A Pilot Study to Assess Solanesol Levels in Exhaled Cigarette Smoke*

by

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SUMMARY

This paper describes the results obtained during the measurement of the level of solanesol in exhaled cigarette smoke from human subjects. The study was performed with three different cigarettes with U.S. Federal Trade Commission (FTC) 'tar' values of 5.0 mg, 10.6 mg, and 16.2 mg. The number of human subjects was ten smokers for each of the evaluated products, each subject smoking three cigarettes within one hour. The exhaled smoke was collected using a vacuum assisted procedure that avoids strain in exhaling, and the solanesol was analyzed using an original high performance liquid chromatography (HPLC) technique. The cigarette butts from the smokers were collected and also analyzed for solanesol. The results obtained for the cigarette butts from the smokers were used to calculate the level of solanesol delivered to the smoker, based on calibration curves. These curves were generated separately by analyzing the solanesol in smoke and in the cigarette butts obtained by machine smoking under different puffing regimes. Knowing the levels of solanesol delivered to the smoker and the exhaled levels it was possible to calculate the retention and retention % of this compound from mainstream smoke for different cigarettes types. The amount of retained solanesol is the lowest for the 5.0 mg 'tar' product, and the highest for the 16.2 mg 'tar' product, although there is not much difference between the 10.6 mg 'tar' product and the 16.2 mg 'tar' product. For the 10.6 mg 'tar' cigarettes the retention % was between 60% and 72%, for the 5.0 mg product the retention % was slightly lower ranging between 53% and 70%, while for the 16.2 mg 'tar' product, the retention % was slightly higher ranging between 62% and 82%. A statistical analysis of the retention % data using ANOVA single factor analysis showed that the 10.6 mg 'tar' ciga-

rette is not different from the 16.2 mg 'tar' product while the retention % for the 5.0 mg 'tar' cigarette was statistically different from the other two products. The values for the retention % of solanesol by human smokers as found in this study were in very good agreement with the few reported results in the literature. [Beitr. Tabakforsch. Int. 23 (2008) 144–152]

ZUSAMMENFASSUNG

Die Solanesolkonzentration im exhalieren Zigarettenrauch von Rauchern wurde gemessen. Für die Untersuchung wurden Zigaretten mit 5.0 mg, 10.6 mg und 16.2 mg Kondensat nach FTC (Federal Trade Commission, FTC) Abrauchnormen verwendet. Jeweils 10 Raucher rauchten die untersuchten Zigaretten, wobei jeder Raucher drei Zigaretten pro Stunde rauchte. Der exhalierter Rauch wurde mit einem Vakuum-unterstützten Verfahren gesammelt, um einen Widerstand beim Exhalieren zu vermeiden und das Solanesol wurde mittels Hochdruck-Flüssigkeitschromatographie (HPLC) bestimmt. Die Zigarettenstummel der Raucher wurden gesammelt und ebenfalls auf ihren Solanesolgehalt untersucht. Das Solanesol in den Zigarettenstummeln der Raucher wurde dazu herangezogen, um die aufgenommene Solanesolmenge auf der Basis von Eichkurven zu berechnen. Diese Kurven wurden separat generiert, indem die Solanesolgehalte im Rauch und auch in den Zigarettenstummeln analysiert wurden, die durch maschinelles Abrauchen unter verschiedenen Abrauchbedingungen erhalten wurden. Weil die Solanesolmenge, die an die Raucher abgegeben wurde und die exhalieren Mengen bekannt waren, war es möglich die Retention dieser Substanz beim Rauchen verschiedener Zigaretten zu berechnen. Die Menge an retiniertem Solanesol ist bei den

Zigaretten mit 5,0 mg Kondensat am niedrigsten und am höchsten bei den Zigaretten mit 16,2 mg Kondensat, wobei der Unterschied zwischen Zigaretten mit 10,6 und 16,2 mg Kondensat gering ist. Bei den Zigaretten mit 10,6 mg Kondensat betrug die prozentuale Retention zwischen 60% und 72%, bei Zigaretten mit 5,0 mg Kondensat war die prozentuale Retention etwas niedriger und lag zwischen 53% und 70%, wohingegen die prozentuale Retention bei Zigaretten mit 16,2 mg Kondensat mit 62% bis 82% etwas höher lag.

