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Differences in the Chemical Composition of the Particulate Phase of Inhaled and Exhaled Cigarette Mainstream Smoke*

by

Serban C. Moldoveanu[1] *and F. Kelley St.Charles*[2]

[1]*R.J. Reynolds Tobacco Co., 950 Reynolds Boulevard, Winston-Salem, NC 27105, USA*
[2]*Consultant, 112 Raven Avenue, Perry, GA 31069, USA*

SUMMARY

In this study, a comparison between the chemical composition of the particulate-phase of exhaled smoke and that of smoke generated with a smoking machine has been performed. For this purpose, eight human subjects smoked a common Lights (10.6 mg 'tar/cig) commercial cigarette and the exhaled particulate-phase smoke from three cigarettes was collected on Cambridge pads for each smoker. The smoke collection from the human subjects was vacuum assisted. The cigarette butts from the smokers were collected and analyzed for nicotine. The machine smoking was performed with a Borgwaldt RM20 CSR smoking machine working under conditions recommended by the U.S. Federal Trade Commission (FTC). The nicotine levels for the cigarette butts from the smokers were used to normalize the level of exhaled smoke condensate to that of the FTC smoking conditions. The smoke condensates from exhaled smoke as well as that from the machine smoking were analyzed by a gas chromatographic technique with mass spectral peak identification. The retention efficiency for 160 compounds was calculated from the ratio of the compound peak areas in the exhaled smoke (normalized by the corresponding butt nicotine level) vs. the areas of the corresponding peaks from the chromatogram of the smoke generated by the smoking machine. In the calculation of the results, it was assumed that the composition of mainstream smoke remains practically constant at different smoking regimes. All compounds found in the machine-generated smoke were also present in the exhaled smoke, but at different levels. About one third of the compounds were retained more than 66% by the smoker. Another third of the compounds were retained between 33% and 66%, and the rest of the compounds were retained very little from the mainstream particulate-phase of the cigarette smoke. The compounds retained more than 66% were in general compounds with lower molecular weight and with higher water solubility, which eluted first from a 5% phenyl dimethyl-polysiloxane (DB-5MS) chromatographic column. The compounds retained less than 33% from smoke were those with higher molecular weights and boiling points, which had longer elution times from the chromatographic column. These compounds consisted mainly of long-chain hydrocarbons (saturated or squalene type) and phytosterol-type compounds. The compounds retained between 33% and 66% had intermediate chromatographic retention times. No attempt was made to evaluate or identify new compounds formed in the exhaled smoke. The results were obtained from a limited number of subjects, but among these the retentions for individual compounds did not show large differences, indicating that the retention process is not very different for the subjects evaluated. An attempt was made to verify whether or not the retention of compounds by the smoker is analogous to a distribution process. Only weak correlations were obtained between the human retention and octanol/water partition coefficients or between the human retention and the chromatographic retention times of individual compounds. [Beitr. Tabakforsch. Int. 2006 (22) 290–302]

ZUSAMMENFASSUNG

Diese Untersuchung vergleicht die chemische Zusammensetzung der Partikelphase von exhaliertem Rauch mit der von Rauch, der mit einer Rauchmaschine erzeugt wurde. Hierfür rauchten acht Testpersonen handelsübliche Light-Zigaretten (10,6 mg Kondensat), wobei die Partikelphase des exhalierten Rauches von insgesamt drei verschiedenen Zigarettenmarken auf Cambridgefiltern gesammelt wurde. Die Sammlung dieser Rauchproben erfolgte vakuumunterstützt. Die Zigarettenstummel der Raucher wurden gesammelt und auf ihren Nikotingehalt untersucht. Das maschinelle Abrauchen erfolgte mit einer Borgwaldt RM20

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CSR Rauchmaschine gemäß der Abrauchnormen der U.S. Federal Trade Commission (FTC). Auf der Basis des Nikotingehalts der von den Rauchern erhaltenen Zigarettenstummel wurde die Menge an exhaliertem Rauchkondensat auf FTC-Bedingungen standardisiert. Das Rauchkondensat des exhalierten und des von der Rauchmaschine erhalten Rauches wurde mittels Gaschromatographie und Massenspektrometrie analysiert. Die Retentionseffizienz für 160 Verbindungen wurde aus dem Verhältnis der Peakflächen der einzelnen Verbindungen im exhalierten Rauch (standardisiert auf den entsprechenden Nikotingehalt im Stummel) zu den entsprechenden Peakflächen im Chromatrogramm des Rauchmaschinen-generierten Rauches berechnet. Bei der Berechnung der Ergebnisse wurde angenommen, dass die Zusammensetzung des Hauptstromrauchs unter verschiedenen Abrauchbedingungen praktisch konstant bleibt. Alle im Rauchmaschinen-generierten Rauch gefundenen Verbindungen befanden sich ebenfalls im exhalierten Rauch, lagen jedoch in unterschiedlichen Konzentrationen vor. Ungefähr ein Drittel der Verbindungen wurde zu mehr als 66% vom Raucher reteniert. Bei einem weiteren Drittel der Verbindungen wurden zwischen 33% und 66% reteniert, der Rest der Verbindungen wurde zu einem geringen Anteil aus der Partikelphase des Hauptstromrauchs von Zigaretten reteniert. Die Verbindungen, die zu mehr als 66% aus dem Rauch reteniert wurden, waren hauptsächlich Verbindungen mit niedrigerem Molekulargewicht und höherer Wasserlöslichkeit, die zuerst von der Säule (DB-5MS) eluiert wurden. Die Verbindungen, die zu weniger als 33% aus dem Rauch reteniert wurden, besaßen ein höheres Molekulargewicht und höhere Siedepunkte und auch längere chromatographische Elutionszeiten. Diese Verbindungen bestanden vorwiegend aus langkettigen Kohlenwasserstoffen (gesättigte oder Squalen-ähnliche Verbindungen) und Phytostyrol-ähnlichen Verbindungen. Die chromatographischen Retentionszeiten der Verbindungen, die zu 33% bis 66% reteniert wurden, lagen dazwischen. Es wurde nicht versucht, neue Verbindungen, die im exhalierten Rauch gebildet wurden, zu charakterisieren oder zu identifizieren. Die Ergebnisse wurden mit einer begrenzten Zahl von Rauchern erhalten, bei denen die individuellen Retentionen einzelner Substanzen keine großen Unterschiede aufwiesen, was darauf hindeutet, dass bei den untersuchten Rauchern die Retention von Substanzen nicht sehr verschieden war. Es wurde der Versuch unternommen zu verifizieren, ob die Retention von Verbindungen beim Raucher sich analog zu einem Verteilungsprozess verhält. Es wurden nur schwache Korrelationen zwischen der Retention von Substanzen im menschlichen Organismus und den Oktanol/Wasser-Verteilungskoeffizienten oder den chromatographischen Retentionszeiten einzelner Verbindungen gefunden. [Beitr. Tabakforsch. Int. 22 (2006) 290–302]

