Comparison of the Yield of Very Low Nicotine Content Cigarettes to the Top 100 United States Brand Styles *

by

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SUMMARY

The objective of this work was to compare the nicotine content and yield of new very low nicotine content cigarettes (VLN™) to the top 100 cigarette brand styles in the United States. Nicotine in tobacco filler and nicotine in smoke were measured. On a dry weight basis, VLN™ cigarettes averaged 0.5 mg of nicotine/g tobacco as compared to 19.4 for the top 100 brand styles in the United States. On a cigarette basis, VLN™ cigarettes contained 0.27 mg of nicotine compared to 12 mg for the top brands. On an ISO smoke yield basis, VLN™ produced 0.03 mg nicotine/cig compared to 0.903 mg nicotine/cig for the leading brands. VLN™ cigarettes contained > 95% less nicotine on a per gram basis of tobacco or per cigarette basis than the top 100 brand styles in the U.S. VLN™ cigarettes yielded > 95% less nicotine in smoke than the top brand styles in the United States. [Beitr. Tabakforsch. Int. 28 (2019) 253–266]

KEYWORDS

Tobacco; cigarettes; nicotine, SPECTRUM®, VLN, VLNC, very low nicotine

ZUSAMMENFASSUNG

Ziel der vorliegenden Arbeit war ein Vergleich von Nikotingehalt und Nikotinausbeute einer neuen Zigarette mit sehr geringem Nikotingehalt (VLN™) mit den 100 führenden Zigarettenprodukten in den USA. Es wurde das Nikotin im Füllstoff und das Nikotin im Rauch gemessen.

Als Trockengewicht ergab sich für die VLN™-Zigaretten durchschnittlich 0,5 mg Nikotin/g Tabak im Vergleich zu 19,4 bei den 100 führenden Produkten in den USA. Pro Zigarette enthielten die VLN™-Zigaretten 0,27 mg Nikotin im Vergleich zu 12 mg bei den wichtigsten Produkten. Hinsichtlich der ISO-Rauchausbeute gaben die VLN™-Zigaretten 0,03 mg Nikotin/Zigarette ab verglichen mit 0,903 mg der führenden Produkte. VLN™-Zigaretten enthielten > 95% weniger Nikotin pro Gramm Tabak und pro Zigarette als die 100 führenden Zigarettenprodukte in den USA. Für VLN™-Zigaretten wurde zudem eine im Vergleich zu den führenden US-Produkten um > 95% geringere Nikotinausbeute im Rauch ermittelt. [Beitr. Tabakforsch. Int. 28 (2019) 253–266]

RESUME

L'objectif de la présente étude était un comparatif de la teneur et du rendement en nicotine de nouvelles cigarettes à très faible teneur en nicotine (VLN™) par rapport aux styles de cigarettes répertoriés dans le top 100 des marques commercialisées aux Etats-Unis. La nicotine fut mesurée dans la matière de remplissage et dans la fumée. Les cigarettes VLN™ affichèrent une moyenne de 0,5 mg de nicotine par gramme de tabac, exprimée en fonction du poids sec, contre 19,4 mg dans le cas des styles de cigarettes du top 100 des marques commercialisées aux Etats-Unis. Considérant l'ensemble de la cigarette, les cigarettes VLN™ contenaient 0,27 mg de nicotine par cigarette, contre 12 mg pour les marques du classement. Selon la méthode ISO de détermination du rendement de la fumée, les cigarettes VLN™ produisaient 0,03 mg de nicotine par cigarette, contre 0,903 mg de nicotine par cigarette dans le

cas des grandes marques. Les cigarettes VLN™ contenaient plus de 95% de nicotine en moins par gramme de tabac et par cigarette que les styles de cigarettes répertoriés dans le top 100 des marques commercialisées aux États Unis. Les cigarettes VLN™ libéraient plus de 95% de nicotine en moins dans la fumée que les produits des grandes marques commercialisées aux USA. [Beitr. Tabakforsch. Int. 28 (2019) 253–266]

INTRODUCTION

It is well known that nicotine is the addictive agent in tobacco. In 1994, Drs. BENOWITZ and HENNINGFIELD proposed reducing nicotine levels of all cigarettes over a 10–15-year period (1). Over the intervening years, literally over a hundred scientific studies were published, evaluating the concept and benefits of very low nicotine cigarettes. In a June 16, 2010 press release, Dr. David Kessler, at the time FDA Commissioner, recommended “*The FDA should quickly move to reduce nicotine levels in cigarettes to non-addictive levels*” (2). Shortly thereafter in a Washington Post newspaper article, Dr. Kessler said that the amount of nicotine in a cigarette should drop from about 10 mg to less than 1 mg (3). In 2017, Dr. Scott Gottlieb, at the time FDA Commissioner, announced a new more comprehensive approach to effective tobacco regulation based on reducing the nicotine levels in cigarettes so that they are minimally or non-addictive (4). As part of the new plan, on March 16, 2018, FDA published an advance notice of proposed rulemaking (ANPRM) announcing an intention to issue a proposed rule that, when finalized and effective, would limit the amount of nicotine in all cigarettes sold in the United States so that they are minimally addictive or non-addictive (5). There are no commercially available cigarettes that meet the proposed standard of 0.2–0.7 mg of nicotine per cigarette.

