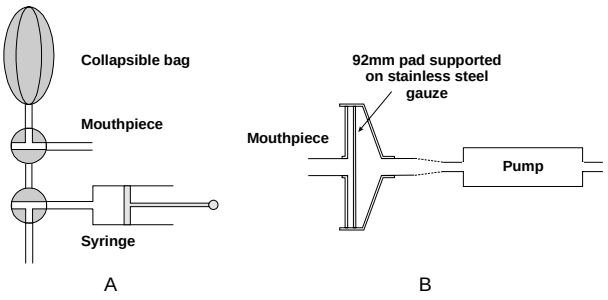


Figure 1. Inhalation apparatus (A) and exhalation apparatus (B)

analyser which recorded the subject's puff volumes, puff durations and puff times. This system was based on the method originally described by CREIGHTON *et al*. (12). Each record was later used to reproduce the subjects' puff volumes and times on a smoking duplicator in order to determine the amounts of nicotine and solanesol in the smoke generated by the subjects in each smoking session.

On completion of smoking, cigarettes were extinguished in solid CO_2 and the butt length measured.

Subjects were asked to take seven puffs, at 60 s intervals, and to prevent any smoke escaping from the mouth after puffing and before exhalation, i.e., no waste smoke. As required by the protocol for a given session, a fixed volume of air was inhaled after each puff. The inhalation was taken from a collapsible anaesthetic bag containing the specified volume of air (Figure 1A), and each breath was held for the specified period.

The experimenter timed the breath-hold duration and instructed the subject when to exhale. The first exhalation after each puff was directed through an acidified 92 mm Cambridge filter pad (Figure 1B) and the air-flow was vacuum-assisted to overcome the effect of filter resistance; this facilitated a more natural exhalation pattern. All seven exhalations from one cigarette were directed through one filter pad. The acidified Cambridge filter pads were prepared in a smoke-free environment by soaking in 1 *N* H_2SO_4 for approximately 15 min. They were air-dried at room temperature for approximately 24 h and stored in an airtight container. The trapping efficiency of the acidified Cambridge pad was assessed prior to the study. Eight puffs of mainstream smoke were blown onto three acidified pads placed in series in the exhaled smoke trap. As much as 97.9% of the total nicotine and 100% of the solanesol (means of duplicate measures) were trapped on the first pad, 2.1% of the nicotine was trapped on the second pad and none of either compound was found on the third pad.

Inhalation depth: Volumes of 75, 250, 500 and 1000 mL were used in the collapsible bag to control the volume of the post-puff inhalation. On each occasion, inhalation was followed by a two second breath-hold. Additionally a zero inhalation (mouth hold) condition was included by instructing the subjects to puff and hold the smoke in their mouth for 2 s prior to exhaling.

The range of inhalation volumes used in this study was chosen to bracket the "normal" inhalation volume range of 413–913 mL, (13). The 75 mL inhalation volume was intended to be a "dead-space" inhalation, i.e., to allow the smoke to penetrate as far as the conducting airways but not into the gas exchange or alveolar region. Previous work on the inhalation of carbon monoxide (14) suggested that alveolar absorption does not occur with inhalation volumes less than 120 mL in adult males.

Breath-hold duration: In addition to the 2 s breath-hold at the range of inhalation volumes, 0 and 10 s breath-hold manoeuvres were performed after a 500 mL inhalation.

It was essential to ensure that the subjects adhered to the protocol for each of the mouth-hold or inhalation procedures. Each subject was given a practice session during which they conducted all of the experimental manoeuvres several times until they were fully familiar with the procedures. Blood samples and data were not collected during these practice sessions. The study operators carefully observed subject behaviour and checked to ensure that smoke did not escape from the mouth during the post-puff period prior to subjects inhaling or exhaling through the test equipment. Many of the study sessions were video-taped and examined to ensure that subjects complied fully with the protocol.

Determination of nicotine and solanesol delivered to human volunteers: The puff volumes, durations and intervals recorded during the smoking of each cigarette were reproduced using a custom-built smoking duplicator at Rothmans International Group Science and Technology Centre, Milton Keynes, UK. However, a standard puff profile was used instead of the exact profile generated by each subject. CREIGHTON and LEWIS (15) reported that there were negligible differences in nicotine delivery between puff shapes for a given puff volume and duration.

The nicotine and solanesol deliveries from the duplicator smoking sessions were determined using the instrumental procedures in International Standard (ISO) Method 10315:1991(10) and American Society for Testing and Materials (ASTM) Method D6271-98 (16), respectively.

