

Published in final edited form as:

Tob Control. 2012 January ; 21(1): 44–48. doi:10.1136/tc.2010.042192.

Carcinogenic Tobacco-Specific *N*-Nitrosamines in U.S. Cigarettes -Three Decades of Remarkable Neglect by the Tobacco Industry

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Abstract

Modification of tobacco curing methods and other changes in cigarette manufacturing techniques could substantially reduce the levels of tobacco-specific nitrosamines (TSNA), a group of potent carcinogens, in cigarette smoke. In 1999, two major U.S. cigarette manufacturers stated their intent to move towards using tobaccos low in TSNA. Since there is no information available on current TSNA levels in tobacco of various cigarettes available in the U.S., we examined the levels of these carcinogens in currently marketed brands. Seventeen brands of cigarettes were purchased in April of 2010 from retail stores in Minnesota. In all brands, the sum of two potent carcinogenic TSNA – 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and *N*'-nitrosonornicotine – in cigarette filler averaged 2.54 (±1.05) µg/g tobacco. This value is virtually identical to the sum of these two carcinogens reported for the tobacco of a U.S. filtered cigarette in 1979. TSNA levels in smoke positively correlated with those in tobacco filler of the same cigarettes. We found no indication that any meaningful attempt was made to reduce or even control TSNA levels in the new varieties of the popular brands Marlboro and Camel introduced over the last decade. In light of the recently granted regulatory authority to the FDA over tobacco products, regulation of TSNA levels in cigarette tobacco should be strongly considered to reduce the levels of these potent carcinogens in cigarette smoke.

Keywords

cigarette tobacco; cigarette smoke; nitrosamines; analysis; carcinogens; smoking

Introduction

Among the multitude of tobacco toxicants and carcinogens, tobacco-specific *N*-nitrosamines (TSNA) are of particular concern due to their abundance and established carcinogenic potency. More than 3 decades ago, a number of studies conclusively demonstrated that TSNA were present in relatively high amounts in both unburned tobacco and tobacco smoke, and that two of these compounds – 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and *N*'-nitrosonornicotine (NNN) – are potent carcinogens in laboratory animals.¹

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Competing Interests: None

The accumulated scientific evidence eventually led to the classification of these compounds as human carcinogens by the International Agency for Research on Cancer (IARC).²

Studies focused on understanding the sources and mechanisms of TSNA formation in tobacco and cigarette smoke have demonstrated that these compounds are formed during tobacco processing^{3,4} and that TSNA levels in processed tobacco depend on tobacco type and nitrate content.⁵ Formation of additional amounts of TSNA may also occur during burning,⁶ however, the results of studies investigating the relative contribution of pyrosynthesis to the total amount of TSNA found in cigarette smoke have been inconsistent^{7,8}. Overall, a number of later studies demonstrate that the amounts of TSNA formed during tobacco burning are not significant and that the levels of pre-formed TSNA in tobacco determine yields in smoke.^{8–12} Thus, the modification of tobacco curing methods and other changes in manufacturing techniques can substantially reduce TSNA levels in cigarette smoke.¹³ The feasibility of implementing such changes is clearly demonstrated by the extremely low TSNA levels in tobacco and smoke of some non-U.S. brands of cigarettes,¹⁴ and by the reported reductions in TSNA levels in tobacco of Canadian cigarettes that were accompanied by corresponding reductions in smoke deliveries.¹⁵

In 1999, RJ Reynolds, one of the major U.S. cigarette manufacturers, disclosed that it possesses a ‘simple, practical way to dramatically reduce TSNA in flue-cured tobacco’, referring to the use of heat exchangers instead of direct-fire burners.¹⁶ The company stated its intention to begin using low-TSNA tobacco in its cigarette blends “as soon as they feasibly can”.¹⁷ Another major U.S. cigarette manufacturer, Philip Morris, reportedly intended to do the same.¹⁸ Over the following decade, a range of new varieties of popular U.S. cigarette brands were launched by both companies. Philip Morris introduced Marlboro Special Blend in 2001, Marlboro Blend No. 27 in the spring of 2003, Marlboro Smooth Menthol and Marlboro Virginia Blend in 2007, and Marlboro Blend No. 54 in June 2009. Similarly, RJ Reynolds was extending their best-selling brand: Camel No. 9 was launched in 2007 and Camel Crush, which contains a menthol capsule within the filter, was introduced in the summer of 2008. The ‘light’ version of Camel Crush, called Camel Menthol Silver, appeared on the market in March, 2010.