Die statische Auswertung der prozentualen Retention mit der einfaktoriellen Varianzanalyse zeigte, dass es zwischen der Zigarette mit 10,6 mg und derjenigen mit 16,2 mg Kondensat keinen Unterschied gibt, während die prozentuale Retention der Zigarette mit 5,0 mg Kondensat statistische Unterschiede zu den anderen beiden Zigaretten aufwies. Die Werte der prozentualen Retention des Solanesols bei Rauchern sind in sehr guter Übereinstimmung mit den wenigen in der Literatur referierten Ergebnissen. [Beitr. Tabakforsch. Int. 23 (2008) 144–152]

RESUME

Cette étude présente les taux mesurés de la solanésol dans la fumée de cigarette exhalée par les fumeurs. L'étude a été conduite avec trois différentes cigarettes à taux de goudron déterminé selon la méthode normalisée de la Federal Trade Commission (FTC) de 5,0 mg, 10,6 mg et 16,2 mg. Les trois types de cigarettes évaluées ont été fumés par dix fumeurs, chacun fumant trois cigarettes par heure. La fumée exhalée a été échantillonnée à l'aide d'une technique sous vide pour éviter une trop grande résistance durant l'exhalation de la fumée. Le solanésol a été analysé par une technique de chromatographie en phase liquide à haute performance (HPLC). Les mégots de cigarette ont été collectés et ensuite également dosé pour leur teneur en solanésol. Ces résultats ont été utilisés pour calculer le taux de solanésol délivré aux fumeurs, basé sur des courbes de calibrage. Ces courbes ont été générées séparément en dosant la teneur en solanésol dans la fumée et dans les mégots de cigarette obtenus par le fumage par machine sous les conditions différentes. La rétention de la solanésol et le pourcentage de rétention à partir de la fumée principale des différents types de cigarettes ont été calculé à partir du taux de solanésol délivré au fumeur et les concentrations de solanésol exhalé. La teneur en solanésol retenue est le plus bas pour la cigarette à 5,0 mg de goudron, et le plus haut pour la cigarette à 16,2 mg de goudron, cependant il y a des petites différences entre les cigarettes à 10,6 mg de goudron et à 16,2 mg de goudron. Pour les cigarettes à 10,6 mg de goudron, le pourcentage de rétention est entre 60% et 72%, pour les cigarettes à 5,0 mg de goudron le pourcentage de rétention est légèrement plus bas allant de 53% à 70%, cependant pour les cigarettes à 16,2 mg de goudron, le pourcentage de rétention est légèrement plus élevé, allant de 62% à 82%.

L'analyse statistique des données obtenues par analyse de la variance Anova à un facteur révèle que la cigarette à 10,6 mg de goudron ne diffère pas de la cigarette à 16,2 mg de goudron, tandis que le pourcentage de rétention de la cigarette à 5,0 mg de goudron est statistiquement différent

de deux autres produits. Les résultats obtenus du pourcentage de rétention du solanésol par le fumeur humain sont en bon accord avec la littérature. [Beitr. Tabakforsch. Int. 23 (2008) 144–152]

INTRODUCTION

Solanesol (2E,6E,10E,14E,18E,22E,26E,30E)-3,7,11,15,19,23,27,31,35-nonamethyl-hexatriacont-2,6,10,14,18,22,26,30,34-nonaen-1-ol) is present in tobacco and in cigarette smoke, and has been considered a good marker for the particulate phase of environmental tobacco smoke (ETS) (1–3). This choice was made in part because the compound is non-volatile (MW = 631.10), and also, because tobacco smoking can be considered the unique source of this substance in the environmental aerosols (although solanesol is present in other plants from *Solanaceae* family). The retention by humans of solanesol from ETS has been previously studied with results showing retention values between 20% and 57% (with an average of $40 \pm 20\%$) in males, and between 10% and 58% (with an average of $27 \pm 14\%$) in females (4). The retention of solanesol was also studied for mainstream cigarette smoke in two recent studies (5, 6). The first of these studies (5) evaluated the retention of solanesol from the mainstream of a 9.6 mg 'tar' cigarette [measured using U.S. Federal Trade Commission (FTC) regimen (7)] on ten smokers using specific inhalation protocols, and the second study (6) evaluated the retention from three experimental cigarettes containing different additives. Both studies evaluated the inhaled level of solanesol using a smoking duplicator. The first study indicated the retention of solanesol of $68.0 \pm 7.5\%$ (for a 2 s breath-hold duration), and the second study showed a retention in the range of 65.1% and 70.7% for a 500 mL inhalation volume for three evaluated cigarettes. Two other studies on solanesol retention from mainstream smoke were referenced in the literature (1), but the results were not published in peer reviewed journals, being only presented at meetings and not being readily available (8, 9). As reported in (1), in one of these studies (8) the average solanesol retention by humans was found to be about $48 \pm 8\%$, while in the other study the average reported retention was 58% for 250 mL inhalation volume and 61% for 500 mL inhalation volume (9).