Determination of exhaled nicotine and solanesol: After use, the acidified Cambridge filter pads were removed from the holder and extracted by shaking in alkali and di-isopropyl ether containing quinoline as internal standard. The aqueous layer was separated from the organic layer and extracted twice more with internal standard solution. The combined extracts were stored at –20 ˚C awaiting analysis. Nicotine was analysed by gas chromatography (based on ASTM Method D5075-96e1 (17)). After evaporation of the extract to dryness and dissolution in methanol, solanesol was measured by high performance liquid chromatography (based on ASTM Method D6271-98 (16)).

Calculation of nicotine and solanesol retention: The respiratory retentions of nicotine and solanesol were calculated as follows:

Retention (%) = 100 × (amount delivered – amount exhaled)/amount delivered

Blood sampling: Blood samples were taken from each subject during the zero, 75 mL and 500 mL inhalation, 2 s breath-hold conditions. A cannula was inserted into an

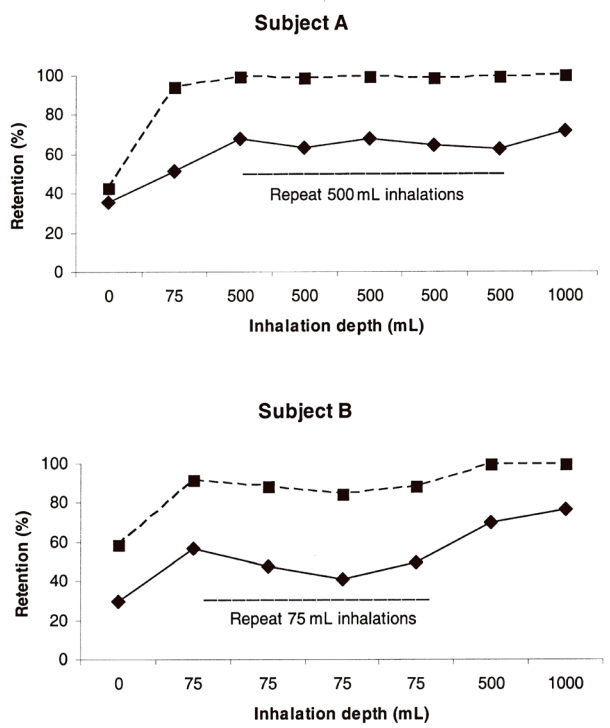


Figure 2. Nicotine (■) and Solanesol (♦) retention data from replicate 500 mL (Subject A) and 75 mL (Subject B) inhalation experiments

ante-cubital vein and 5 mL blood samples were taken at the following times:

- 1 min before the lighting puff (puff 1);
- 3.5 min after lighting (30 s after puff 4);
- 7 min after lighting (1 min after last puff);
- 10, 15, 20, 40 and 60 min after lighting.

Blood samples were collected into heparinised tubes and centrifuged. Plasma was transferred to polypropylene tubes and stored at −20 ˚C to await nicotine analysis, which was completed within three months.

Additional 5 mL blood samples were taken pre-smoking and at 10 min after lighting. These were collected into EDTA anticoagulated tubes for carboxyhaemoglobin (COHb) determination.

Determination of plasma nicotine: Nicotine was extracted from plasma samples by mixing with 35% ammonia solution, dichloromethane and quinoline internal standard solution and analysed by gas chromatography (based on ASTM Method D5075-96e1 (17)).

Where plasma samples contained nicotine levels between the limit of detection (LoD, 2 ng/mL), and the limit of quantification (LoQ, 5 ng/mL), a value of 3.5 ng/mL (midway between LoD and LoQ) was assumed. These low plasma nicotine values occurred primarily in the mouth-hold condition.

Determination of COHb in whole blood: All assays were performed within one hour of sample collection using a blood gas analyser (Instrumentation Laboratory Synthesis 35). Carboxyhaemoglobin boost was determined by subtracting the pre-smoking value from the post-smoking value.

Statistical methods: Statistical evaluation was done by one way analysis of variance (ANOVA) and additionally by Fisher's test for the comparison of means (individual error rate 0.05) when a significant ($p < 0.05$) ANOVA result was obtained. Minitab Version 13.1 (Minitab Inc, State College, Pennsylvania, USA) was used. No outlier testing was undertaken.

Pharmacokinetics: Pharmacokinetic parameters were derived from the plasma nicotine data using WinNonlin Version 1.5 (Pharsight Corporation, Mountain View, California, USA).

RESULTS

Protocol compliance

The protocol required familiarisation sessions and 70 study sessions. It was conducted over a five-week period. The following deviations from protocol occurred:

- two subjects could not be cannulated on one occasion each, thus the 75 mL inhalation plasma data set is based on 8 rather than 10 subjects
- the smoking behaviour records for two sessions could not be duplicated because the records were corrupted.