In 2011, 22nd Century developed the SPECTRUM® line of research cigarettes in collaboration with the National Institute on Drug Abuse (NIDA), the FDA, the National Cancer Institute and the Centers for Disease Control and Prevention. These cigarettes are made available to researchers through NIDA’s Drug Supply Program. The SPECTRUM® product line consists of a series of cigarette styles that vary in nicotine content - from very low (~0.4 mg/g tobacco) to relatively high nicotine contents and yields. The nicotine smoke yields range from 0.02 to 1.2 mg nicotine/cig and from 3 to 16 mg of “tar”/cig (6). SPECTRUM® products are available in 24 styles, in both regular and menthol versions, with 8 levels of nicotine in their tobacco. The amount of nicotine in the various styles of SPECTRUM® cigarettes is controlled by blending conventional nicotine content tobacco and tobacco that has been genetically modified¹ and selected to have low levels of nicotine. The lowest nicotine level SPECTRUM® ciga-

rettes contain only very low nicotine tobacco. As nicotine production in tobacco is controlled by multiple genes in the plant, 22nd Century’s technology involves blocking or downregulating several genes in the tobacco plant and then selecting for tobacco plant lines that produce the lowest levels of nicotine. SPECTRUM® cigarettes are made with the Company’s proprietary Burley tobacco (strain Vector 21-41) at a target nicotine specification level of 0.5 mg/g of tobacco filler (dry weight). The low nicotine level cigarettes are often stated to contain a nominal level of 0.4 mg of nicotine/g of tobacco. These research cigarettes have been produced since 2011 with a target nicotine level of 0.5 mg nicotine/g of dry tobacco filler weight basis accounting for variations in water content.

22nd Century’s proprietary low nicotine tobacco was developed in 1998 and has since been used in many cigarettes under different names, including X-22, Xodus, Magic, MAGIC 0, MAGIC 2, Quest®, and the SPECTRUM® research cigarettes. Quest® cigarettes were developed and marketed by Vector Tobacco, Inc. under license from 22nd Century from 2002 to 2009. The family of Quest® products included Quest®1 (a cigarette made of conventional tobacco), Quest®2 (a cigarette containing a blend of conventional tobacco and very low nicotine tobacco), and Quest®3 (a cigarette made exclusively with very low nicotine tobacco). Quest cigarettes were marketed to reduce nicotine uptake and nicotine dependency. In 2006, Vector Tobacco filed an Investigational New Drug (IND) Application (number 69,185) with the U.S. FDA for the Quest products with very low nicotine tobacco. Step-down reduction in nicotine levels in test cigarettes followed by nicotine replacement therapy (patches) did not affect abstinence at 3 and 6 months when compared to smoking the usual brand followed by nicotine replacement therapy (7). When Vector stopped the sales of Quest, 22nd Century developed X-22 cigarettes with the intent of obtaining a smoking cessation claim. In 2011, 22nd Century conducted a clinical study with X-22 under IND 103,589. The 4-week study failed to demonstrate cessation efficacy and the clinical development was paused.

22nd Century has developed a version of the SPECTRUM® research cigarette for commercial distribution. This product, trademarked VLN, is exactly the same as the lowest level of nicotine versions of SPECTRUM®. The SPECTRUM® Nicotine Research Cigarettes (NRC) 102 and 103 will be commercially available (after FDA approval) as VLN™ Kings and VLN™ Menthol Kings, respectively. The goal of this research was to evaluate how the nicotine content and yield of VLN™ cigarettes compared to currently marketed cigarettes.

METHODS

Cigarettes

The top 100 cigarette brand styles in the U.S. were determined by purchasing sales data from Management Science Associates, Inc. (MSA). A one-time aggregate of 52 weeks of data for calendar year 2017, comprised of the week ending January 7, 2017, to the week ending December 30, 2017, was obtained. The data was U.S. nationwide sales

¹ The tobacco used in SPECTRUM® and VLN™ cigarettes is derived from a low alkaloid strain of Burley 21 tobacco. The Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA) prepared an environmental assessment of tobacco line Vector 21-41 and granted a petition to designate this product as non-regulated (33).

data (in cartons) for warehouse-delivered products in the convenience store channel made up of both chain and independent stores. The United States convenience store channel universe is made up of 154,000+ stores. MSA collects data from over 60,000 stores. From these 60,000, a sub-set of approximately 30–32,000 convenience stores, both chain and independent, are used to project data to the size of the total convenience store channel. The raw sales data (in cartons) from MSA was categorized at the SKU (stock keeping unit; the bar code on the side of the pack) level. Identical products having different SKUs (because they were purchased with a coupon, or another form of discount, or if they have a slight difference in description) were combined. For testing purposes, the commercial cigarettes were purchased from retail outlets in the Richmond, VA metropolitan area during July 2018. VLNTM cigarettes were provided by 22nd Century Group, Inc. The top 100 brand styles represented more than 80% of all cigarettes sold in the U.S. The brand styles tested are listed in Table 1. Some of the brand styles were discontinued. The next product on the list was tested.

VLNTM King and VLNTM Menthol King are the same cigarettes as the SPECTRUM[®] NRC102 and NRC103 research cigarettes produced by 22nd Century for NIDA. The only difference between VLNTM King and VLNTM Menthol King is that menthol has been added to the filter of the Menthol King product. All other tobacco, materials, and ingredients are the same in the products. Table 2 shows a comparison of the measured physical properties of VLNTM and SPECTRUM[®] cigarettes.

Sample analysis

Mainstream smoke collection and analysis of cigarettes were performed using an SM 450 20-port linear smoking machine (Cerulean, Milton Keynes, UK) under ISO conditions of 35 mL volume, 2 s duration with a 60 s puff period. Twenty replicates were analyzed for “tar”, nicotine, and carbon monoxide yields. Analysis was conducted following the relevant ISO standards for the analysis of nicotine, water, CO and Nicotine Free Dry Particulate Matter (NFDPM) in mainstream cigarette smoke (8–12). The ISO method for the analysis of nicotine in mainstream smoke, ISO 10315, does not cover VLNTM tobacco products because of the low nicotine levels. The work by BROWN *et al.* has demonstrated that with the addition of lower calibration standards, the method is suitable for the analysis of nicotine in the mainstream smoke of VLN products (13). At the time of the analyses, all analytical methods were accredited to the ISO 17025 standard (14, 15). Cigarette filler analysis was conducted from tobacco removed from conditioned cigarettes. Tobacco was composited from 200 cigarettes per brand style. Before analysis, tobacco was ground to a uniform particle size. Nicotine content of the commercial brands was determined by gas chromatography/flame ionization detector (GC-FID) following the CDC method (16) while nicotine content of the VLN products was determined by gas chromatography/mass spectrometry (GC-MS) following CORESTA Recommend method 87 (17). Tobacco water content was determined by following the three-hour CDC oven volatiles method (16).