RESUME

Cette étude compare la composition chimique de la phase particulaire exhalée par le fumeur avec celle de la fumée générée par une machine à fumer. Pour cela, huit fumeurs ont fumé une cigarette légère habituelle et la phase particulaire exhalée de trois types de cigarette de chacun des fumeurs a été recueillie sur des filtres Cambridge. La collecte de fumée des fumeurs humains a été effectuée par des dispositifs sous vide. Les mégots des cigarettes obtenus par les fumeurs ont été retirés et dosés pour leur teneur en nicotine. Le fumage a été réalisé sur machine à fumer Borgwaldt RM20 CSR en conditions normalisées de la Federal Trade Commission (FTC). Le rendement du condensat de la fumée exhalée a été normalisé selon les conditions FTC à la base de la teneur en nicotine des mégots retirés des fumeurs. Le condensat de la fumée exhalée et celui obtenu sur machine à fumer ont été analysés par chromatographie en phase gazeuse et spectrométrie de masse. L'efficacité de rétention de 160 composants a été calculée en fonction du ratio entre la surface des pics des composés de la fumée exhalée (normalisée en fonction de la teneur en nicotine correspondante dans le mégot) et la surface des pics correspondants de la fumée générée sur machine à fumer. Dans le calcul des résultats il a été supposé que la composition de la fumée principale reste constante sous les différents régimes de fumage. Tous les composés trouvés dans la fumée générée sur machine ont également été trouvés dans la fumée exhalée, mais sont présents en teneurs différentes. Un tiers environ des composés a été retenu de plus de 66% par le fumeur. Un autre tiers des composés a été retenu de 33% à 66% et les autres composés ont été retenus de façon réduite à partir de la phase particulaire de la fumée de cigarette. Les composés retenus de plus de 66% sont en général des composés de faible poids moléculaire et solubilité dans l'eau élevée, éluant le premier de la colonne chromatographique avec du phényldiméthyl-polysiloxane (DB-5MS). Les composés retenus de moins de 33% à partir de la fumée sont des composants de poids moléculaires et de points d'ébullition plus élevés ayant une élution de la colonne moins rapide. Ces composés sont surtout des hydrocarbures à longue chaîne (saturés ou de type squalène) et des composés de type phytostyrol. Les composés retenus de 33% à 66% ont un temps de rétention de la colonne intermédiaire. L'évaluation et l'identification des composés nouvellement formés dans la fumée exhalée n'ont pas été considérées dans l'étude. Les résultats ont été obtenus à partir d'un nombre limité de fumeurs, mais parmi ceux-ci il n'existent pas de grandes différences dans les temps de rétention des composés particuliers. Ceci indique que le processus de rétention ne diffère pas de façon importante chez les fumeurs examinés. Il a été également étudié si la rétention des composés par les fumeurs se trouve en analogie avec un processus de distribution. De faibles corrélations ont été observées entre la rétention humaine et les coefficients de partage entre le décanol et l'eau ou entre la rétention humaine et les temps de rétention chromatographique des composés particuliers. [Beitr. Tabakforsch. Int. 22 (2006) 290–302]

INTRODUCTION

Although the interest in the retention by the smoker of cigarette smoke constituents is about 100 years old (1), most of this interest has been focused on nicotine (2–7) and on whole particulate matter (8–14). As reported in an excellent recent review (15), very few published papers evaluated other individual compounds in exhaled smoke.

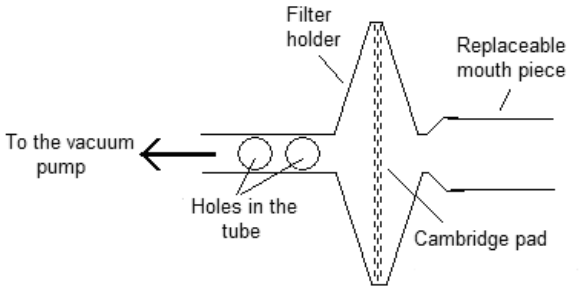

Figure 1. Schematic drawing of the device used for the collection of exhaled mainstream cigarette smoke

An early paper published in 1909 included ammonia and pyridine (8). One study published in 1951 reported the retention of nicotine, pyridine, ammonia, and of several groups of compounds such as aldehydes plus ketones, phenols, neutral substances, etc. (16). Another study published in 1959 evaluated nitrogen oxides (17), two studies published in 1968 evaluated acetaldehyde, acetone, acetonitrile, isoprene, toluene, and CO (13, 14), and another study published in 1970 evaluated the retention of inhaled acetaldehyde (18). More recently, the retention of nitric oxide was revisited in a paper published in 1995 (19) and a study published in 1989 evaluated phenol, triacetin, propylene glycol, 3-hydroxypyridine, neophytadiene, hydroquinone and glycerol (20). Two other studies evaluated solanesol (together with nicotine) (6, 7). Several older British American Tobacco (BAT) internal studies give results on three carbonyl compounds, sum of phenols, isoprene, and coumarin (15). On the other hand, much more attention has been given to breath analysis (21–28). Differences in the content in the breath between smokers and nonsmokers were reported for compounds such as benzene, 1,3-butadiene, and 2,3-dimethylfuran (21), prostaglandin E2 (22), nitrite (23), and NO (24). A considerable number of other studies evaluated the composition of breath related to illnesses such as asthma and chronic obstructive pulmonary disease (COPD) (25–28). One paper indicating in the title the analysis of exhaled tobacco smoke (29) evaluated in fact simulated environmental tobacco smoke, as a sum of sidestream smoke and exhaled smoke. The study reported herein is intended to generate further information regarding the chemical composition of the particulate-phase of exhaled cigarette smoke. However, given that smoking behaviors, i.e. puff volume, puff frequency, potential vent-blocking, inhalation behaviors are known to vary for an individual smoker, as well as from one subject to the another, the study provides a limited 'snapshot' of exhaled cigarette smoke composition. Also, a larger variety of cigarette styles and specific analytical techniques for particular classes of compounds would be desirable for a more in-depth evaluation of the quantitative composition of exhaled cigarette smoke.

EXPERIMENTAL

Smoke collection

The first step in the analysis of exhaled smoke is the smoke collection from the human subjects. For this purpose, a simple device schematically shown in Figure 1 has been used. The device consists of a 92-mm Cambridge holder and pad having at one opening a replaceable mouth piece (Atlantic Medical Solutions, Charlotte, NC 28217), and at the other opening being connected to a diaphragm vacuum pump, which aspirates 2.2 m^3/h (Vacuum brand GMBH, Wertheim, Germany). The tube connecting the pad holder to the pump has two large holes to the exterior, which can be covered with the fingers. When no smoke is exhaled, the holes in the tube to the vacuum pump are kept open such that air from the surrounding is aspirated by the pump without passing the Cambridge filter. During smoke exhalation, the smoker blows the smoke through the replaceable mouth piece. At the same time the holes in the tube are covered, 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 was used by eight human subjects selected to smoke their preferred brand.

The cigarette was a filter Lights commercial product of 83 mm, with American blend tobacco, 10.6 mg target 'tar' ['tar' is defined as total particulate matter (TPM) minus water and nicotine]. The cigarette had 10.4 mg CO yield, 0.680 g tobacco, 27 mm filter, and 32% ventilation. Each subject smoked three cigarettes within one hour, and the exhaled smoke was collected. The smoking was performed in an environment familiar to the smoker (office) with as little as possible change from typical conditions. The cigarettes were previously conditioned under U.S. Federal Trade Commission (FTC) recommendations (30). The cigarette butts from the smokers were collected for nicotine analysis.

Mainstream particulate-phase smoke from ten cigarettes was also generated and collected with a Borgwaldt RM20 CSR smoking machine working under FTC type conditions (30) i.e. 35-mL puff volume, 2-sec puff duration, 60-sec puff interval, calibration of the smoking machine to a specific 'tar' level. This machine-generated particulate-phase smoke was used for the comparison with the exhaled smoke.

Smoke analysis

The particulate-phase from each pad was extracted with 25 mL acetonitrile, for 30 min on a mechanical shaker. The solution from the exhaled smoke was added in three aliquots of 5 mL onto 400 mg Tenax® (Tenax® GC, 35/60 mesh, Alltech, Deerfield, Illinois 60015), and gently evaporated after each addition. The evaporation was done at 40 ˚C under a current of nitrogen and lasted about 15 min for each aliquot. The study was done exclusively for particulate-phase compounds from smoke, and the very volatile compounds that may evaporate in these conditions were not of immediate interest. The solution from the machine-smoke pad was diluted in the ratio 3:10 to account for the difference in the number of cigarettes smoked by the human and by the machine and was further processed identically to the solution from the exhaled smoke.