Reproducibility of nicotine and solanesol retention results

Two subjects performed a series of repeat inhalation procedures (Subject A: 500 mL; Subject B: 75 mL) in addition to the inhalation procedures outlined in the protocol. The results of these repeat procedures are displayed in Figure 2.

The solanesol retention levels for the repeat inhalations (48.7 ± 6.7% for the 75 mL and 65.0 ± 2.5% for the 500 mL inhalations) fell above the levels obtained from the lower, and below the levels obtained from the higher inhalation depths for both subjects. The nicotine retentions (99.1 ± 0.2%) obtained from the repeat 500 mL inhalations in subject A were higher than the retentions obtained with the zero and 75 mL inhalations. Those obtained following repeat 75 mL inhalations in subject B (88.0 ± 3.0%) were all consistently higher than the nicotine retention with the zero inhalation, and lower than retentions obtained under the 500 mL and 1000 mL conditions.

Effect of inhalation volume on nicotine and solanesol retention within the respiratory tract

Five inhalation volumes with a 2 s breath-hold were studied for the test cigarette. The mean values for the amounts of nicotine and solanesol delivered and retained are shown in Table 2 and Figure 3.

The retention of nicotine, expressed as a percentage of the delivered amount, was consistently higher than the corresponding values for solanesol in all conditions. Nicotine retention with zero inhalation (46.5 ± 8.6%) was lower than at all other inhalation volumes ($p < 0.05$). Nicotine retention at 75 mL inhalation (89.8 ± 11.4%) was less than at higher volumes ($p < 0.05$). There were no statistically significant differences between nicotine retentions at 250, 500 and 1000 mL inhalation volumes (96.4 ± 1.5%, 99.0 ±

Table 2. The influence of inhalation depth at constant breath-hold (2 s) on the deliveries and retentions of nicotine and solanesol

Parameters investigated	Zero inhalation	75 mL inhalation	250 mL inhalation	500 mL inhalation	1000 mL inhalation	ANOVA *p* value
Total puff volume (mL)	382 ± 51 [a b]	365 ± 56 [b]	453 ± 80 [a]	411 ± 72 [a b]	443 ± 101 [a]	0.047
Nicotine inhaled (µg)	1043 ± 165 [a]	1036 ± 181 [a]	1236 ± 212 [a]	1116 ± 233 [a]	1251 ± 288 [a]	0.086
Nicotine retained (µg)	494 ± 144 [c]	925 ± 185 [b]	1191 ± 204 [a]	1105 ± 235 [a]	1244 ± 287 [a]	0.0001
Nicotine retained (%)	46.5 ± 8.6 [c]	89.9 ± 11.4 [b]	96.4 ± 1.5 [a]	99.0 ± 0.5 [a]	99.5 ± 0.3 [a]	0.0001
Solanesol inhaled (µg)	372 ± 77 [a]	349 ± 72 [a]	423 ± 60 [a]	387 ± 83 [a]	432 ± 100 [a]	0.119
Solanesol retained (µg)	133 ± 77 [c]	204 ± 66 [b]	220 ± 42 [b]	268 ± 86 [a b]	312 ± 83 [a]	0.0001
Solanesol retained (%)	34.2 ± 15.9 [c]	58.0 ± 11.5 [b]	52.1 ± 7.6 [b]	68.0 ± 7.5 [a]	71.9 ± 5.9 [a]	0.0001

Values are mean ± standard deviation (SD) from 10 subjects and *p* values relate to a one way analysis of variance (ANOVA) test. The suffixes indicate significant differences (Fisher test $p < 0.05$) between inhalation conditions. Values share the same suffix when the difference between conditions is not statistically significant (Fisher test $p > 0.05$).

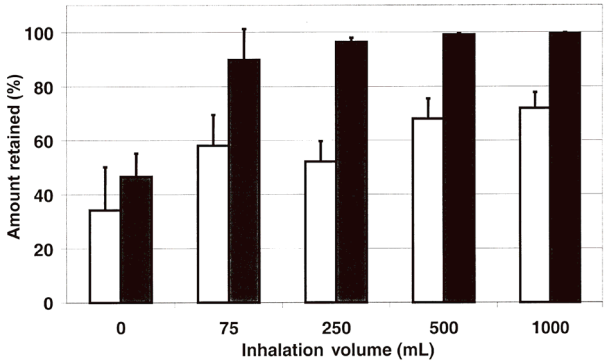


Figure 3. Effect of inhalation volume on the mean + 1 SD amounts of solanesol (open bars) and nicotine (solid bars) retained in 10 subjects