Determination of “tar”, nicotine, and water

Total particulate matter (TPM) from cigarette smoke was collected on a Cambridge filter pad and extracted in 20 mL of 2-propanol (MilliporeSigma, Burlington, MA, USA, OmniSolv grade, CAS #67-63-0) containing 0.5 mL/L of n-heptadecane (Alfa-Aesar, Ward Hill, MA, USA, 99%, CAS #629-78-7) and 5 mL/L of ethanol (Sigma-Aldrich, St. Louis, MO, USA, 200 proof anhydrous, CAS #64-17-5) as internal standards. An aliquot of the sample solution was analyzed with an Agilent (Santa Clara, CA, USA) 6890N gas chromatograph equipped with a flame ionization detector (FID) and a thermal conductivity detector. The nicotine was analyzed on the FID channel using a custom stainless steel Supelco (Bellefonte, PA, USA) column measuring 6 ft × 1/8 in × 2.1 mm with 7% Carbowax, 3% OS-138, 2% KOH, 80/100 Chromosorb WHP packing. The water was analyzed on the TCD channel using a custom stainless steel Supelco column measuring 10 ft × 1/8 in × 2.1 mm with 80/100 Porapak QS packing. The “tar” (nicotine free dry particulate matter - NFDPM) was calculated by subtracting resulting nicotine and water from the TPM. The limit of detection under ISO 3308 for nicotine and water was 1.2 µg/cig and 80 µg/cig respectively while the limit of detection under ISO 10315 for nicotine and water was 2.0 µg/cig and 133 µg/cig respectively.

Determination of carbon monoxide by non-dispersive infrared absorption

The vapor phase of the smoke was collected in gas sampling bags attached to the smoking machine. A Cerulean non-dispersive infrared absorption detector (NDIR) was used to measure the CO concentration in the vapor phase in percent by volume (% vol). The NDIR detector was calibrated using specialty gases from ARC3 (Richmond, VA) with certified concentrations of 1%, 3%, and 6% (vol) CO in nitrogen. Using the number of cigarettes, the cigarette puff count, the puff volume, and ambient temperature and humidity conditions, the % CO was converted to mg/cig.

Determination of nicotine in tobacco filler by GC-FID

5 mL of 2N sodium hydroxide (Fluka, Morristown, NJ, USA, CAS #1310-73-2) was added to the tobacco (1.00 g ± 0.1 g) and allowed to stand for 15 min. 50 mL of methyl tert-butyl ether (Macron, Allentown, PA, CAS #1634-04-4) containing 0.4 mg/mL of the internal standard quinoline (Alfa-Aesar, 98%, CAS #91-22-5) was then added and the sample was placed on an orbital shaker for 2 hours at 200 RPM. An aliquot of the organic layer was analyzed with an Agilent 7890 gas chromatograph equipped with a flame ionization detector. Analyte separation was achieved using an Agilent J&W HP-5 30 m × 320 µm × 0.25 µm column. The limit of detection for this method was approximately 150 µg/g tobacco.

Table 1. Top 100 brands in U.S. by unit sales.

Manufacturer	Brand family	Item description	Rank	Notes
Altria Group	Marlboro	Marlboro Gold King	1	
Altria Group	Marlboro	Marlboro Red King	2	
Reynolds American, Inc	Newport	Newport Menthol Green 100	3	
Reynolds American, Inc	Newport	Newport Menthol Green King	4	
Altria Group	Marlboro	Marlboro Special Blend Gold King	5	
Altria Group	Marlboro	Marlboro Gold 100	6	
Reynolds American, Inc	Pall Mall	Pall Mall Red 100	7	
Reynolds American, Inc	Camel	Camel Blue King	8	
Altria Group	Marlboro	Marlboro Red 100	9	
Altria Group	Marlboro	Marlboro Special Blend Gold 100	10	
Altria Group	Marlboro	Marlboro Silver King	11	
Altria Group	Marlboro	Marlboro Special Blend Black 100	12	
Altria Group	Marlboro	Marlboro Special Blend Red 100	13	
Altria Group	Marlboro	Marlboro Special Blend Red King	14	
Altria Group	Marlboro	Marlboro Special Blend Black King	15	
Reynolds American, Inc	Pall Mall	Pall Mall Blue (Light) 100	16	
Altria Group	Marlboro	Marlboro Menthol Gold King	17	
Reynolds American, Inc	Camel	Camel Crush King	18	
Altria Group	Marlboro	Marlboro Green (Menthol) King	19	
Altria Group	Marlboro	Marlboro 72 Red	20	
Reynolds American, Inc	Camel	Camel Menthol Silver King	21	Disc. ¹
Altria Group	L&M	L&M Full Flavor King	22	
Altria Group	L&M	L&M 100 (Full Flavor)	23	
Reynolds American, Inc	Pall Mall	Pall Mall Blue (Light) King	24	
Reynolds American, Inc	Pall Mall	Pall Mall Red King	25	
Reynolds American, Inc	Newport	Newport Non-Menthol 100	26	
Reynolds American, Inc	Camel	Camel Menthol Green King	27	Disc. ²
Altria Group	Marlboro	Marlboro Silver 100	28	
Reynolds American, Inc	Camel	Camel Full Flavor Filter King	29	
ITG Brands Llc	Kool	Kool Menthol King	30	
Reynolds American, Inc	Pall Mall	Pall Mall Menthol 100	31	
Altria Group	Marlboro	Marlboro Blend 27 King	32	
Altria Group	L&M	L&M 100 Menthol	33	
Altria Group	Marlboro	Marlboro 72 Gold	34	
Altria Group	L&M	L&M Blue King	35	
ITG Brands Llc	Winston	Winston Red King	36	
Altria Group	Marlboro	Marlboro Nxt King	37	
Altria Group	Marlboro	Marlboro Menthol 100	38	
ITG Brands Llc	Maverick	Maverick Menthol 100	39	
Reynolds American, Inc	Camel	Camel 99 Full Flavor Bx	40	
ITG Brands Llc	Winston	Winston Gold King	41	
Santa Fe Tobacco	Natural Am Spirits	Natural American Spirit Yellow Mellow King	42	
Altria Group	L&M	L&M Blue 100	43	
Altria Group	L&M	L&M Menthol King	44	
Reynolds American, Inc	Pall Mall	Pall Mall Orange (Ultra Light) 100	45	
Reynolds American, Inc	Newport	Newport Non-menthol King	46	
Altria Group	Marlboro	Marlboro Smooth Menthol King	47	
Santa Fe Tobacco	Natural Am Spirits	Natural American Spirit Blue King	48	
Altria Group	Marlboro	Marlboro Menthol Gold 100	49	
Altria Group	Marlboro	Marlboro Special Blend Black Menthol 100	50	
Altria Group	Parliament	Parliament White King	51	
ITG Brands Llc	Kool	Kool Green 100	52	
Reynolds American, Inc	Camel	Camel 99 Blue	53	
Altria Group	Marlboro	Marlboro Special Blend Black Menthol King	54	
Reynolds American, Inc	Pall Mall	Pall Mall White Menthol 100	55	
Altria Group	Marlboro	Marlboro 72 Silver	56	
ITG Brands Llc	Winston	Winston Gold 100	57	
Altria Group	Marlboro	Marlboro Red Label King	58	
Altria Group	Marlboro	Marlboro 72 Menthol Green	59	
Reynolds American, Inc	Camel	Camel Turkish Blend Royal Filter King	60	