This present study reexamined the retention of solanesol from three commercial cigarettes with 5.0 mg 'tar', 10.6 mg 'tar', and 16.2 mg 'tar'. Each brand was evaluated by ten smokers, the human subjects being a smoker of the particular tested brand (three different groups of ten smokers participated in the study). The smoking was performed in an environment familiar to the smoker (office) with as little change as possible from typical smoking conditions and with no control on the puffing, vent blocking, inhalation, or exhalation patterns. Also, no attempt was made to evaluate in which part of the respiratory tract the retention occurred. The solanesol level delivered to the smoker was calculated based on the solanesol in the cigarette butts from the human subjects, using calibration curves obtained in advance. Only a few other studies were done on solanesol retention from mainstream cigarette smoke (1). The advantages of this present study compared

Table 1. Description of tested cigarettes

Descriptor	16.2 mg 'tar'	10.6 mg 'tar'	5.0 mg 'tar'
FTC 'tar' (mg/cig)	16.2	10.6	5.0
Cigarette length (mm)	83	83	83
Filter type	CA ^a	CA	CA
Filter length (mm)	21	27	27
Filter ventilation (%)	23	32	54
Filter pressure drop (mm H ₂ O)	62	97	126
Blend type	American	American	American
Nicotine (mg/cig)	1.31	0.92	0.50
CO (mg/cig)	13.9	10.7	7.4

^a CA = Cellulose acetate.

to the previous ones included a larger number of smokers, the evaluation of three cigarettes with different 'tar' levels, and smoking in conditions as close as possible with those familiar for the smoker.

EXPERIMENTAL

Present study has been performed with three cigarettes with different 'tar' levels. The description of the cigarettes tested is given in Table 1. To achieve the purpose of this study it was necessary to find the levels of solanesol in the exhaled smoke and in the smoke delivered to the human subject. Knowing these two levels of solanesol it was possible to calculate the retention of this compound from mainstream smoke. The analysis of solanesol from the pads used for smoke collection and from 1 cm cut portion of the cigarette butts was performed by an high performance liquid chromatography (HPLC) procedure. This HPLC procedure was original, but in several respects similar with other procedures reported in the literature for solanesol analysis (10, 11). The experimental part of this study consists of several steps, which include: 1) collection of particulate matter on Cambridge pads from the mainstream cigarette smoke obtained with a smoking machine using eight different smoking conditions; the cigarette butts of the smoked cigarettes were retained for further solanesol analysis, 2) collection of particulate matter from the exhaled cigarette

smoke; the cigarette butts of the cigarettes smoked by humans were also retained for solanesol analysis, 3) measurement of solanesol using an HPLC method in the pads and in 1 cm cut portion of the cigarette butts, and 4) calculation of the results. Each of these steps are discussed in detail in sections that follow.