While TSNA levels in the smoke of U.S. cigarettes are reported occasionally,^{9,19} the information on TSNA levels in the tobacco filler of various brands of cigarettes currently marketed in the U.S. is relatively scarce¹⁴. In light of the cigarette manufacturers’ stated intentions to reduce TSNA levels in cigarette tobacco by modifying tobacco processing techniques, the new varieties that have been recently introduced to the market are of particular interest. We here analyzed a current sample of seventeen brands of cigarettes, including some of the varieties launched in the past decade. Both the filler and the smoke were analyzed. To investigate whether cigarette brand and/or filter design affect the transfer rate of TSNA from tobacco to smoke, we also added known amounts of deuterium-labeled NNN and NNK to the filler of each cigarette brand and subsequently analyzed the labeled TSNA in the smoke generated by these cigarettes.

Materials and Methods

Cigarettes

The cigarettes were purchased in April 2010 from retail stores in Minnesota. One pack of each brand was purchased from three different communities in Minnesota: Golden Valley, St. Louis Park, and Minneapolis. The tobacco of cigarettes from each pack was analyzed separately, and the mean of three analyses was determined for each brand. The addition of deuterated NNN and NNK to the cigarettes was carried out with a specially designed microsyringe applicator system which uniformly distributed 20 μ l of spiking solution

containing [pyridine-D₄]NNN and [pyridine-D₄]NNK at a concentration of 10 ng/μl each, along the tobacco rod of the cigarette.

Analyses

Cigarettes were smoked under FTC standard conditions with a 35-ml puff volume and 2-s puff duration at a frequency of one puff per minute, as previously described.⁹ Mainstream smoke was collected on Cambridge filter pads pre-treated with ascorbic acid to prevent artifact formation of TSNA. The four commonly reported TSNA – NNN, NNK, *N'*-nitrosoanatabine (NAT), and *N'*-nitrosoanabasine (NAB) – were analyzed as previously described.²⁰ Briefly, both cigarette filler samples and smoke filter pads were extracted with citrate-phosphate buffer, the extracts were purified on ChemElut cartridges (Varian, Harbor City, CA), followed by solid-phase extraction on Sep-Pak Plus silica cartridges (Waters Corp., Milford, MA). The purified cigarette filler samples were analyzed by gas chromatography (GC) interfaced with a Thermal Energy Analyzer (TEA) (Orion Research, Beverly, MA). The smoke samples were analyzed by liquid chromatography (LC)-tandem mass-spectrometry (MS/MS) in positive ion electrospray mode with selected reaction monitoring for *m/z* 178 → 148 for NNN, *m/z* 182 → 152 for [pyridine-D₄]NNN, *m/z* 208 → 178 for NNK, *m/z* 212 → 182 for [pyridine-D₄]NNK, *m/z* 190 → 160 for NAT, and *m/z* 192 → 162 for NAB. The internal standards 5-methyl-*N'*-nitrosonornicotine (used for quantitation of NNN, NAT, and NAB) and 5-(methylnitrosamino)-1-(3-pyridyl)-1-pentanone (used for quantitation of NNK) were monitored at *m/z* 192 → 162 and *m/z* 222 → 192, respectively. Moisture content was analyzed as previously described.²¹

Results

The results of the TSNA analyses in cigarette filler are summarized in Table 1. The amount of NNN in all brands averaged 1.96 (±0.92) μg/g tobacco, ranging from 0.33 μg/g tobacco in Marlboro Virginia Blend to 4.03 μg/g tobacco in Marlboro Smooth Menthol. The amount of NNK in the tobacco filler of all brands averaged 0.58 (±0.17) μg/g tobacco, ranging from 0.34 μg/g tobacco in Marlboro Virginia Blend to 0.91 μg/g tobacco in Marlboro Blend # 54. The sum of four TSNA, referred to as total TSNA, in all brands averaged 4.03 (±1.58) μg/g tobacco, ranging from 1.14 μg/g tobacco in Marlboro Virginia Blend to 7.00 μg/g tobacco in Marlboro Smooth Menthol.