Table 1. Continued

Manufacturer	Brand family	Item description	Rank	Notes
Reynolds American, Inc	Pall Mall	Pall Mall Menthol King	61	
ITG Brands Llc	Maverick	Maverick Full Flavor 100	62	
Reynolds American, Inc	Pall Mall	Pall Mall Black Menthol 100	63	
Reynolds American, Inc	Camel	Camel Wides Red King	64	
Liggett Vector Brands	Pyramid	Pyramid Red 100	65	
Liggett Vector Brands	Eagle 20's	Eagle 20's Red 100	66	
Reynolds American, Inc	Camel	Camel Crush Menthol Silver King	67	
Altria Group	Marlboro	Marlboro Southern Cut King	68	
ITG Brands Llc	Winston	Winston Red 100	69	
Reynolds American, Inc	Newport	Newport Menthol Gold King	70	
Altria Group	Marlboro	Marlboro Silver Menthol King	71	
Reynolds American, Inc	Pall Mall	Pall Mall Orange 85	72	
Reynolds American, Inc	Camel	Camel Crush Menthol King	73	
Altria Group	Marlboro	Marlboro Edge King	74	
ITG Brands Llc	Kool	Kool Blue (Mild) King	75	
Altria Group	Marlboro	Marlboro Smooth Menthol 100	76	
Santa Fe Tobacco	Natural Am Spirits	Natural American Spirit Organic Turquoise King	77	
ITG Brands Llc	USA Gold	USA Gold Red King	78	
Altria Group	Marlboro	Marlboro Black Menthol 100	79	
Altria Group	Marlboro	Marlboro Red Label 100	80	
Altria Group	Marlboro	Marlboro Black Menthol King	81	
ITG Brands Llc	Salem	Salem Menthol Gold King	82	
Reynolds American, Inc	Misty	Misty 120 Menthol (Light Green)	83	
Santa Fe Tobacco	Natural Am Spirits	Natural American Spirit Menthol Light Green King	84	
Liggett Vector Brands	Pyramid	Pyramid Blue 100	85	
ITG Brands Llc	Salem	Salem Slim Menthol 100	86	
ITG Brands Llc	Maverick	Maverick Gold (Light) 100	87	
Altria Group	Marlboro	Marlboro Silver Menthol 100	88	
Reynolds American, Inc	Camel	Camel Turkish Blend Gold King	89	
Altria Group	Marlboro	Marlboro 83	90	
Altria Group	Marlboro	Marlboro Midnight Menthol King	91	
ITG Brands Llc	USA Gold	USA Gold (Light) King	92	
Reynolds American, Inc	Misty	Misty 120 Blue	93	
Altria Group	Marlboro	Marlboro 72 Menthol Blue	94	Disc. ³
Reynolds American, Inc	Newport	Newport Menthol Gold 100	95	
Santa Fe Tobacco	Natural Am Spirits	Natural American Spirit Organic Mellow Gold King	96	
ITG Brands Llc	USA Gold	USA Gold Red 100	97	
Altria Group	L&M	L&M Bold Menthol 100	98	
Reynolds American, Inc	Camel	Camel Turkish Blend Jade Silver King	99	
ITG Brands Llc	Salem	Salem Green King	100	
ITG Brands Llc	Winston	Winston White King	101	
Reynolds American, Inc	Newport	Newport Menthol Smooth Select 100	102	
Altria Group	Marlboro	Marlboro Menthol Blue King	103	

¹ Disc. = Discontinued - cannot test. The non-crush versions of the varieties of Camel Menthol products have been phased out and are no longer available for purchase. The sales of Camel Menthol Silver King (non-crush) was ranked #21 of 100 in 2017. The sales of Camel Crush Menthol Silver King were ranked #67 of 100, so the crush version of Camel Menthol Silver King was evaluated. Since item #21 on the list no longer exists in the market, the next item down on the list, ranked #102, Newport Menthol Smooth Select 100 size was chosen.