Collection of samples from smoking machine

Particulate matter was collected from five cigarettes on Cambridge pads using machine smoking under various conditions. These included conditions recommended by the US Federal Trade Commission (FTC) (7) on a Borgwaldt rotary machine RM20/CSR (Schnackenburgallee 15, D-22525 Hamburg, Germany) (puff volume 35 mL, with a duration of 2 s and each puff taken at 60 s interval), and intensive conditions with 60 mL puff volume, a puff duration of 2 s and each puff taken at 60 s interval (indicated as 60/60 conditions), 45 mL puff volume, a puff duration of 2 s and each puff taken at 30 s interval (indicated as 45/30 conditions), 60 mL puff volume, a puff duration of 2 s and each puff taken at 30 s interval (indicated as 60/30 conditions), 35 mL puff volume, a puff duration of 1.5 s and each puff taken at 75 s interval (indicated as 35/75 conditions), 50 mL puff volume, a puff duration of 2 s and each puff taken at 30 s interval (indicated as 50/30 conditions), 55 mL puff volume, a puff duration of 2 s and each puff taken at 80 s interval (indicated as 55/80 conditions), and 45 mL puff volume, a puff duration of 1.5 s and each puff taken at 40 s interval (indicated as 45/40 conditions). This variety of smoking conditions was selected in order to cover a range of flow rates in the cigarette filter that covers, at least in part, the flow rates similar to those noticed in human smoking (13). The cigarette butts from the cigarettes were also collected for the analysis of solanesol content. The butts were cut at 1 cm length from the mouth end, and this portion was used for the analysis of solanesol. The length of the cut portion of the cigarette butt was measured for ensuring that the length is precisely 1 cm. A correction factor was applied if a small difference of the cut length was noticed.

Exhaled smoke collection

The collection of the exhaled smoke has been done using a simple device schematically shown in Figure 1. The device

Figure 1. Device for the collection of exhaled cigarette smoke

Table 2. High performance liquid chromatography solvent gradient program

Time min	Methanol %A	Ethanol %B	Heptane %C	Flow mL/min
0.0	100	0	0	1.0
7.0	100	0	0	1.0
9.0	0	100	0	0.8
11.0	0	100	0	0.8
14.0	0	0	100	0.8
18.5	0	0	100	0.8
19.0	0	100	0	0.8
20	0	100	0	0.8
21	100	0	0	1.0

consisted of a 92 mm Cambridge pad holder and a pad having at one opening a replaceable mouth piece, and at the other opening being connected to a diaphragm vacuum pump, which can aspirate 2.2 m³/h (Vacuumbrand GMBH, Wertheim, Germany). Through the pad and the connecting tubing, the pump achieves about 250 mL/s flow. The tube connecting the pad holder to the pump had two large holes to the exterior, which can be covered with two fingers. When no smoke is exhaled, the holes in the tube to the vacuum pump were kept open such that surrounding air is aspirated by the pump without passing through the Cambridge filter. During exhalation of smoke, the smoker blows the smoke through the replaceable mouth piece. At the same time the two holes in the tube should be covered with two fingers, such that the exhaled smoke is aspirated through the Cambridge pad. This allows the exhaled smoke to be collected on the pad, without additional strain on the smoker. Considerable strain would be necessary otherwise to overcome the flow resistance of the Cambridge pad. The device shown in Figure 1 has been used by ten human subjects for testing each of the evaluated cigarettes. Three cigarettes were smoked for each trial within one hour, and the exhaled smoke was collected on one pad. The cigarette butts were retained for solanesol analysis that was performed on 1 cm cut from the mouth end of the cigarette.

HPLC analysis

The analysis for solanesol in the particulate phase smoke collected on pads or on the cut portion of the cigarette butts

started with an extraction in *n*-heptane. For this purpose, the samples to be extracted were placed in an extraction vial (with the volume of about 125 mL). To each vial was added 100 µL of a solution containing 1000 µg/mL pyrene used as a chromatographic standard, and then 20 mL heptane. The extraction was performed on a wrist action mechanical shaker (Burrell Scientific Co., PA 15219) for 30 min. About 1.5 mL solution of each sample was transferred into an HPLC vial for analysis. The HPLC analysis was done using UV detection on an Agilent instrument (1050 HPLC with a diode array UV detector, Agilent, Wilmington, Delaware 19808). The HPLC instrument was equipped with a Spherisorb 5µ ODS (2) column 250 × 4.6 mm (Phenomenex, Torrance, CA 90501-1430). The injection volume was 20 µL. The elution was done using a gradient of three solvents, A- methanol, B- ethanol, C- heptane (ethanol is needed because heptane and methanol are not miscible). The parameters for the HPLC gradient (linear) are given in Table 2.