Table 1. GC-MS operating parameters [a]

Parameter	Description	Parameter	Description
GC column	DB-5MS	Carrier gas	Helium
Column dimensions	60 m long, 0.32 mm i.d.	Flow mode	Constant flow
Film thickness	0.50 mm	Flow rate	1.1 mL/min
Initial oven temperature	37 ˚C	Nominal initial pressure	4.88 psi
Initial time	4.0 min	Purge valve off	1 min
Oven ramp rate	2 ˚C/mm	Split flow	20.0 mL/min
Oven final first ramp	60 ˚C	GC outlet	MSD
Final time first ramp	0 min	Outlet pressure	Vacuum
Oven ramp rate	5 ˚C/mm	MSD transfer line heater	300 ˚C
Oven final temperature	320 ˚C	Ion source temperature	230 ˚C
Final time	20 min	Quadrupole temperature	150 ˚C
Total run time	87.5 min	MSD EM offset	200 V
Inlet temperature	310 ˚C	MSD solvent delay	2.0 min
Inlet mode	Splitless	MSD acquisition mode	TIC
Injection type	Desorption from 5 mg Tenax®	Mass range	35 amu – 550 amu

[a] Abbreviations: MSD = mass selective detector; MSD EM = mass selective detector electron multiplier; TIC = total ion chromatogram.

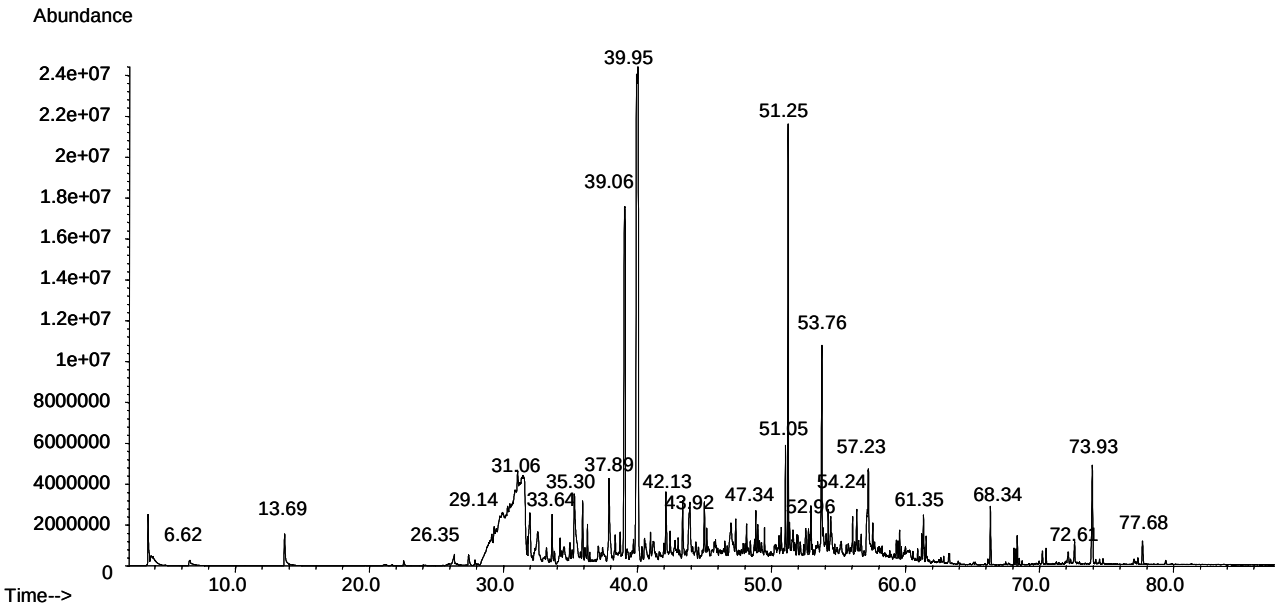

Figure 2. Chromatogram of the particulate-phase machine generated mainstream smoke

The smoke deposited on 5 mg of Tenax® was desorbed at 340 ˚C for 30 sec into a gas chromatographic-mass spectrometric (GC-MS) instrument for analysis. The desorption was done with a Pyroprobe 1000 insert with a platinum coil (CDS Analytical, Oxford, PA 19363) interfaced to the GC injection port. The GC-MS instrument was an Agilent 6890/5973 system (Agilent, Wilmington, Delaware 19808). The parameters for the analysis are given in Table 1.

The desorption procedure used to load the sample into the GC-MS system was preferred to the solution injection for several reasons. One reason was that the relatively high dilution of the pad extract did not produce chromatographic peaks of acceptable intensity. The use of 5 mg Tenax® for desorption allowed the transfer to the GC-MS system of considerably more material than in 1 µL or 2 µL extracting solution, which can be injected in a GC system. Therefore, the analysis was extended to compounds in low concentration in smoke. Another reason was the intent to protect the injection port and the column of the GC instrument from loading compounds soluble in acetonitrile but not volatile. These compounds may accumulate in the GC injection port and generate decomposition products that will potentially interfere in the chromatographic separation.

With the previously described conditions, a chromatogram of the particulate-phase extract as shown in Figure 2 was obtained for the machine-smoked cigarettes, and as shown in Figure 3 for exhaled smoke. The glycerol peak (at 31.06 min) and those of a few other compounds, obtained with the chromatographic column used in this study, have poor shapes. However, the chosen column allows heating up to 320 ˚C without considerable bleed and therefore, the detection of high boiling point compounds can be easily

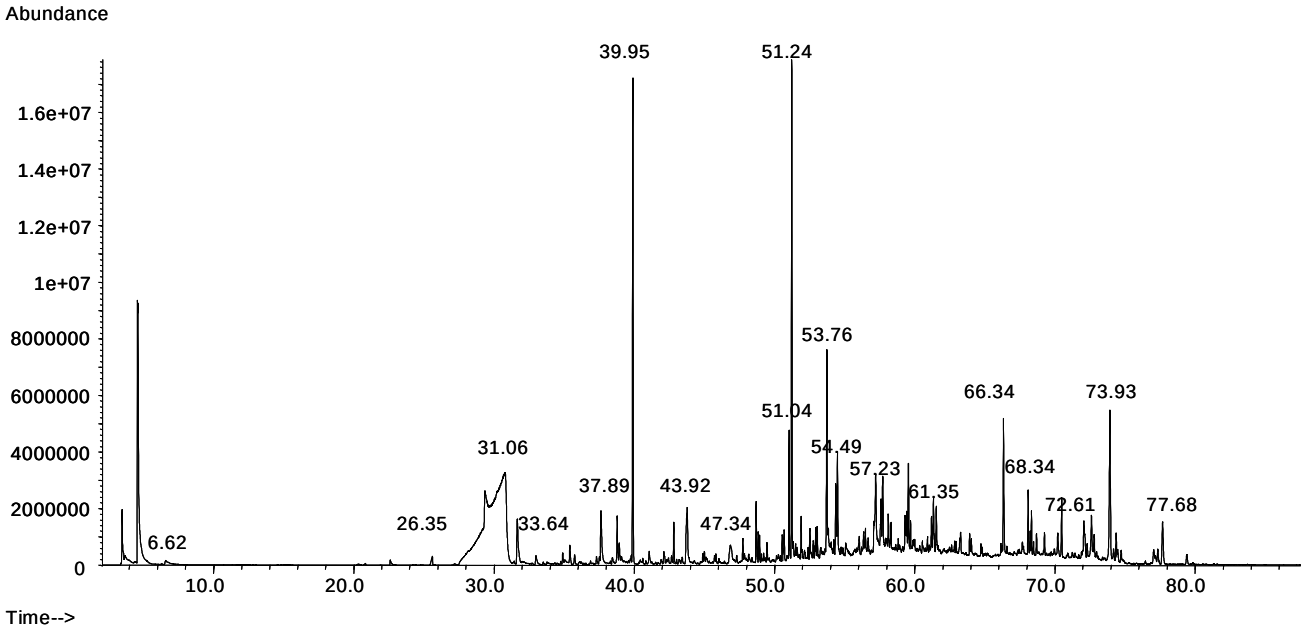

Figure 3. Chromatogram of the particulate-phase from exhaled cigarette smoke

achieved. This is not possible with other columns that generate better peak shapes for glycerol (such as Carbowax type columns). The peak areas from the chromatograms were integrated with the aid of the data processing capability of the mass spectral instrumentation. Peaks having an area exceeding a specified threshold level were identified by mass spectral library searches with Wley275 and NIST'98 libraries. No attempts were made to obtain further verification of peak identity.