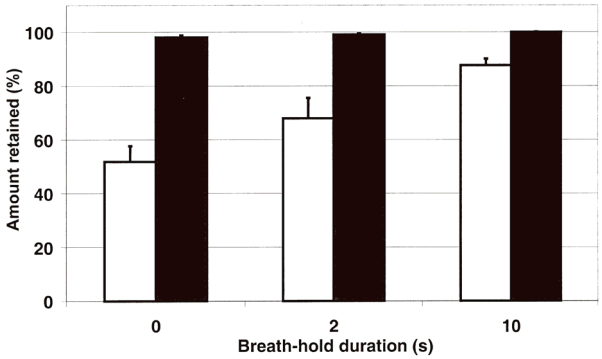


Figure 4. Effect of breath-hold duration at constant 500 mL inhalation depth on the mean + 1 SD amounts of solanesol (open bars) and nicotine (solid bars) retained in 10 subjects

0.5% and 99.5 ± 0.3%, respectively). Solanesol retentions ranged from 34.1% with no inhalation to 70.9% with an inhalation volume of 1000 mL. The solanesol retentions at 75 and 250 mL inhalation volumes did not differ significantly, nor did those at 500 and 1000 mL inhalation volumes. All other differences were statistically significant ($p < 0.05$).

Effect of breath-hold duration on nicotine and solanesol retentions within the respiratory tract

The effect of breath-hold duration (0, 2 and 10 s) was studied at an inhalation volume of 500 mL (Table 3 and Figure 4).

With a 0 s breath-hold, 98.0 ± 0.8% of the nicotine delivered was retained. Nicotine retention increased to 99.9 ± 0.1% with a 10 s breath-hold. Although the arithmetic differences between nicotine retention at the different breath-hold durations were small they were statistically significant ($p < 0.05$). Solanesol retention increased significantly ($p < 0.05$) as breath-hold duration increased from 0 sec (51.8 ± 5.9%) to 2 sec (68.0 ± 7.5%) to 10 sec (87.6 ± 2.4%).

Effect of inhalation volume on plasma nicotine levels

Pre-smoking plasma nicotine levels were not significantly different for the three inhalation volumes (Table 4 and Figure 5).

Table 3. The influence of breath-hold duration at constant inhalation depth (500 mL) on the deliveries and retentions of nicotine and solanesol

Parameters investigated	Zero breath-hold	2 s breath-hold	10 s breath-hold	ANOVA *p* value
Total puff volume (mL)	441 ± 78 [a]	411 ± 72 [a]	415 ± 61 [a]	0.599
Nicotine inhaled (µg)	1229 ± 182 [a]	1116 ± 233 [a]	1170 ± 181 [a]	0.583
Nicotine retained (µg)	1204 ± 178 [a]	1106 ± 235 [a]	1168 ± 181 [a]	0.547
Nicotine retained (%)	98.0 ± 0.8 [c]	99.0 ± 0.5 [b]	99.9 ± 0.1 [a]	0.0001
Solanesol inhaled (µg)	408 ± 63 [a]	387 ± 83 [a]	405 ± 61 [a]	0.771
Solanesol retained (µg)	211 ± 39 [c]	268 ± 86 [b]	355 ± 55 [a]	0.0001
Solanesol retained (%)	51.8 ± 5.9 [c]	68.0 ± 7.5 [b]	87.6 ± 2.4 [a]	0.0001

Values are mean ± standard deviation (SD) from 10 subjects and *p* values relate to a one way analysis of variance (ANOVA) test. The suffixes indicate significant differences (Fisher test $p < 0.05$) between inhalation conditions. Values share the same suffix when the difference between conditions is not statistically significant (Fisher test $p > 0.05$).

Table 4. The effect of inhalation depth on plasma nicotine levels

	Time (min)							
Inhalation volume (mL)	0	3.5	7	10	15	20	40	60
0	5.8 ± 2.7	6.0 ± 2.5	5.9 ± 2.8	5.9 ± 2.6	6.2 ± 2.4	5.1 ± 2.1	5.2 ± 2.1	4.8 ± 1.9
75	5.5 ± 2.4	8.7 ± 3.6	13.7 ± 4.6	10.8 ± 3.9	9.3 ± 2.9	8.7 ± 2.8	6.7 ± 2.3	5.7 ± 2.6
500	7.1 ± 2.7	11.3 ± 3.3	18.5 ± 4.3	13.6 ± 3.0	11.7 ± 2.5	10.0 ± 2.7	8.6 ± 3.4	6.6 ± 2.0

Values are mean ± standard deviation (SD) plasma nicotine concentrations (ng/mL) from 10 subjects (n = 8 for 75 mL inhalation).