All new expansions of the Marlboro brand, except for Marlboro Virginia Blend, had higher levels of total TSNA than the regular Marlboro cigarettes (Table 1). Expansions of the Camel brand had total TSNA levels similar to those found in the filler of the regular Camel cigarettes (Table 1).

The results of the TSNA analyses in cigarette smoke are summarized in Table 2. The amount of NNN in all brands averaged 140 (±55) ng/cigarette, ranging from 20 ng/cigarette in Marlboro Virginia Blend to 232 ng/cigarette in Marlboro Blend #54. The amount of NNK in all brands averaged 75 (±32) ng/cigarette, ranging from 26 ng/cigarette in Marlboro Virginia Blend to 133 ng/cigarette in Marlboro Blend # 54. Total TSNA in all brands averaged 336 (±123) ng/cigarette, ranging from 76 ng/cigarette in Marlboro Virginia Blend to 572 ng/cigarette in Marlboro Smooth Menthol.

Overall, with the exception of Marlboro Virginia Blend, Philip Morris cigarettes had relatively higher levels of total TSNA than RJ Reynolds cigarettes: 4.91 (±1.96) compared to 3.39 (±0.98) μg/g tobacco, respectively in the filler, and 381 (±157) compared to 301 (±92) ng/cigarette, respectively in smoke.

Transfer rate of the deuterated NNN and NNK added to the tobacco filler of the studied cigarettes varied slightly among brands: [pyridine-D₄]NNN measured in smoke accounted for an average 10.7% of the amount added to filler (ranging from 7.0 to 13.1%), and [pyridine-D₄]NNK accounted for an average 11.1% of the amount added to filler (range, 7.5–14.6%) (Table 2).

Discussion

The carcinogenic potency of the tobacco-specific nitrosamines NNN and NNK is well established, and the existing evidence indicates that these carcinogens can be nearly eliminated from tobacco products.^{13,14} A meaningful effort from the tobacco industry to make the necessary changes in their manufacturing approaches and dramatically reduce TSNA levels in cigarette filler, thus reducing smoke deliveries of these carcinogens,¹⁵ was anticipated to take place about a decade ago. We here analyzed a sample of U.S. cigarettes purchased in the spring of 2010 to examine whether there are any reductions in TSNA levels in the filler and smoke of currently sold brands, including some varieties introduced to the U.S. market over the past decade.

The analytical method used in this study was first described in 1979, when NNN and NNK were quantified in the tobacco of an unidentified commercial filtered U.S. cigarette at 1.4 µg/g tobacco and 0.7 µg/g tobacco, respectively.⁵ If the values reported then are taken as a starting point and compared to those reported for the tobacco of U.S. commercial filtered cigarettes at two 15-year intervals – in 1995²² and 2010 (this report) – the conclusion is that despite the available technology to produce cigarettes low in TSNA, the levels of these carcinogens in the tobacco of popular U.S. cigarette brands have remained essentially the same (Figure 1). Similarly, there is no apparent significant reduction in smoke TSNA levels over the past decade: NNN levels in the smoke of 26 brands of cigarettes analyzed as a part of the 1999 Massachusetts Benchmark Study ranged from 100 to 317 ng/cigarette, and NNK ranged from 54 to 226 ng/cigarette (summarized in ²³). Another major observation in our study is that the TSNA levels in the recently introduced new varieties of existing cigarette brands do not reflect any attempt to reduce, or at least control, the levels of these carcinogens. Thus, Marlboro Blend # 54 contains much higher levels of total TSNA than the regular Marlboro variety: 7.00 µg/g tobacco vs. 4.63 µg/g tobacco in the filler, and 572 ng/cigarette vs. 378 ng/cigarette in smoke (Tables 1 and 2). The levels of TSNA in the expansions of the Camel brand are also somewhat similar to the levels in regular Camel cigarettes (Tables 1 and 2). The overall slightly lower levels of TSNA in the cigarettes produced by R.J. Reynolds, as compared to Philip Morris' brands, could be a consequence of the promised strategy to use low-TSNA flue-cured tobaccos.¹⁷ However, since flue-cured tobacco comprises only part of a cigarette blend, TSNA reduction in only this type of tobacco leads to not more than a modest change in total TSNA levels. In this respect, Marlboro Virginia Blend cigarettes are an exception. These cigarettes are made from a single type of flue-cured bright tobacco, and as a result, have the lowest NNN and NNK levels among all Philip Morris brands (Tables 1 and 2).