Determination of nicotine in the cigarette butts

One additional analysis performed for this study was that of cigarette butts for nicotine. For the analysis of butt nicotine, the smoked butts were collected and cut into 1-cm lengths. The 1-cm mouth portions of the three cigarettes from each smoker were extracted with 25 mL methanol containing an internal standard (dodecanol). The level of nicotine was measured by a standard procedure (31) within one day from collection. Other compounds were not measured in the cigarette butts.

RESULTS AND DISCUSSION

The first step in this study was the identification of the compounds from the smoke condensate by the gas chromatographic separation and mass spectral peak identification. The compounds identified in the particulate-phase smoke from the commercial Lights cigarette evaluated in this study are listed in Table 2. These compounds were identified initially in the smoke collected from 10 cigarettes on a Cambridge pad from a smoking machine and then in the exhaled-smoke condensate. The number of compounds in smoke is known to be considerably higher than those reported, but the experimental conditions in the study allowed the detection of only a limited number of compounds. Some of the compounds known to be present in cigarette smoke were not detected either because their volatility was different from that of the compounds seen in the chromatogram, or because their level in smoke was too low to assure detection. Some compound identifications by the mass spectra were certain, while other identifications were only tentative. The compounds tentatively identified are denoted in Table 2 by a question mark (?) following the compound name. A few compounds identified only by their mass spectrum were not previously reported to be present in cigarette smoke and are denoted in Table 2 by an asterisk (*). Their identification is also questionable. However, the questionable compounds were not eliminated from Table 2 since they were used to provide information regarding the correlation between the retention of smoke components in a chromatographic column and the retention by the human subjects. All compounds found in the machine-generated smoke were also present in the exhaled smoke, but at different levels.

The comparison between the machine smoke and the exhaled smoke was done by a normalization of the quantity of exhaled smoke condensate to that of FTC smoking conditions. For the cigarettes evaluated in this study the average level of nicotine obtained under machine smoking in FTC conditions was found to be 0.186 mg/(cig butt). The results for butt nicotine measurements from various smokers are given in Table 3. These results were used to generate the normalization factors, which were obtained as the ratio of butt nicotine level from the smoker vs. butt nicotine for the cigarettes machine smoked under FTC conditions.

The nicotine in the cigarette butt (1 cm from the mouth end) has a linear dependence on the amount of nicotine collected on the Cambridge pad as previously reported (32, 33). This correlation also has been verified for the cigarettes used in this study, for various smoking condi-

Table 2. Compounds identified in the particulate-phase mainstream smoke of a Lights cigarette listed in the order of their retention times on a DB-5 type chromatographic column [a]

No.	Compound	MW	CAS Reg. No.	Ret. time	Ave. ret. %
1	Acetic acid	60	64-19-7	6.62	75.8
2	Hexane, 3-methyl-	100	589-34-4	9.80	74.8
3	Heptane	100	142-82-5	11.08	73.2
4	Cyclohexane, methyl-	98	108-87-2	12.71	74.2
5	1,2-Propanediol (propylene glycol)	76	57-55-6	13.69	98.9
6	Butanoic acid, 2-methyl-	102	116-53-0	21.70	73.9
7	2-Cyclohexen-1-one	96	930-68-7	22.54	52.2
8	2,5-Cyclohexadiene-1,4-dione (*p*-benzoquinone)	108	106-51-4	24.70	74.4
9	Pentanoic acid, 3-methyl- (β-methylvaleric acid)	116	105-43-1	26.35	71.7
10	Phenol	94	108-95-2	27.41	96.5
11	Benzene, 1,2,4-trimethyl- (pseudocumene)	120	95-63-6	27.89	77.5
12	2-Cyclopenten-1-one, 2-hydroxy-3-methyl- (methylcyclopentenolone)	112	80-71-7	29.14	74.9
13	Cyclohexene, 1-methyl-4-(1-methylethyl)-, R- (*d*-limonene)	136	5989-27-5	29.32	47.7
14	Phenol, 2-methyl- (*o*-cresol)	108	95-48-7	30.32	98.5
15	3(2*H*)-Furanone, 4-hydroxy-2,5-dimethyl (furaneol)	128	3658-77-3	30.49	99.3
16	Phenol, 4-methyl- (*p*-cresol)	109	106-44-5	31.03	98.7
17	1,2,3-Propanertiol (glycerol)	92	56-81-5	31.06	52.4
18	Phenol, 2-methoxy- (guaiacol)	124	90-05-1	31.34	74.5
19	1,2,3-Propanetriol, monoacetate (monoacetin)	134	26446-35-5	31.81	54.3
20	1,2,3-Propanetriol, diacetate (diacetin)	176	25395-31-7	31.99	88.1
21	3- Pyridinol	95	109-00-2	32.57	95.4
22	2,5-Pyrrolidinedione (succinimide)	99	123-56-8	33.22	81.6
23	4*H*-Pyran-4-one, 2,3-dihydro-3,5-dihydoxy-6-methyl-	144	28564-83-2	33.64	91.0
24	2,4-Imidazolidinedione, 5-ethyl- (5-ethylhydantoin)	128	15414-82-1	33.82	74.7
25	Phenol, 4-ethyl-	122	123-07-9	34.23	92.1
26	2*H*-Pyran-2-one, 5,6-dihydro-6-propyl-	140	16400-69-4	34.36	86.1
27	Benzoic acid	122	65-85-0	34.54	98.7
28	4*H*-Pyran-4-one, 5-hydroxy-2-methyl-	126	644-46-2	34.97	99.4
29	2(3*H*)-Furanone, dihydro-5-propyl- (γ-heptalactone)	128	105-21-5	35.14	75.5
30	1,2-Benzenediol (catechol)	110	120-80-9	35.30	93.2
31	Benzofuran, 2,3-dihydro- (coumaran)	120	496-16-2	35.92	90.1
32	2-Furancarboxaldehyde, 5-hydroxymethyl-	126	67-47-0	36.10	53.1
33	Propanetriol diacetate isomer ?	176	?	36.28	89.0
34	Phenol, 3-ethyl-5-methyl- (5-ethyl-*m*-cresol)	136	698-71-5	36.45	99.0
35	Benzeneacetic acid (phenylacetic acid)	136	103-82-2	37.09	99.5
36	1,2-Benzenediol, 4-methyl- (4-methylcatechol)	124	452-86-8	37.41	98.6
37	1,4-Benzenediol (hydroquinone)	110	123-31-9	37.89	82.9
38	1*H*-Indole	117	120-72-9	38.36	70.4
39	Ethanone, 1-(2-hydroxy-5-methylphenyl)-	150	1450-72-2	38.72	98.5
40	Pentanedioic acid, 3-methyl-	146	626-51-7	38.89	99.4
41	1,2,3-Propanetriol, triacetate (triacetin)	218	102-76-1	39.06	98.8
42	Pyrrolidine, diethyl-	125	71607-78-8	39.23	98.5
43	Phenol, 2,6-dimethoxy- (syringol)	154	91-10-1	39.71	80.4
44	Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (*S*)- (nicotine)	162	54-11-5	39.95	84.6
45*	1,2-Benzenediol monoacetate	152	102-29-4	40.34	84.4
46	1,2-Benzenediol, 4-ethyl-	138	1124-39-6	40.54	89.0
47	1,4-Benzenediol, 2,3-dimethyl-	138	608-43-5	40.62	82.3
48	1*H*-Indole, 2-methyl-	131	95-20-5	40.98	74.5
49	Benzaldehyde, 4-hydroxy-3-methoxy- (vanillin)	152	121-33-5	41.21	89.6
50	Anhydrosugar	162 ?		41.27	71.1
51	Benzeneethanol, 3-hydroxy- (3-hydroxyphenethyl alcohol)	138	13398-94-2	41.99	98.7
52	Pyridine, 3-(3,4-dihydro-2*H*-pyrrol-5-yl)- (myosmine)	146	532-12-7	42.13	89.1
53	Benzene, 4-ethenyl-1,2-dimethoxy-	164	6380-23-0	42.44	81.7
54	Cyclohexanone, 2-methyl-5-(1-methylethenyl)- (dihydrocarvone)	152	7764-50-3	42.81	81.9
55	Unknown	160 ?		43.04	85.4
56	Pyridine, 3-(1-methyl-1*H*-pyrrol-2-yl)- (nicotyrine)	158	487-19-4	43.38	77.9
57	β-D-Glucopyranose, 1,6-anhydro- (levoglucosan)	162	498-07-7	43.92	71.7
58	Unknown	182 ?		44.13	98.8
59*	Pyrimidine, 5-hydroxy-4-phenyl-	172	88070-43-3	44.37	98.8
60	1-Naphthalenol (1-naphthol)	144	90-15-3	44.56	98.7
61	2,3'-Bipyridine (2,3'-dipyridyl)	156	581-50-0	45.00	74.7
62*	Benzaldehyde, 2,5-dimethoxy-4-methyl-	180	4925-88-6	45.18	79.7
63	Mixture			45.75	68.7
64	1,2-Benzenedicarboxylic acid, diethyl ester (diethyl phthalate)	222	84-66-2	45.83	69.0