² Disc. = Discontinued - cannot test. The non-crush versions of the varieties of Camel Menthol products have been phased out and are no longer available for purchase. The sales of Camel Menthol Green King (non-crush) was ranked #27 of 100 in 2017. The sales of Camel Crush Menthol King were ranked #73 of 100, so the crush version of Camel Menthol Green King was evaluated. Since item #27 on the list no longer exists in the market, the next item down on the list, ranked #101, Winston White King size was chosen.

³ Disc. = Discontinued - cannot test. Marlboro 72 Menthol Blue was discontinued in 2017. It ranked #94 of 100 for sales on the 2017 list of products. Since it is not available for purchase it was replaced it with #103 on the list, Marlboro Menthol Blue King Size.

Table 2. Comparison of the measured physical properties of SPECTRUM and VLN cigarettes.

Parameter		SPECTRUM King NRC102	VLN™ King	SPECTRUM Menthol King NRC103	VLN™ Menthol King
Cigarette length (mm)	Mean	82.8	82.9	82.9	82.9
	SD	0.1	0.1	0.1	0.1
Tipping length (mm)	Mean	30.2	30.2	30.1	30.3
	SD	0.4	0.3	0.4	0.3
Filter length (mm)	Mean	25.0	25.1	25.0	25.2
	SD	0.0	0.2	0.0	0.3
Rod length (mm)	Mean	57.8	57.9	57.9	57.7
	SD	0.1	0.1	0.1	0.3
Permeability banded (CU)	Mean	13.6	13.5	15.0	14.5
	SD	4.0	2.6	4.5	5.4
Permeability non-banded (CU)	Mean	77.6	81.5	79.2	83.3
	SD	6.4	4.7	12.5	10.4
Cigarette weight (g)	Mean	0.900	0.936	0.963	0.932
	SD	0.018	0.021	0.026	0.017
Tobacco weight (mg)	Mean	650	687	647	650
	SD	21	23	16	20
Circumference (mm)	Mean	24.9	24.8	24.8	25.0
	SD	0.1	0.1	0.1	0.1
Diameter (mm)	Mean	7.90	7.89	7.91	7.95
	SD	0.03	0.02	0.03	0.02
Tip ventilation (%)	Mean	14.1	12.5	14.8	14.0
	SD	1.5	1.1	1.7	1.3
Pressure drop "open"	Mean	121.9	119.0	119.0	113.0
	SD	5	4	4	4
Plug resistance to draw	Mean	98.7	91.6	88.8	92.2
	SD	5.5	4.2	5.1	6.0
Filter weight (g)	Mean	0.200	0.222	0.220	0.220
	SD	0.003	0.003	0.003	0.003

Determination of nicotine in tobacco filler by GC/MS

4 mL of 5N NaOH (Fisher Chemical, Fair Lawn, NJ, USA) was added to the tobacco ($0.5 \text{ g} \pm 0.1 \text{ g}$) into a 50 mL conical tube and allowed to stand for 30 min. 40 mL of methanol (Fisher, distilled in glass) containing 50 µg/mL of the internal standard quinoline (Alfa-Aesar P/N 43225) was added to the conical tube along with 2 grinding stones (US Stoneware, East Palestine, OH, USA, P/N Brun050-090). The sample was then placed on the 2010 Geno-grinder (Spex, Metuchen, NJ, USA) for 30 min at 1000 RPM. Samples were removed from the grinder and placed into a centrifuge (Sorvall Legend XT by Thermo Fisher Scientific, Waltham, MA, USA) for 10 min at 3000 RPM. An aliquot of the methanol extract was analyzed with an Agilent 7890 GC equipped with a 5975C MSD. Analyte separation was achieved on an Agilent Cam column ($30 \text{ m} \times 0.250 \text{ mm} \times 0.25 \text{ µm}$). The limit of detection for nicotine was 0.88 µg/g tobacco.

RESULTS

Cigarettes were analyzed for the amount of nicotine in the tobacco filler and also for the amount of nicotine in the smoke. "Tar" and carbon monoxide in smoke were also analyzed. The results of the analyses are presented in Table 3. Tobacco filler has varying amounts of water dependent on how the product was manufactured and on the humectants used. Nicotine in the tobacco filler is

presented as it was measured ("as is" basis) and then corrected to a dry weight basis. As expected, there was no difference in the nicotine content or yield between VLN™ Kings and VLN™ Menthol Kings since the only difference between the two products is addition of a menthol solution to the filter. On an "as is" basis the average nicotine in the tobacco filler for the top 100 brand styles in the U.S. was 17.2 mg nicotine/g tobacco filler. The amount of nicotine in VLN™ was 0.41 mg/g tobacco on an "as is" basis. When the results were corrected for variations in the water content, the top 100 brand styles averaged 19.4 mg nicotine/g tobacco filler dry weight (range 14.7–33.2). VLN™ cigarettes averaged 0.47 mg of nicotine /g tobacco filler. Figure 1 shows the dramatic differences between VLN™ products and the top 100 brand styles sold in the U.S. A more relevant comparison is on a cigarette basis. The weight of tobacco varies between commercial cigarettes. On a per-cigarette basis the average nicotine in the tobacco filler for the top 100 brand styles in the U.S. was 12.0 mg nicotine/cig (range 8.2–24.1). The amount of nicotine in VLN™ was 0.27 mg/cig. Figure 2 illustrates the differences between VLN™ products and the top 100 brand styles sold in the U.S. on a per-cigarette basis. The smoke from the cigarettes was also analyzed under ISO smoking conditions to see if design differences might affect the theoretical amount of nicotine delivered to the smoker. The average nicotine yield was 0.903 mg/cig (range: 0.38–1.82) for the top 100 brand styles. VLN™ cigarettes yielded 0.03 mg nicotine/cig under ISO smoking conditions.