The measuring wavelength starts at 335 nm and changes at 14.5 min to 205 nm, switching back to 335 nm at 18 min. The chromatogram for a pad extract is shown in Figure 2a, with solanesol eluting at 15.165 min, and pyrene at 4.802 min. The gradient conditions for the solvents and for the change in wavelength can be shortened by 5 min to every time parameter, without altering the quality of the separation. The chromatogram generated for the same sample in shortened conditions is shown in Figure 2b, with solanesol eluting at 10.719 min and pyrene at 5.226 min. However, since a reduced run time for generating the chromatograms was not critical for this study, all the measurements were performed using the conditions shown in Table 2 (conditions not shortened).

Calculation of the results

Quantitation of solanesol was performed using a calibration curve relating the peak area from the chromatogram and the solution concentration. For this purpose, ten standards with concentrations between 5 µg/mL and 300 µg/mL solanesol (Aldrich/Sigma, Saint Louis, MO 63178-9916) were prepared in heptane and analyzed by the previously described HPLC procedure. Each solution also contained the chromatographic standard (pyrene 50 µg/mL), but the pyrene

Figure 2. Chromatograms of a pad extract showing the peaks of solanesol and of the chromatographic standard – Chromatogram A (solanesol at 15.165 min) gives the separation in conditions shown in Table 2; Chromatogram B (solanesol at 10.719 min) shows the shortened separation

Table 3. Results from the measurement of solanesol from calibration and from standard addition procedure for the 10.6 mg 'tar' cigarette smoked under FTC regimen

Sample	Added solanesol $\mu\text{g/cig}$	Level from calibration $\mu\text{g/cig}$	Level calculated from standard addition $\mu\text{g/cig}$	RSD %
1	0	218.57	213.86	1.54
1	100	320.77		
2	0	238.48	235.75	0.81
2	200	440.80		
3	0	273.92	284.61	2.71
3	400	658.91		
4	0	242.56	243.54	0.29
4	200	441.76		
1a	0	222.90	225.07	0.68
1a	100	321.94		
2a	0	240.14	238.97	0.35
2a	200	441.13		
3a	0	274.87	284.87	2.53
3a	400	660.84		
4a	0	242.93	244.49	0.45
4a	200	441.66		

peak was used only to verify the reproducibility of the chromatography and not for normalizing solanesol peak areas. The calibration curve for solanesol quantitation was linear with $R^2 = 0.9997$, and the line intercept was zero. Since the analytical procedure for solanesol measurement is new, a validation step was performed for solanesol determination from cigarette smoke. Smoke collection being known to have relative large variability, the precision of the procedure was evaluated without this step, by extracting pads spiked with 20 μg of solanesol (such that after the extraction with 20 mL heptane the solution contains 1 $\mu\text{g/mL}$ added compound). The obtained standard deviation for ten measurements was $\text{SD} = 0.063 \mu\text{g/mL}$ leading to a calculated limit of detection ($\text{LOD} = 3 \times \text{SD}$) of 0.19 $\mu\text{g/mL}$ and a calculated limit of quantitation ($\text{LOQ} = 10 \times \text{SD}$) of 0.63 $\mu\text{g/mL}$ solanesol. The extraction efficiency for the same ten measurements was between 97% and 107%, proving the very good recovery for the procedure when the solanesol is added on the Cambridge pads. The levels of solanesol measured in various samples used for the evaluation of the retention by smokers were considerably higher than LOD value. The accuracy of the measurement was verified by standard addition method. This procedure was applicable since the calibration curve of solanesol had a zero intercept. For the standard addition method, four replicates each consisting of five 10.6 mg 'tar' cigarette were smoked using the FTC regimen. The pads were extracted by the previously described procedure, and the solution analyzed (in duplicate). To each sample was added a given amount of

solanisol and the samples were analyzed after this addition (in duplicate). The calculation of the x -axes intercept allows to evaluate the amount of solanesol in the initial sample [see e.g. (12)]. The results are shown in Table 3. As seen from this table, the calculated level of solanesol using the calibration curves and the standard addition procedure are very close with RSD% values lower than 3%. This proves the good accuracy of the procedure.