Table 2 (*cont.*)

No.	Compound	MW	CAS Reg. No.	Ret. time	Ave. ret. %
65	2-Buten-1-one, (1-(3-hydroxy-2,6,6-trimethyl-1-cyclohexen-1-yl)- (3-hydroxy-β-damascone)	208	102488-09-5	46.53	84.0
66*	1*H*-midazole, 1,4-dimethyl-5-phenyl-	172	1131-16-4	46.70	98.7
67	Mixture			46.99	75.7
68	α-Ionol, 3-oxo-	208	N/A	47.15	99.8
69	2-Cyclohexen-1-one,4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl- [R-[R*,R*-(E)]]	208	52210-15-8	47.34	88.3
70	Dodecanol	186	112-53-8	48.05	39.3
71	Ethanone, 1,1',1''-(1,3,5-benzenetriyl)tris- + 2-phenylphenol	204 + 170	779-90-8	48.16	67.9
72	Phenol, 2,6-dimethoxy-4-(2-propenyl)-	194	6627-88-9	48.44	98.8
73	2-Cyclohexen-1-one,4-(3-hydroxybutyl)-3,5,5-trimethyl- [(9R)-9-hydroxy-4-megastigmen-3-one]	210	36151-02-7	48.75	9.1
74*	Cycloundecane, 1,1,2-trimethyl-	196	62376-15-2	48.84	61.7
75	2-Pyrrolidinone, 1-methyl-5-(3-pyridinyl)- (cotinine)	176	486-56-6	48.99	71.1
76*	1*H*-Indole, 1,3-dibutyl-	186	55191-12-3	49.12	18.7
77*	1,1'-Biphenyl-2,3-diol	186	1133-63-7	49.27	82.9
78	Tetradecanoic acid (myristic acid)	228	544-63-8	49.49	65.8
79	Cyclohexane, 1,5-diisopropyl-2,3-dimethyl-	196	N/A	50.23	72.1
80	1,4-Naphthalenedione, 2,3,6-trimethyl-	200	20490-42-0	50.35	43.6
81*	Phenol, 3-methoxy-, acetate	166	5451-83-2	50.56	73.7
82	1-Pyrrolidinecarboxaldehyde, 2-(3-pyridinyl)-, (*S*)- (*N*-formylnornicotine)	176	3000-81-5	50.73	68.5
83*	Propanoic acid, 3-(ethoxycarbonyl)-3-(1-cyclohexenyl)- ?	226	82546-67-6	51.05	73.7
84	1-Hexadecene, 3-methylene-7,11,15-trimethyl- (neophytadiene)	278	504-96-1	51.25	45.7
85	2-Pentadecanone, 6,10,14-trimethyl- (hexahydrofarnesylacetone)	268	502-69-2	51.36	72.9
86	2-Cyclohexen-1-one, 4-(1,3-butadienyl)-3,5,5-trimethyl- (E)-, (megastigmatrienone)	190	38818-55-2	51.52	1.2
87	2,3-Naphthalenediol, 1,2,3,4,5,6,7,8-octahydro-1-methyl-7-(1-methylethenyl)- (rishitin)	222	18178-54-6	51.62	99.1
88	1,2-Benzenedicarboxylic acid, butyl-, 2-ethylhexyl ester	334	85-69-8	51.91	1.0
89	Phytol type	296	150-86-7 ?	52.15	63.1
90	1*H*-Purine-2,6-dione, 3,7-dihydro-3,7-dimethyl- (theobromine)	180	83-67-0	52.56	67.0
91	Hexadecanone	240	18787-63-8	52.77	63.7
92	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl- (farnesol isomer A)	222	N/A	52.96	84.3
93	Hexadecanoic acid, methyl ester	270	112-39-0	53.03	1.4
94	1,4-Pentanediol, 3-[1S,2S]-2-(2-hydroxyethyl)-1,3,3-trimethyl-cyclohexyl-	272	N/A	53.32	43.0
95*	Benzoin acetate	254	574-06-1	53.45	99.6
96	*n*-Hexadecanoic acid (palmitic acid)	256	57-10-3	53.76	60.5
97	Pyrrolo[1,2a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	210	5654-86-4	53.87	42.2
98	Benzofuran-2,3-dione, 4,7-dimethyl-2,3-dihydro-	176	31297-30-0	54.09	23.6
99*	Octadecadiynoic acid, methyl ester	290	56847-03-1	54.24	82.0
100	Hexadecanoic acid, ethyl ester (ethyl palmitate)	284	628-97-7	54.37	0.6
101	2*H*-1-Benzopyran-2-one, 7-hydroxy-6-methoxy- (scopoletin)	192	92-61-5	54.42	9.1
102	Eicosane	282	112-95-8	54.50	19.1
103	9*H*-Pyrido[3,4-*b*]indole, 1-methyl- (harman)	182	486-84-0	54.73	60.1
104	9*H*-Pyrido[3,4-*b*]indole (norharman)	168	244-63-3	55.14	23.4
105	Dioxobenzofuran, 2,3-dihydro-4,6,7-trimethyl-	190	31297-33-3	55.21	99.0
106	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl- (farnesol)	222	4602-84-0	55.58	86.0
107	Propanedioic acid, (phenylmethyl)-, diethyl ester ? (diethyl benzylmalonate)	250 ?	607-81-8	55.74	82.0
108	4,8,13-Cyclotetradecatriene-1,3-diol, 1,5,9-trimethyl-12-(1-methylethyl)- (4,8,13-duvatriene-1,3-diol, isomer 1)	306	7220-78-2	56.06	76.4
109*	4*H*-Pyrido[1,2-*a*]pyrimidine-3-acetic acid, 9-hydroxy-4-oxo-, ethyl ester	248	50609-61-5	56.21	61.9
110	4,8,13-Cyclotetradecatriene-1,3-diol, 1,5,9-trimethyl-12-(1-methylethyl)- (4,8,13-duvatriene-1,3-diol, isomer 2)	306	57605-80-8	56.37	83.0
111	9,12,15-Octadecatrienoic acid, methyl ester (methyl linolenate)	292	301-00-8	56.51	21.1
112	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl- (phytol)	296	150-86-7	56.70	68.5
113	9,12-Octadecadienoic acid (linoleic acid)	280	60-33-3	57.06	74.8
114	9-Octadecenoic acid (oleic acid)	282	112-80-1	57.17	77.0
115	9,12,15-Octadecatrienoic acid (Z,Z,Z) (α-linolenic acid)	278	463-40-1	57.23	30.0
116	Octadecanoic acid (stearic acid)	284	57-11-4	57.56	4.1
117	1-Docosene	308	1599-67-3	57.61	1.7
118	Ethyl oleate	310	111-62-6	57.70	1.6
119	Octadecanoic acid, ethyl ester (ethyl stearate)	312	111-61-5	58.12	1.0
120	Docosane	310	629-97-0	58.32	3.3
121	2,7,11-Cyclotetradecatrien-1-ol, 4-(1-methylethyl)-1,7,11-trimethyl- (isocembrol)	290	25269-17-4	58.83	0.8