We found a positive correlation between TSNA levels in tobacco filler and smoke of the same cigarettes (Figure 2), which is consistent with previously published data.⁹ Moreover, the studied brands did not differ drastically in the transfer rate of deuterium-labeled NNN and NNK from cigarette filler to smoke (Table 2). These findings indicate that TSNA levels in the smoke of cigarettes investigated here are driven primarily by the corresponding TSNA levels in cigarette filler, once again stressing the importance of changes in tobacco processing and blending approaches for the reduction of smoke deliveries of these carcinogens. As was shown for commercial Canadian and Australian cigarettes, TSNA levels in cigarette smoke can be reduced to just a few nanograms per cigarette, or even

nearly eliminated^{15,18}. Thus, the sum of NNN and NNK in the filler of six brands of Canadian cigarettes decreased from an average 1.2 µg/g tobacco in 2003 to an average 0.35 µg/g tobacco in 2005 (71% reduction).¹⁵ This resulted in comparable reductions in TSNA levels in the smoke of these cigarettes, and NNN and NNK levels in cigarettes sold in 2005 averaged as low as 7 ng/cigarette and 11 ng/cigarette, respectively. U.S. cigarette manufacturers possess the necessary knowledge and tools to achieve similar reductions.

TSNA are not the only carcinogens found in cigarette smoke, and it is unclear whether the reduction of their levels alone will lead to a reduction in risk of developing smoking-induced cancers. However, NNN is the most prevalent esophageal carcinogen in cigarette smoke,²⁴ while the evidence is strong that NNK and polycyclic aromatic hydrocarbons are causative agents for lung cancer in smokers.²⁵ Based on its historical levels in cigarette smoke and its ability to induce adenocarcinoma of the lung in three commonly used rodent models, as well as comparative lung cancer death rates from the U.S. and Australia, NNK may be partially responsible for adenocarcinoma becoming the leading type of lung cancer in the United States.^{26,27} Two recent reports demonstrated that higher levels of total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), a biomarker of NNK exposure in smokers, are associated with a higher risk of lung cancer.^{28,29} Furthermore, a recent study has documented a clear relationship between urinary NNAL levels and mouth-level exposure to TSNA, showing that smokers of low-TSNA cigarettes have lower levels of NNAL in their urine.³⁰ Taken together, the results of these studies imply that higher levels of TSNA in cigarette smoke are associated with a higher risk of cancer in smokers.

In summary, despite the available knowledge and tools, there appears to be a remarkable lack of any reduction in the levels of known human carcinogens in products being sold to millions of customers. In light of the recently granted regulatory authority to the U.S. FDA over tobacco products, regulation of TSNA levels in cigarette tobacco should be strongly considered.

Acknowledgments

Funding: This study was supported by NIH Grants P50 DA-13333 as part of the Tobacco Harm Reduction Network and CA-81301.

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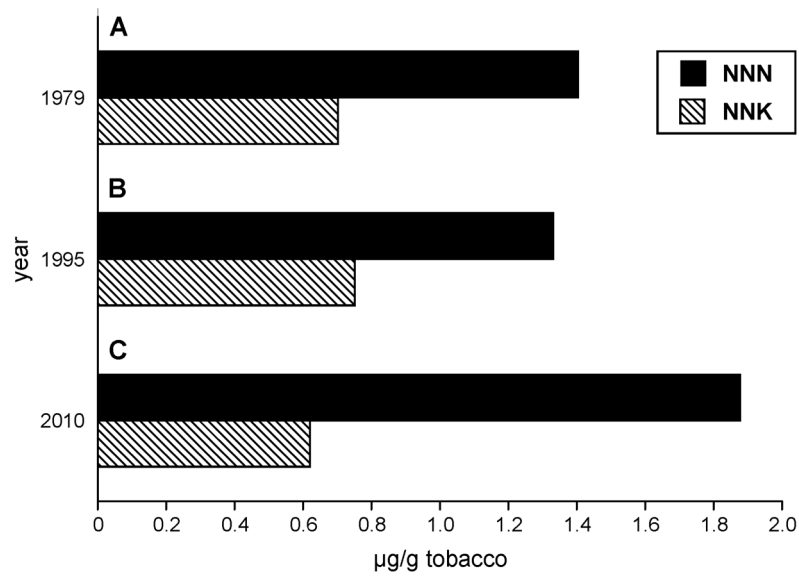


Figure 1.