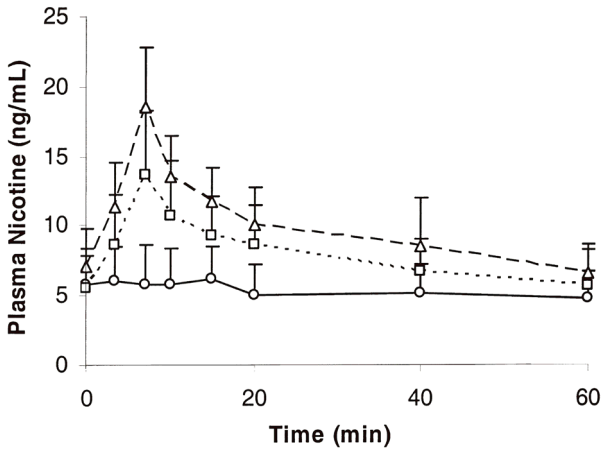


Figure 5. Effect of inhalation volume on plasma nicotine concentrations. Ordinate = mean ± 1 SD plasma nicotine concentration in 10 subjects (n = 8 for 75 mL inhalation). Mouth-hold (○), 75 mL inhalation (□) and 500 mL (△) inhalation conditions. Smoking occurred during the zero to 7-min period.

Plasma nicotine levels did not change significantly from the pre-smoking values following the mouth-hold (zero inhalation) manoeuvre. Following the 75 mL inhalation, plasma nicotine levels were significantly higher ($p < 0.05$) than after the mouth-hold except at 40 and 60 min. Plasma nicotine levels at every time point after the 500 mL inhalation were significantly higher ($p < 0.05$) than those following the mouth-hold manoeuvre. Plasma nicotine levels following the 500 mL inhalation were consistently higher than those following the 75 mL inhalation; however, the difference was statistically significant ($p < 0.05$) only at the 7-min sample point. This was the point of maximum plasma nicotine concentration.

Effect of inhalation volume on COHb levels

Carboxyhaemoglobin levels increased significantly after the 75 and 500 mL inhalations (boosts: 0.40% ± 0.28% and 0.83% ± 0.30%, respectively; $p < 0.05$), but there was no significant change after the mouth-hold (zero inhalation) (boost: −0.03% ± 0.31%; not significant).

Smoking behaviour

The experimental procedure for this study controlled all aspects of smoking behaviour except puff volume and, of course, nicotine metabolism.

Subjects were very consistent in their individual total puff volumes per cigarette across the seven experimental smoking sessions. For the ten subjects, mean puff volumes across the sessions ranged from 40.9 ± 2.2 mL to 87.4 ± 16.2 mL. The mean puff volume for all subjects and sessions was 59.7 ± 10.7 mL which was within the range of published mean puff volumes (range 21 mL to 66 mL (13)).

DISCUSSION

The principal findings of this study are that: 1) nicotine retention was greater than solanesol retention in all manoeuvres; 2) 90% or more of the nicotine was retained even at an inhalation volume as low as 75 mL and 3) about 50% retention of nicotine during the mouth-hold manoeuvre did not result in an increase in plasma nicotine concentration.

The first finding has implications for the mechanisms of retention of nicotine and solanesol during smoking. Solanesol does not evaporate from aerosol particles at body temperature, even at high dilution. It can, therefore, be retained in the airways and lungs only by deposition of the smoke particles. This deposition results from inertial impaction, Brownian diffusion and gravitational sedimentation (18). The greater retention of nicotine demonstrates that it does not all remain in the aerosol particles with solanesol, otherwise the retentions of the two compounds would be the same, and supports the hypothesis that nicotine evaporates from the smoke particles, diffuses to and is absorbed into the surface of the airways. This is consistent with the data reported by FROST *et al*. (6) for a smaller number of subjects using similar methodology, and by the *in vitro* experiments on the evaporation of nicotine from particles by LEWIS *et al*. (2). Our finding that there is greater retention of nicotine than solanesol in the mouth suggests that evaporation of nicotine from particles has already started at this point.

Our finding that the percentage retention of nicotine in the respiratory tract was higher than solanesol is consistent with the work of BLACK *et al* (19). They used an inhaled-exhaled technique in human volunteers exposed to aged and diluted sidestream tobacco smoke and reported respiratory retentions of 70–80% for nicotine and 40% for solanesol. BLACK *et al* concluded that nicotine and its metabolites in biofluids are not representative markers of exposure to environmental tobacco smoke (ETS) particulates. Our results indicate that this conclusion also applies to mainstream cigarette smoke.

The second finding, that 46.5 ± 8.6% of the nicotine was retained in the mouth-hold manoeuvre and 89 ± 12% in the 75 mL inhalation manoeuvre, strongly suggests that substantial amounts of nicotine can be retained within the upper respiratory tract and will not reach the alveolar region of the lung for rapid uptake into the arterial circulation. This is consistent with observations of nicotine absorption by ROSE *et al*. (7) who showed that rises in

arterial levels of nicotine are more than 10 times lower than would be expected if the nicotine were absorbed rapidly into the arterial circulation. ROSE *et al.* (7) claimed that a slowing of the entry of nicotine into the arterial system resulting from an initial distribution of nicotine into respiratory tissue was a plausible explanation of their findings.