Table 2 (*cont.*)

No.	Compound	MW	CAS Reg. No.	Ret. time	Ave. ret. %
122	Hexadecanoic acid, 2-hydroxy-1-(hydroxy-methyl)ethyl ester (2-monopalmitin)	330	23470-00-0	59.31	13.3
123	Phytosterol-type		N/A	59.44	3.9
124	1,1',3'-Terphenyl-2'-ol	246	2432-11-3	59.57	4.4
125	Farnesol type	288	N/A	59.71	5.4
126	Tricosane	324	638-67-5	60.01	1.1
127	2-Eicosanol	298	4340-76-5	60.92	23.0
128*	4,8,12,16-Tetramethylheptadecan-4-olide ?	324 ?	96168-15-9	61.06	1.5
129	2,6,10,14,18-Eicosapentaene, 2,6,10,14,18- pentamethyl-?	342	75581-03-2	61.21	5.1
130	2,6,10,14,18-Eicosapentaene, 2,6,10,14,18- pentamethyl- isomer	342	N/A	61.35	18.1
131*	1,3-Isobenzofurandione, 5-(1,1-dimethyl-1-heptyl)- ?	274 ?	N/A	61.55	36.5
132*	2,2':6',2"-Terpyridine	233	1148-79-4	62.65	10.5
133	2,6,10,14,18-Eicosapentaene, 2,6,10,14,18- pentamethyl- isomer	342	N/A	62.85	1.4
134	Pentacosane	352	629-99-2	63.28	3.1
135	1,2-Benzenedicarboxylic acid, diisooctyl ester	390	27554-26-3	63.93	1.4
136	Heptacosene ?	378	N/A	64.05	1.0
137	Docosanoic acid, ethyl ester (ethyl behenate)	368	5908-87-2	64.74	27.4
138	Sterol type	400		6615	28.3
139	Nonacosane	408	630-03-5	66.34	2.4
140	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-	410	111-02-4	68.09	0.6
141	2,6,10,14,18-Eicosapentaene, pentamethyl-2,6,10,14,18- isomer	342	N/A	68.24	0.9
142	Farnesol type	424	N/A	68.34	6.0
143	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl- isomer	410	111-02-4	68.49	36.1
144	Hentriacontane	436	630-04-6	68.70	4.5
145	Hentriacontane isomer	436	N/A	69.27	1.3
146	Cholesta-3,5-diene (cholesterylene)	368	747-90-0	70.22	1.2
147	Dotriacontane	450	544-85-4	70.49	0.9
148	Cholest-4-en-3-ol, 4-methyl-, (3α)	400	96443-01-5	72.14	1.4
149	β-Tocopherol	416	148-03-8	72.30	14.3
150	Stigmasta-3,5,22-triene	394	81531-12-6	72.61	11.6
151	Dotriacontane isomer	450	N/A	72.80	1.6
152	α-Tocopherol	430	59-02-9	73.93	20.3
153	Sterol type	394	N/A	74.19	15.2
154	Tritriacontane	464	630-05-7	74.37	1.4
155	Cholest-5-en-3-ol (3β)- ? (cholesterol)	386	57-88-5	74.48	13.3
156	Sterol type	400	N/A	74.73	1.2
157	Ergost-5-en-3-ol, (3β,24R)- (campesterol)	400	474-62-4	77.05	1.4
158	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl- isomer	410	N/A	77.34	1.1
159	Stigmasta-5,22-dien-3-ol (3β,22R)- (stigmasterol)	412	83-48-7	77.68	1.2
160	Stigmasta-5-en-3-ol (3β)- (sitosterol)	414	83-47-6	79.41	1.0

[a] The compounds tentatively identified are denoted by a question mark (?) following the compound name. A few compounds identified only by their mass spectrum were not previously reported to be present in cigarette smoke and are denoted by an asterisk (*).

Table 3. Nicotine levels in the human-smoked cigarette butts and the corresponding correction factor used for the normalization to the condensate obtained with FTC conditions

Smoker	Butt nicotine in mg/cig	Correction factor
No. 1	0.179	1.0307
No. 2	0.177	1.0423
No. 3	0.318	0.5811
No. 4	0.202	0.9149
No. 5	0.242	0.7633
No. 6	0.207	0.8928
No. 7	0.180	1.0250
No. 8	0.241	0.7674

tions (FTC as well as with increased puff volume and frequency). The curve showing the dependence of mainstream nicotine level as collected on a Cambridge pad vs. butt nicotine generated for the studied cigarette is shown in Figure 4. The R^2 value for the linear dependence is 0.946 indicating a strong correlation. Also, the TPM on the pad had a linear correlation with the butt nicotine. The linear curve showing this dependence is given in Figure 5, the R^2 value being 0.950.

In the present study, all compounds in the smoke condensate were assumed to have a linear dependence on the level of nicotine in the cigarette butt (and consequently to the level of TPM on the pad). This linearity assumption is only an approximation, and its validity was evaluated for a number of compounds by machine smoking the cigarettes in three different regimes including FTC and two specific intensive smoking conditions. These intensive conditions used 2sec puff duration, 45-mL puff volume, and 30-sec puff interval (indicated as 45/30), and 2-sec puff duration, 60-mL puff volume, and 30-sec puff interval (indicated as 60/30). The smoking has been performed in duplicate and the TPM average values for the three smoking regimes were 12.4 mg (FTC), 26.9 mg (45/30) and 36.8 mg (60/30).

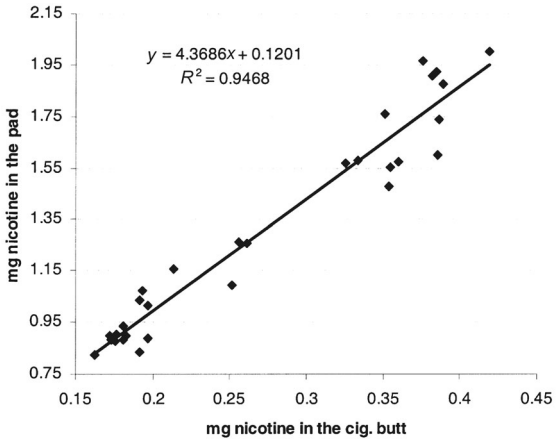

Figure 4. Nicotine levels on the Cambridge pad as a function of the nicotine level in the cigarette butt for the Lights commercial cigarette evaluated in this study

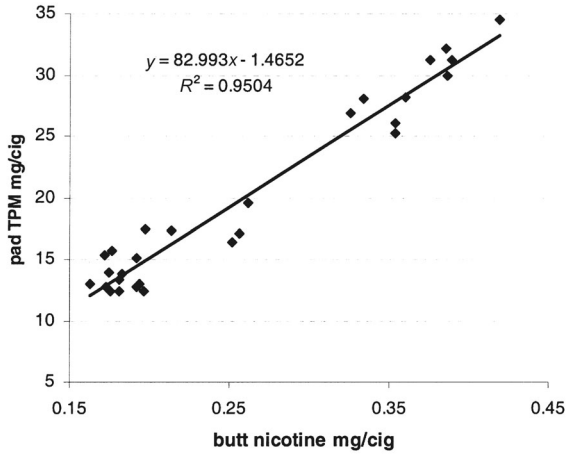

Figure 5. Cambridge pad total particulate matter (TPM) as a function of the nicotine level in the cigarette butt for the Lights commercial cigarette evaluated in this study

The ratio of TPM values for 45/30 vs. FTC was found to be 2.17 and that for 60/30 vs. FTC was 2.97. The ratios of the peak areas of corresponding compounds in the chromatograms obtained by the two intensive smoking regimes vs. FTC smoking were further calculated and are shown in Figure 6 (for selected compounds). As seen in Figure 6 the ratios for the two intensive smoking regimes vs. FTC smoking are very close to the corresponding TPM ratios. Within the expected variability, this indicates that the majority of the evaluated compounds increase in a linear manner with the TPM. Similar results regarding the linear dependence (with a positive slope) of the level of various compounds on pad nicotine (and on 'tar' levels where 'tar' = TPM – nicotine – water) have been found in the 1999 Massachusetts Benchmark Study (34). The results in the Massachusetts Benchmark Study were generated from a variety of cigarette brands smoked under Massachusetts recommended conditions, and not from the same cigarette smoked in different smoking regimes. Also, not all compounds from the particulate-phase showed linear dependence. Nevertheless, the results from the Massachusetts Benchmark Study provide additional confirmation for considering as a first approximation that the variation in the levels of many compounds from smoke condensate is proportional to that of nicotine yields and TPM levels.