RESULTS AND DISCUSSION

The analysis of solanesol was done initially for machine smoked cigarettes using different puffing conditions. At the same time with the analysis of the smoke pad, the cut portion of the cigarette butts was analyzed. Correlation charts between the level of solanesol in smoke as a function of the level in the cigarette butts were obtained. Using these charts the level of solanesol in the cigarette smoke delivered to the human subject was calculated based on the measured level of solanesol in the cigarette butt from the smoker. After this part of the study had been completed, the human subjects smoked the cigarettes and the exhaled smoke collected as previously described was analyzed for solanesol. The cigarette butts from each smoker were collected and the solanesol from 1 cm cut portion was analyzed. The level of solanesol in exhaled smoke was then compared with that in the smoke delivered to the human subject. The amount of solanesol retained by the smoker can be calculated from the following difference:

$$\text{Retention amount } (\mu\text{g/cig}) = \text{Delivered level} - \text{Exhaled level} \quad [1]$$

The retention efficiencies (%) can also be calculated for each smoker using the expression:

$$\text{Retention \%} = 100 - \frac{\text{Exhaled level}}{\text{Delivered level}} \cdot 100 \quad [2]$$

In order to determine the amount of solanesol in the smoke delivered to the human, the regression lines between the level of solanesol in smoke as a function of solanesol in 1 cm cut portion of the cigarette butts were generated. The correlation charts were obtained using eight puffing regimens. Special attention was given to the comparison of the results obtained including or not including the FTC smoking regimen. The flow rate of smoke in the cigarette butt for FTC smoking regimen is 17.5 mL/s, which is lower than most values of flow rates measured for human smoking behavior (13). However, the results regarding solanesol levels in the cigarette butt and on the Cambridge pad are only marginally affected by the inclusion of FTC smoking regimen in the calculations. The dependence curves for each of the evaluated cigarettes are given in Table 4. Two graphs showing the

Table 4. Equations of the dependence lines between solanesol in smoke vs. solanesol in 1 cm cut of the cigarette butt for the three cigarettes with low, medium and high 'tar' levels, including or excluding from the calibration the FTC smoking regimen

Cigarette	Equation with FTC	R^2	Equation without FTC	R^2
5.0 mg 'tar'	$y = 3.8503 x - 22.3180$	0.9722	$y = 3.7638 x - 12.4680$	0.9740
10.6 mg 'tar'	$y = 4.4937 x + 3.5144$	0.9616	$y = 4.2931 x + 30.2520$	0.9713
16.2 mg 'tar'	$y = 4.8926 x + 22.1100$	0.9645	$y = 4.8009 x + 37.7730$	0.9736

Figure 3. Calibration line of solanesol level in smoke vs. solanesol in the cigarette butt for the 5.0 mg 'tar' cigarette including FTC and other smoking regimens

Figure 4. Calibration line of solanesol level in smoke vs. solanesol in the cigarette butt for the 5.0 mg 'tar' cigarette for several smoking regimens not including FTC regimen

calibration curves for the 5.0 mg 'tar' cigarette including FTC smoking regimen and excluding it are shown in Figures 3 and 4, respectively.

A linear dependence has been previously reported between the nicotine level in the cigarette butt and that collected on a Cambridge pad (13, 14). Other studies (15) have shown that a linear dependence is also maintained between the nicotine level in the cigarette butt and the level of other compounds from smoke, such as aldehydes and ketones. Also, linear dependencies were observed between the level of solanesol in the cigarette butt, and compounds in the mainstream smoke such as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), N-nitrosornicotine (NNN), carbon monoxide, isoprene, ethylene and acetaldehyde (16). In this present study it was shown that the linear dependence is also valid between the solanesol in the cigarette butt (1 cm cut) and the solanesol in mainstream smoke (as collected on a Cambridge pad). This linear dependence should still be viewed as an empirical observation, although explainable by the fact that the cigarette filter is not selective for non-volatile compounds in the

mainstream smoke and therefore retains a fixed proportion of the particulate phase. The good R^2 values of the dependence show that the calculation of the level of solanesol in smoke can be done with good accuracy, once the level of solanesol retained in 1 cm cut of the cigarette butt is known.

