# NICOTINE AND TOBACCO-SPECIFIC NITROSAMINE VARIATION IN FILLER FROM INDIVIDUAL CIGARS

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## Introduction

Even with the recent 2016 deeming of cigars by the US Food and Drug Administration (FDA) and the increase in interest in cigar science, few published studies exist on HPHCs in cigar filler and the cigar-to-cigar variability of HPHCs in cigar tobacco filler. Cigar construction inherently has more variation than cigarettes due to a wide range of filler, binder, and wrapper tobacco options. Long filler cigars are inherently non-uniform since they are constructed from individual leaves of tobacco. Cigar tobaccos are also grown and cured to produce a variety of specific smoking characteristics. Soil type and fertilization rate during the growth process may influence nicotine and TSNA formation. The curing process also has an effect on nicotine content and TSNA formation.<sup>2</sup> In this study we attempted to quantify how these (and other) factors affect the overall variability of nicotine and TSNAs (NNN, NNK, NAT, NAB) in cigar filler on a per cigar basis, from both machine-made and handmade cigars. Eight total brands were analyzed and products included both natural and reconstituted wrappers with cut filler and long-leaf style cigars.



Brand	Cigar Name	Avg Weight (g)	Avg Nicotine Content (mg/cig)	Avg Total TSNA Content (µg/cig)	Construction
1	Outlaws Double Barrel Rum™	2.30	35.2	45.4	Hand-rolled
2	Quorum Shade Churchill™	13.5	245	585	Hand-rolled
3	Gurkha Warpig Robusto™	14.5	232	813	Hand-rolled
4	White Owl Grape™	2.41	17.9	24.0	Machine- made
5	Game BLUE – Natural Leaf™	2.40	20.7	18.4	Machine- made
6	Black & Mild JAZZ™	2.54	21.8	20.2	Machine- made
7	Backwoods Sweet Aromatic™	2.69	45.9	31.6	Machine- made
8	Phillies Blunt™	7.02	62.4	113	Machine- made

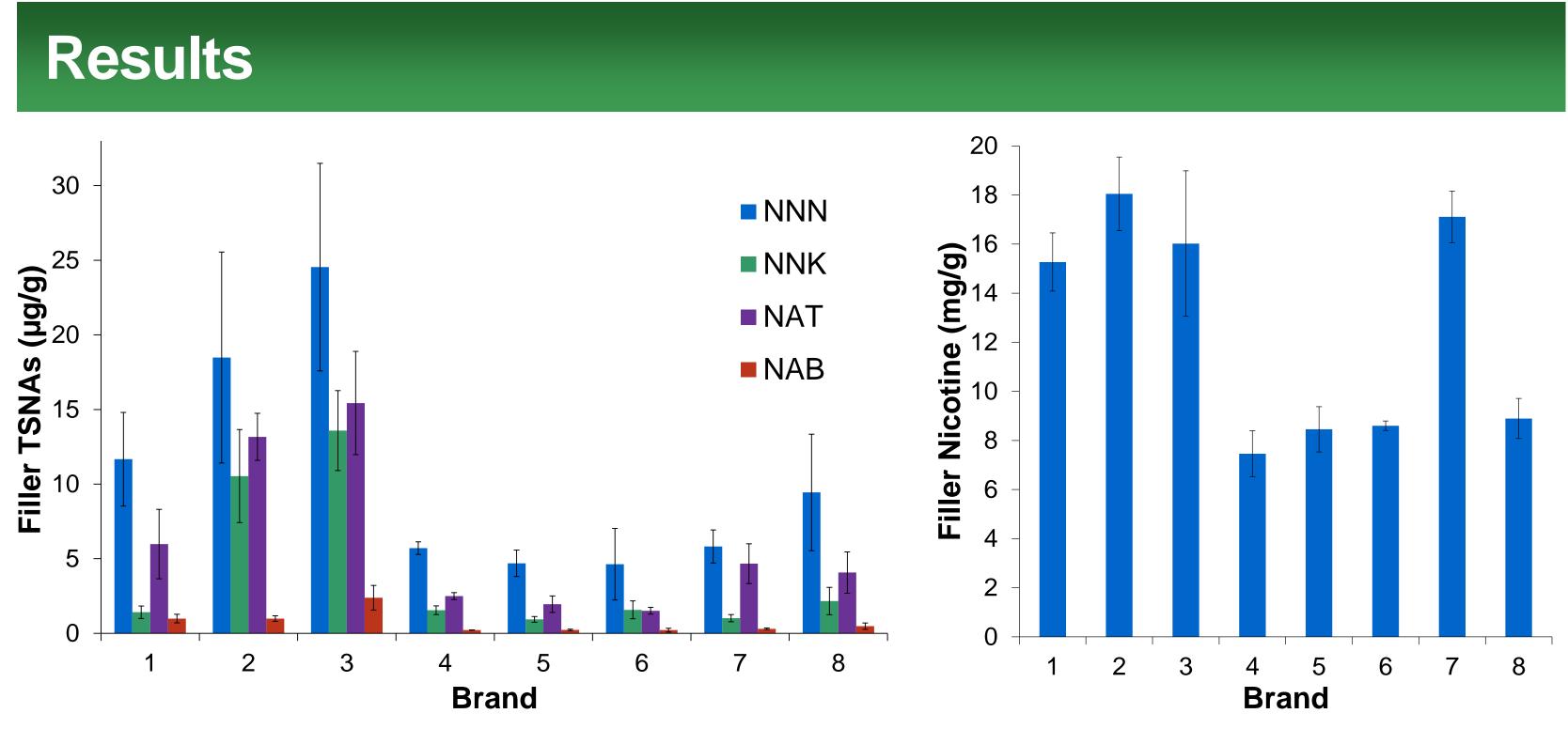
**Figure 1.** Sample pictures and legend describing sample names, weights, average nicotine and TSNA content, and construction type.