One other question regarding exhaled smoke composition is related to the variation of retention as a function of human smoking characteristics, such as inhalation volume and breath-hold duration. The influence of these factors has been only recently reported for nicotine and solanesol (7), although a few older studies not published in the peer-reviewed literature existed (15). From the results previously reported (7), the ratio (%) for the retention of nicotine vs. solanesol as a function of inhalation volume does not vary

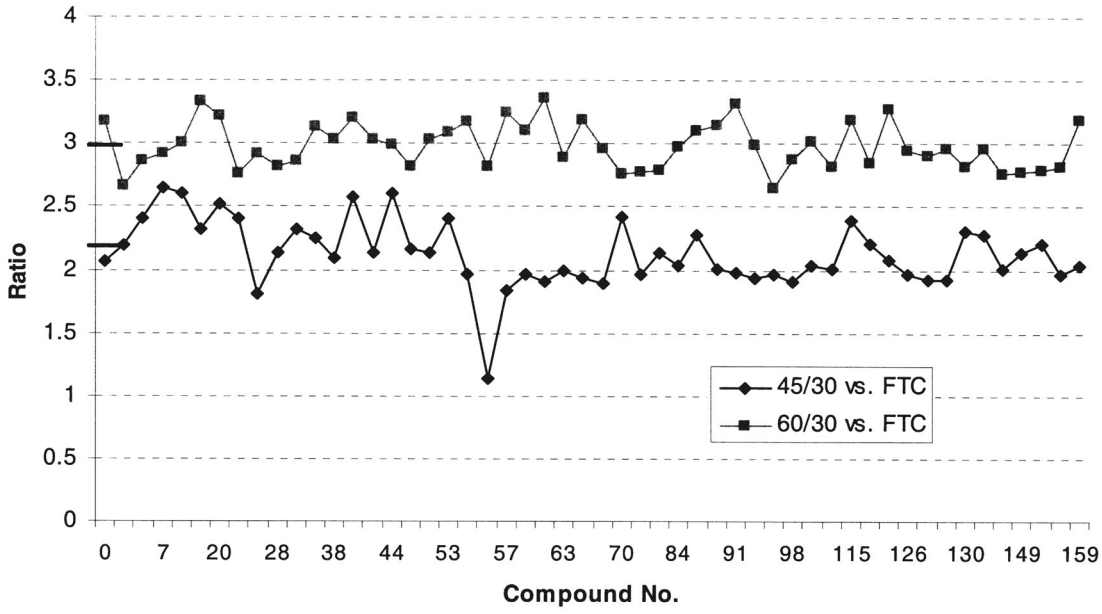

Figure 6. The ratios of the peak areas of corresponding compounds in the chromatograms obtained using 45/30 vs. FTC and 60/30 vs. FTC smoking regimes. The ratios for the TPM values of 45/30 vs. FTC and of 60/30 vs. FTC smoking regimes are indicated as bars at the beginning of each plot.

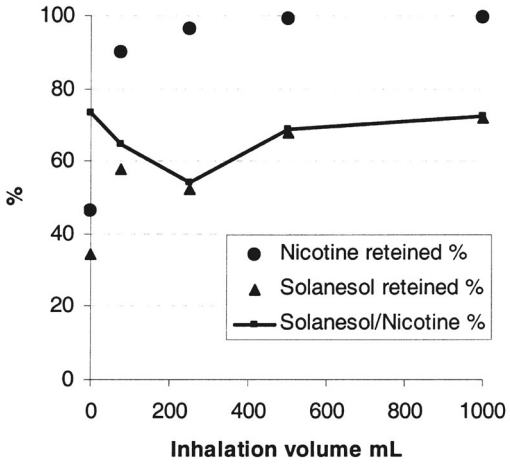

Figure 7. Results reported in the literature (7) regarding the retention of nicotine and solanesol as a function of inhalation volume and the ratio (%) of the two retention values

significantly beyond a certain inhalation volume. This is shown in Figure 7 based on literature data. Breath-hold duration seems to influence the retention of different compounds more, as shown for nicotine and solanesol (7). In the present study, the inhalation volume and breath-hold duration were not monitored. The smoking was done with as little as possible change from typical for each smoker, and the variability in the results obtained in the present study may reflect in part this type of variation.

The comparison of the chemical composition of the particulate-phase of exhaled mainstream smoke with that of smoke collected with a Borgwaldt RM20 CSR smoking machine working under FTC conditions is shown in Figures 8a and 8b. The results are presented as retention percentage. The retention percentage was calculated from peak area ratios in exhaled smoke (corrected with the factor shown in Table 3) vs. machine smoke for each compound analyzed, using the expression:

$$\text{Retention \%} = (1 - \text{Peak area exhaled/Peak area machine smoked}) \times 100$$

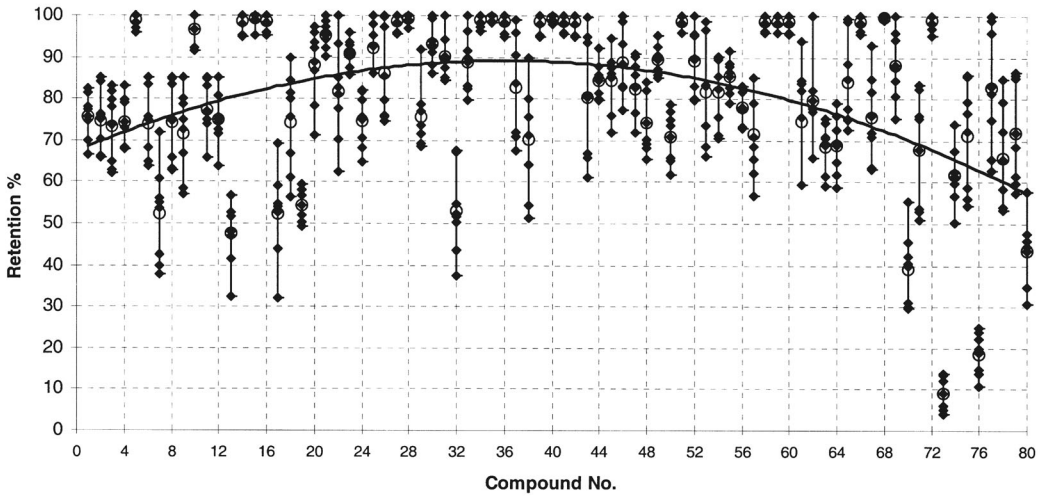

Figure 8a. Retention of mainstream smoke components by eight human subjects as obtained by comparing exhaled smoke composition (normalized to butt nicotine for FTC smoking) with that of machine-generated smoke (first 80 compounds from Table 2.) The eight points for each compound represent different smokers. The trendline is generated for the average points (shown as a circle)