Levels of NNN and NNK in tobacco filler of a U.S. filtered cigarette at three time-points over the 30-year period. **A**, levels reported for an unidentified U.S. commercial filtered cigarette⁵; **B**, average of NNN and NNK levels reported for tobacco filler of 5 unidentified leading U.S. cigarette brands²²; **C**, average levels of NNN and NNK in all brands reported in the current study (Table 1).

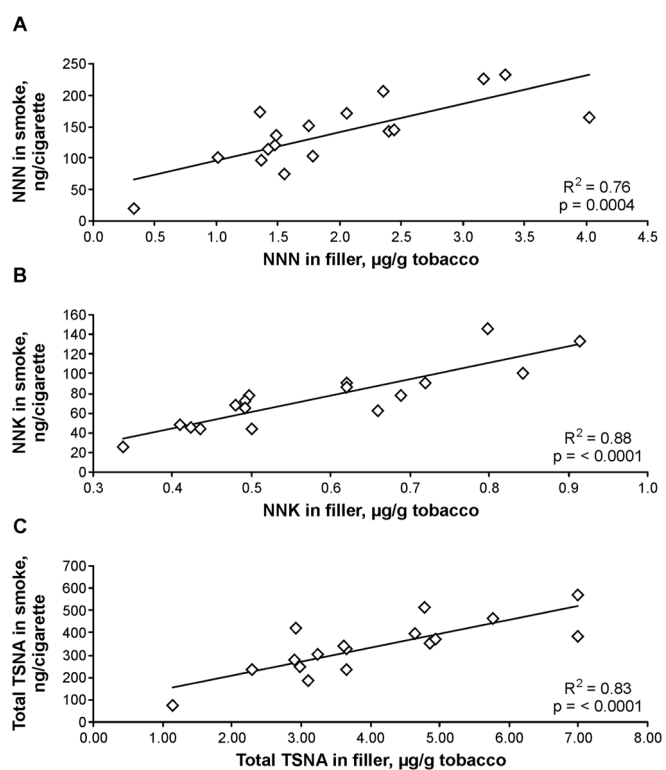


Figure 2. Correlation between tobacco-specific nitrosamine levels in tobacco filler and smoke of cigarettes analyzed in this study: **A**, NNN; **B**, NNK; **C**, Total TSNA (sum of NNN, NNK, NAT, and NAB).

Table 1

Tobacco-specific *N*-nitrosamines in tobacco filler of some U.S. cigarettes marketed in 2010^{a,b}.

Cigarette brand	Filler weight, g	Moisture content, %	µg/g tobacco (wet weight)					
			NNN	NAT	NAB	NNK	NNN + NNK	Total TSNA
Philip Morris (Altria Group, Inc.)								
Marlboro Full Flavor	0.675	12.8	2.06	1.74	0.11	0.72	2.78	4.63
Marlboro Special Blend	0.616	12.5	2.40	1.69	0.08	0.69	3.09	4.86
Marlboro Blend # 27	0.627	11.3	2.44	1.77	0.10	0.62	3.06	4.93
Marlboro Blend # 54	0.638	11.7	3.34	2.60	0.14	0.91	4.25	6.99
Marlboro Smooth Menthol	0.599	9.4	4.03	2.22	0.13	0.62	4.65	7.00
Marlboro Virginia Blend	0.635	11.6	0.33	0.44	0.04	0.34	0.67	1.14
Basic Full Flavor	0.664	11.9	2.35	1.54	0.10	0.80	3.15	4.79
Average for Philip Morris brands	0.636	11.6	2.42	1.71	0.10	0.67	3.09	4.91
SD	0.026	1.1	1.15	0.67	0.03	0.18	1.28	1.96
Lorillard Tobacco Company								
Newport Menthol	0.664	14.11	1.75	1.29	0.08	0.49	2.24	3.62
RJ Reynolds (Reynolds American, Inc.)								
Camel Full Flavor	0.769	13.8	1.48	1.20	0.08	0.48	1.96	3.24
Camel # 9	0.675	11.8	1.78	1.30	0.08	0.50	2.28	3.66
Camel # 9 Menthol	0.634	12.8	1.56	1.06	0.05	0.43	1.99	3.09
Camel Silver	0.610	11.6	1.01	0.80	0.05	0.42	1.43	2.28
Camel Crush	0.650	12.1	1.36	1.14	0.08	0.41	1.77	2.99
Winston Full Flavor	0.659	12.0	1.35	1.01	0.07	0.50	1.85	2.93
Kool Filter Kings	0.660	13.9	1.49	1.43	0.08	0.66	2.15	3.65
Pall Mall Full Flavor	0.778	11.8	1.42	0.93	0.06	0.49	1.91	2.90
Doral Full Flavor	0.672	8.83	3.17	1.67	0.09	0.84	4.01	5.77
Average for RJ Reynolds brands	0.679	12.1	1.62	1.17	0.07	0.53	2.15	3.39
SD	0.057	1.5	0.61	0.27	0.01	0.14	0.74	0.98