## Methods

Ten cigars from each brand were individually conditioned by CORESTA Recommended Method (CRM) 46, weighed, and ground. Tobacco from each cigar was analyzed in triplicate for nicotine, NNN, NNK, NAT, and NAB content. Analytical results were reported on a per gram basis and corrected to a per cigar basis using the original cigar weight.

Nicotine was extracted in MTBE and analyzed with a GC-FID utilizing the procedure outlined in CRM 62.

TSNAs were extracted in a 100 mM ammonium acetate solution, filtered, and analyzed via LC-MS/MS utilizing the procedure outlined in CRM 72.



**Figure 2.** Average TSNA and nicotine levels presented per gram of filler. The error bars represent one standard deviation for the 10 cigars tested.

Wrapper

Filler

Table 1. Nicotine vs. total TSNA levels displayed for overall brand averages. No strong correlation was found between nicotine and individual or total TSNA levels in these cigars.

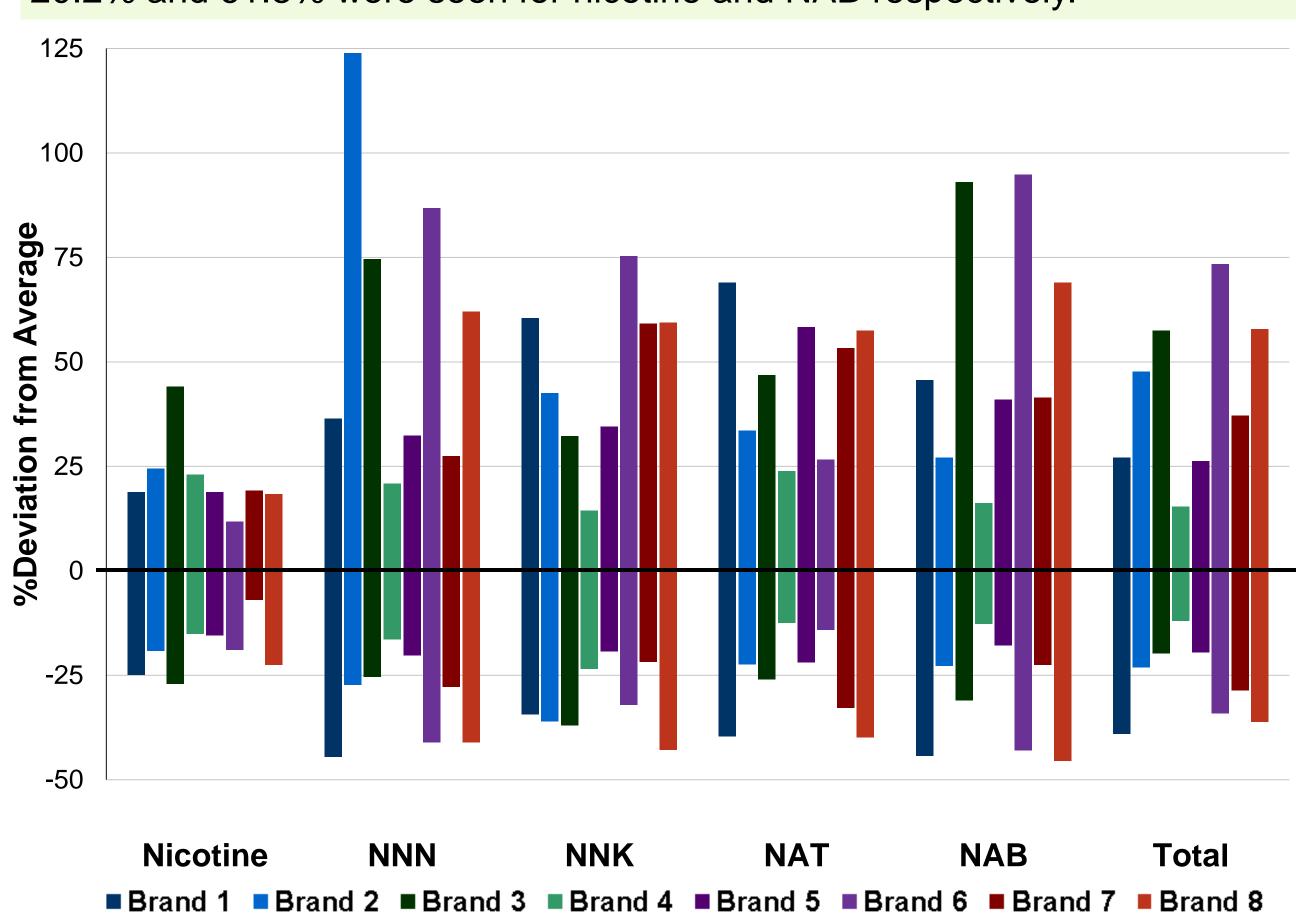
				(mg/g)	Dev	(µg/g)	Dev
	1	Natural	Cut Leaf	15.3	1.18	20.1	5.25
	2	Natural	Long Leaf	18.1	1.50	43.2	7.39
	3	Natural	Long Leaf	16.0	2.96	55.9	12.0
	4	Reconstituted	Cut Leaf	7.46	0.93	9.98	0.44
	5	Natural	Cut Leaf	8.46	0.92	7.80	1.50
	6	Reconstituted	Pipe Tobacco	8.60	0.19	7.93	3.29
	7	Natural	Cut Leaf	17.1	1.05	11.8	2.43
	8	Reconstituted	Cut Leaf	8.89	0.82	16.2	6.34