Table 3. Nicotine content and yield.

Product	ISO smoke analysis (mg/cig)			Filler analysis		
	"Tar"	Nicotine	CO	Nicotine (mg/g)	Nicotine (mg/g) dry weight basis	Nicotine (mg/cig)
VLN King	7.12	0.030	11.3	0.41	0.47	0.27
VLN Menthol King	6.90	0.030	11.0	0.41	0.47	0.27
Camel Blue King	9.84	0.837	10.9	17.2	19.7	11.7
Marlboro Gold King	8.97	0.670	10.2	15.9	18.2	10.3
Marlboro Menthol Gold King	9.44	0.741	9.9	16.6	19.0	10.5
Marlboro Red King	14.00	0.956	13.3	15.8	17.4	11.0
Marlboro Special Blend Gold King	8.87	0.675	9.9	15.9	18.1	10.2
Newport Menthol Green King	15.40	1.080	15.1	17.9	20.3	12.0
Camel 99 Blue	12.30	0.890	11.9	17.3	19.5	13.6
Camel 99 Full Flavor Bx	16.30	1.230	15.2	16.8	18.9	13.2
Camel Crush King	11.90	0.941	12.2	17.2	19.7	10.8
Camel Crush Menthol King	13.50	1.070	12.5	17.5	20.0	10.9
Camel Crush Menthol Silver King	11.90	0.916	12.1	17.0	19.2	10.9
Camel Full Flavor Filter King	15.10	1.210	14.1	17.3	19.7	12.2
Winston White King	4.50	0.383	6.8	16.1	18.3	10.4
Newport Menthol Smooth Select 100	12.30	1.010	13.6	17.8	20.3	13.6
Camel Turkish Blend Gold King	9.64	0.727	11.5	15.6	17.4	9.3
Camel Turkish Blend Jade Silver King	9.69	0.755	10.4	16.6	18.3	9.6
Camel Turkish Blend Royal Filter King	14.10	1.000	13.7	16.4	18.8	10.2
Camel Wides Red King	17.50	1.220	18.3	17.7	20.1	14.6
Eagle 20'S Red 100	14.40	0.996	16.9	17.3	19.6	12.1
Kool Blue King	10.10	0.762	10.5	16.8	19.2	10.5
Kool Green 100	13.50	1.000	14.8	17.1	19.6	12.3
Kool Menthol King	14.80	1.050	14.1	17.0	19.4	11.3
L&M 100	11.70	0.707	14.5	13.2	14.7	9.3
L&M 100 Menthol	14.10	1.020	16.5	17.5	20.0	12.5
L&M Blue 100	9.11	0.599	13.0	13.9	15.9	9.8
L&M Blue King	9.15	0.584	12.1	13.9	15.7	8.2
L&M Bold Menthol 100	17.20	1.220	16.7	17.7	20.1	13.2
L&M Full Flavor King	12.10	0.678	13.0	13.5	15.2	9.0
L&M Menthol King	13.60	0.973	13.7	18.6	21.2	12.5
Marlboro 72 Gold	9.12	0.675	9.6	16.8	19.2	9.8
Marlboro Menthol Blue King	11.20	0.802	12.1	17.8	20.3	10.9
Marlboro 72 Menthol Green	14.00	0.863	13.3	17.7	20.3	10.0
Marlboro 72 Red	12.10	0.812	11.6	15.7	17.9	9.3
Marlboro 72 Silver	5.34	0.488	6.3	20.2	23.0	10.2
Marlboro 83	14.20	0.985	13.3	16.4	18.7	11.5
Marlboro Black Menthol 100	16.00	1.100	16.3	16.6	18.8	11.8
Marlboro Black Menthol King	15.10	0.959	14.4	16.2	18.6	10.1
Marlboro Blend 27 King	11.90	0.829	12.7	16.0	18.0	9.8
Marlboro Edge King	15.00	0.987	14.4	16.2	18.5	11.0
Marlboro Gold 100	8.72	0.679	10.8	16.2	18.5	11.9
Marlboro Green (Menthol) King	14.40	0.980	13.5	16.9	18.9	11.8
Marlboro Menthol 100	14.50	1.020	15.6	17.3	19.7	13.3
Marlboro Menthol Gold 100	9.09	0.731	11.3	17.4	19.8	12.9
Marlboro Midnight Menthol King	15.50	0.980	14.0	16.4	18.3	10.7
Marlboro Nxt King	8.08	0.664	7.8	16.3	18.2	9.4
Marlboro Red 100	15.30	1.090	15.4	16.7	18.8	12.8
Marlboro Red Label 100	13.10	0.952	13.5	16.5	18.8	12.0
Marlboro Red Label King	14.40	0.978	13.5	16.4	18.7	10.9
Marlboro Silver 100	4.50	0.404	6.5	16.9	19.3	11.6
Marlboro Silver King	4.97	0.424	6.5	17.3	19.7	10.2
Marlboro Silver Menthol 100	5.91	0.493	8.3	17.1	19.4	12.3
Marlboro Silver Menthol King	5.13	0.427	6.7	16.9	18.9	10.3
Marlboro Smooth Menthol 100	13.00	0.952	14.4	17.7	20.2	12.7
Marlboro Smooth Menthol King	14.20	0.936	14.2	17.4	19.9	10.4
Marlboro Southern Cut King	15.50	0.995	13.9	16.4	18.5	10.8