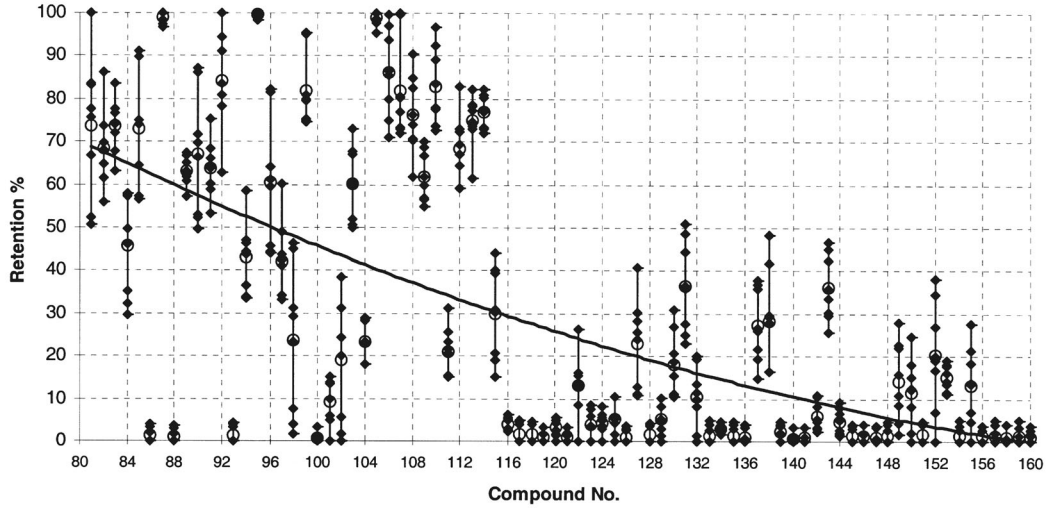

Figure 8b. Retention of mainstream smoke components by eight human subjects as obtained by comparing exhaled smoke composition (normalized to butt nicotine for FTC smoking) with that of machine-generated smoke (last 80 compounds from Table 2.) The eight points for each compound represent different smokers. The trendline is generated for the average points (shown as a circle)

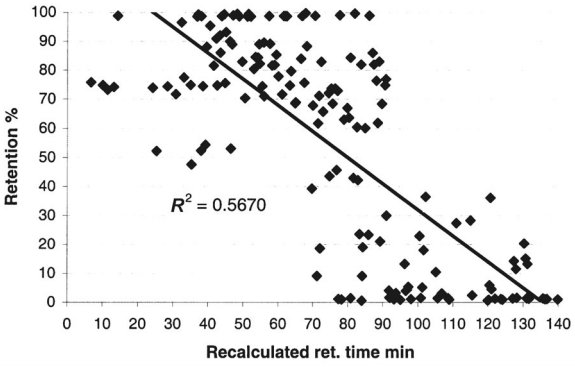

Figure 9. The dependence between the retention of smoke components by the human subjects and the recalculated chromatographic retention time (on a DB5 column) of several compounds from smoke

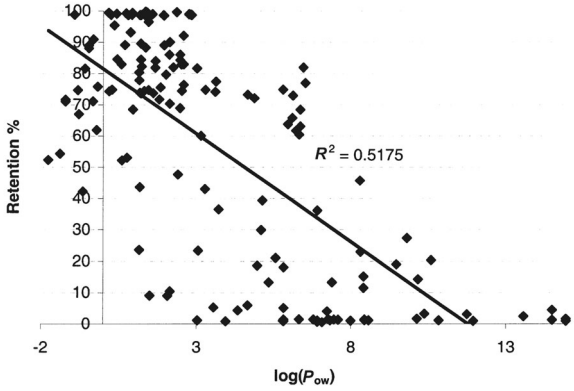

Figure 10. The dependence between the retention of smoke components by the human subjects and the partition coefficients log(P_{ow}) of several compounds from smoke

The compounds are arranged in the order of their elution from the chromatographic column, the numbers on the *x*-axis in Figures 8a and 8b correspond to the compound number given in Table 2. Eight values, corresponding to each smoker, were generated for each compound, and the graphs show also the average values (as points). The averages of the retentions for each compound are also given in Table 2. For some compounds the differences between the average value and the extremes were less than 5%. However, the typical variability in the chromatographic analysis in this study is around 5% as found by performing repeated chromatographic runs from the same sample (data not presented).

Figures 8a and 8b show a very interesting pattern regarding the retention of the compounds from cigarette smoke. As seen in these figures, about one third of the compounds were retained by more than 66%. These were in general lower molecular weight compounds, which were eluted early from the chromatographic column. The trendline (for the average values) shows a slightly lower retention % for the earlier compounds with a maximum around the 35th compound. It is not clear if this is a real trend, or an artifact. If real, this trend may show that compounds with a higher propensity to stay in the vapor phase are not as well retained as other small molecules of more polar compounds with high water solubility. Although evaluated on a limited number of subjects, the results for individual compounds did not show a very large scatter, indicating that the retention process is not very different among the evaluated subjects.

Another third of the compounds were retained between 33% and 66%. These are compounds eluting in the mid range of the retention times for the chromatographic separation. They have lower volatility than the first group. The last third of the compounds are even less retained from smoke. They were heavier compounds, with higher boiling points, which were eluted late from the chromatographic column. This group includes mainly long-chain hydrocarbons (saturated or squalene type) and phytosterol-type compounds. Polycyclic aromatic hydrocarbons were at too low levels to be measured in this study.

The comparison of the retentions determined in this study with the data available in the literature shows very good agreement for most compounds. Nicotine shows in this study retention between 80% and 92% (84.6% middle point), which is in good agreement with the results reported in the literature (15). Also, the results for phenol in this report show a retention between 91% and 99% (95.6% middle point), which is in good agreement with the value of 100% reported in the literature (20). Excellent agreement was also obtained for the retention of triacetin with 98.7% as compared to the reported value of 99 ± 0.2% (20), propylene glycol with 98.9% as compared to 99 ± 0.4% (20), pyridinol with 95.5% as compared to 94 ± 5% (20), and for hydroquinone with 82.9% middle point retention as compared to reported 87 ± 10% (20). Disagreement with the data from the literature was noticed only for neophytadiene, which was found in this study to be retained at a level of 45.7% compared to the reported 92 ± 6% (20).

The study clearly shows that the retention of different compounds from cigarette smoke by human subjects differs from compound to compound. Although considerable variability was seen between the smokers, the same trend was found for all eight subjects. An attempt has been made to find similarity between the human retention of smoke components and the compounds retention in a chromatographic column. The separation in the chromatographic column is based on the compound distribution between the mobile phase and the stationary phase. The retention time on a DB-5 column (which is a dimethylpolysiloxane with 5% phenylpolysiloxane units) depends mainly on the boiling point of the compounds, and indirectly on the polarity of each compound (which affects the boiling point). The analogy between the retention of smoke components by the human subject and the chromatographic retention time is shown in Figure 9. A modification of the chromatographic retention times was performed to render all compounds as eluting at the same temperature (35). The graph from Figure 9 shows only a modest linear correlation (R^2 = 0.5670).

The second attempt was made to correlate the human retention efficiency of individual compounds to their partition coefficient between octanol and water (P_{ow}). The log(P_{ow}) values were either obtained from the literature (36) or calculated (37, 38). The graph showing the dependence of the retention on log(P_{ow}) is given in Figure 10. The linear correlation in this case is even weaker (R^2 = 0.5175) than that on retention times. These results indicated that human retention of smoke components is not a simple distribution process, but a complex one, depending on the nature of the

compounds and possibly on their concentration in smoke. The study of specific group of compounds with a larger number of subjects would generate more precise information on exhaled mainstream smoke composition.

CONCLUSIONS

A study has been performed to evaluate the retention of 160 compounds from mainstream cigarette smoke by eight human subjects, each smoking three cigarettes. The study showed that the retention of different compounds from cigarette smoke differs from compound to compound in a range from 5–10% to 90–100%. About one third of the evaluated compounds, including molecules with lower molecular weight and relatively good solubility in water were retained more than 66%. Another third of the measured compounds, which are found in the middle retention time range for a slightly polar chromatographic column are retained between 33% and 66%. The last group which has even longer retention times in the chromatographic column consists mainly of long-chain hydrocarbons and sterol-type compounds. This group of compounds was retained much less by the smokers. An attempt was made to verify whether or not the retention of compounds by the smoker is analogous to a distribution process. Only weak correlations were obtained between the human retention and octanol/water partition coefficients or between the human retention and the chromatographic retention times of individual compounds.

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Address for correspondence:

Serban Moldoveanu
R.J. Reynolds Tobacco Co.
950 Reynolds Boulevard
Winston-Salem, NC 27105
E-mail: moldovs@rjrt.com