^aAll the cigarette brands analyzed here are king size filtered cigarettes packaged in hard packs.

^bEach value is the mean of three analyses.

Table 2

Tobacco-specific *N*-nitrosamines in smoke of some U.S. cigarettes marketed in 2010^{a,b}.

Cigarette brand	ng/cigarette						% Transfer from filler to smoke	
	NNN	NAT	NAB	NNK	NNN + NNK	Total TSNA	[pyridine-D ₄]NNN	[pyridine-D ₄]NNK
<i>Philip Morris (Altria Group, Inc.)</i>								
Marlboro Full Flavor	171.0	119.7	16.6	90.3	261.3	397.7	11.5	9.0
Marlboro Special Blend	141.6	118.4	15.3	77.3	219.0	352.7	11.4	11.6
Marlboro Blend # 27	145.2	118.4	16.3	91.2	236.3	371.1	9.4	10.7
Marlboro Blend # 54	232.1	183.1	22.6	133.7	365.8	571.6	12.3	14.6
Marlboro Smooth Menthol	164.2	117.1	18.1	86.4	250.6	385.7	11.1	13.7
Marlboro Virginia Blend	19.5	27.2	3.9	25.6	45.1	76.2	10.8	8.0
Basic Full Flavor	207.1	138.7	22.6	146.1	353.1	514.4	13.0	14.0
<i>Average for Philip Morris brands</i>	154.4	117.5	16.5	92.9	247.3	381.3	11.4	11.7
<i>SD</i>	67.9	46.4	6.3	39.4	105.9	157.2	1.2	2.6
<i>Lorillard Tobacco Company</i>								
Newport Menthol	151.8	109.3	13.6	65.6	217.3	340.3	13.1	14.6
<i>RJ Reynolds (Reynolds American, Inc.)</i>								
Camel Full Flavor	120.2	97.8	15.6	67.5	187.7	301.1	9.5	9.2
Camel # 9	102.8	77.2	12.4	44.4	147.2	236.7	9.2	9.0
Camel # 9 Menthol	75.2	60.2	8.9	43.4	118.6	187.7	7.0	7.4
Camel Silver	100.3	77.6	12.4	45.6	145.9	235.9	7.9	9.0
Camel Crush	96.9	90.0	15.1	48.1	145.0	250.1	9.8	12.2
Winston Full Flavor	172.8	144.4	27.9	78.5	251.3	423.7	12.0	10.4
Kool Filter Kings	135.8	111.3	17.0	63.0	198.8	327.1	12.6	14.2
Pall Mall Full Flavor	114.3	80.6	11.5	72.8	187.1	279.2	12.2	13.8
Doral Full Flavor	225.9	124.8	15.7	100.4	326.3	466.8	8.5	7.5
<i>Average for RJ Reynolds brands</i>	127.1	96.0	15.2	62.6	189.8	300.9	9.8	10.3
<i>SD</i>	46.2	26.6	5.4	19.4	64.5	91.8	2.0	2.5

^a All the cigarette brands analyzed here are king size filtered cigarettes packaged in hard packs.

^b Each value is the mean of two analyses.