Table 3. Continued

Product	ISO smoke analysis (mg/cig)			Filler analysis		
	"Tar"	Nicotine	CO	Nicotine (mg/g)	Nicotine (mg/g) dry weight basis	Nicotine (mg/cig)
Marlboro Special Blend Black 100	14.80	1.000	15.3	16.8	19.0	12.2
Marlboro Special Blend Black King	14.60	0.938	14.1	16.7	18.5	10.3
Marlboro Special Blend Black Menthol 100	15.40	0.994	16.0	16.3	18.5	11.8
Marlboro Special Blend Black Menthol King	15.20	0.978	14.0	16.9	19.3	10.5
Marlboro Special Blend Gold 100	9.90	0.766	12.3	17.0	19.4	12.6
Marlboro Special Blend Red 100	12.80	0.892	13.5	16.4	18.6	12.0
Marlboro Special Blend Red King	10.80	0.750	11.1	16.3	18.4	10.1
Maverick Full Flavor 100	14.20	1.000	15.3	15.2	17.3	12.2
Maverick Gold 100	7.76	0.584	9.4	15.5	17.7	12.2
Maverick Menthol 100	16.60	1.160	16.2	15.7	17.8	13.2
Misty 120 Blue	11.60	1.020	10.8	16.2	18.4	14.0
Misty 120 Menthol (Green)	10.20	0.809	10.1	14.8	16.9	12.4
Natural American Spirit Blue King	17.00	1.820	13.0	21.9	24.8	18.6
Natural American Spirit Menthol Green King	16.40	1.780	12.8	24.3	27.2	20.2
Natural American Spirit Organic Mellow Gold King	7.72	1.110	9.7	29.3	33.2	23.8
Natural American Spirit Organic Turquoise King	14.00	1.820	12.0	28.9	32.6	24.1
Natural American Spirit Yellow Mellow King	13.10	1.410	11.5	22.6	25.6	18.9
Newport Menthol Gold 100	12.00	0.889	12.0	17.8	19.8	14.2
Newport Menthol Gold King	8.38	0.662	9.2	18.6	21.2	11.4
Newport Menthol Green 100	18.20	1.500	15.5	18.9	21.4	14.8
Newport Non-Menthol 100	18.80	1.490	15.9	18.8	21.4	15.0
Newport Non-Menthol King	14.90	1.050	15.2	18.3	20.7	11.8
Pall Mall Black Menthol 100	14.50	1.110	14.2	17.6	19.6	12.4
Pall Mall Blue 100	10.20	0.854	10.3	16.0	18.2	12.8
Pall Mall Blue King	10.20	0.828	9.9	16.3	18.5	11.0
Pall Mall Menthol 100	10.40	0.838	11.0	15.9	18.0	13.0
Pall Mall Menthol King	10.40	0.819	10.9	15.9	17.9	10.9
Pall Mall Orange 100	5.04	0.438	7.0	15.8	17.8	12.9
Pall Mall Orange 85	5.01	0.435	7.1	16.0	18.1	10.7
Pall Mall Red 100	14.10	1.090	13.2	16.1	18.3	14.1
Pall Mall Red King	13.80	1.080	11.3	16.0	18.1	12.3
Pall Mall White Menthol 100	7.89	0.661	9.5	18.5	21.0	13.4
Parliament White King	8.40	0.579	9.7	16.8	19.1	9.8
Pyramid Blue 100	8.90	0.693	11.3	15.8	17.1	10.8
Pyramid Red 100	14.50	0.971	15.7	17.2	19.4	12.1
Salem Green King	15.70	0.958	15.5	16.1	18.2	11.1
Salem Menthol Gold King	8.14	0.595	10.8	16.2	18.4	10.1
Salem Slim Menthol 100	6.92	0.570	7.9	15.9	18.0	10.1
USA Gold King	10.40	0.699	11.7	17.7	19.8	9.4
USA Gold Red 100	14.20	1.030	13.9	18.7	21.2	11.9
USA Gold Red King	12.50	0.902	11.8	18.1	20.6	10.3
Winston Gold 100	9.19	0.757	12.8	16.8	19.1	12.3
Winston Gold King	9.33	0.760	11.1	17.2	19.5	10.9
Winston Red 100	13.70	1.100	15.0	17.7	19.3	13.0
Winston Red King	12.90	0.983	13.6	17.2	19.6	12.3
Mean	11.96	0.903	12.37	17.2	19.4	12.0
SD	3.38	0.276	2.66	2.3	2.6	2.6
Min	4.50	0.380	6.26	13.20	14.70	8.20
Max	18.80	1.820	18.30	29.30	33.20	24.10
VLN King	7.12	0.030	11.3	0.41	0.47	0.27
VLN Menthol King	6.90	0.030	11.0	0.41	0.47	0.27

Figure 3 graphically presents the differences between VLN™ cigarettes and the top brand styles. On a per-cigarette basis VLN™ cigarettes contained 0.27 mg of nicotine in the tobacco and yielded 0.03 mg of nicotine in the smoke, a conversion efficiency of 11.1%. The commer-

cial cigarettes contained on average 12 mg of nicotine per cigarette and yielded 0.903 mg of nicotine in smoke, a conversion efficiency of 7.5%. These differences in efficiency are not unexpected since yield is dependent on the amount of tobacco, the type of filter, and the amount of

Figure 1. Comparison of nicotine content in tobacco filler on a dry weight basis.

Figure 2. Comparison of nicotine content on a per-cigarette basis.

Figure 3. Comparison of nicotine in smoke on a per-cigarette basis.

ventilation. VLN™ cigarettes contain about 650 mg of tobacco which is consistent with the amount of tobacco normally used in conventional king size cigarettes. Some of the marketed cigarettes in the top 100 list were 100's² which generally contain more tobacco and generally have slightly higher "tar" yields on a per-cigarette basis. Most commercial filter cigarettes in the U.S. use a single cellulose acetate filter with or without added carbon. The filter used in VLN™ was a dual filter made up of a cellulose acetate segment on the mouth end and a crepe paper filter on the tobacco end. This different filter design and different degrees of ventilation (ventilation was not measured in this study for the commercial cigarettes) may account for some of the differences in the nicotine yield.