The levels of nicotine retained in the mouth during the mouth-hold manoeuvre were surprisingly high, and this raises the question as to whether these high retention values were genuine or occurred as consequence of the subjects' non-compliance with the protocol. An apparent high level of mouth retention could have been obtained if the subjects had lost significant amounts of smoke from the mouth prior to exhaling through the Cambridge pad. The experimenters observed each of the subjects specifically for the appearance of waste or lost smoke and this was not detected in any of the subjects and hence was discounted as a source of error. A second problem could have been an inadvertent small inhalation during the mouth-hold period resulting in significant amounts of nicotine retention in the respiratory tract rather than in the mouth region. We have ruled out the possibility of inadvertent inhalation for the following reasons: 1) subjects practiced all manoeuvres prior to the study sessions. 2) COHb levels fell marginally following the mouth-hold manoeuvres. Inadvertent inhalations would have resulted in an increase in COHb levels as was the case for the shallow (75 mL) inhalations. Additionally, inhalations would have resulted in an increase in the venous blood levels of nicotine. Even the shallow (75 mL) inhalations resulted in increased blood levels of nicotine. However the mouth-hold manoeuvres were associated with small reductions in pre- to post-smoking blood levels of nicotine. Thus it is highly likely that the high retention levels of nicotine within the mouth are genuine and did not occur as a result of protocol non-compliance.

Our finding that, even with a modest inhalation volume of 250 mL (the "normal" inhalation volume range is 413–913 mL (13)) and 2 s breath-hold, 96.4 ± 1.5% of nicotine was retained, means that there is little scope for further nicotine retention by increasing inhalation volume or breath-hold time. This observation is consistent with the work of ZACNY *et al*. (20) who found that increasing inhalation volume from 10% to 60% vital capacity, or post inhalation breath-hold time from zero to 16 s did not increase nicotine "boosts" (post minus pre-smoking venous nicotine levels). In contrast, solanesol retention in the respiratory tract increased significantly with increasing inhalation volume and breath-hold duration.

The third finding relates to the uptake of nicotine from the mouth into the systemic circulation. In this study 46.5 ± 8.6% nicotine was retained after a 2 s mouth-hold. This is consistent with results from FROST *et al*. (6) who found 32.6 ± 15.7% retention after puffing with immediate exhalation. Our observations that venous nicotine levels did not increase following the mouth-hold (zero inhalation) condition confirm results previously reported by GORI *et al*. (21) and ZACNY *et al*. (20); however neither group measured retention. Our data are consistent with work using inhaled nicotine vapour, where the nicotine is found primarily in the mouth, oesophagus and stomach (22,23). These and related studies, (e.g., references 24–26), indicate that nicotine transfers poorly from the mouth to the systemic circulation. Nicotine retained within the mouth following a puff of cigarette smoke will initially dissolve into saliva which is typically slightly acidic (about pH of 6.5). It is unlikely that nicotine and other smoke components will overcome the local buffering capacity of saliva, thus most of the nicotine in saliva will be in the protonated form. As the principal mechanism whereby nicotine enters tissues is by a process of passive diffusion of nonprotonated nicotine across cell membranes (27) the movement of nicotine from saliva into the lining of the mouth will be relatively slow. Additionally, saliva containing nicotine will be swallowed and then subjected to a very slow absorption route due to the acidic environment of the stomach. A somewhat different situation exists with oral tobacco use, e.g., moist snuff, which does result in an increase in systemic levels of nicotine (28). The likely explanations why systemic absorption of nicotine occurs with moist snuff are a) a relatively large amount of nicotine is present in the mouth (typical products have a nicotine content in excess of 10 mg/g of tobacco (29,30); b) most moist snuff products produce pH values in excess of 7.5 when extracted in aqueous solution (29); c) a pinch or a sachet of slightly alkaline oral tobacco placed in the mouth may be sufficient to overcome the buffering capacity of saliva and increase the absorption rate of nicotine.

Our finding that there is greater retention of nicotine than solanesol in the mouth suggests that evaporation of nicotine from particles has already started at this point.

Particles of the size present in cigarette smoke might be expected to be deposited downstream from the mouth and upper airways in the more distal regions of the lungs, however, MARTONEN and MUSANTE (31) concluded that cigarette smoke exhibits cloud motion, a phenomenon which increases deposition in the upper airway region. This may be the explanation for the high levels of solanesol retention within the mouth and upper airways found in our study.