Nicotine Std

# Discussion

No strong correlation was seen between nicotine and TSNA levels. The premium cigars tested did have higher overall amounts of nicotine and TSNAs, both per gram and per cigar, but TSNA formation does not appear to be influenced strongly by the amount of nicotine in the filler leaves. Tobacco leaves chosen for nicotine content alone do not appear to fall within any sort of anticipated TSNA ranges. Product consistency does seem to correlate with expected variability as the natural wrapper and/or long-leaf filler variants produced the most inconsistent results seen for this study. On a filler basis, nicotine averages ranged from 7.46 - 18.0 mg/g and the brands returned RSD values of 2.25 - 18.5% (avg = 9.5%). Total TSNA averages ranged from

7.80 – 55.9 ug/g and brand RSD values were 4.42 – 41.2% (avg = 23.7). Converting filler values to per cigar values provides the most accurate estimation of overall variability as it includes weight fluctuation. This is especially important for cigar smoke data as typically only one cigar is analyzed per port due to the large amount of particulate matter generated. On a per cigar basis, RSD values for all brands rose to an average of 12.3% for nicotine and 24.2% for total TSNAs. Individual brand RSD values of 20.2% and 61.3% were seen for nicotine and NAB respectively.



**Figure 3.** The maximum and minimum deviations from average shown for each brand. Each bar represents the overall range obtained during analysis for each analyte.

### Conclusion

Total

Std

Cigars are highly variable products, even at the filler stage. Nicotine ranges were as high as 71% of brand average and in the case of TSNAs, several ranges were larger than the average itself. In this study, the cigars with long filler construction had the most overall analyte variation and the highest nicotine content. The increased nicotine content did not necessarily correlate to an increased amount of TSNA formation as other cut leaf style cigars had similar nicotine concentrations but less TSNA formation and similar variability. A higher nicotine concentration in a given cigar could potentially facilitate greater TSNA development but nitrosamine formation appears to be influenced more by factors in the tobacco growing conditions, curing method, cigar design, and blend selection. Growth and curing conditions appear to affect TSNA formation in individual tobacco leaves differently as the least homogenized products in this study had the most variability.

#### References

<sup>1</sup>Analysis of Variability in Curing Conditions and TSNA within Barns of Dark Air-Cured Tobacco. Richmond, M; Bailey, W; Goff, B; Pearce, R. Tobacco Science, Vol 54. Jan 2017.

<sup>2</sup>Role of Oxides of Nitrogen in Tobacco-Specific Nitrosamine Formation in Flue-Cured Tobacco. Nestor, et al. Beiträge zur Tabakforschung International, Vol 20, No 7. Nov 2003.