"Tar" and carbon monoxide were also measured in the cigarette smoke. The average "tar" yield in smoke for the top brand styles was 11.96 mg/cig (range 4.5–18.0). The average "tar" yield for VLN™ was 7 mg/cig. "Tar" yield is dependent on the amount of tobacco, the type of filter, and the amount of ventilation which may be different between VLN™ cigarettes and the top brands. The average CO yield for the top brands was 12.4 mg/cig (range 6.3–18.3). The average CO yield for VLN™ was 11.2 mg/cig. The amount of CO in smoke is determined mostly by the amount of ventilation in the tipping paper.³ VLN™ uses a tipping paper with about 13% ventilation. The amount of ventilation in commercial cigarettes can range from 0 to 70% (18).

DISCUSSION

VLN™ cigarettes are substantially lower in nicotine than any other cigarettes currently sold to consumers in the United States - whether measured on a cigarette, tobacco dry weight, or cigarette smoke yield basis. VLN™ cigarettes contain a target level of 0.5 mg of nicotine/g of tobacco filler (dry weight). The actual measured amount was 0.47 mg of nicotine/g of tobacco. Analysis of the top 100 leading cigarette brands in the U.S. showed that, on average, these cigarettes contained 19.4 mg of nicotine/g of tobacco on a dry weight basis. Accordingly, the nicotine content of VLN™ cigarette tobacco is 97.6% lower than the average nicotine content of the tobacco filler in the top 100 leading brands. When compared to the lowest nicotine content product of any of the top 100 leading US brands, "L&M 100", the nicotine content of VLN™ is 96% lower. When compared on a cigarette basis, the top 100 brands average 12.0 mg of nicotine and VLN™ averages 0.27 mg. Therefore, without exception, VLN™ cigarettes contain at least 95% less nicotine than the top 100 brands in the U.S.. Similarly, the amount of nicotine in smoke (yield) for VLN™ cigarettes is 0.03 mg/cig, which is also 97% lower than the top 100 cigarette brand style's nicotine smoke yields (0.903 mg/cig). Because of the unique technology

used to reduce nicotine and the absence of the technology in any other commercial cigarette on the market, it is believed that the nicotine level in VLN™ is reduced by at least 95% when compared to all other cigarettes available in the U.S. marketplace irrespective of the comparison basis.

Clinical studies indicate that use of VLN™ cigarettes can lead to substantial reductions in nicotine exposure (19–23) as a result of the lower nicotine delivery and also due to an overall reduction in cigarettes consumed per day (20, 23–27). In the longest (20 weeks) and largest (1,250 subjects with no intention to quit) study to date on SPECTRUM®/VLN™ cigarettes, HATSUKAMI *et al.* observed more than a 50%-reduction in cigarettes per day (28). The "gold standard" for measuring reduced nicotine exposure is the amount of total nicotine equivalents (TNE) in the urine. HATSUKAMI *et al.* observed a 57% reduction in TNE in the 20-week study with SPECTRUM®/VLN™ cigarettes. There is clear evidence that consumption of cigarettes that have very low levels of nicotine in the tobacco will result in reductions in the nicotine in the smoke and the smoker will be exposed to significantly lower levels of nicotine as indicated by the total nicotine equivalents in the urine. This is due to reduced nicotine intake and cigarette consumption over the duration of use. KAMENS *et al.* (29) compared the pharmacokinetic profile of SPECTRUM®/VLN™ cigarettes to usual brand after a single use in twelve daily cigarette smokers. Nicotine area under the curve was reduced by 95%. In most clinical studies with VLN™ there is a tendency for subjects to "cheat" and use non-study cigarettes (30–32). This probably accounts for the only 57% reduction in TNE observed by HATSUKAMI *et al.* after 20 weeks of use compared to the 95% reduction in nicotine plasma levels after single use under confined circumstances. There is very little nicotine in VLN™ cigarettes and compensation has not been observed after long term use (19, 20, 21, 23, 28).

Premarket Tobacco Applications (PMTA) and Modified Risk Tobacco Applications (MRTPA) were submitted to the United States Food and Drug Administration for VLN™ cigarettes in December of 2018. Section 911(h)(2)(B) of the Tobacco Control Act states that the Secretary may require that the percent (or fraction) of change and identity of the reference tobacco product and a quantitative comparison of the amount of the substance claimed to be reduced shall be stated in immediate proximity to the most prominent claim. The principal reduced exposure claim for the VLN™ King and VLN™ Menthol King products will be "95% Less Nicotine". This claim is based on the nicotine content of the filler in the cigarette irrespective of the basis of comparison (per g of tobacco filler, per g of tobacco filler calculated on a dry weight basis, or per cigarette). The tobacco filler target nicotine level is 0.5 mg nicotine/g of tobacco filler (dry weight). The top 100 brand styles in the U.S. were identified and analyzed. These results demonstrated that VLN™ cigarettes had reduced nicotine levels of 95% less in the tobacco filler and smoke compared to the top 100 brands representing over 80% of all cigarettes sold in the U.S. Because of the unique technology used to reduce nicotine and the absence of the technology in any other cigarette on the market, it is believed that the nicotine level in VLN™ is

² King size cigarettes are generally considered to be about 85 mm long and 100's are about 100 mm long. While these terms are used as general indicators of the length of the cigarettes, manufacturers may make cigarettes with slightly different lengths that are sold as belonging to either the category "Kings" or "100's".

³ The focus of this research was the nicotine content and nicotine yield of the cigarettes. Ventilation was not measured.

reduced by at least 95% when compared to all other cigarettes in the marketplace and a comparator statement such as “leading brands”, “usual brand”, “typical brands”, or “top three brands” is not required. Analysis of the smoke yield of VLN™ cigarettes also demonstrated that the amount of nicotine in smoke was reduced at least 95% also.

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