GUYATT *et al.* (14) suggested that alveolar absorption of carbon monoxide does not occur with inhalation volumes less than 120 mL in adult males. It was, therefore, somewhat surprising that we found clear COHb boosts after 75 mL inhalations. One possible explanation is that, in our study, subjects employed a two second breath-hold whereas this was not the case in the GUYATT *et al.* investigation. This breath-hold may have given the opportunity for CO to diffuse towards the lungs, to avoid immediate exhalation and to be carried into the lungs by the next inhalation.

Recently, PANKOW (32) has discussed the mechanisms by which moderately volatile compounds such as nicotine would be deposited in the respiratory tract during inhalation. He identified four different processes and considered the likely contribution of each of these to the total nicotine uptake. One process he considered is the deposition of nicotine that is initially in the gaseous phase. PANKOW correctly states that the contribution of this mechanism must be small because there is very little nicotine in the gaseous phase when the aerosol leaves the cigarette (1,3). The second is the uptake of nicotine that evaporates from the aerosol particles during inhalation. The other two processes he discussed involve the deposition of nicotine from aerosol particles after they have deposited in the

respiratory tract, one involving the evaporation of nicotine from deposited particles and the other resulting from direct diffusion of nicotine from deposited particles into tissues. PANKOW stated that the relative contributions of the various deposition mechanisms have not been determined for major brands when the smoke is inhaled according to typical smoker inhalation patterns. The results reported in our paper provide evidence that nicotine evaporation makes a major contribution to total nicotine deposition.

CONCLUSION

Our finding that there is much greater retention of nicotine than solanesol with shallow inhalations is consistent with the view that nicotine evaporates from the cigarette smoke aerosol particles in the upper respiratory tract.

There is significant retention of nicotine in the mouth but any absorption via the mouth did not increase nicotine levels in the systemic circulation .

Even a shallow inhalation (<250 mL) results in 96% nicotine retention.

There is minimal capacity for smokers to increase nicotine retention by employing longer breath-hold durations or increased inhalation volumes.

In contrast, the particulate-based compound solanesol showed progressively increasing retention with breath-hold time and with inhalation volume.

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REFERENCES

1. Houseman, T.H.: Studies of cigarette smoke transfer using radio-isotopically labelled tobacco constituents. Part II: The transference of radio-isotopically labelled nicotine to cigarette smoke; Beitr. Tabakforsch. 7 (1973) 142–147.
2. Lewis, D.A., I. Colbeck, and D.C. Mariner: Dilution of mainstream tobacco smoke and its effects upon the evaporation and diffusion of nicotine; J. Aerosol. Sci. 26 (1995) 841–846.
3. Stevens, N.A. and M.F. Borgerding: GC-AED studies of nicotine fate in a burning cigarette; Anal. Chem. 72 (1999) 2179–2185.
4. Chen, B.T., J. Namenxi, H.C. Yen, J.L. Mauderly, and R.G. Cuddihy: Physical characterization of cigarette smoke aerosol generated from a Walton smoke machine; Aerosol Sci. Technol. 12 (1990) 364–375.
5. Russell, M.A.H. and C. Feyerabend: Cigarette smoking: a dependence on high-nicotine boli; Drug. Metab. Rev. 8 (1978) 29–57.
6. Frost, B.E., D.C. Mariner, and N.M. Sinclair: Factors relating to nicotine physio-chemistry and retention in human smokers; *in*: Proceedings of the CORESTA Congress, October 1998, Brighton, UK, 211–218.
7. Rose, J.E., F.M. Behm, E.C. Westman, and R.E. Coleman: Arterial nicotine kinetics during cigarette smoking and intravenous nicotine administration: implications for addiction; Drug Alcohol Dependence 56 (1999) 99–107.
8. Sterling, E.M., C.W. Collett, and J.A. Ross: Assessment of non-smokers exposure to environmental tobacco smoke using personal-exposure and fixed location monitoring; Indoor Built Environment 5 (1996) 112–125.
9. ISO 4387: Methods for chemical analysis of tobacco and tobacco products. Determination of total and nicotine-free dry particulate matter using a routine analytical smoking machine; International Organisation for Standardisation, Geneva, 1991.
10. ISO 10315: Methods for chemical analysis of tobacco and tobacco products. Determination of nicotine in smoke condensate of cigarettes (gas-chromatographic method); International Organisation for Standardisation, Geneva, 1991.
11. ISO 8454: Methods for chemical analysis of tobacco and tobacco smoke products. Determination of carbon monoxide in the vapour phase of cigarette smoke (NDIR method); International Organisation for Standardisation, Geneva, 1995.
12. Creighton, D.E., M.J. Noble, and R.T. Whewell: Instruments to measure, record and duplicate human smoking patterns; *in*: Smoking Behaviour, edited by R.E. Thornton, Churchill Livingstone, Edinburgh, London and New York, 1978, pp. 277–288.
13. US Department of Health and Human Services (USDHHS): The health consequences of smoking: Nicotine addiction; Public Health Services, Washington DC, 1988.
14. Guyatt, A.R., M.A. Holmes, and G. Cumming: Can carbon monoxide be absorbed from the upper respiratory tract in man? Eur. J. Respir. Dis. 62 (1981) 383–390.
15. Creighton, D.E. and P.H. Lewis: The effect of smoking pattern on smoke deliveries; *in*: Smoking Behaviour, edited by R.E. Thornton, Churchill Livingstone, Edinburgh, London and New York, 1978, pp. 289–314.
16. American Society for Testing and Materials: D6271-98, Standard test method for estimating contribution of environmental tobacco smoke to respirable suspended particles based on solanesol, 1998.
17. American Society for Testing and Materials: D5075-96e1, Standard test method for nicotine and 3-ethenylpyridine in indoor air, 1996.
18. Dixon, M., K. Lambing, and J.I. Seeman: On the transfer of nicotine from tobacco to the smoker. A brief review of ammonia and “pH” factors; Beitr. Tabakforsch. Int. 19 (2000) 103–113.