Calculation of the retention of solanesol from the three evaluated cigarettes

The levels of solanesol in the exhaled smoke as obtained from the analysis of the Cambridge pads used for the collection of smoke from three cigarettes for each smoker are given in Table 5 for the 5.0 mg 'tar' cigarette, in Table 6 for the 10.6 mg 'tar', and in Table 7 for 16.2 mg 'tar' cigarette. A wide range of values for the exhaled solanesol level were noticed among different smokers, and cigarettes with different 'tar' levels showed considerable overlapping of these values. The level of solanesol in the smoke delivered to the human subject were calculated from the level of solanesol measured in 1 cm cut of the cigarette

Table 5. The levels of solanesol in exhaled smoke, the delivered levels to the smoker, the amount retained (all in µg/cig), and retention % for 5.0 mg ‘tar’ cigarette

Smoker ^a	Exhaled µg/cig	Delivered µg/cig	Amount retained µg/cig	Retention %
1	171.3	362.7	191.4	52.8
2	167.7	366.8	199.1	54.3
3	73.4	206.7	133.3	64.5
4	78.0	216.3	138.3	63.9
5	170.6	374.8	204.3	54.5
6	104.9	271.6	166.7	61.4
7	132.2	293.8	161.6	55.0
8	103.2	257.1	154.0	59.9
9	131.8	286.5	154.7	54.0
10	61.3	205.3	144.1	70.2
Average			164.7	59.0
RSD%			15.4	9.9

^a The group of ten smokers was different for each cigarette style.

Table 6. The levels of solanesol in exhaled smoke, the delivered levels to the smoker, the amount retained (all in µg/cig), and retention % for 10.6 mg tar cigarette

Smoker ^a	Exhaled µg/cig	Delivered µg/cig	Amount retained µg/cig	Retention %
1	193.4	481.7	288.4	59.9
2	168.4	509.9	341.5	67.0
3	189.4	500.1	310.7	62.1
4	97.9	262.9	165.1	62.8
5	151.2	538.7	387.5	71.9
6	140.5	456.8	316.3	69.2
7	184.4	464.3	279.9	60.3
8	98.5	279.2	180.8	64.7
9	151.7	534.0	382.3	71.6
10	141.2	461.2	319.9	69.4
Average			297.2	65.9
RSD%			25.0	6.5

^a See Table 5.

Table 7. The levels of solanesol in exhaled smoke, the delivered levels to the smoker, the amount retained (all in µg/cig), and retention % for 16.2 mg ‘tar’ cigarette

Smoker ^a	Exhaled µg/cig	Delivered µg/cig	Amount retained µg/cig	Retention %
1	128.8	431.2	302.3	70.1
2	71.0	343.6	272.7	79.3
3	127.1	337.9	210.8	62.4
4	132.4	334.9	202.5	60.5
5	72.2	391.4	319.2	81.5
6	121.8	425.0	303.2	71.4
7	105.8	333.1	227.3	68.2
8	187.7	594.9	407.2	68.4
9	171.1	576.9	405.7	70.3
10	104.8	532.6	427.8	80.3
Average			307.9	71.3
RSD%			27.0	10.1

^a See Table 5.

Table 8. *P*-values in ANOVA single factor analysis for 96% confidence level ($\alpha = 0.05$) of retention % for different cigarettes

Cigarette	10.6 mg ‘tar’	16.2 mg ‘tar’
5.0 mg ‘tar’	0.00906	0.00586
10.6 mg ‘tar’	—	0.06198

butts of each smoker (average from three cigarettes), and are given in Table 5 for the 5.0 mg ‘tar’ cigarette, in Table 6 for the 10.6 mg ‘tar’, and in Table 7 for 16.2 mg ‘tar’ cigarette. The equations used for these calculations (see Table 4) did not include the values for the FTC smoking regimens. The range of solanesol delivered to each smoker, also covered a wide range and again it can be noticed that cigarettes with different ‘tar’ levels showed considerable overlapping in the levels of solanesol.