19. Black, A., J.J. McAughey, D.A. Knight., C.J. Dickens, and J.C. Strong: Estimation of ETS retention in volunteers from measurements of exhaled smoke composition; *in*: Indoor Air '93, edited by M. Jantunen, P. Kalliokoski, E. Kukkonen, K. Saarela, O. Seppanen, and H. Vuorelma, Indoor Air 1993, Vol. 3, Helsinki, Finland, pp. 41–46.
20. Zacny, J.P., M.L. Stitzer, F.J. Brown, J.E. Yingling, and R.R. Griffiths: Human cigarette smoking: Effects of puff and inhalation parameters on smoke exposure; J. Pharm. Exp. Ther. 240 (1987) 554–564.
21. Gori, G.B., N.L. Benowitz, and C.J. Lynch: Mouth versus deep airway absorption of nicotine in cigarette smokers; Pharmacol. Biochem. Behav. 25 (1986) 1181–1186.
22. Lunell, E., M. Bergstrom, G. Antoni, B. Langstrom, and A. Nordbert: Nicotine deposition and body distribution from a nicotine inhaler and a cigarette studied with positron emission tomography; Clin. Pharmacol. Ther. 59 (1996) 593–594.
23. Bergstrom, M., A. Nordberg, E. Lunell, G. Antoni, and B. Langstrom: Regional deposition of inhaled ^{11}C-nicotine vapor in the human airway as visualized by positron emission tomography; Clin. Pharmacol. Ther. 57 (1995) 309–317.
24. Russell, M.A.H., M.J. Jarvis, G. Sutherland, and C. Feyerabend: Nicotine replacement in smoking cessation; J. Amer. Med. Assoc. 257 (1987) 3262–3265.
25. Schuh,K.J., L.M. Schuh, J.E. Henningfield, and M.L. Stitzer: Nicotine nasal spray and vapor inhaler: Abuse liability assessment; Pyschopharmacology 130 (1997) 352–361.
26. Molander, L., E. Lunell, S.-B. Anderson, and F. Kuylenstierna: Dose released and absolute bioavailability of nicotine from a nicotine vapour inhaler; Clin. Pharmacol. Ther. 59 (1996) 394–400.
27. Schievelbein, H: Nicotine, resorption and fate; *in*: International Encyclopedia of Pharmacology and Therapeutics, Section 114: Nicotine and the tobacco smoking habit, edited by D.J.K. Balfour, Pergamon Press , 1984, pp. 1–15
28. US Department of Health and Human Services (USDHHS): The health consequences of using smokeless tobacco: Public Health Services, Washington DC, 1986.
29. Henningfield, J.E., A. Radzius, and E.J. Cone: Estimation of available nicotine content of six smokeless tobacco products; Tob. Control 4 (1995) 57–61.
30. Djordjevic, M., D. Hoffman, T. Glynn, and G. Connolly: US commercial brands of moist snuff, 1994. Assessment of nicotine, moisture and pH; Tob. Control 4 (1995) 62–66.
31. Martonen, T.B. and C.J. Musante: Importance of cloud motion on cigarette smoke deposition in the lung; Inhal. Toxicol. 12 (2000) 261–280.
32. Pankow, J.F: A consideration of the role of gas/particle partitioning in the deposition of nicotine and other tobacco smoke compounds in the respiratory tract; Chem. Res. Toxicol. 14 (2001) 1465–1481.

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