From the levels of exhaled solanesol and that of calculated inhaled solanesol it was possible to calculate using Eqn. [1] the amount of solanesol retained by each smoker. The results are shown in each table for the three analyzed cigarettes. The amount of solanesol retained is the lowest for the 5.0 mg ‘tar’ product, and the highest for the 16.2 mg ‘tar’ product, although there is not much difference between the 10.6 mg ‘tar’ product and the 16.2 mg ‘tar’ product. The retention % of solanesol using Eqn. [2] is given in Table 5 for the 5.0 mg ‘tar’ cigarette, in Table 6 for the 10.6 mg ‘tar’, and in Table 7 for 16.2 mg ‘tar’ cigarette. As seen from these tables, solanesol retention % varies between 53% and 82%, from smoker to smoker including all cigarette styles, but some differences in the range can be noticed for cigarettes with different ‘tar’ level. For the 10.6 mg ‘tar’ cigarettes the retention was between 60% and 72%, for the 5.0 mg product the retention was slightly lower ranging between 53% and 70%, while for the 16.2 mg ‘tar’ product, the retention was slightly higher ranging between 62% and 82%. The results were further analyzed using single factor ANOVA technique. The results showing the *P*-values for 95% confidence level are given in Table 8. As seen from Table 8, statistical analysis of the retention % data show that the 10.6 mg ‘tar’ cigarette is not different from the 16.2 mg ‘tar’ product ($P > 0.05$). On the other hand, the retention % for the 5.0 mg ‘tar’ cigarette was statistically different from the other two products.

The results regarding the retention were very little affected if the calibration curves for the solanesol levels delivered to the smoker were obtained including the FTC regimen. Table 9 shows the comparison between the results obtained as previously described, and the results using calibrations including the data from FTC smoking regimen.

Since the smoking behavior (i.e. puff volume, puff frequency, puff duration, potential vent-blocking, and other various inhalation behaviors) vary considerably from one subject to another, this study provides only a limited ‘snapshot’ on the retention efficiency of solanesol from mainstream cigarette smoke, and the finding that the retention % from 16.2 mg ‘tar’ cigarette is higher than that of 10.6 mg ‘tar’ cigarette, which is slightly higher than that

Table 9. Comparison of the results regarding solanesol retention without using FTC smoking regimen data for the calibrations curves, and including FTC regimen data for the calibrations

Retention	5.0 mg 'tar'		10.6 mg 'tar'		16.2 mg 'tar'	
	Without FTC	Including FTC	Without FTC	Including FTC	Without FTC	Including FTC
Average	59.0	59.9	65.9	67.3	71.3	71.7
Minimum	52.8	53.8	59.9	61.5	60.5	61.1
Maximum	70.2	70.7	71.9	73.1	81.5	81.8

for 5.0 mg 'tar' cigarette may be valid only for the particular group of smokers participating in this study. Only a study involving a considerably larger number of subjects may clarify the question if this finding is valid for a larger population.

The values for the retention % of solanesol by human smokers as found in this study were in very good agreement with the data reported previously in the literature (5), with which a direct comparison is possible. The results for a 9.6 mg 'tar' cigarette with American blend were reported to be $(52.1 \pm 7.6)\%$ for 250 mL inhalation depth, $(68.0 \pm 7.5)\%$ for 500 mL inhalation depth, and $(71.9 \pm 5.9)\%$ for 1000 mL inhalation depth (5). The retention % levels described in other reports are more difficult to compare with the present data. Nevertheless, these previously reported data (5, 6) or referenced from meeting presentations (1) are in overall good agreement with the findings from this study.

CONCLUSIONS

This study reports the level of solanesol in exhaled cigarette smoke for three cigarettes with different 'tar' levels including a 5.0 mg 'tar', a 10.6 mg 'tar' and a 16.2 mg 'tar' product. The study was done on ten human subjects for each style of the analyzed cigarette. The human subjects were smokers of the respective brand and style. The results showed that the amount of solanesol retained is the lowest for the 5.0 mg 'tar' product, and the highest for the 16.2 mg 'tar' product, although there is no much difference between the 10.6 mg 'tar' product and the 16.2 mg 'tar' product. The solanesol retention % varies between about 53% and 82%, from smoker to smoker, but some differences in the range can be noticed for cigarettes with different 'tar' levels. The retention % from 16.2 mg 'tar' product being the highest and from the 5.0 mg 'tar' product the lowest. The results on retention % were in very good agreement with those previously reported in the literature. This present study extends the knowledge on solanesol retention % to cigarettes of different 'tar' level.

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